

# Does the age of fine root carbon indicate the age of fine roots in boreal forests?

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Received: 13 October 2009 / Accepted: 1 June 2010  
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**Abstract** To test the reliability of the radiocarbon method for determining root age, we analyzed fine roots (originating from the years 1985–1993) from ingrowth cores with known maximum root age (1–6 years old). For this purpose, three Scots pine (*Pinus sylvestris* L.) stands were selected from boreal forests in Finland. We analyzed root  $^{14}\text{C}$  age by the radiocarbon method and compared it with the above-mentioned known maximum fine root age. In general, ages determined by the two methods (root  $^{14}\text{C}$  age and ingrowth core root maximum age) were in agreement with each other for roots of small diameter (<0.5 mm). By contrast, in most of the samples of fine roots of larger diameter (1.5–2 mm), the  $^{14}\text{C}$  age of root samples of 1987–1989 exceeded the ingrowth core root maximum age by 1–10 years. This shows that these roots had received a large amount of older stored carbon from unknown sources in addition to atmospheric  $\text{CO}_2$  directly from photosynthesis. We conclude that the  $^{14}\text{C}$  signature of fine roots, especially those of larger diameter, may not always be indicative of root age, and that further studies are needed

concerning the extent of possible root uptake of older carbon and its residence time in roots.

**Keywords** Fine root age · *Pinus sylvestris* · Radiocarbon · Root carbon · Ingrowth cores · Tree ring

## Introduction

Changes in fine root dynamics in boreal forests in response to climate change could be a major link between plant responses to climate change and longer-term changes in soil organic matter and ecosystem C balance (Norby and Jackson 2000). Consequently, global predictions of the response of boreal forest ecosystems to atmospheric and climatic change will be improved with greater understanding of fine root dynamics. Although there is wide consensus in the scientific community that fine roots of trees and understorey vegetation play an important role in the carbon and nutrient dynamics of forest soils, not enough quantitative data exist about their contribution to the carbon (C) and nutrient budgets (Gower et al. 1994; Bartelink 1998; Matamala et al. 2003; Trumbore and Gaudinski 2003; Majdi and Andersson 2004). The sink strength of the fine roots for C depends on root longevity (Joslin et al. 2006), but the published estimates of fine root longevity (or turnover time) differ more than fivefold (a review of Guo et al. 2008).

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Because fine roots are increasingly recognized as a key parameter for the accurate assessment of ecosystem carbon budgets (Guo et al. 2008; Pritchard and Strand 2008; Strand et al. 2008), such large discrepancies in root longevity and turnover estimates lead to uncertainty in assessing terrestrial C cycles (Trumbore and Gaudinski 2003; Högberg and Read 2006).

Fine root turnover and longevity have been obtained through sequential coring and measurement of root growth into root-free ingrowth cores (e.g. Fairley and Alexander 1985; Vogt et al. 1986; Powell and Day 1991; Fahey and Hughes 1994; Makkonen and Helmisaari 2001; Helmisaari et al. 2002; Ostonen et al. 2005), nutrient budgets (Nadelhoffer et al. 1985), or minirhizotrons allowing in situ observations of root growth and mortality (e.g. Hendrick and Pregitzer 1993; Fahey and Hughes 1994; Eissenstat and Yanai 1997; Johnson et al. 2000; Majdi and Öhrvik 2004). All these techniques have their advantages and shortcomings (Majdi et al. 2005; Hendricks et al. 2006), and the method of investigation has been considered to significantly influence the estimate of root longevity. Gill and Jackson (2000), in a meta-analysis comprising 59 studies, reported that the estimated fine root turnover times may vary from 5 months to 2 years. Measurements of the mean age of fine root C using the radiocarbon method (Gaudinski et al. 2001) indicated that  $^{14}\text{C}$ -values measured in live, dead, and mixed fine roots from temperate deciduous and coniferous forests corresponded to an average age of 3–18 years, longer than the estimates of root lifetime previously reported using other methods. If fine root C age corresponds to fine root age, and fine roots live many years, as these studies suggested, then the proportion of net primary production (NPP) allocated to support fine root turnover must be substantially less than previously assumed (Matamala et al. 2003).

At present, there is a debate concerning the effectiveness of different methods for investigating fine root longevity (Strand et al. 2008). Earlier estimates using the minirhizotron (MR) method were claimed to be superior to those from other methods (Fahey et al. 1999; Majdi et al. 2005), but the development of isotopic methods, including the radiocarbon ( $^{14}\text{C}$ ) method has raised questions about the accuracy of the MR method (Tierney and Fahey 2002; Vargas and Allen 2008; Gaul et al. 2009). Similarly, the accuracy of the  $^{14}\text{C}$  method needs thorough investigation.

