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Research

Dynamics of heterorhizic root systems: protoxylem groups within the fine-root system of *Chamaecyparis obtusa*

Takuo Hishi and Hiroshi Takeda

Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

Author for correspondence:
Takuo Hishi
Tel: +81 75 753 6080
Fax: +81 75 753 6080
Email: tak@kais.kyoto-u.ac.jp

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Summary

• To understand the physiology of fine-root functions in relation to soil organic sources, the heterogeneity of individual root functions within a fine-root system requires investigation. Here the heterogeneous dynamics within fine-root systems are reported.

• The fine roots of *Chamaecyparis obtusa* were sampled using a sequential ingrowth core method over 2 yr. After color categorization, roots were classified into protoxylem groups from anatomical observations.

• The root lengths with diarch and triarch groups fluctuated seasonally, whereas the tetrarch root length increased. The percentage of secondary root mortality to total mortality increased with increasing amounts of protoxylem. The carbon : nitrogen ratio indicated that the decomposability of primary roots might be greater than that of secondary roots. The position of diarch roots was mostly apical, whereas tetrarch roots tended to be distributed in basal positions within the root architecture.

• We demonstrate the heterogeneous dynamics within a fine-root system of *C. obtusa*. Fine-root heterogeneity should affect soil C dynamics. This heterogeneity is determined by the branching position within the root architecture.

Key words: Cupressaceae, fine-root dynamics, heterorhizy, protoxylem, root architecture, secondary growth.

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Introduction

In forest ecosystems, fine roots play an important role in carbon and nutrient dynamics (Vogt *et al.*, 1982; Hendricks *et al.*, 1993), and may account for ≈40% of total net primary production (Vogt *et al.*, 1996). In previous studies of root dynamics, all individual roots in a fine-root system have been treated as physiologically identical units in an ambiguous diameter size class, such as <1 or <2 mm. Recent studies have shown that there is a wide range of variability in seasonality, longevity and chemical composition among individuals within fine-root systems, according to branching position or diameter size class in tenths of millimetres (Wells & Eissenstat, 2001; King *et al.*, 2002; Pregitzer *et al.*, 2002). Moreover, the substantial differences among individual roots within a fine-root system may lead to differences in their contribution to soil systems in terms of C and nutrient dynamics (Guo *et al.*, 2004; Langley & Hungate, 2003). However, there have been few studies on the dynamics of individual roots within fine-root systems in relation to sources of dead soil organic matter.

Fine root systems contribute organic material to soil systems through high mortality and decomposition rates. Differences in the longevity of individual roots (e.g. ephemeral or perennial) should affect soil organic dynamics through root mortality, as should differences in decomposition rates. The C : N ratio is an important index for determining the decomposition rate in the earliest growth phase (Ostertag & Hobbie, 1999; Berg & McClaugherty, 2003). Pregitzer *et al.* (2002) found considerable differences in the C : N ratio among the branching positions of fine-root systems, and they suggested that ephemeral individuals may have a lower C : N ratio. Moreover, ephemeral roots disappeared more quickly than did perennial roots in a mini-rhizotron study (Ruess *et al.*, 2003). Therefore variations in root longevity may be related not only to root mortality, but also to individual root decomposition rates.

Differences in the longevity of individuals may be the result of secondary growth. Secondary growth should be an important event, not only in terms of anatomical and functional characteristics (Esau, 1964; McKenzie & Peterson, 1995b), but also possibly in terms of the longevity of individual roots (Hishi & Takeda, 2005). Secondary roots have a continuous cork layer and secondary xylem, consisting of well developed secondary tissues. The cork layer is an effective barrier against external stresses such as drought, disease or herbivory, but also causes a considerable decrease in absorptive ability. Primary roots lack a cork layer or secondary vascular system, and absorb nutrients or water through their passage cells, which are living primary tissues (Esau, 1964; McKenzie & Peterson, 1995a, 1995b). Brown roots are often used as an index of secondary growth and are considered to have greater longevity (López *et al.*, 2001), whereas Wells & Eissenstat (2001) have reported that root browning has no significant effect on the longevity of individual roots. From these studies, secondary roots may be expected to have greater longevity than primary roots, because secondary roots have greater stress tolerance than primary roots. Nonetheless, roots that die after secondary growth should have slower decomposition rates than those that die before secondary growth, because secondary roots consist of a greater amount of secondary tissue, which resists decomposition. Fig. 1 shows individual roots that died before or after secondary growth.

The earliest xylem in a given plant organ is called protoxylem (Esau, 1964). In a wide range of conifers, the amount of protoxylem in root cross sections that does not change throughout the life cycle of individual fine roots indicates whether individual fine roots advance ontogenetically to secondary growth (Noelle, 1910). This ontogenetic difference in the functions of individual roots is called heterorhizy. In *Chamaecyparis obtusa*, individual roots with more protoxylem tend to develop a greater proportion of secondary growth before they die than do individuals with less protoxylem (Hishi & Takeda, 2005). Moreover, individual roots with more protoxylem tend to have a larger vascular diameter, and the frequency of greater amounts of protoxylem tends to be greater in the basal parts of a root system than in its apical parts (Wilcox, 1962; Esau, 1964). Therefore protoxylem amounts may be related to fine-root system dynamics and architecture, and to their interrelationship.

We tested the following hypotheses: (1) the length dynamics of roots with greater amounts of protoxylem fluctuate less than those of roots with lesser amounts of protoxylem, because roots with a larger amount of protoxylem tend to develop secondary growth roots; (2) the proportion of mortality in secondary roots to the total mortality is greater in roots with a higher amount of protoxylem than in roots with a lower amount of protoxylem; (3) the potential decomposition rates of roots with greater amounts of protoxylem are lower than those of

