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Research paper

Age-related changes in survival and turnover rates of balsam fir (*Abies balsamea* (L.) Mill.) fine roots

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Fine-root (≤2 mm) demographics change as forests age, but the direction and extent of change are unknown. Knowledge of the change and understanding of causes will improve predictions of climate change impacts. We used minirhizotrons at three young and three mature balsam fir (*Abies balsamea* (L.) Mill.) sites to measure median lifespan (MLS) for each site and for annual cohorts. We computed turnover rate from the inverse of MLS (T_{inv}) and calculated a second turnover rate (T) from annual mortality, annual production and previous year-end standing crop. Median lifespan at mature sites (436 days) was half that at young sites (872 days). Median lifespan of annual cohorts varied widely at all sites. Age-class distributions of fine roots seen by minirhizotrons changed with increasing years of observation, with older age classes accumulating more slowly at mature sites. Our findings highlight the need to determine whether the proportional contributions of absorbing and transporting fine roots to annual production and their median lifespans change during stand development. Due to its variation among annual cohorts, we believe robust estimates of MLS at our sites require 5–7 years of observation, and reliable estimates of T_{inv} are reached earlier than T.

Keywords: carbon cycling, fine-root mortality rate, median lifespan, minirhizotron, root ecology.

Introduction

Production of fine roots comprises a substantial fraction of annual net primary production in terrestrial ecosystems, and their death contributes significantly to carbon (C) and nutrient cycling (Gill and Jackson 2000, Matamala et al. 2003, Hendricks et al. 2006, Yuan and Chen 2012, McCormack et al. 2015*b*). Moreover, impacts of changing climate on fine-root dynamics affect C sequestration and ecosystem functioning (Norby and Jackson 2000) to extents that remain uncertain (Smithwick et al. 2014, McCormack et al. 2015*a*). Focusing on C sequestration in soils, mortality of fine roots is an important detrital input, annually contributing an amount similar to that of foliage (Gill and Jackson 2000). Despite its importance, current understanding of environmental and intrinsic effects on fine-root mortality is limited (McCormack and Guo 2014), and data do not exist for many tree species (McCormack et al. 2013, Iversen et al. 2017).

Efforts to understand fine-root mortality usually focus on its rate to avoid the confounding effect of differing standing crop sizes when comparing sites, years or experimental treatments (Lauenroth and Gill 2003, Majdi et al. 2005, Yuan and Chen 2012, Chen and Brassard 2013). In addition, ecosystem modelers require these rate variables (McCormack et al. 2013, 2015*a*, Smithwick et al. 2014). Median lifespan (MLS) and turnover rate are commonly used to quantify annual fine-root mortality rates (Eissenstat and Yanai 1997, Lauenroth and Gill 2003, McCormack et al. 2014). Median lifespan is the time taken for half of fine roots to die. Turnover rate is quantified in several ways (McCormack et al. 2014): most use annual mortality divided by a measure of

standing crop, and one is calculated as the inverse of MLS. Values of turnover rates vary in the first approach due to differences in the standing crop parameter used in their denominator, but when used with the appropriate measure of standing crop, each should give similar estimates of annual mortality. Median lifespan and turnover rate calculated as the inverse of MLS are the mortality rate variables most commonly used with minirhizotron data.

Early studies of root systems defined fine roots simply by a diameter threshold and assumed their functional role to be absorbing water and nutrients, but this view proved too simplistic. A portion of fine roots uptake water and nutrients, but the primary function of other fine roots is transport (see review by McCormack et al. 2015*b*). Transport fine roots tend to have larger mean diameters than absorbing fine roots, but there is also much overlap in their ranges of diameters (McCormack et al. 2015*b*). The branching structure of roots also affects their functional role; as a result, first- to third-order fine roots predominantly function as absorbing roots, whereas higher order fine roots function predominantly in a transport role (Eissenstat et al. 2000, Pregitzer 2002, Guo et al. 2008, McCormack et al. 2015*b*). The morphology and chemical composition of absorbing roots contribute to them being more vulnerable to environmental stresses and more susceptible to grazing and, therefore, short lived, whereas transporting roots are more robust, contributing to their being more long lived (Eissenstat and Yanai 1997, Withington et al. 2006, Xia et al. 2010, McCormack et al. 2015*b*). In support of this distinction, some studies reported smaller diameter fine roots having shorter lifespans than larger diameter fine roots (Baddeley and Watson 2005, Gu et al. 2011, Hansson et al. 2013). Other studies reported shorter lifespans for fine roots of low branching order than for those of a higher order (Guo et al. 2008, Xia et al. 2010, Gu et al. 2011, Sun et al. 2012). Lastly, studies using isotopic ratios identified short-lived and long-lived pools of fine roots (Matamala et al. 2003, Joslin et al. 2006, Gaudinski et al. 2010, Keel et al. 2012, Ahrens et al. 2014). This view of fine roots suggests separate estimates of MLS and turnover rates for absorbing and transporting fine roots.

Median lifespan and turnover rate vary due to several endogenous and exogenous causes (Chen and Brassard 2013). For example, MLS or turnover rate varied among years in multiyear studies due to climatic variation (Anderson et al. 2003, Kern et al. 2004, Fukuzawa et al. 2013). They also varied among sites occupied by similar vegetation in some studies (Burton et al. 2000, Finér et al. 2011, Olesinski et al. 2012*a*, McCormack and Guo 2014) but not in others (Pinno et al. 2010) and they varied among studies of a species (for example, Peek (2007) found three reports of fine-root MLS for *Picea abies* (L.) Karst. ranging from 256 to 730 days). Moreover, MLS and turnover rate responded to treatments in some manipulative studies (Johnson et al. 2000, Kern et al. 2004, Majdi and Öhrvik 2004, Leppälammi-Kujansuu et al. 2014, Kou et al. 2017) but not in others (Tingey et al. 2000, Norby et al. 2004, McCormack et al. 2010, Repo et al. 2014). This variety of findings provides opportunities to learn about environmental and endogenous regulation of mortality rate (for example, see reviews by Gill and Jackson 2000, Wells and Eissenstat 2002, Guo et al. 2008, Chen and Brassard 2013) and illustrates the challenge of finding representative values of MLS or turnover rate for species included in some ecosystem models (see discussions in Smithwick et al. (2014) and McCormack et al. (2013)). The duration of most minirhizotron studies is only 2–3 years and, as a result, they risk poorly estimating mortality rates if longer-lived fine roots viewed by the minirhizotron underrepresent the population in the bulk soil, or if environmental factors deviated widely from their norms during the few observation years. Longer-term studies are needed to better assess interannual variation of turnover rates and address concerns about the accuracy of estimates from short-term studies.