$^{14}\text{C}$  is a radioactive isotope of carbon that naturally accounts for only a small fraction of the atmospheric carbon available for photosynthesis. However, the atmospheric  $^{14}\text{C}$  value was dramatically enhanced by thermonuclear weapons testing during the 1950s and 1960s and has been decreasing since then as  $^{14}\text{C}$  is taken up by the oceans and terrestrial organisms. The  $^{14}\text{C}$  method compares  $^{14}\text{C}$  levels in organic matter to recorded changes in atmospheric  $^{14}\text{C}$  levels, to estimate the mean age of organic matter as the mean elapsed time since the carbon was fixed from the atmosphere (Trumbore 1993). Radiocarbon isotopes allow a direct estimation of the age of live root C (structural C components, such as cellulose and lignin). The  $^{14}\text{C}$  method assumes that the  $^{14}\text{C}$  signature of root carbon corresponds well with root age, although this relationship has not been fully verified (Tierney and Fahey 2002). Previous studies have shown that recently assimilated carbon is used to produce fine root cellulose (Gaudinski et al. 2001; Matamala et al. 2003; Joslin et al. 2006; Trumbore et al. 2006). Because the structural carbon (cellulose) is not replaced once deposited in the root, the root cellulose C age should represent the real age of the root. However, recycling of older C to support the growth of new roots could cause C to persist in the fine root pool much longer than the individual roots themselves (Strand et al. 2008). In fact, some recent studies in temperate and tropical forests have shown that not only the recently assimilated carbon is used to produce new fine roots, but older carbon stored in nonstructural carbon (NSC) pools may also be transferred for the production of these structures (Luo 2003), especially during ecosystem development (Vargas et al. 2009) and in systems with frequent and severe disturbances (Langley et al. 2002; Wurth et al. 2005; Guo et al. 2004). In these cases, the  $\Delta^{14}\text{C}$  value of fine roots may not always be an indicator of the real age of the roots. More studies from different regions, climates and ecological situations are needed to determine whether C retranslocation to fine roots is a common phenomenon or an exception. Failure to take into account the mobilization of older stored carbon to produce new fine roots could lead to inaccurate estimates of fine root longevity and below-ground net primary productivity (Luo et al. 2004). However, little is known about the sizes, ages and ecological roles of NSC pools (Körner 2003; Wurth et al. 2005; Poorter and Kitajima 2007).

Due to the above-mentioned controversy concerning the possible root uptake of older carbon, in the present study we analysed the  $^{14}\text{C}$  content of the cellulose component of fine roots collected from ingrowth cores of known maximum age. In addition, bulk roots (i.e. without removal of any structural or non-structural compounds from the roots) were also analysed for their  $\Delta^{14}\text{C}$ , in order to make comparisons of  $\Delta^{14}\text{C}$  values between both bulk and cellulose fine roots. If the radiocarbon method is a reliable method for root age determination, then the  $\Delta^{14}\text{C}$  values of the cellulose of roots sampled (at least for the <0.5 mm diameter roots) should be consistent with the  $\Delta^{14}\text{C}$  values in the atmosphere as well as more precisely with those of the tree stemwood annual rings. Therefore, we also performed a set of control measurements on tree ring cellulose.

The goal of the present study was to test the reliability of the  $^{14}\text{C}$  method by using fine roots of known maximum age from boreal forests of Finland. Our main objectives were (i) to date fine roots of known maximum age by the  $^{14}\text{C}$  method, (ii) to assess the variation in fine root  $^{14}\text{C}$  longevity with root diameter, and (iii) to determine the seasonal variations of root  $^{14}\text{C}$ .

## Materials and methods

### Site descriptions

The research was carried out in three Scots pine stands in Finland. The Punkaharju site (61°48'N, 29°19'E) is situated in south-eastern Finland, the Jämijärvi site (61°45'N, 22°40'E) in western Finland and the Mekrijärvi site (62°47'N, 30°58'E) in eastern Finland.

The soil type in all the stands is a podzol, relatively poor in available nutrients. All sites have a thin mor organic layer, 2–3 cm thick at Jämijärvi, and 6 and 4 cm thick at Mekrijärvi and Punkaharju, respectively. The site type at Punkaharju is between *Vaccinium vitis-idaea* and *Calluna* types, Jämijärvi is *Calluna* type, and the Mekrijärvi site is *Vaccinium vitis-idaea* type according to the classification of Cajander (1949). The field layer vegetation is dominated by dwarf shrubs, such as *Vaccinium vitis-idaea* L., *Vaccinium myrtillus* L. and *Calluna vulgaris* (L.) Hull. Mosses such as *Pleurozium schreberi* (Brid.) Mitt. are

dominant in the bottom layer, but *Cladina* species are also common, especially at the Jämijärvi site. All stands had closed canopies. The age of the Punkaharju stand was around 35, Jämijärvi 45 and Mekrijärvi 100 years at the time of ingrowth core placing. The Jämijärvi stand is described in detail in Derome et al. (2009), and the Mekrijärvi stand in Helmisaari et al. (2002).

The sites are located in a boreal climate region. The long-term (1961–1990) mean annual temperature sum (equaling the sum of differences between daily mean temperatures and the threshold of +5°C) and precipitation were 1280°C and 593 mm for Punkaharju, 1163°C and 600 mm for Jämijärvi, and 1066°C and 649 mm for Mekrijärvi.