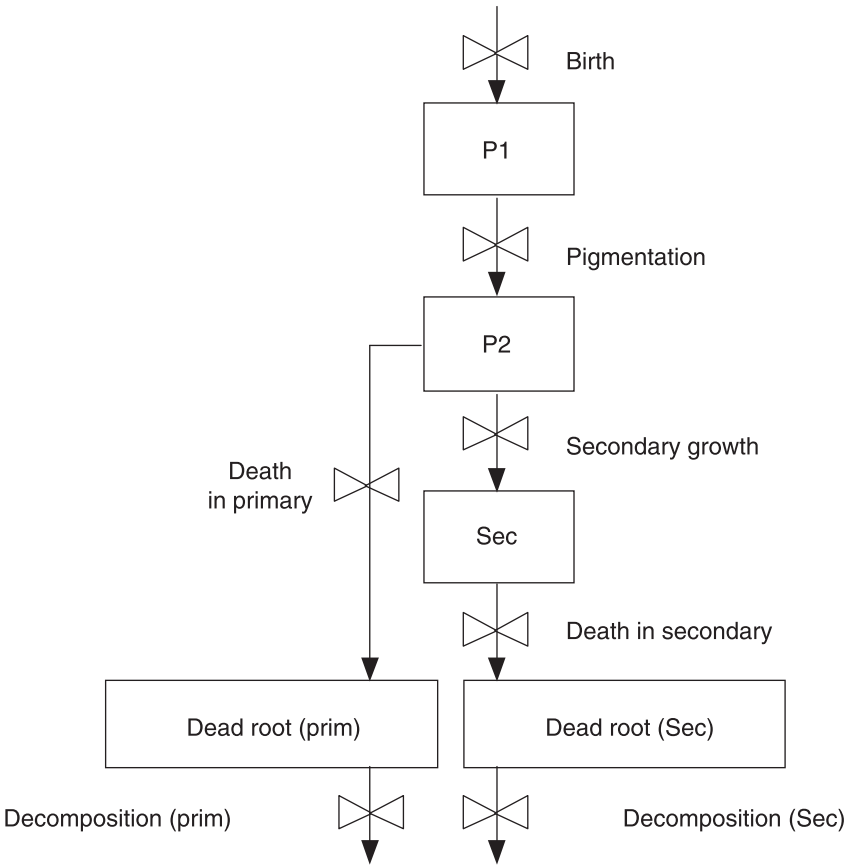

Fig. 1 The compartment-flow model in relation to individual root life cycles. Compartments are the states of variables: length of light-colored primary roots (P1); red-pigmented primary roots (P2); dark brown secondary roots (Sec); dead root in primary growth (prim); dead root in secondary growth (Sec), linked by seven flows, birth, pigmentation, secondary growth, death in primary, death in secondary roots, decomposition in primary and decomposition in secondary roots. Butterfly values regulate these flows. Individual roots have two alternative life cycles, dying as primary or as secondary roots.

roots with lesser amounts of protoxylem; (4) roots with lesser amounts of protoxylem are positioned more apically than roots with greater amounts of protoxylem within the root-system architecture. All these factors should affect the functioning of fine roots, both as an absorptive organ of water and nutrients, and as soil organic substrates. Nonetheless, we wanted to understand the relationship between the maintenance processes of fine-root architecture and ecosystem functions. To clarify these hypotheses, we monitored fine-root dynamics and estimated the mortality of primary and secondary roots in each of the protoxylem groups. The root dynamics of each protoxylem group were examined using an anatomical method. The chemical compositions of primary and secondary roots were also analyzed to assess potential decomposition rates, and then the contributions of ephemeral and perennial roots to the supply of organic material to the soil system were estimated. The distribution of protoxylem groups within the root-system architecture was also examined.

Materials and Methods

Chamaecyparis obtusa

Chamaecyparis obtusa Sieb. et Zucc. is a primitive gymnosperm (conifer) species that associates with arbuscular mycorrhizal fungi and exhibits phi thickenings in root cross sections. Primitive conifers such as the Cupressaceae and Taxodiaceae have not been as well studied as the Pinaceae because primitive gymnosperms are not dominant in the world. However, primitive coniferous forests are important plantation forests, representing up to 40% of the total forest area in Japan. The heterorhizy of primitive gymnosperms, compared with other tree groups, is almost unidentifiable by morphological traits alone (Brundrett *et al.*, 1990).

Study site

This study was carried out in a natural, warm temperate, evergreen coniferous forest of *C. obtusa* at Kamigamo Field Station of Kyoto University, ≈12 km north of Kyoto City (35°40′N, 135°43′E), from March 2002 to March 2004. The mean annual precipitation was 1526 mm during the study period. The mean monthly temperature ranged from 1.3°C (January) to 27.6°C (August), with a mean temperature of 14.9°C over the 2 yr study period. Precipitation at this location is high in early summer and autumn; droughts in the summer season are severe because of high temperatures and the low water retention of the organic layer (Takeda, 1987). The understorey consisted of shrubs of *Cleyera japonica* Thunb., *Eurya japonica* Thunb. and *Lyonia ovalifolia* Wall., and *C. obtusa* saplings. The form of the soil humus in the study area was a Moder, with a very poorly developed A horizon (<1 cm). The boundary between the organic horizon and mineral soil was distinct. The thickness of the organic layer ranged from 2.5 to 6.0 cm with a mean of ≈5 cm. Inorganic N pools were 2.4 ± 1.2 and 0.9 ± 0.2 N mg^{-1} kg^{-1} in dry soil, and N mineralization rates in laboratory incubation were 55 ± 16 and 0.2 ± 0.2 N mg kg^{-1} soil per month in the organic layer and surface (0–5 cm in depth) mineral soil, respectively (M. Hirobe, unpublished). Fine roots of *C. obtusa* were distributed densely in the upper organic layer and constituted the root mat at the study site. The fine roots of *C. obtusa* are thick and orange in apical roots, and quite different from those of the other understorey broadleaved species at this site, which have fibrous, white apical roots. The study plot was 10×10 m in area and was located in a *C. obtusa* forest; it was divided into 16 subplots, each 2.5×2.5 m in area. The above-ground productivity of this study area is ≈2.5 t ha^{-1} (R. Tateno, unpublished).

Sequential ingrowth core method

The dynamics of fine roots were studied by the ingrowth core method (Vogt & Persson, 1991). The sequential ingrowth core method is advantageous when observing the growth processes of fine-root systems of the same maximum age (Eissenstat, 1991); the sequential harvest ingrowth core method allows measurement of the timing of root growth and mortality (Majdi, 1996; Cheng & Bledsoe, 2002). The organic soil near the study plot was collected and sieved through a 2 mm mesh; the soil substrate was then air-dried. All living roots and branched or long dead roots (>1 cm) were removed. Sieved organic soil contains large amounts of unbranched dead roots <1 cm long (≈10% of organic substrates and >95% of natural dead roots in weight; Hishi & Takeda, unpublished data), which are termed 'fragmented dead roots'. Fragmented roots were retained as part of the organic soil. The organic soil was air-dried, then used as the substrate for the ingrowth cores. The ingrowth cores were made of a cylindrical mesh bag (3 cm diameter, 8 cm height, 2 mm mesh size). Each ingrowth core was stuffed with ≈7 g organic substrate up to 5 cm in depth, which was similar to the density of the natural organic layer at this plot. Ten ingrowth cores were buried in each subplot on 10 March 2002.

For each sampling event, 14 ingrowth cores were collected at 1 (April 2002), 3 (June 2002), 4 (July 2002), 5 (August 2002), 6 (September 2002), 7 (October 2002), 9 (December 2002), 12 (March 2003), 16 (July 2003), 19 (October 2003), and 24 (March 2004) months after the date of core burial (0 months is represented by March 2002). The selection of the 14 ingrowth cores each month was determined randomly. In total, 154 cores were collected during the study period. Ingrowth cores were packed in polyethylene bags for transport to the laboratory.