Better understanding of changes in fine-root turnover during stand development can improve predictions of C sequestration and responses to changing climate (Yuan and Chen 2012). Some studies found turnover rates varied with tree age, but their findings are inconsistent. Finér et al. (2011) reported fine-root turnover rate declined with stand age in their meta-analyses, whereas Baddeley and Watson (2005) found no difference in fine-root turnover between young and old wild cherry (*Prunus avium* L.) trees. Olesinski et al. (2012*b*) found turnover rates increased with stand age for a chronosequence of balsam fir (*Abies balsamea* (L.) Mill.) sites, and Yuan and Chen (2012) found turnover rates increased with age in a boreal forest chronosequence.

We report on a longer-term study of fine-root dynamics using the minirhizotron method at balsam fir sites in eastern Canada. Earlier reports from these sites, using shorter time series, mainly investigated environmental and endogenous regulation of fine-root production (Olesinski et al. 2011, 2012*a*). Another study found differences in fine-root dynamics between young and mature stands (Olesinski et al. 2012*b*). We focus on fine-root mortality rate in the present investigation, using longer time series of minirhizotron observations than in earlier studies. Our first objective is determining whether fine-root MLS differs between young and mature balsam fir sites and discussing the implications of our findings to the two-pool approach. Our second objective is using long-term measurements to gain insights about factors affecting the accuracy and bias of mortality rate estimates, particularly those arising from interannual variability of mortality rate and from limitations of the minirhizotron method itself. We analyze mortality rates of annual cohorts at three young and three mature balsam fir sites with 6–11 years of minirhizotron data.

Materials and methods

Study sites

Minirhizotron observations of fine roots were collected at three young and three mature balsam fir sites (Table 1). Each site is occupied by an even-aged stand originating after harvest. Aerial extents of sites ranged from 4 to 60 ha. Olesinski et al. (2012*a*, 2012*b*) described these sites previously. Briefly, four

sites (Y1–3 and M1) are located in central New Brunswick, M2 is located in northwestern New Brunswick, and M3 is in central Quebec. These three geographic areas have distinctly different climates. M3 is located on a steep slope (20–30%), and all other sites are on modest slopes (~5%). Aspects vary among sites. Soils at all sites are moist, well-drained podzol soils. Balsam fir dominated overstories at all sites, representing >90% of overstories. All forests regenerated naturally after clearcutting, and understory vegetation was sparse at all sites. M1 was precommercially thinned 11 years before this study began, and the overstory was closed at the time this study began. M1 was commercially thinned in 2005 with removal of 30% of basal area, mostly from parallel trails ~5 m wide, spaced ~30 m apart. M2 was precommercially thinned in 1978. Y3 was precommercially thinned in autumn of 2003, immediately prior to beginning this study. Y1 was clearcut during late autumn of 2004.

Minirhizotron observations

Table 2 summarizes minirhizotron observations used in this study. Methods of collecting and analyzing minirhizotron observations were described in detail by Olesinski et al. (2011, 2012*a*, 2012*b*). Measurements used by Olesinski et al. are supplemented by measurements taken in 2010–12 using the same methods. Minirhizotron measurements are briefly described below. Five clear acrylic tubes (5.1 cm in diameter) were installed at M2 and M3 in 1997. Data were collected from 1998 to 2008 at M3. Data were collected without interruption from 2004 to 2012 at M2. Ten acrylic tubes were installed at M1, Y2 and Y3 in 2004 and at Y1 in 2005. This study uses only seven tubes installed in areas not disturbed by skid trails at Y1. One tube at each of Y2, Y3 and M1 were lost due to disturbance by wildlife or frost heaving. Measurements continued to 2010 at young sites and until 2012 at M1. Three to four tubes were located in the vicinity of each of three permanent sample plots (0.04 ha) established at each site. Images were collected in access tubes with a portable image acquisition system (Bartz Technologies Ltd, Santa Barbara, CA, USA) consisting of a digital camera and an indexed handle. Images were collected at 15-mm intervals along the tube and the indexed handle ensured images were collected from the same locations on every date. Images were collected monthly when tubes were accessible, generally from May to November. Digital images were analyzed with WinRhizotron MF 2005a (Regent Instruments, Quebec, QC, Canada). We recorded the location of roots intersecting acrylic tubes for roots ≤2 mm in diameter, and their diameters at the point of intersection. Dates of birth and death of fine roots were calculated as midpoints between the observation date at which they appeared or disappeared and the previous observation date. Observations from every image were combined to determine a total for the tubes, and tubes were added together to calculate a total for the site. Thus, our data consist of root numbers and their diameters at the point of intersection with the tube at each observation date, numbers of fine roots produced and dying during each measurement interval, lifespan of roots dying in each interval, and current lifespans of all living roots on each measurement date.

Annual fine-root production (often referred to as an annual cohort) and annual mortality are sums of all roots appearing (production) or disappearing (mortality) between one year-end measurement (November) and the next year-end measurement. Roots first appearing in images taken at our first observation of the year are assigned to the current year. Similarly, roots first disappearing as of the first measurement of the year are also assigned to the current year. Our measure of standing crop is fine roots existing at the last observation date of each year. This metric suits our modeling approach (Olesinski et al. 2012*a*), which uses an annual time step and maintains mass balance of standing crop, production and mortality from one year to the next.

Consistent with our modeling approach, we identify fine roots available to die in a year as including the standing crop existing at the end of the previous year plus fine roots produced during the year. Based on this, we calculate annual turnover rate (T) as

$$T_i = M_i/(SC_{i-1} + P_i) \quad (1)$$

where i is year, M is annual mortality, SC is year-end standing crop and P is annual production. In the first year of minirhizotron measurements, SC_{i-1} does not exist, so T is reported beginning in the second year.