### Ingrowth cores, root sampling and sorting

In the *Punkaharju* study site, the ingrowth cores were placed in soil on 1.8.1987 and sampled after 2–6 years: 30.5.1989, 19.9.1989, 13.6.1991 and 30.9.1993. In the *Jämijärvi* site, the ingrowth cores were placed in soil in September 1992 and sampled after one year on 20.9.1993. In the *Mekrijärvi* site, the ingrowth cores were placed in the soil on 1.8.1985 and sampled after 1–3 years: 24.9.1986, 3.6.1987, 22.9.1987, 9.6.1988 and 7.10.1988.

An ingrowth core consists of a cylindrical gauze bag (mesh bag) with a specified volume filled with root-free soil (Makkonen and Helmisaari 1999). It is inserted into a suitably drilled hole in the soil and left to become colonized by the roots of neighboring plants. The technique enables measurement of root growth into the ingrowth core within a known period (Polonski and Kuhn 2002).

At each location, 30 cm long root-free mesh bags (Ø 5.7 cm) were systematically placed into holes made with a soil corer in the research stands. Mesh bags were filled with homogenous sieved mineral soil from the same site. The mesh size of the bags was 5.5 mm. The ingrowth cores were sampled using a special spade, and roots grown into them were removed and transported to the laboratory. In the laboratory, the ingrowth cores were separated into 10 cm thick layers. Roots from each layer were washed free of soil and separated into Scots pine roots and understorey roots, and both groups also into living or dead. Roots were then separated into two diameter classes: <2 mm and >2 mm, and the first group further into small fine roots

<0.5 mm and large fine roots 1.5–2 mm. These groups from the mineral soil layer 0–10 cm were then used for  $^{14}\text{C}$  analyses. There was a total of 51 samples, the replicate number of samples being 2–3 except for five samples  $n = 1$  (Tables 1, 2). Each root sample was a mixture of many roots and the root sample weight varied between 15 and 50 mg. All classified root samples were dried at 70°C for 5 days.

### Stemwood annual ring sampling

The atmospheric  $\Delta^{14}\text{C}$  values were from a German site. However, the atmospheric  $\Delta^{14}\text{C}$  values of Finnish atmosphere may differ slightly from those in Germany. Therefore, to be more precise, we also analyzed the  $^{14}\text{C}$  signature of the tree stemwood annual rings from one of our studied sites, Jämijärvi, which should represent the corresponding atmospheric  $^{14}\text{C}$  signatures in Finland.

One tree with a breast height (1.3 m from the soil surface) diameter of 16 cm and a tree height of 14 m was felled in the Jämijärvi stand on 17.4.2009. After felling the tree, one 2–3 cm thick stem disc was cut at breast height. One wood sample from each annual ring formed during 1985–1995 was cut out in the laboratory for  $^{14}\text{C}$  analyses.

### Radiocarbon approach

The radiocarbon contents of the roots as well as wood samples were measured with the AMS (Accelerator Mass Spectrometry) technique. All the samples were first chemically pretreated as follows. The mechanically cleaned samples were acid washed (2 h) with 2% HCl at 80°C to remove possible carbonate contaminants. During the treatment, the samples were also treated twice in an ultrasonic bath for 10 s to speed up the removal of possible dirt. After neutralization, organic acids were removed from the samples by performing a hot (80°C) wash in 2% NaOH (30 min) two times. Another acid wash was then performed, after which the samples were again neutralized and eventually dried at 50°C for 48 h.

If cellulose was to be extracted from the samples, the last drying was omitted and the treatment continued as follows. The samples were placed in hot (80°C) distilled water and HCl + NaClO<sub>2</sub> were added until the residual cellulose was completely bleached. The cellulose samples were acid washed at

80°C to ensure total removal of NaClO<sub>2</sub>, leaving a residue of completely white cellulose. All the samples were then neutralized with distilled water and dried at 50°C for 48 h.

The original dried root sample sizes varied from 5.1 to 60 mg whereas the tree ring sample sizes were consistently better controlled and larger. Typically, we used only 1 mg to minimize potential effects of contamination from the treatment. Hence, an amount corresponding to  $\sim 1$  mg of carbon of each sample was packed inside an evacuated and torch-sealed glass ampoule with an excess of CuO. The packed samples were combusted at 520°C overnight. The released CO<sub>2</sub> was collected and purified with liquid nitrogen and ethanol-dry ice traps at  $-196$  and  $-78^\circ\text{C}$ , respectively. After purifying and measuring the sample  $\delta^{13}\text{C}$  value, the CO<sub>2</sub> samples were converted to graphite targets in the presence of zinc powder and iron catalyst (Slota et al. 1986). After the sample pretreatment, combustion and graphitization, we obtained 0.1–2.1 mg graphite targets (average  $0.8 \pm 0.5$  mg) from fine root samples and  $>1$  mg from the stemwood tree ring samples. AMS measurements were performed at the Uppsala Tandem Laboratory. The results are expressed as  $\Delta^{14}\text{C}$  (Stuiver and Polach 1977).