The laboratory measurement procedures were as follows: the root samples from 14 ingrowth cores were first classified into three visual classes. Root length and the mean diameters of 14 ingrowth cores were measured for each visual class. Of the ingrowth cores, nine of 14 samples were used to determine root dry weight and C : N ratios, then specific root length (SRL) and tissue density were calculated using root length

and diameter data. The remaining five core samples were then used for anatomical observations and to determine the mean proportions of the protoxylem groups in each visual class. The root length of each protoxylem group for each visual class was calculated from the root length of each visual class multiplied by the proportion of each protoxylem group. For the 4- and 16-month periods only, samples were obtained to estimate the distribution patterns of protoxylem groups within the fine-root-system architecture. Thirty fine-root clusters from root clusters in nine ingrowth cores were used for estimating dry weight before visual classification. After sampling anatomical specimens, these samples were restored to the root samples before visual classification. These methods are detailed in the following sections.

Visual type classification

After harvesting the ingrowth cores, visual classification procedures were completed within 10 days. The contents of the ingrowth cores were first separated into living fine roots and organic soil. Living fine roots were vigorous and not fragile. Living root samples were examined for color under a white light. Visual color categorization corresponds directly to anatomical features (Hishi & Takeda, 2005). Almost all (>98%) roots that were brown and shrunken in texture had a continuous cork layer and a secondary vascular system (secondary roots). Of the roots that were orange or red and fresh in texture, 97% had passage cells but no cork cambium (primary roots). Therefore the living fine roots were classified into three visual classes, and were named P1 (less-pigmented primary roots); P2 (well pigmented primary roots); and Sec (secondary roots), for orange, red and brown roots, respectively. The procedures of visual classification are detailed in Hishi & Takeda, 2005).

Morphological and chemical measurements

Following classification, root images of each visual class were captured as 256-level gray-scale TIF images in 0.085 mm pixel^{-1} (300 dpi resolution). From the images, root length and mean diameter were determined using the NIH IMAGE program ROOT LENGTH version 1.80 (Kimura & Yamasaki, 2001). The fresh weight of each visual class was also determined. Nine ingrowth cores were randomly selected for dry weight measurements from the 14 ingrowth cores taken during each sampling event; the remaining five samples were used for anatomical observations. The nine samples were dried at 70°C for 48 h and weighed. The dry weights of anatomical samples were calculated from the water content of each visual root class. Then the SRL and tissue density were calculated.

The dried roots of each visual class were pooled from replicates on a single harvest date. In the initial periods of the experiment (1, 3 and 4 months), P2 and Sec roots were rarely found, and could not be used for chemical analysis. P1 roots were also not found in the 1 month period. Therefore the chemical analysis sample sizes were 10, 8 and 8 for P1, P2 and Sec, respectively. Samples were ground, and C and N concentrations determined using an NC analyzer (Shimadzu Co., Tokyo).

Protoxylem groups

After visual classification, the root samples of each visual class from five ingrowth cores used for anatomical observations were kept in 80% (v/v aqueous) ethanol. Five root parts were randomly selected from each visual class root sample of five replications for anatomical observation (25 root parts for each visual root class). Root parts were cut at branching points when the root parts consisted of several individual roots. Cut individual root samples were pooled, spread out on a plastic dish with 70% (v/v, aqueous) ethanol, then mixed. For anatomical measurements, 30–40 samples were selected for each visual class in each month. Cross-sectional specimens of fine roots were made by hand-sectioning at the center of an individual root under a dissecting microscope (×10). The observations of unstained root cross sections were carried out under fluorescence microscopy (365 nm wavelength). The amount of protoxylem was counted for each visual root class. In *C. obtusa* most of the fine roots consisted of roots with two (diarch), three (triarch) and four (tetrarch) protoxylem groups (Hishi & Takeda, 2005).

Data analysis

The fate of individual roots can be described using a compartment model with five components and seven flows (Fig. 1). To calculate root-length dynamics for each protoxylem group, the compartment flow model can be considered to consist of three components and five flows. The lengths of each developmental stage (P1, P2, Sec) are three known compartments of living roots. Using temporal changes in these components, the following five flow processes can be calculated: birth, pigmentation, mortality in primary, secondary growth, and mortality in secondary. We assumed that the state of individual roots changes in the order P1, P2, dead root in primary roots; and P1, P2, Sec, dead root in secondary roots. Stages within this order may be skipped, but never reversed.

The proportion of the protoxylem group ($P_{i,j}$) of visual class i was determined by the number of cross sections with protoxylem j divided by the total number of cross sections. The length of protoxylem group j for a certain visual class i (L_{ij}) was calculated using the equation $L_{i,j} = L_i P_{i,j}$, where L_i is the length of visual class i.

During the interval from $t-1$ to t, root-length production [$B_j(t)$] and mortality [$M_j(t)$] for each protoxylem group j were calculated using a decision matrix, where t refers to the sampling occasion, not the month (Table 1). For example, in this study the sampling occasions, t, in months 1, 3, 4, 5, 6, 7, 9,

Table 1 Decision matrix illustrating estimation of fine-root length production [$B_j(t)$] and mortality [$M_j(t)$] in each interval time

	Production [$B_j(t)$]		Mortality [$M_j(t)$]	
	ΔP1 > 0	ΔP1 < 0	ΔP1 > 0	ΔP1 < 0
ΔP2, ΔSec > 0	ΔP1 + ΔP2 + ΔSec	ΔP1 + ΔP2 + ΔSec or 0	0	−(ΔP1 + ΔP2 + ΔSec) or 0
ΔP2 > 0, ΔSec < 0	ΔP1 + ΔP2	ΔP1 + ΔP2 or 0	−ΔSec	−(ΔP1 + ΔP2 + ΔSec) or −ΔSec†
ΔP2 < 0, ΔSec > 0	ΔP1 + ΔP2 + ΔSec or 0	ΔP1 + ΔP2 + ΔSec or 0	−(ΔP2 + ΔSec)	−(ΔP1 + ΔP2 + ΔSec) or 0
ΔP2, ΔSec < 0	ΔP1	0	−(ΔP2 + ΔSec)	−(ΔP1 + ΔP2 + ΔSec)

The appropriate quadrant is selected according to the direction of change in P1, P2, Sec root length during the interval between two sampling times. $\Delta P1 = L_{P1j}(t) - L_{P1j}(t-1)$, $\Delta P2 = L_{P2,j}(t) - L_{P2j}(t-1)$, and $\Delta Sec = L_{Sec,j}(t) - L_{Sec,j}(t-1)$, where j, t and L indicate protoxylem groups, sampling occasions and root length, respectively.
†If ΔP1 + ΔP2 = 0, the mortality is −ΔSec, otherwise, −(ΔP1 + ΔP2 + ΔSec).