Table 1. Locations and tree attributes at three young (Y) and three mature (M) balsam fir sites used in this study. Sites are located in central New Brunswick (Y1–Y3, M1), northwestern New Brunswick (M2) and central Quebec (M3). Tree measurements as of 2008.

Site	Location	Elevation (m)	Mean annual temp[1] (°C)	Age (years)	Average tree		Stand density (trees ha^{-1})
					DBH[2] (cm)	Height (m)	
Y1	46°27′42′N, 67°04′12′W	349	3.4	4	1.5	1.8	23,000
Y2	46°28′53′N, 67°07′01′W	322	3.4	19	4	4	39,500
Y3	46°28′53′N, 67°06′53′W	322	3.4	19	9	5	3000
M1	46°28′19′N, 67°05′60′W	350	3.4	41	16	13	2800
M2	47°44′10′N, 68°09′00′W	475	2.0	58	18	16	2300
M3	47°19′00′N, 71°06′00′W	700	0.3	78	17	17	2100

[1]1971–2000 normals for the nearest Environment Canada weather station.
[2]Diameter at breast height, measured 1.3 m aboveground.

Table 2. Summary of minirhizotron data at three young (Y) and three mature (M) balsam fir sites in eastern Canada.

Site	Observation years	No. of site-years	No. tubes	Total no. of roots observed
Y1	2005–2010	6	7	351
Y2	2004–2010	7	9	380
Y3	2004–2010	7	9	332
M1	2004–2012	9	9	582
M2	2004–2012	9	5	293
M3	1998–2008	11	5	367

Data analysis

Median lifespan, defined as the time taken for 50% of roots to die, for each site was estimated by the Kaplan–Meier method using R (R Development Core Team 2016). We calculate a turnover rate, referred to as T_{inv}, as the inverse of MLS. In addition, MLS and T_{inv} of annual cohorts were estimated by linear interpolation between measurement dates before and after passing the 50% mortality threshold, and variability among these values provided an uncertainty measure of site-level MLS. We assessed differences in T and T_{inv} between young and mature stands using linear mixed modeling with the lme4 package in R (Bates et al. 2017). Ecoclimatic region, mean annual temperature for each site-year and year of observation were included as random effects in initial runs. We made other simple comparisons of young and mature stands with *t*-tests or Mann–Whitney tests when the normality assumption failed, using Sigmastat v. 3.5 (Systat Software Inc., San Jose, CA, USA).

Results

The T_{inv} of fine roots at mature sites (0.84 year^{-1}) was two times faster than at young sites (0.42 year^{-1}) (Table 3). Linear mixed modeling found a significant difference in T_{inv} between young and mature sites ($t = 2.81$, $n = 29$, $P < 0.005$), whereas the random effect of ecoclimatic regions accounted for little variation among sites (s(ecoclimatic region) $<10^{-5}$). Similar to T_{inv}, MLS of fine roots at mature sites (436 days) was half that at young sites (872 days), and statistical comparisons are the same as for T_{inv} as one is the inverse of the other (Figure 1; Table 3). In mature stands, survival declined rapidly in the first 4 years for each annual cohort, reaching 0.15 ± 0.023 (SE) (Figure 2). It changed slowly in subsequent years (Figure 1d–f). In contrast, survival of annual cohorts at the end of their fourth year was higher in young stands than in mature stands ($P < 0.001$), averaging 0.38 ± 0.025 (SE), and continued declining in subsequent years more noticeably than in mature stands (Figure 1a–c). Similar to T_{inv}, T was significantly greater ($t = 4.22$, $n = 38$, $P < 0.0001$) at mature sites (0.33 ± 0254) than at young sites (0.19 ± 0.034) and the random effect of ecoclimatic region accounted for little of the variation between young and mature sites (Figure 3).

For the obvious reason that it is the only cohort in view, the current-year cohort accounted for all mortality in the first year of observation at each site, and the contribution of current-year roots to total mortality declined in subsequent years of observation (Figure 4). The decline in subsequent years of the contribution of current-year roots to annual fine-root mortality was greater in young than mature stands (Figure 4). For example, for the fifth year of observation and subsequent years, the current-year cohort contributions to annual mortality in mature stands (0.20 ± 0.04) was greater ($P = 0.022$) than that in young stands (0.064 ± 0.014). Similarly, for these later years, current-year cohorts were a larger proportion ($P = 0.004$) of standing crops in mature stands (0.46 ± 0.034) than in young stands (0.30 ± 0.025) (Figure 5). Annual cohorts older than 4 years begin contributing to mortality only in the fifth year of observation at each site, and their contribution tends to increase in the latter years (Figure 4). Beginning with the sixth year of observation, the contribution of cohorts living more than 4 years to annual mortality at mature sites (0.10 ± 0.020) was less ($P = 0.001$) than at young sites (0.26 ± 0.040) (Figure 4). Similarly, roots living more than 4 years were a lower ($P = 0.002$) proportion of year-end standing crops at mature sites (0.20 ± 0.022) than at young sites (0.34 ± 0.023) (Figure 5).

Table 3. Fine root median lifespans, estimated using the Kaplan–Maier method using minirhizotron observations at three young (Y) and three mature (M) balsam fir sites in eastern Canada. Years of observation ranged from 6 (Y1) to 11 years (M3).

Site	Lifespan (days)			Turnover (year^{-1})
	Median	Lower 95%	Upper 95%	
Y1	1116	982	1380	0.33
Y2	942	736	1102	0.39
Y3	730	668	792	0.50
M1	550	486	622	0.66
M2	521	464	581	0.70
M3	236	196	312	1.56

The range of annual T (highest–lowest) at 0.14 year^{-1} was least at Y1, and at 0.42 year^{-1} was greatest at M3 (Figure 6a). The coefficient of variation for T among years ranged from a low of 27% at Y3 to a high of 46% at Y2. Likewise, the range of MLS among annual cohorts (highest–lowest), at 465 days, was least at M3 and, at 703 days, was greatest at Y2. Coefficients of variation ranged from a low of 32% at Y2 to a high of 63% at M3 (Figure 6b).

Mean diameter of fine roots observed by minirhizotron was greater ($P = 0.003$) at young sites than mature sites (Figure 7a). There was no relationship between mean diameter of dying roots and their age at time of death (Figure 7b).