The ages of samples were obtained by comparing the measured  $\Delta^{14}\text{C}$  values to the atmospheric values measured during recent decades (Levin and Kromer 2004). In particular, we chose the yearly averaged values of Levin and Kromer (2004) for comparison.

## Results

Our results (Figs. 1, 2, 3, 4) indicate that tree ring and atmospheric  $\Delta^{14}\text{C}$  values were similar to each other, although tree rings tended to be slightly more  $^{14}\text{C}$  enriched than the atmosphere. This is due to the slight increase in atmospheric  $^{14}\text{C}$  during the growing season compared to the yearly average—the tree ring cellulose absorbs this elevated amount during its formation. Overall, these reference measurements confirm the reliability of the performed  $^{14}\text{C}$  analyses.

In the *Punkaharju* study site, the ingrowth cores were placed in the soil in 1987 and the fine roots of both diameter classes were sampled from the cores in 1989, 1991 and 1993. The mean radiocarbon content of bulk live fine roots ( $\varnothing < 0.5$  mm) grown into the ingrowth cores during 1987–1993 was in the range of

**Table 1**  $\Delta^{14}\text{C}$  values for atmosphere (monthly maximum–minimum) for the period between ingrowth core placing and sampling, mean ( $\pm$ SD)  $\Delta^{14}\text{C}$  values in bulk roots and root cellulose, and comparison of ingrowth core maximum root age and  $^{14}\text{C}$  root age in bulk roots and root cellulose of roots <0.5 mm in diameter

Root	Ingrowth core placing	Ingrowth core sampling time	Atmos. $\Delta^{14}\text{C}$ (‰) range between years, max–min	Bulk root $\Delta^{14}\text{C}$ (‰)	Root cellulose $\Delta^{14}\text{C}$ (‰)	Maximum ingrowth core root age (years)	Bulk root $^{14}\text{C}$ age (years)	Root cellulose $^{14}\text{C}$ age (years)
Live roots	Punkaharju 1.8.1987	May 1989	187–158	187	262 ( $\pm$ 1.4)	<2	3	8 <sup>a</sup>
		September 1989	187–158	147 ( $\pm$ 2.5)	144 ( $\pm$ 5.7)	2	0	0
		June 1991	187–133	159 ( $\pm$ 11)	143 ( $\pm$ 0.7)	4	2	0
		September 1993	187–120	150 ( $\pm$ 2.6)	152 ( $\pm$ 0.7)	6	3	3
	Jämijärvi 1.8.1992 Mekrijärvi 1.8.1985	September 1993	139–120	114 ( $\pm$ 2.3)	135 ( $\pm$ 40)	1	0	2
		September 1986	204–174	No sample	193 ( $\pm$ 5.0)	<1		<1
		June 1987	204–166	No sample	191 ( $\pm$ 1.41)	2		2
		September 1987	204–166	No sample	191 ( $\pm$ 6.5)	2		2
		June 1988	204–163	No sample	181 ( $\pm$ 1.0)	3		2
		October 1988	204–163	No sample	178 ( $\pm$ 3.0)	3		1
Dead roots	Punkaharju 1.8.1987	September 1993	187–120	173 ( $\pm$ 17)	151 ( $\pm$ 1.4)	6	6	3

Live and dead bulk roots,  $n = 3$  (except on May 1989,  $n = 1$ ), cellulose,  $n = 2$  (except in September 1987, June 1988 and October 1988,  $n = 3$ )

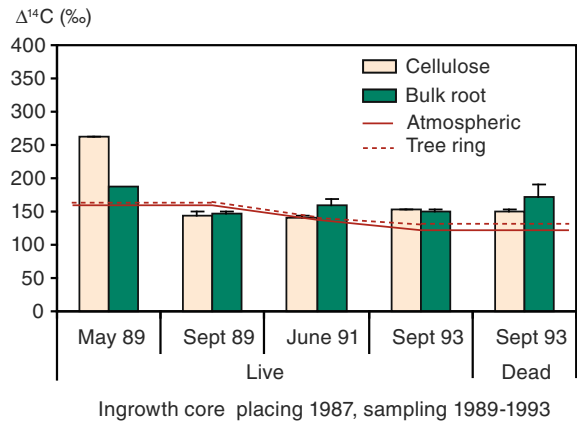
<sup>a</sup> Do not support ingrowth core root age values

**Table 2**  $\Delta^{14}\text{C}$  values for atmosphere (monthly maximum–minimum) for the period between ingrowth core placing and sampling, mean ( $\pm\text{SD}$ )  $\Delta^{14}\text{C}$  values in bulk roots and root cellulose, and comparison of ingrowth core maximum root age and  $^{14}\text{C}$  root age in bulk roots and root cellulose of roots 1.5–2 mm in diameter