12, 16, 19 and 24 were labeled 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11, respectively. This matrix followed Fig. 1, but mortality was pooled for primary and secondary growth. The total length production and mortality for each protoxylem group during study periods can be calculated by summing monthly estimates.

$$\text{Total length production} = \sum_{t=1}^{11} B_j(t) \quad \text{Eqn 1}$$

$$\text{Total length mortality} = \sum_{t=1}^{11} M_j(t) \quad \text{Eqn 2}$$

When the length of Sec ($L_{sec,j}$) increases, the length of shifting secondary growth during interval $t-1$ to t[S(t)] is an increment of the length of Sec roots [$L_{sec,j}(t+1) - L_{sec,j}(t)$], and mortality in secondary roots is 0. When the length of Sec decreases, mortality in secondary roots [$M_{sec}(t)$] is $L_{sec,j}(t) - L_{sec,j}(t+1)$, and the length of shifting secondary growth is 0.

$$\text{Length shifting secondary growth} = \sum_{t=1}^{11} S_j(t) \quad \text{Eqn 3}$$

$$\text{Length mortality in secondary roots} = \sum_{t=1}^{11} M_{sec,j}(t) \quad \text{Eqn 4}$$

The mortality of primary roots can be calculated as follows:

$$\text{Length mortality in primary} = \sum_{t=1}^{11} M_j(t) - \sum_{t=1}^{11} M_{sec,j}(t) \quad \text{Eqn 5}$$

The SRL of each visual class was used to convert length data into mass data. To simplify calculations, we assumed that the SRL values of P1 and P2 were the same, and defined the SRL values of primary roots (SRL_{prim}) as the mean of the SRL values of P1 and P2. We assumed that there were no differences in SRL values among protoxylem groups. The first appearance of roots is as primary roots, and primary root mass production is length production divided by SRL_{prim}.

$$\text{Total mass production of primary roots} = \sum_{t=1}^{11} B_j / SRL_{prim} \quad \text{Eqn 6}$$

When roots shift to secondary growth, the ratio of weight to length (inverse of SRL) increases. The effect of weight increment per length is as follows:

$$\text{Weight increment per length in secondary growth} = [(1/SRL_{sec}) - (1/SRL_{prim})] \quad \text{Eqn 7}$$

Therefore, the total weight increment by secondary growth and total mass productivity is as follows:

$$\text{Total weight increment by secondary growth} = \sum_{t=1}^{11} S_j(t)\left(\frac{1}{SRL_{sec}} - \frac{1}{SRL_{pim}}\right) \quad \text{Eqn 8}$$

$$\text{Total mass production} = \sum_{t=1}^{11} B_j(t)/SRL_{prim} + \sum_{t=1}^{11} S_j(t)\left(\frac{1}{SRL_{sec}} - \frac{1}{SRL_{prim}}\right) \quad \text{Eqn 9}$$

Mass mortality was calculated as length mortality divided by SRL, according to visual root class.

$$\text{Total mass mortality in primary} = \left(\sum_{t=1}^{11} M_j(t) - \sum_{t=1}^{11} M_{sec,j}(t)\right) \Big/ SRL_{prim} \quad \text{Eqn 10}$$

$$\text{Total mass mortality in secondary} = \sum_{t=1}^{11} M_{sec,j}(t) \Big/ SRL_{sec} \quad \text{Eqn 11}$$

The amount of dead root matter was not used to calculate mortality because, in this experiment, many dead roots were already present in the organic substrates.

Distribution of protoxylem groups in a fine-root system

For the 4- and 16-month periods, the 30 root systems with the highest branching rates (highest number of tips) were selected before visual classification from nine ingrowth core samples to estimate dry weight; these were not used to determine proportions of protoxylem groups (i.e. not for anatomical observations). From these root samples, first-order, second-order, third-order and basal roots were selected. First-order samples were collected from roots having the highest elevation with an apical meristem. Second-order roots were connected to first-order roots. Third-order roots supported more than two second-order roots and were not in a basal position. Basal roots were located at the entry point of the ingrowth core. Thirty cross sections were made by hand-sectioning for each branching position and each sampling time. Then the numbers of protoxylem groups in each cross section were counted.

After sampling anatomical specimens, the roots were returned to the root samples before visual classification. These processes affected fewer than 1% of the values of total length, mean diameter, fresh weight and dry weight for each visual class.

Statistical analysis

To compare visual root types and sampling dates, one-way ANOVA and Fisher's protected least significant difference (PLSD) were used. To compare the proportions of protoxylem groups within root systems, a contingency table analysis was used. Groups consisting of fewer than five specimens were excluded from the analysis. All analyses were performed using STATVIEW version 3.1 (SAS Institute Inc.).

Results

Morphological characteristics of each visual class

The SRL and tissue density changed significantly with changes among visual classes (Table 2). The SRL values of Sec were significantly lower than those of P1 and P2, and the SRL values of P1 and P2 were not significantly different. Therefore the weight increment per unit length of secondary growth (the difference between P1 or P2 and Sec) was 45–53%, with a mean of 49% in primary P1 or P2 roots. The tissue density of Sec was greater than that of P1 and P2, and the tissue density of P2 was greater than that of P1. Diameters differed significantly, but were not linearly distributed, among visual classes: P1 = Sec > P2.

Table 2 Mean (±1 SD) specific root length (SRL), diameter and tissue density of each visual root type of *Chamaecyparis obtusa*

	SRL (m g^{-1})	Diameter (mm)	Tissue density (g cm^{-3})
P1	25.8 ± 8.9^{a}	0.44 ± 0.07^{a}	0.50 ± 0.12^{c}
P2	24.6 ± 5.1^{a}	0.39 ± 0.04^{b}	0.63 ± 0.20^{b}
Sec	16.9 ± 4.3^{b}	0.42 ± 0.06^{a}	0.80 ± 0.21^{a}

SRL, tissue density: $n = 99$, 72 and 72 for P1, P2 and Sec, respectively. Diameter: $n = 140$, 112 and 112 for P1, P2 and Sec, respectively.
[a,b,c] Values significantly different at $P < 0.05$.

Summary of fine-root dynamics

The total fine-root length and dry weight showed similar trends during the study period (Fig. 1a,b). Fine roots were first found in months 1–3. The total living fine-root length and dry weight increased during months 3–7; stabilized during months 7–16; increased again in months 16–19; and finally decreased during months 19–24. The total length and weight of living fine roots peaked in month 19 at 8329 m m^{-2} in length and 413 g m^{-2} weight. Total living fine roots were 5682 and 6803 m m^{-2} long, and 257 and 362 g m^{-2} in weight, in months 12 and 24, respectively. The total root length increased during the first 7 months and during months 16–19 of the study period. The total lengths and weights of fine roots sampled in months 7, 9, 12, 16 and 24 were not significantly different.

P1 roots comprised more than 80% of total roots during the first 5 months and remained dominant until month 12. Sec roots increased and accumulated until month 19 and decreased only during months 19–24.