Discussion

Comparing T and T_{inv}

Estimates of T from the early years of minirhizotron observation may be biased because older age classes of fine roots are underrepresented in minirhizotron images (Figure 5). Older age classes

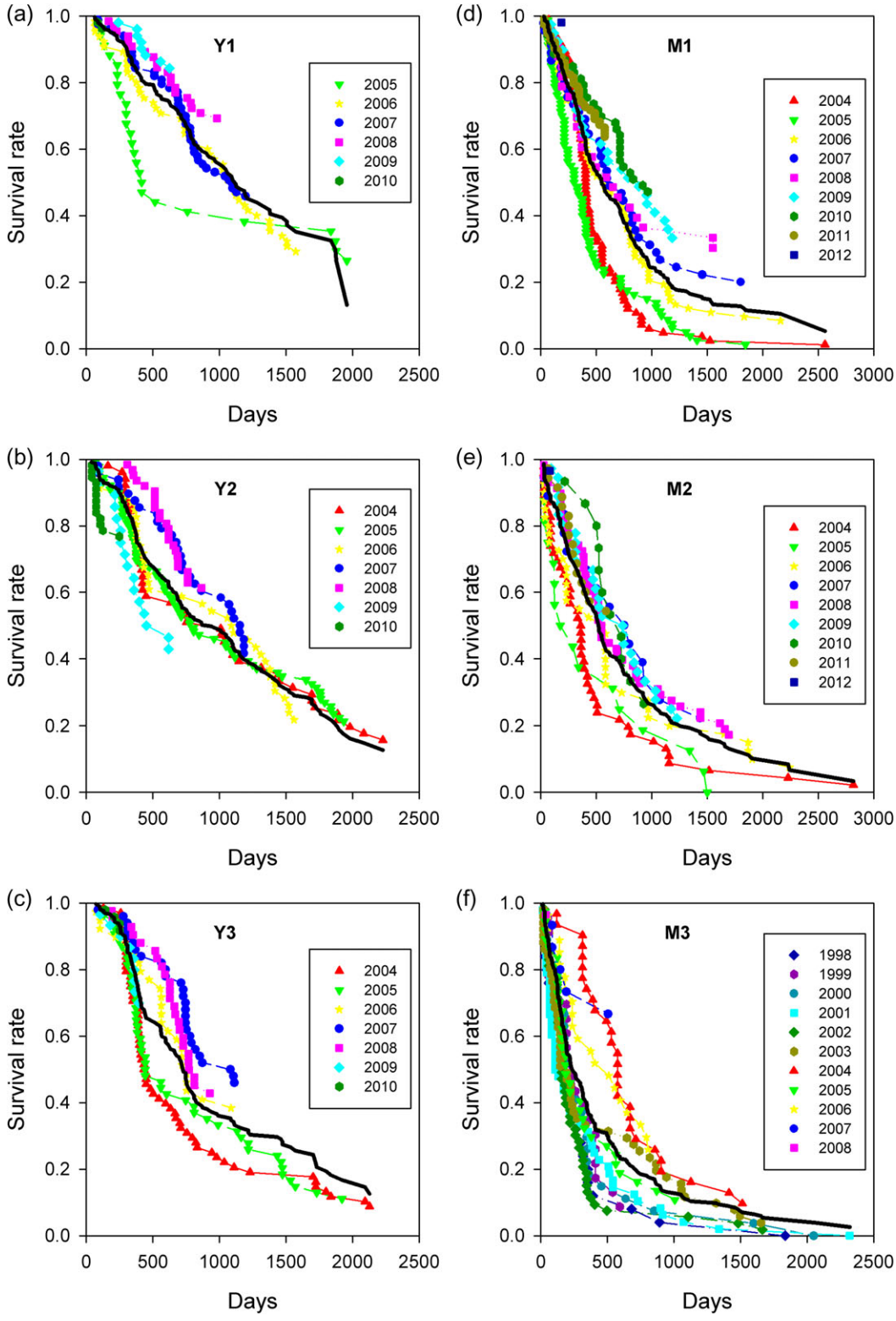

Figure 1. Survivorship of fine roots in yearly cohorts at three young (Y) and three mature (M) balsam fir sites in eastern Canada. Solid black lines depict results of Kaplan–Maier analysis of survival for all years combined.

of fine roots accumulated more rapidly in the standing crops of young sites than mature sites, as a result making greater contributions to annual mortality within a few years of observation (Figure 4). Hence, annual values of T at young stands may become representative of the site earlier than T at mature stands. An advantage of T is the ease of using it in ecosystem models. When T is known, for

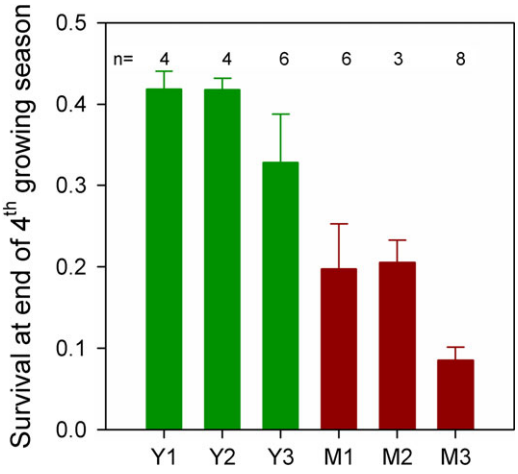

Figure 2. Cumulative mortality at the end of the fourth growing season averaged for cohorts defined by birth year at three young (Y) and three mature (M) balsam fir sites in eastern Canada. Capped bars represent one standard error of the mean (*n* is number of cohorts used to calculate the mean). A statistical comparison of young vs mature is reported in the text.