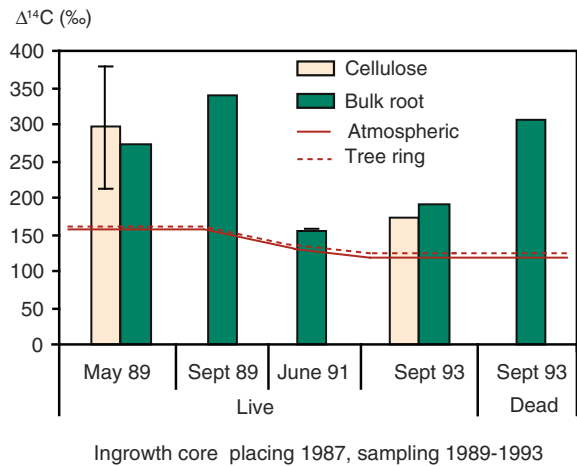
Root	Ingrowth core placing	Ingrowth core sampling time	Atmos. $\Delta^{14}\text{C}$ (‰) range between years, max–min	Bulk root $\Delta^{14}\text{C}$ (‰)	Root cellulose $\Delta^{14}\text{C}$ (‰)	Maximum ingrowth core root age (years)	Bulk root $^{14}\text{C}$ age (years)	Root cellulose $^{14}\text{C}$ age (years)
Live roots	Punkaharju	1.8.1987	187–158	274 <sup>a</sup>	296 ( $\pm 83$ ) <sup>a</sup>	<2	9 <sup>a</sup>	10 <sup>a</sup>
		May 1989	187–158	339 <sup>a</sup>	No sample	2	12 <sup>a</sup>	No sample
		September 1989	187–133	156 ( $\pm 1.4$ )	No sample	4	2	No sample
		June 1991	187–120	190	172	6	7	6
Dead roots	Punkaharju	September 1993	187–120	306	No sample	6	14	No sample

Live and dead roots,  $n = 1$  (except values with SD,  $n = 2$ )

<sup>a</sup> Do not support ingrowth core root age values



**Fig. 1**  $\Delta^{14}\text{C}$  values of live and dead roots (diameter <0.5 mm) of the Punkaharju site ( $n = 1$ –3; see Table 1, error bars represent SD of means). Comparison of mean annual atmospheric (solid line,  $\pm\text{SD}$ ), stemwood annual ring (broken line,  $n = 1$ ) and root (histogram column)  $\Delta^{14}\text{C}$  values

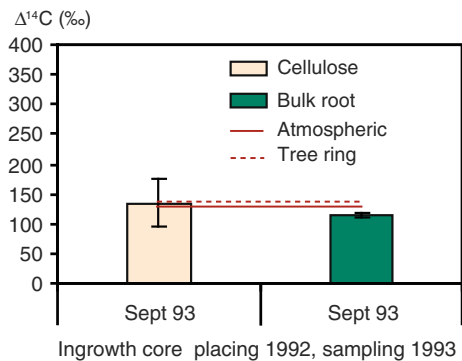


**Fig. 2**  $\Delta^{14}\text{C}$  values of live and dead roots (diameter 1.5–2 mm diameter) of the Punkaharju site ( $n = 1$ –2; see Table 1, error bars represent SD of means). Comparison of mean annual atmospheric (solid line,  $\pm\text{SD}$ ), stemwood annual ring (broken line,  $n = 1$ ) and root (histogram column)  $\Delta^{14}\text{C}$  values

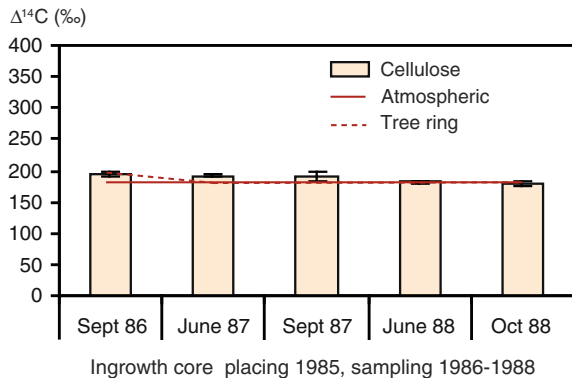
187–147‰, consistently with that of recorded atmospheric levels (187–120‰) and the tree rings (181–131‰) in the corresponding years, thus supporting the ingrowth core root age range (Fig. 1 and Table 1).

The radiocarbon content of bulk live fine roots of the larger diameter class (1.5–2 mm) placed in 1987 and sampled during the same years as above (1989, 1991 and 1993), matched both the atmospheric and tree ring levels in samples from 1991 and 1993, but in





**Fig. 3**  $\Delta^{14}\text{C}$  values of live and dead roots (diameter <0.5 mm) of the Jämijärvi site ( $n = 2-3$ , error bars represent SD of means). Comparison of mean annual atmospheric (solid line,  $\pm$ SD), stemwood annual ring (broken line,  $n = 1$ ) and root (histogram column)  $\Delta^{14}\text{C}$  values



**Fig. 4**  $\Delta^{14}\text{C}$  values of live and dead roots (diameter <0.5 mm) of the Mekrijärvi site ( $n = 2-3$ , error bars represent SD of means). Comparison of mean annual atmospheric (solid line,  $\pm$ SD), stemwood annual ring (broken line,  $n = 1$ ) and root (histogram column)  $\Delta^{14}\text{C}$  values

1989 (May and September) the samples considerably exceeded (339–274‰) the recorded atmospheric (187–158‰) and tree ring (181–161‰) levels. This also means that the observed  $^{14}\text{C}$  enriched carbon in larger diameter fine roots in 1989 was much older (up to 10 years older) than the known maximum age of roots (Fig. 2 and Table 2).