Dynamics of each protoxylem group

The root-length dynamics differed among protoxylem groups (Fig. 2). The root lengths of the diarch and triarch groups fluctuated, whereas the length of the tetrarch roots increased steadily until month 19. For all protoxylem groups, the length of Sec roots increased until month 19. The total lengths of diarch and triarch roots had two peaks, while that of tetrarch roots had one peak. The peak diarch root lengths were 2658 and 2727 m m^{-2} in months 7 and 19, respectively. The peak triarch root lengths were 2190 and 2630 m m^{-2} in months 6 and 16, respectively. The peak tetrarch root length measured 1901 m m^{-2} in month 19.

The relationship between the total length of diarch and triarch roots changed seasonally over the 2 yr study period (Fig. 3). The slopes of the correlation lines between the lengths of diarch and triarch roots in spring–summer (4–6 and 16 months) were significantly lower than the slopes in autumn–winter (7–12 and 19–24 months) (ANCOVA; slope difference, $df = 1$, $F = 18.7$, $P < 0.0001$). Although significant correlations were found between the lengths of diarch and tetrarch roots and between triarch and tetrarch roots for most sampling dates, no common seasonal changes were found in either of the study period years. In total, 85% of the length of primary roots died before 19 months, and 76% of secondary roots died during months 19–24.

Production in terms of the length and weight of each protoxylem group is shown in Tables 3 and 4, respectively.

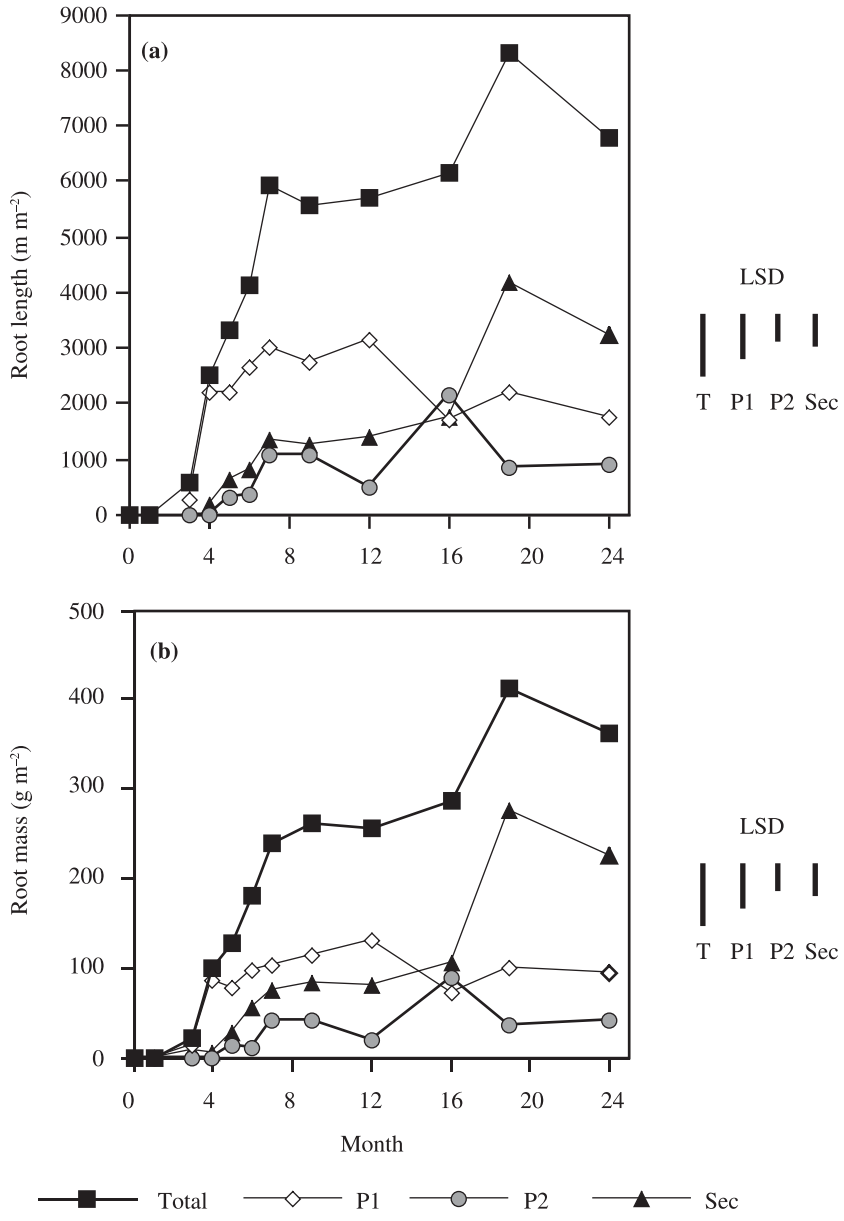

Fig. 2 Mean root length (a) and mass (b) of each visual root class in *Chamaecyparis obtusa* with Fisher's LSD ($n = 14$, $P < 0.05$).

Secondary growth as a length percentage of total production was 21, 41 and 69% in diarch, triarch and tetrarch roots, respectively (Table 3); most diarch and triarch roots did not shift to secondary growth. In weight productivity, Table 4 shows the percentage of weight production of secondary roots to total production. As a result, total secondary root production was 28, 50 and 76% of total production in diarch, triarch and tetrarch roots, respectively, with a mean of 48%. Therefore the decreases in diarch and triarch root lengths were largely attributed to a decrease of primary roots (e.g. P1 and P2) before month 19.

The mortalities in the length and weight of each protoxylem group are shown in Tables 5 and 6, respectively. The total mortality of tetrarch roots was less than that of diarch and

Table 3 Length production of each protoxylem group in *Chamaecyparis obtusa* during the 2 yr study period and the contribution of secondary roots to production

	Total production (m m^2 2 yr^{-1})	Secondary root production (m m^2 2 yr^{-1})	% Sec
Diarch	4258	915	21
Triarch	3679	1503	41
Tetrarch	2217	1519	69
Total	10 154	3937	39

%Sec indicates proportion of secondary root length production to length production of each protoxylem group.