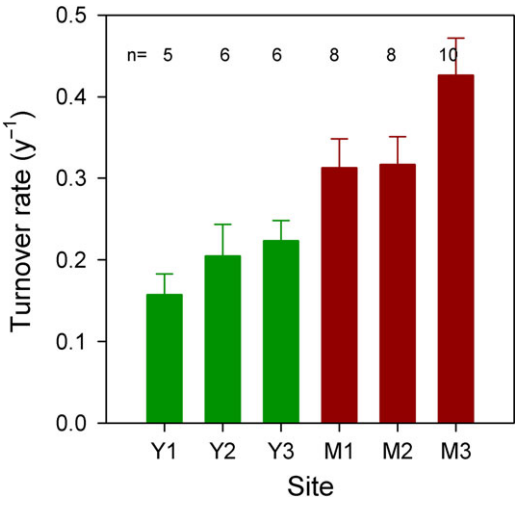

Figure 3. The average turnover rate (annual mortality)/(standing crop at the end of the previous year plus annual production) at three young (Y) and three mature (M) balsam fir sites in eastern Canada. Capped bars represent one standard error of the mean (*n* is the number of years for which *T* is averaged). A statistical comparison of young vs mature is reported in the text.

example from a minirhizotron study such as this one, and SC_{i-1} is known, for example from soil cores collected in late autumn or from the previous annual iteration of a model, estimating annual mortality is accomplished simply by inverting Eq. (1). Moreover, effects of environmental factors on annual values of *T* may account for much of the interannual variation in annual mortality.

In contrast to *T*, the age-class distribution of fine roots in view of minirhizotrons does not affect estimates of T_{inv}. Hence, the earliest estimates of T_{inv} are as valid as those in later years. A difficulty with T_{inv} is using it to estimate annual mortality of fine root biomass in field studies and in models. Unlike *T* and an inversion of Eq. (1) using measured or modeled SC_{i-1}, T_{inv} cannot be multiplied by a readily accessible measure of standing crop to estimate annual fine-root mortality. Moreover, identifying environmental influences on T_{inv} is more difficult than for *T*, because T_{inv} is based on mortality over variable time intervals that are often greater than 1 year, hence the low estimated random effects in this study. Most minirhizotron studies are of limited duration for practical reasons, giving an advantage to the use of T_{inv} and MLS for comparing sites or experimental treatments. However, *T* may be of more interest to modelers. An alternative approach that reduces the time taken to obtain valid estimates of *T* is using the minirhizotron method only to estimate population dynamics of the short-lived, absorptive subpopulation of fine roots, primarily of first to third branching order, and another method to estimate relative rates for the long-lived, transporting subpopulation (Ahrens et al. 2014, McCormack and Guo 2014, Smithwick et al. 2014). Recent advances in understanding of the roles of fine-root traits make this approach increasingly feasible (McCormack et al. 2012, 2015*a*, 2015*b*, 2017, Iversen et al. 2017).

The virtue of the minirhizotron method is using observations of individual fine-root production and death to better understand processes and estimate proportional rates of production and mortality (Eissenstat and Yanai 1997). In contrast, the greatest challenge of the minirhizotron method is estimating standing crop due to the very small proportion of fine roots observed, and lack of accepted method to scale up from tube to areal estimates (Rytter and Rytter 2012, Taylor et al. 2014). An alternative approach is using the soil coring method to measure standing crops and the minirhizotron method to measure proportional rates of production and mortality (Rytter and Rytter 2012).

Young vs mature

The higher MLS of fine roots in young stands, compared with mature stands, has implications for estimates of C cycling. The average MLS for all our balsam fir sites (Table 1) underestimates site-specific MLS estimates by 7–63% at young sites and overestimates MLS by 19–65% at mature sites. Assuming fine-root mortality contributes approximately half of annual detrital inputs to soil C (Gill and Jackson 2000), the estimates of these inputs using the study-wide average MLS overestimate annual detrital production at young sites by 3–30% and underestimate annual detrital production at mature sites by 10–30%. As a result, using a constant value for MLS during stand development may overestimate sequestration of soil C in young stands and underestimate it in mature stands.

We use the following simple model, based on four population characteristics regulating MLS, to explore possible reasons for differences in MLS between young and mature stands.

$$\mathrm{MLS} = \mathrm{MLS}_{\text{short-lived}} \times (N_{\text{short-lived}}/N_{\text{tot}}) + \mathrm{MLS}_{\text{long-lived}} \times (N_{\text{long-lived}}/N_{\text{tot}}) \quad (2)$$