The live root cellulose radiocarbon content of both root diameter classes was in accordance with the atmospheric and tree ring values of  $\Delta^{14}\text{C}$  with the exception of those roots (both diameter classes) sampled in May 1989 with clearly higher values of 262‰ (for the roots <0.5 mm) and 296‰ (for the roots

1.5–2 mm diameter). These values substantially exceeded the atmospheric (187–158‰) and tree ring (181–161‰)  $\Delta^{14}\text{C}$  values. Thus fine root cellulose contained older (6–8 years older) carbon than the known maximum root age (Figs. 1, 2 and Tables 1, 2).

Regarding the dead bulk fine roots (Punkaharju site), the radiocarbon content of fine roots of smaller diameter (<0.5 mm) fell within the range (in 1993:  $\text{Ø} < 0.5 \text{ mm} = 173 \pm 16.7\%$ ) of the radiocarbon values of the corresponding atmosphere (187–120‰) and tree rings (181–161‰) (Fig. 1 and Table 1), whereas the dead fine roots of larger diameter (1.5–2 mm) showed remarkably higher radiocarbon values ( $\text{Ø} 1.5-2 \text{ mm} = 306\%$ ) in comparison to the corresponding atmospheric (187–120‰) and tree ring (181–161‰) values, indicating greater age of carbon in the thicker bulk dead roots (Fig. 2 and Table 2). We have only the data of the cellulose of the dead roots of <0.5 mm diameter (Punkaharju site) from one sample from 1993. This sample has a similar range of  $\Delta^{14}\text{C}$  values ( $151 \pm 1.4\%$ ) to those of atmospheric (187–120‰) and tree ring (181–161‰) radiocarbon values of the sampling years. This limited amount of data indicates that the dead bulk roots are more enriched in  $\Delta^{14}\text{C}$  values than the live bulk roots, although the cellulose of dead roots does not differ much from the live cellulose.

For the Jämijärvi site, ingrowth cores were placed in 1992 and the roots were sampled after 1 year in 1993. The bulk fine roots (diameter <0.5 mm) sampled during 1993 had rather similar  $\Delta^{14}\text{C}$  values ( $114 \pm 2.3\%$ ) to that of the current year's atmosphere (1993 = 123‰) and tree rings (131‰). Similarly, the cellulose in the same samples showed a range of  $\Delta^{14}\text{C}$  values ( $135 \pm 40\%$ ) in accordance with that of atmospheric (139–120‰) and tree ring (131‰)  $\Delta^{14}\text{C}$  values indicating no addition of older carbon (Fig. 3 and Table 1).

At the Mekrijärvi site, the ingrowth cores were placed in the soil in 1985 and fine roots of both diameter classes were sampled from the cores in 1986, 1987 and 1988. The results from this site indicate that the radiocarbon content of cellulose (bulk roots not analyzed) of live fine roots ( $\text{Ø} < 0.5 \text{ mm}$ ), sampled during 1986–1988, was in the range of 193–178‰, almost consistently with the recorded atmospheric (204–163‰) and tree ring (209–180‰) levels in the corresponding years, supporting the ingrowth core root age range (Fig. 4 and Table 1).

The radiocarbon concentration of fine roots (both bulk and cellulose) of the Punkaharju site increased with the increase in the root diameter (compare Figs. 1, 2 and Tables 1, 2). Thus roots with 1.5–2 mm in diameter tended to have older carbon than those with <0.5 mm diameter. The  $\Delta^{14}\text{C}$  values of bulk fine roots <0.5 mm diameter (147–187‰) were on average more  $^{14}\text{C}$ -depleted than those of roots between 1.5 and 2 mm in diameter (156–339‰). Except for the June 1991 sampling with similar bulk root  $\Delta^{14}\text{C}$  values between diameter classes, the difference of  $\Delta^{14}\text{C}$  values between larger and smaller diameter fine bulk roots was in the range of 40–192‰, which corresponds to a difference in mean  $^{14}\text{C}$  age range of 3–12 years. For the fine root cellulose, the difference in  $\Delta^{14}\text{C}$  values between the two diameter classes was smaller (2–3 years).