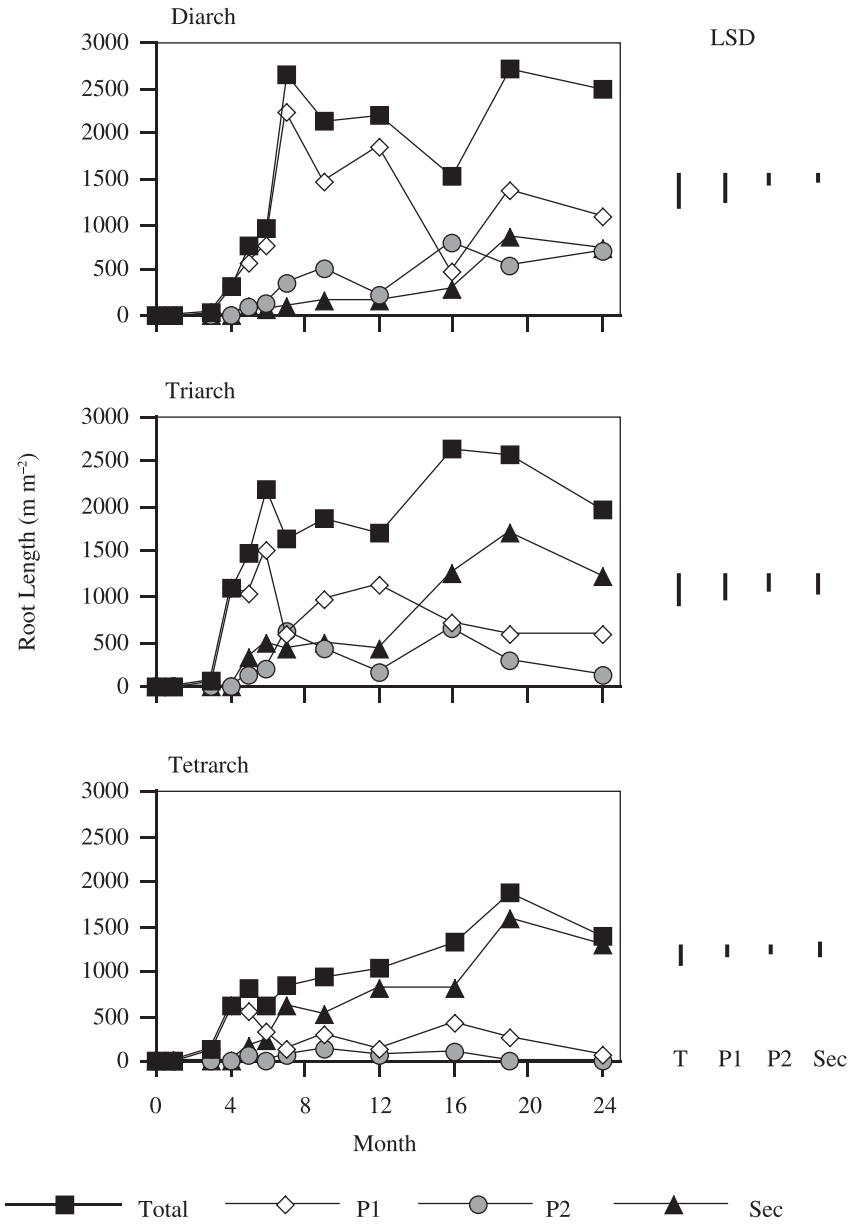

Fig. 3 Mean root length of each visual root class for each protoxylem group in *Chamaecyparis obtusa* with Fisher's LSD ($n = 14$, $P < 0.05$).

Table 4 Weight production of each protoxylem group in *Chamaecyparis obtusa* during the 2 yr study period

	Total production (g m^{-2} 2 yr^{-1})	Primary root production (g m^{-2} 2 yr^{-1})	Increment by secondary growth (g m^{-2} 2 yr^{-1})	Secondary root production (g m^{-2} 2 yr^{-1})	% Sec
Diarch	190	173	17	54	28
Triarch	177	150	28	89	50
Tetrarch	118	90	28	90	76
Total	485	413	73	233	48

Total production could be calculated by primary root production plus weight increment by secondary growth. Secondary root production is production as primary roots plus its increment by secondary growth of secondary roots.
%Sec indicates proportion of secondary root length production to length production of each protoxylem group.

Table 5 Length mortality and contribution of primary and secondary roots to mortality for each protoxylem group in *Chamaecyparis obtusa* during the 2 yr study period

	Total mortality ($m\ m^{-2}\ 2\ yr^{-1}$)	Primary ($m\ m^{-2}\ 2\ yr^{-1}$)	Secondary ($m\ m^{-2}\ 2\ yr^{-1}$)	% Sec
Diarch	1767	1643	124	7
Triarch	1727	1119	608	35
Tetrarch	907	411	496	54
Total	4401	3173	1228	28

%Sec indicates proportion of secondary roots to total mortality.

Table 6 Mass mortality and contribution of primary and secondary roots to mortality for each protoxylem group in *Chamaecyparis obtusa* during the 2 yr study period

	Total mortality ($g\ m^{-2}\ 2\ yr^{-1}$)	Primary ($g\ m^{-2}\ 2\ yr^{-1}$)	Secondary ($g\ m^{-2}\ 2\ yr^{-1}$)	% Sec
Diarch	74	67	7	10
Triarch	81	45	36	44
Tetrarch	46	17	29	63
Total	201	129	72	36

%Sec indicates proportion of secondary roots to total mortality.

Table 7 Mean (± SD) carbon and nitrogen concentrations, and C : N ratio of each visual root type in *Chamaecyparis obtusa*

	n	C ($mg\ g^{-1}$)	N ($mg\ g^{-1}$)	C : N
P1	10	48.8 ± 1.1^{a}	1.35 ± 0.05^{a}	36.3 ± 1.6^{c}
P2	8	49.3 ± 0.9^{a}	1.18 ± 0.09^{b}	41.9 ± 3.3^{b}
Sec	8	49.3 ± 1.0^{a}	0.92 ± 0.06^{c}	53.1 ± 3.0^{a}

Each sample collected on a single harvest date pooled for each visual type. ^{a,b,c} Values significantly different at $P < 0.05$.

triarch roots. The percentage of primary root mortality and the total mortality of diarch and triarch roots were similar. The contributions of primary root mortality to total mortality were 93, 65 and 46% in length, and 90, 54 and 35% in weight for diarch, triarch and tetrarch roots, respectively. Diarch and triarch roots represented 86% of the primary root mortality, and triarch and tetrarch roots represented 90% of secondary root mortality.

Chemical characteristics of each visual class

The C concentration showed no differences among visual classes (Table 7). The N concentration decreased (P1 > P2 > Sec) and the C : N ratio increased (P1 < P2 < Sec) as roots became darker in color.

Root-system architecture

At 4 months the fine-root system was immature (Fig. 4a), and at 16 months it was well developed and branched (Fig. 4b).