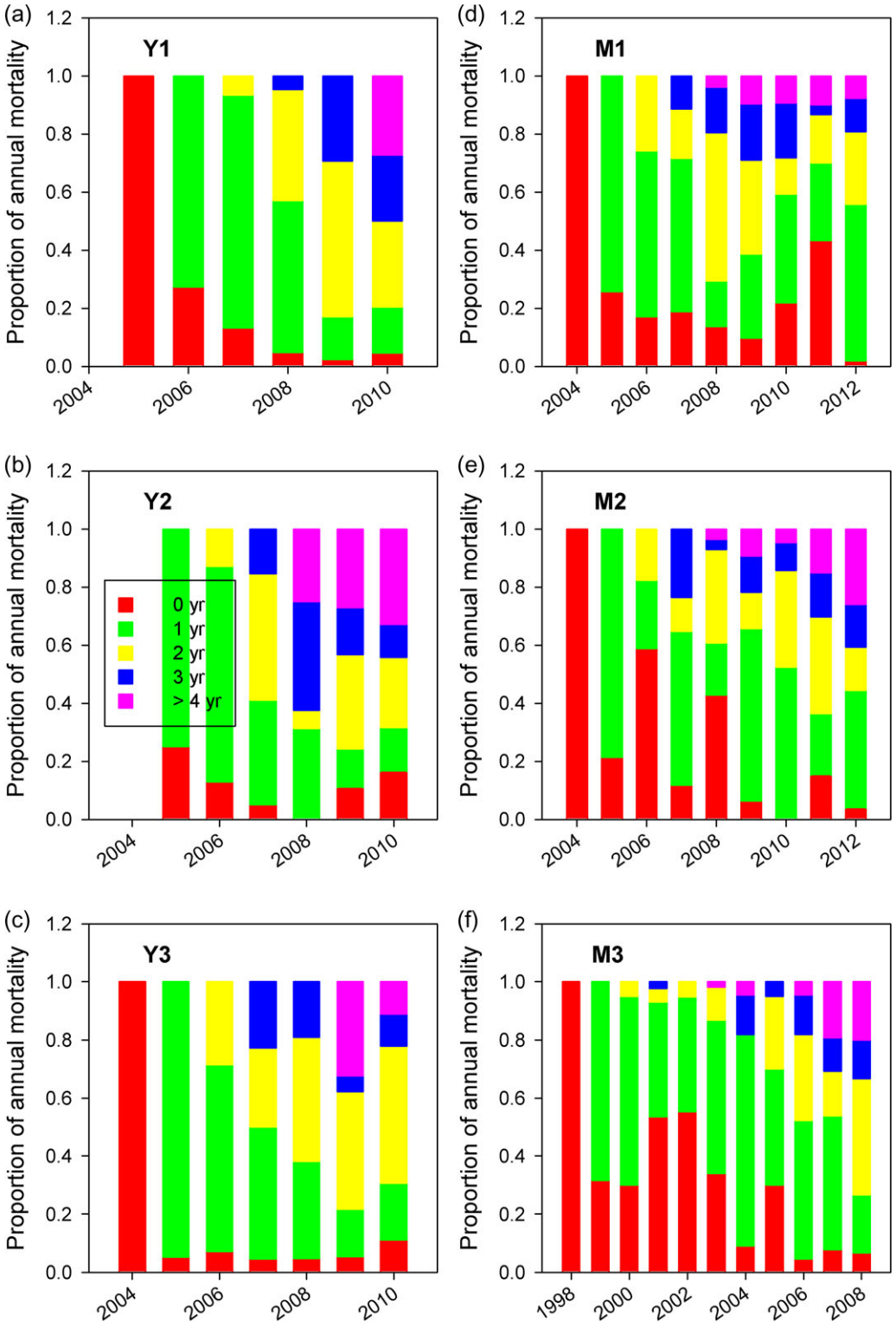

Figure 4. Proportional contributions of yearly cohorts to annual mortality of fine roots at three young (Y) and three mature (M) balsam fir sites in eastern Canada. Years are sequential from the beginning of observations at each site and calendar years vary among sites.

where N refers to numbers of fine roots intersecting minirhizotron tubes in an annual cohort and $N_{tot} = N_{short\text{-}lived} + N_{long\text{-}lived}$. This model is based on the notion that each annual cohort consists of two subpopulations having distinctly different MLS. Eq. (2) suggests two plausible explanations for the differences in MLS between young and mature stands. One possibility is that $MLS_{short\text{-}lived}$ and $MLS_{long\text{-}lived}$ do not change during stand development; therefore, differences between sites arise because

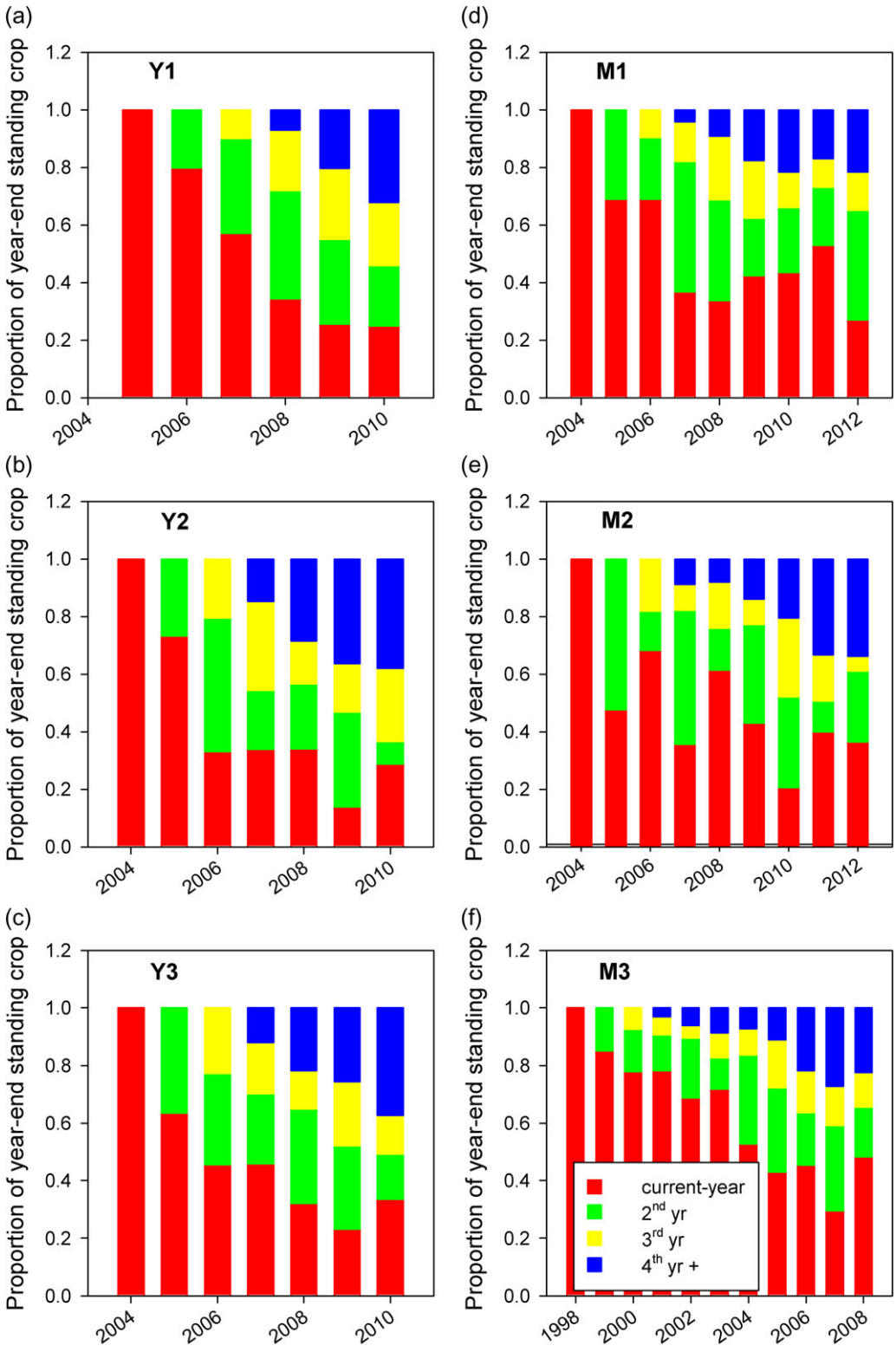

Figure 5. Proportional contributions of yearly cohorts to year-end standing crops of fine roots at three young (Y) and three mature (M) balsam fir sites in eastern Canada. Years are sequential from the beginning of observations at each site and calendar years vary among sites.