Seasonal variations of the  $\Delta^{14}\text{C}$  values of fine roots were also studied and, although some variations were observed, the results indicated no definite trend of seasonality (Figs. 1, 2, 3). For the Punkaharju site, the bulk fine root and the root cellulose of the smaller diameter class (<0.5 mm) were remarkably more  $^{14}\text{C}$  enriched in roots sampled in the spring season (May 1989 = 187‰ for bulk root and 262‰ for the cellulose) than in those sampled in the autumn (September 1989 =  $147 \pm 2.5\%$ ‰ for the bulk root and  $144 \pm 5.7\%$ ‰ for the cellulose). By contrast, the fine roots of diameter <0.5 mm and bulk fine roots with diameter 1.5–2 mm (Fig. 2) were substantially more  $^{14}\text{C}$  enriched in the autumn samples (339‰) than in the spring samples (274‰). For the cellulose, we have no samples available from the same year. For the Mekrijärvi site, we have no data from bulk roots but the root cellulose of fine roots <0.5 mm diameter did not show any remarkable differences between sampling seasons (Fig. 4).

The results of the ingrowth core maximum root age and root  $^{14}\text{C}$  age were in agreement except for the Punkaharju site in the year 1989 (for both fine root diameter classes). For the smaller diameter roots,  $^{14}\text{C}$  age of the root cellulose samples of May 1989 exceeded by 8 years the values of the ingrowth core maximum root age. Similarly, for the larger diameter root samples (both bulk roots and cellulose) of the same site and of the same sampling time as well as for September 1989, the root  $^{14}\text{C}$  age exceeded the ingrowth core maximum root age by 7–10 years. Hence, our data of the Punkaharju site from 1989

indicate older C input into the roots of both fine root diameter classes.

## Discussion

The radiocarbon contents of all Scots pine fine root samples (bulk fine roots, root cellulose, live or dead) of known maximum age and corresponding atmospheric and tree ring  $\Delta^{14}\text{C}$  values were in agreement with each other in most cases for fine roots of smaller diameter (<0.5 mm), and inconclusive in some cases of larger diameter (1.5–2 mm) fine roots.

The radiocarbon based age of most of the larger diameter bulk and cellulose fine roots (both live and dead) exceeded the known maximum age of roots by 7–10 years. For the larger diameter fine roots, for most of the dates only a single sample was analyzed. The observed extreme values in single samples could not be confirmed with more analyses due to the lack of sufficient fine root material. Based on the results, we conclude that the  $^{14}\text{C}$  signature of fine roots may not always be indicative of root age, either because of addition of carbon from unknown sources such as storage reserves or some other process.

A few earlier studies have already reported the possible root uptake of storage carbon from unknown sources. For example, Gaudinski et al. (2009) reported the mean age of storage C in new roots ranging between 1 and 2 years from a study in temperate forests (two coniferous, one deciduous). In their study, C reserves were assumed to cycle quickly and their impact on fine root C age was considered to be small, particularly for roots that live for many years (Gaudinski et al. 2009). In this case, root uptake of older carbon should have no impact on the real root age determination by the  $^{14}\text{C}$  method. However, the radiocarbon approach might overestimate fine root longevity if new roots were built with much older C from senescing tissues or storage pools (Luo 2003; Strand et al. 2008). In tropical forests, Vargas et al. (2009) provided evidence that newly produced roots are constructed from increasingly older carbon during forest ecosystem development; they reported that, at young sites (6- and 10-year of age), new roots are produced with recently fixed stored carbon (~2 years of age), whereas in older forests, new roots are produced with older stored carbon (up to 10 years of age). Our Punkaharju site was the



youngest of the studied sites, and thus tree age was unlikely to contribute to the elevated root  $^{14}\text{C}$  values.

The use of storage C may even be higher in boreal forests compared to temperate and tropical forests. For example, a few studies in boreal black spruce forests in Alaska and Canada (Czimczik et al. 2006; Schuur and Trumbore 2006; Carbone et al. 2007) found the mean age of stored C pools supplying root respiration, and therefore presumably root growth, to be >3–5 years, based on the fact that root respired  $\text{CO}_2$  showed  $\Delta^{14}\text{C}$  values significantly higher than those of recent photosynthetic products. This suggests that NSC pools of several years of age are used in root metabolism and/or growth. Environmental disturbances such as fire, drought or pest outbreaks in the forest may also result in root uptake of older C (reviewed by Chapin et al. 1990), but such environmental disturbances did not occur in the recent history of our studied sites.

Our limited data, however, indicate that the dead bulk roots were more enriched in  $\Delta^{14}\text{C}$  values than live bulk roots, although the cellulose of dead roots did not differ much from the live cellulose. Trumbore et al. (2006) reported different results: dead root cellulose was slightly more depleted in  $\Delta^{14}\text{C}$  values than live root cellulose, which was assumed to be due to the fact that most of the flux of C through roots is probably via shorter-lived components such as root tips. Although root death is probably not a random process, we still know very little about what causes roots to die (Pregitzer 2002).