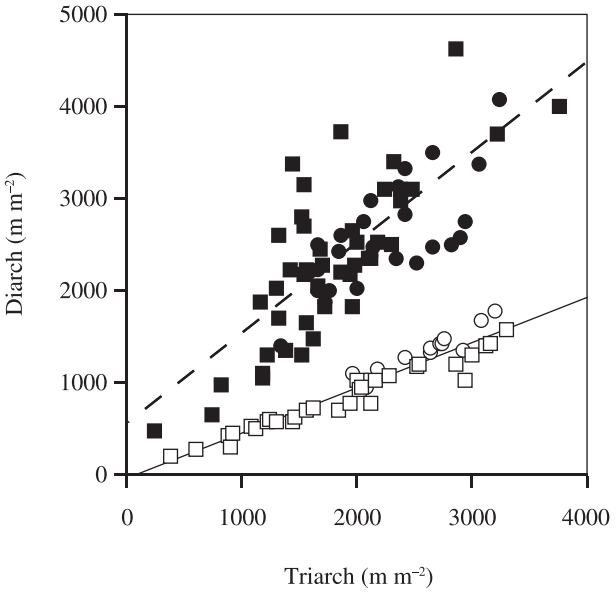

Fig. 4 Relationship between total lengths of diarch and triarch roots in *Chamaecyparis obtusa*. Open squares and open circles indicate spring–summer of the first year (months 4, 5 and 6) and spring–summer of the second year (month 16), respectively. Closed squares and closed circles indicate autumn–winter of the first year (months 7, 9 and 12) and autumn–winter of the second year (months 19 and 24). Solid line represents the regression line of spring–summer [(diarch) = –37.119 + 0.50(triarch), $R^2 = 0.91$, $P < 0.0001$]; broken line represents the regression line of autumn–winter [(diarch) = –517 + 0.98(triarch), $R^2 = 0.60$, $P < 0.0001$].

The distributions of protoxylem groups differed significantly among root branching orders and sampling times (Fig. 4c). The distribution of protoxylem in basal roots at 4 months was similar to that in basal positions at 16 months. The distribution of protoxylem in first-order roots at 4 months was similar to that in second- and third-order roots at 16 months. The diarch structure was distributed mostly in apical positions at 16 months. The tetrarch structure was distributed in basal positions relative to the triarch structure.

Discussion

Total fine-root production and mortality

In this study the regrowth rates of living fine-root masses in months 0–12 and 12–24 were 257 and 106 $g\ m^{-2}\ yr^{-1}$, respectively (Fig. 1a,b), but dead roots were not considered in this productivity estimate. Using the estimate of production of protoxylem groups, the total productivity should be 434 $g\ m^{-2}\ 2\ yr^{-1}$ (Table 4). The production per year, which was estimated from the 2-yr production divided by two, was 217 $g\ m^{-2}\ yr^{-1}$. In warm temperate coniferous forests, fine-root mass production ranges from 254 to 409 $g\ m^{-2}$ (Vogt *et al.*, 1996). Fine-root production was less in this study than in other warm temperate coniferous forests. In a *C. obtusa* site within 1 km

of our study plot, one study estimated fine-root production, using a modified N-budget technique, to be from 149 to 267 g m^{-2} yr^{-1} (Tokuchi *et al.*, 2002). Our estimate of fine-root production in the organic layer was within this range.

There are some problems in estimating fine-root production using the ingrowth core method. The ingrowth cores might not be representative of intact soil, which initially has a root-free condition. Delay in root colonization may occur. Moreover, in this study the method of estimating production did not formally follow the ingrowth core method used in previous studies (Vogt & Persson, 1991). For example, the interval time was not uniform, and changes in the amounts of dead roots were not measured, which could have led to errors in estimates of production (Vogt *et al.*, 1986). The developmental stages in root architecture may affect the processes of fine-root productivity and mortality. In our study, fine-root systems seemed to colonize and make the frame architecture during months 0–7, and dynamically maintained the fine-root architecture with exchanges near the apical roots (Figs 2, 4). The former processes may contribute to an increase in living roots, and the latter may contribute to mortality in fine roots. In natural undisturbed soil, root systems have various developmental stages or ages, whereas the root systems in ingrowth cores are of a similar developmental stage or age, as in this study. To estimate fine-root productivity, we should caution that the manner of recruitment of individual roots within the fine-root system changes along with root system development.

Classification into protoxylem groups

In this study, individual perennial and ephemeral roots were defined according to whether the roots advanced to secondary growth after primary growth (Fig. 1), because secondary growth is one of the most dramatic events defining individual anatomical and functional root characteristics (Esau, 1964; McKenzie & Peterson, 1995a, 1995b). In some tree species, identification of secondary growth roots is often difficult when considering appearance alone (McKenzie & Peterson, 1995a), but in *C. obtusa* brown roots clearly correspond with the anatomical characteristics of secondary roots (Hishi & Takeda, 2005). We consider our method of visual classification, rather than using diameter, to be appropriate in this study because diameter does not reflect the visual class (Table 2).

Protoxylem groups are an important index in identifying the life cycles of individual roots, regardless of whether they experience secondary growth (Noelle, 1910; Hishi & Takeda, 2005). In this study, the differences in protoxylem group life cycles could not be completely classified using the leaves and branches of the shoot system. For example, ≈20% of the diarch length turned into secondary roots, and half the tetrarch length died before becoming secondary growth (Tables 3 and 4). In conifer species, Noelle (1910) proposed the heterorhizy among protoxylem groups. This exception may be the reason why Wilcox (1962) rejected the existence of heterorhizy among protoxylem groups, in his study of incense cedar. This is because the ranges of diameters among protoxylem groups largely overlapped: Wilcox called this a 50-yr-old conflict. However, the difference in life cycles among protoxylem groups was statistically significant (Hishi & Takeda, 2005). Also, the differences in length dynamics by secondary growth (Fig. 3) and in architectural position (Fig. 5) among protoxylem groups indicates that classification of protoxylem groups may be a good indicator of their life cycles and physiological functions, at least for *C. obtusa*. Our study of *C. obtusa* supports the concept of heterorhizy among protoxylem groups that Noelle (1910) proposed 100 years ago.

Dynamics of protoxylem groups

As expected, the dynamics of tetrarch roots fluctuated less than those of diarch or triarch roots. This may be because most tetrarch roots develop secondary growth roots (Fig. 2a–c). The dynamics of root length for each protoxylem group were characterized by dynamic primary roots and by an accumulation of secondary roots during the study period. The lengths of diarch and triarch roots changed not only with incubation time, but also with season, while the length of tetrarch roots changed with incubation time and not with season. King *et al.* (2002) reported that, in the loblolly pine (*Pinus taeda*), ephemeral roots show seasonal variation but perennial roots do not. We suggest that the seasonal effect on the dynamics of ephemeral roots may be stronger than that on perennial roots.