$N_{short\text{-}lived}/N_{tot}$ and $N_{long\text{-}lived}/N_{tot}$ change as trees age. The second possibility is that $N_{short\text{-}lived}/N_{tot}$ and $N_{long\text{-}lived}/N_{tot}$ do not change during stand development, therefore differences in MLS between young and mature sites arise because short-lived roots live longer at young than at mature balsam fir sites. We did not attempt to assign fine roots to subpopulations in this study, thus

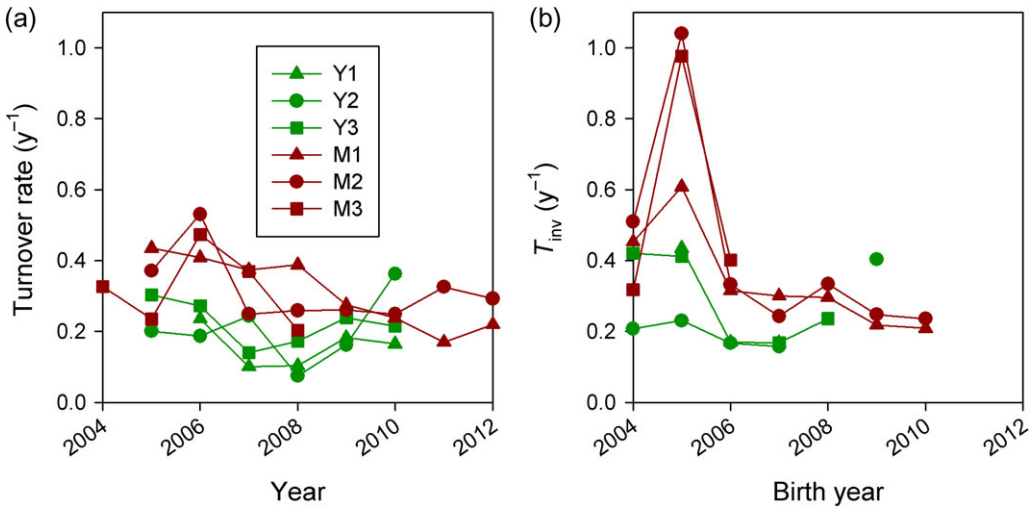

Figure 6. (a) Annual turnover rates (annual mortality)/(standing crop at the end of the previous year plus annual production) and, (b) median lifespans of each annual cohort observed at three young (Y) and three mature (M) balsam fir stands.

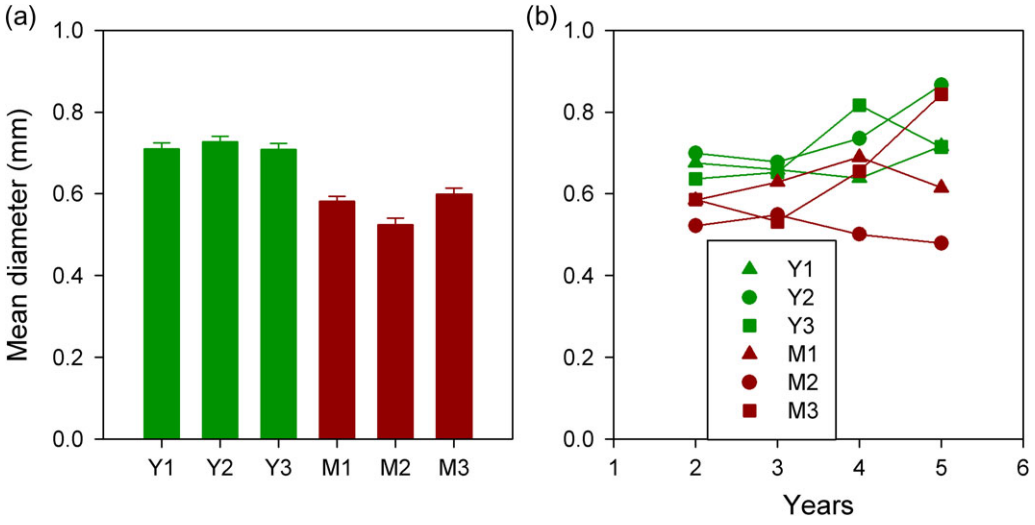

Figure 7. (a) Mean diameters of fine roots observed by minirhizotron. Capped bars represent one standard error of the mean. Number of roots used to calculate each mean is reported in Table 2. A statistical comparison of young vs mature is reported in the text. (b) Relationships between mean diameters of fine roots dying and their age at time of death at three young (Y) and three mature (M) balsam fir stands in eastern Canada.

we cannot determine their proportions of the population or their MLS. Nevertheless, using the present results to discuss merits of the alternative hypotheses can highlight the importance of identifying subpopulations in future studies.

The constant MLS of subpopulations hypothesis Assuming $MLS_{short\text{-}lived}$ and $MLS_{long\text{-}lived}$ do not change during stand development, the observed difference in MLS between young and mature stands implies lower $N_{short\text{-}lived}/N_{tot}$ and correspondingly higher $N_{long\text{-}lived}/N_{tot}$ in annual cohorts produced in young stands than those of mature stands. We are not aware of previous studies investigating age-related changes in $MLS_{short\text{-}lived}$ and $MLS_{long\text{-}lived}$. Nevertheless, there is circumstantial evidence supporting this view.

We found higher mean diameter among annual fine-root cohorts in young stands than mature stands (Figure 7a), possibly due to a larger proportion of the higher order, mainly transporting fine roots. The transporting fine roots tend to be longer lived than the smaller diameter, first- to third-order, mainly absorptive fine roots. In terms of Eq. (2), the higher mean diameter of annual cohorts implies $N_{long\text{-}lived}/N_{tot}$ is higher in young stands and $N_{short\text{-}lived}/N_{tot}$ is higher in mature stands. Root systems of young trees are expanding into unoccupied soil volume as young forests recover from stand-replacing disturbances (Makkonen and Helmisaari 2001, Claus and George 2005, Børja et al. 2008, Yuan and Chen 2012, Olesinski et al. 2012*b*). In this circumstance, greater allocation to long-lived, transport roots may be required to create the 'skeleton' to which absorbing

roots are attached. Conversely, after this 'skeleton' provides access to the entire available soil volume, a larger proportion of short-lived, absorbing roots may emerge from existing transport roots and fewer transporting fine roots need to be produced to maintain full occupancy of the soil.