In our current study, the difference in  $\Delta^{14}\text{C}$  values between the two diameter classes was smaller for the root cellulose (2–3 years) than for the bulk live fine roots (up to 10 years). Our findings of root  $^{14}\text{C}$  age pattern are consistent with the results of several other studies (Matamala et al. 2003; Joslin et al. 2006; Gaul et al. 2009). For example, Gaul et al. (2009) reported that roots >0.5 mm in diameter tended to live longer than those <0.5 mm in diameter. In the organic layer, the  $\Delta^{14}\text{C}$  values of fine roots <0.5 mm in diameter were on average 9‰ lower than those of roots between 0.5 and 1 mm in diameter, reflecting a difference in mean age of  $\sim 2$  years. Wells and Eissenstat (2001) reported that root survivorship may vary markedly between fine roots differing in diameter by only a few tenths of a millimeter. Furthermore, Riley et al. (2009), through their  $^{14}\text{C}$  labeled temperate forest study, predicted live root turnover times <1 year for the

short-lived root pools (<0.5 mm in diameter) and 10 years for the long-lived root pools (0.5–2 mm in diameter). It is also rational that high-order roots (i.e. larger diameter) live longer than low-order roots (smaller diameter) because the death of a high-order root entails the loss of all the adjacent lower-order roots (Guo et al. 2008).

The causes of enhanced fine root longevity with increasing root diameter are not fully clear. The smallest diameter roots with their mycorrhizal root tips typically contain the highest concentrations of N (Pregitzer et al. 1997, 2002; Helmisaari et al. 2007, 2009). As a consequence, these tissues are more efficient in nutrient uptake but also more susceptible to herbivores and root parasitism than thicker fine roots (Graham 1995). In addition, smaller diameter roots were shown to be more vulnerable to abiotic stress factors such as soil drought (Espeleta et al. 1999). Our roots <0.5 mm in diameter included ectomycorrhizal short roots, that were not encountered on roots >1 mm in diameter (Helmisaari et al. 2009). The formation and elongation of smaller diameter ectomycorrhizal roots represents a strong carbon sink. In the course of time, some of the fine roots become older and grow additionally in diameter. It is possible that the formation of ectomycorrhizal fine roots uses proportionally more carbon directly from current photosynthesis than the diameter growth of larger (and older) roots. Therefore, as the root grouping clearly affects the  $^{14}\text{C}$  results, in fine root longevity/turnover studies more emphasis should be placed on root sorting and more careful grouping, as many authors (e.g. Tierney and Fahey 2002) have pointed out.

Seasonal variations of the  $\Delta^{14}\text{C}$  values of fine roots did not show any consistent pattern. Although the smaller diameter fine roots (both bulk fine roots and the root cellulose) of the Punkaharju site were more  $^{14}\text{C}$  enriched in the spring samples than in those sampled in the autumn, larger diameter fine roots were substantially more  $^{14}\text{C}$  enriched in autumn samples than in the spring samples. Hypothetically, the  $^{14}\text{C}$  enrichment of the fine roots of the spring season may be due to insufficiency of current carbon available for root cellulose formation during the early spring. Therefore the roots take older carbon either from tree storage or  $^{14}\text{C}$  enriched  $\text{CO}_2$  respired from soil (discussed by Hobbie et al. 2002). However, as the roots sampled in spring most probably originated

from the autumn or summer of the previous year, this hypothesis should be further tested. Thus, we still need more data to determine whether there are any seasonal trends in  $^{14}\text{C}$  values.

## Conclusions

Several studies have supported the assumption that the  $^{14}\text{C}$  method reflects the turnover rates of long-lived roots, resulting in the overestimation of root longevity (Gaudinski et al. 2001; Guo et al. 2008; Strand et al. 2008). On the basis of our results, it can be concluded that the  $^{14}\text{C}$  age of fine roots was in accordance with the known root age of the very fine roots <0.5 mm in diameter. By contrast, for the fine roots of larger diameter (1.5–2 mm), the reliability of the radiocarbon method for root age is still questionable due to our inadequate information and understanding of the dynamics of storage C.

In some cases, the  $^{14}\text{C}$  age of the fine roots (especially of larger diameter) was even a decade older than the known age of roots. Although we had only a limited number of samples representing larger fine roots, the maximum root age in our study was known and hence we can confirm that these older  $^{14}\text{C}$  ages could not be related to root age, indicating that the radiocarbon method must be used with caution. It is probable that the  $^{14}\text{C}$  signature of fine roots is not always indicative of root age, at least in the case of the boreal forest fine roots of larger diameter, e.g. because of addition of C from storage. Further studies are needed concerning the extent of possible root uptake of older carbon and its residence time in roots.

**Acknowledgements** We are grateful to forest engineer Erkki Salo for sampling and preparing the tree ring samples. We are also grateful to persons contributing to sampling and sorting of the original root material: Dr. Kirsi Makkonen, forest engineers Pekka Välikangas and Reijo Hautajärvi, and the staff of the Salla Office of the Finnish Forest Research Institute. We gratefully acknowledge the help of the personnel of the Dating Laboratory, Finnish Museum of Natural History, University of Helsinki, Finland for their technical assistance in radiocarbon analysis. This work was supported by the Academy of Finland.

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