Differences in MLS between Y1 and the other young sites (Figure 1a–c; Table 3) provide additional circumstantial support for shifting allocation of fine root production to absorbing roots as root system occupancy of soil increases. Higher MLS at Y1 than at Y2 and Y3 may be due to Y1 being younger (Table 1), hence experiencing greater expansion rate of root systems than at Y2 and Y3. These circumstances favor rapid expansion of tree root systems. We speculate root systems at Y1 were in an early, rapid expansion phase during our measurements, 1–6 years following clearcutting, whereas root systems at Y2 and Y3 were in later, slower stages of root system expansion during measurements 15–21 years after clearcutting. As a result, MLS in Y2 and Y3 are intermediate to Y1 and mature sites.

A difference in MLS of annual cohorts produced before and after thinning at one of our sites provides additional circumstantial evidence for higher proportional allocation of fine-root production to long-lived roots when tree root systems are expanding to occupy soil volume recently made unoccupied. Median lifespans of annual cohorts produced in 2006 and later were longer than those of cohorts produced before commercial thinning at M1 (Figure 1d). Greater $N_{long\text{-}lived}/N_{tot}$ after thinning can explain these differences in MLS.

The constant proportions of subpopulations hypothesis We found no information about the influence of tree or forest stand age on longevity of short- or long-lived roots or on their ratios, or proportions of the total. The literature suggests that short-lived roots die within 1–2 years (Pregitzer 2002, Trumbore and Gaudinski 2003, Joslin et al. 2006, Xia et al. 2010). The survivorship of roots in annual cohorts at our oldest site reasonably conforms to this notion. However, survival in many annual cohorts at the other sites declined steadily for more than 2 years without a distinct change in mortality rate (Figure 1). We hypothesize that short-lived roots in annual cohorts of M3 died early and that the remaining roots were the long-lived roots. Annual cohorts at the other mature sites reached this stage later than M3. Our observations in young stands might have ended before all the short-lived roots in many cohorts died. If the long, gently sloping tail of the right-skewed curve at M3 represents demography of long-lived roots, then their proportion in the total must be low and $MLS_{short\text{-}lived}$ decisively influences the overall MLS. We assume that proportional dominance of short-lived roots remains relatively steady regardless of tree or stand age. In the spirit of this notion, Espeleta et al. (2009) proposed focusing studies of fine-root demography on lower order, short-lived roots.

Some authors (e.g., Schoettle and Fahey 1994, Espeleta and Donovan 2002) suggest that prolonging the lifespan of fine roots improves the efficiency of utilization of invested C. Fine roots show high plasticity in their demographic and architectural traits in response to environmental stimuli (Hodge 2006), although it remains unknown how much plasticity is in proportions of roots of different branching orders and functions. Our hypothesis considers it possible that longevity of short-lived roots varies in response to physiological differences between young and mature plants (Wells and Eissenstat 2002) and to changing site conditions during forest stand development (Oliver and Larson 1996).

Within-site variability of MLS, T_{inv} and T

Variations in MLS and T_{inv} among annual cohorts and interannual variation of T (Figure 6) are inevitable due to interannual variation of weather and endogenous factors (Anderson et al. 2003, Norby et al. 2004, Olesinski et al. 2011, 2012*a*, Fukuzawa et al. 2013). As examples, Olesinski et al. (2011) found extended drought during summer increased turnover rates at our balsam fir sites, and Olesinski et al. (2012*b*) reported that heavy cone crops affect production and turnover of balsam fir fine roots for 2 years. Johnson et al. (2000) found roots born in autumn had longer MLS than those born in spring, hence interannual variation in seasonal distribution of fine root production can also contribute to variation of MLS among annual cohorts. Interannual variation in factors affecting turnover rates may be largely responsible for disparate values of MLS reported among short-term studies of some species. We used the Fine-Root Ecology Database (Iversen et al. 2017) to find tree species with more than one study reporting MLS or T_{inv}, in order to find examples of the ranges of values reported for some species. Two studies in sugar maple (*Acer saccharum* Marsh.) forests reported MLS ranging from 324 to 698 days (Hendrick and Pregitzer 1993, McCormack et al. 2012), and three studies in *P. abies* forests reported values ranging from 304 to 1158 days (Majdi 2001, Withington et al. 2006, Hansson et al. 2013). This raises the question of how many years of observation are needed to estimate values of T and T_{inv} representative of sites over longer terms. Based on the variations we observed, we suggest estimates of MLS and T_{inv} for four to five annual cohorts are required for a reasonably reliable estimate for balsam fir sites.

Climate affects fine root demography also, although effects on both population size and turnover rate need to be considered. The findings in this study suggest climatic differences had modest influence on turnover rate among mature balsam fir sites. Similarly, Burton et al. (2000) found climatic differences did not affect MLS of sugar maple fine roots on a latitudinal transect. These studies did not report on differences in sizes of fine root populations at climatically different sites. However, a review of literature by Finér et al. (2011) found climate to have a greater impact on fine root biomass than turnover rate. Hence, climate may affect population size more than T or T_{inv}.

Conclusions

Greater MLS at young balsam fir sites than mature sites affects the temporal course of C sequestration in living biomass and soil C. Knowledge of C sequestration during stand development has practical implications in some circumstances, for example, using ecosystem C cycle models to evaluate C mitigation strategies. This finding highlights the need to better understand both partitioning of annual fine-root production between short-lived, absorbing roots and longer-lived, transporting ones, and factors controlling MLS of these fine-root pools.

Our findings of substantial interannual variation in the annual turnover rate calculated as a proportion of standing crop (T), and of variation among annual cohorts in turnover rate calculated as the inverse of MLS, illustrate the importance of longer-term minirhizotron studies to obtaining accurate estimates of mortality rate variables. Longer-term studies may be particularly important for T, as it is affected by the age-class distribution of roots used for its calculation, and the age-class distribution in view of minirhizotrons in the early years differs from that in the bulk soil. Moreover, this characteristic of minirhizotron may limit its use to determining mortality rate variables of the short-lived, absorbing pool of fine roots.

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Conflict of interest

None declared.

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