The lengths of diarch and triarch roots showed dynamic growth and death, whereas total fine-root length was relatively stable after month 7. The lengths of diarch and triarch roots had rhythmic phenology (Fig. 3), whereas the total fine-root length density differed between the first and second years with root-system development (Fig. 1a). In both the first and second years, triarch roots grew during late spring and early summer, and diarch roots grew during mid-autumn. In many studies, two peaks of living fine-root biomass have been found (e.g. Hendrick & Pregitzer, 1993; Son & Hwang, 2003). An interesting finding of our study is that different fine-root types within the root life cycle, such as diarch and triarch, alternately emerged in spring and autumn without conforming to the root system developmental stage. We suggest that this shifting phenology may contribute to differences in the longevity of individual roots with differences in first appearance dates, as previously reported (Hendrick & Pregitzer, 1992; Ruess *et al.*, 2003). At this study site summer droughts are severe (Takeda, 1987), and moisture conditions are moderate in autumn, winter and spring. The fine roots of *C. obtusa* grow in spring and autumn. The secondary growth of individual roots increases their stress tolerance (McKenzie & Peterson, 1995b), so in spring the fine roots of *C. obtusa* extend their organ longevity by constructing triarch roots with the developmental potential of secondary growth.

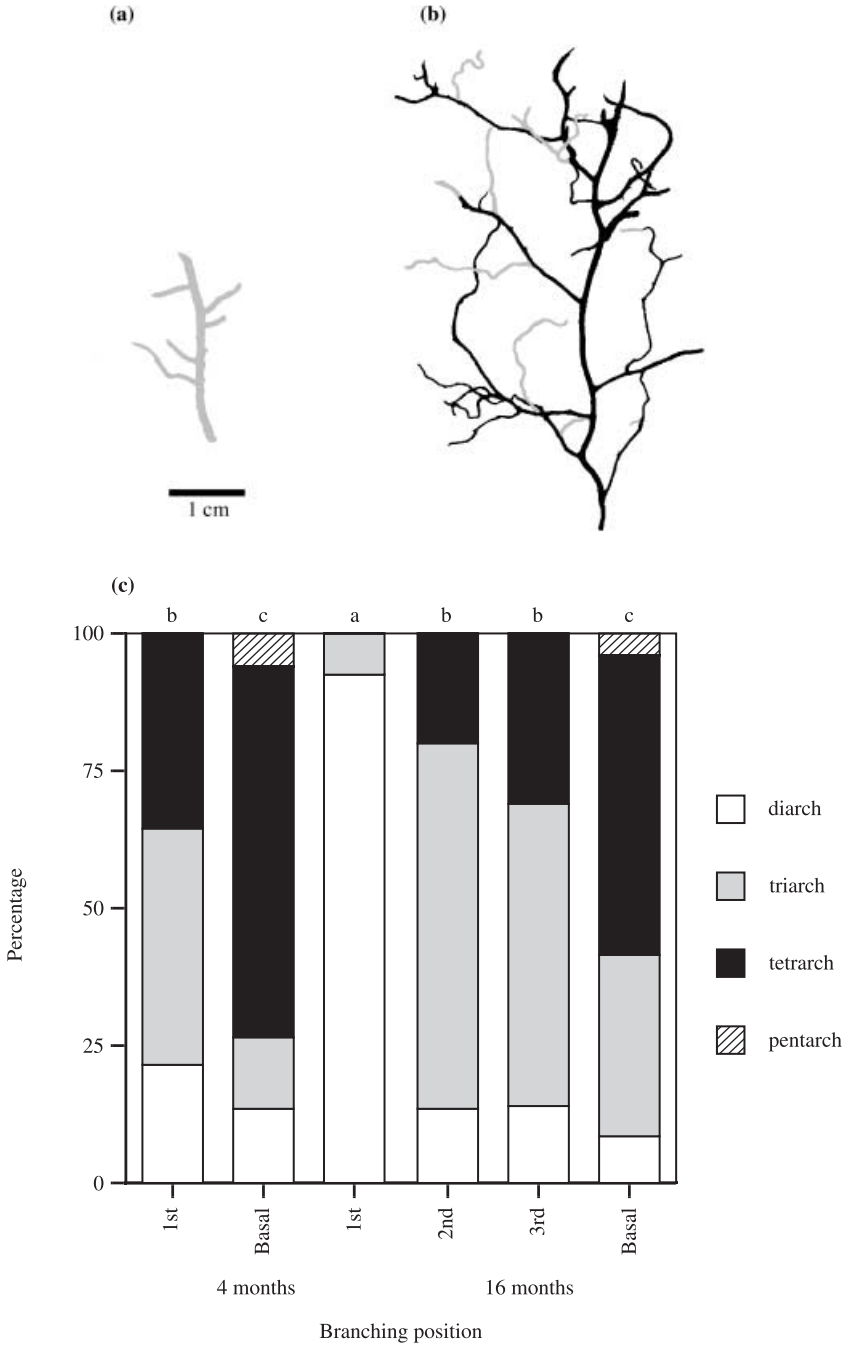

Fig. 5 Scanned images of the root system of *Chamaecyparis obtusa* at (a) 4 months; (b) 16 months. Gray regions indicate primary roots; black areas, secondary roots. (c) Distributions of protoxylem groups within fine-root architecture at 4 and 16 months. Different letters indicate values that differ significantly at $P < 0.05$, based on sequential Bonferroni analysis after contingency table analysis. 1st, a first-order root with apical meristem; 2nd, a second-order root; 3rd, a third-order root. Basal, root at entry point of ingrowth cores, and the most distal roots to apical first order.

The process of root death differed temporally among ephemeral and perennial roots. The majority of diarch roots died as primary roots during months 7–16, and most tetrarch roots died as secondary roots during months 19–24 (Fig. 2). Triarch roots died as primary roots during months 6 and 12 and as secondary growth roots during months 19–24. We propose that the decrease in secondary roots may be reflected in the death of the whole fine-root system that occurred during months 19 and 24.

Ephemeral and perennial roots as soil organic matter

Fine-root litter has been considered an important source of soil organic matter (Vogt *et al.*, 1982). As we expected, the proportion of root death occurring before secondary growth to total death differed among the protoxylem groups; the ranking was tetrarch > triarch > diarch (Tables 5 and 6).

The C : N ratio is an important index in determining the decomposition rate of the earliest decomposition phase of fine

roots (Ostertag & Hobbie, 1999; Silver & Miya, 2001; Berg & McClaugherty, 2003). The C : N ratio was greater in secondary roots (Sec) than in primary roots (P1 and P2) (Table 6). Moreover, secondary roots consist mainly of secondary tissues such as lignin and suberin. These chemicals strongly resist decomposition (Berg & McClaugherty, 2003), indicating that the decomposition rate may be less in secondary than in primary roots. In our decomposition experiment with the fine roots of *C. obtusa* using the litter-bag method ($n = 6$, Hishi *et al.*, unpublished data), the initial decomposition rates of primary roots were significantly faster (14 ± 2 and $21 \pm 5\%$) than those of secondary roots (9 ± 1 and $12 \pm 3\%$) in both the 3- and 6-month periods. These results indicate that the ranking of decomposition rates may be diarch < triarch < tetrarch roots, because of the differences in the proportion of secondary roots to total mortality among the protoxylem groups. Ruess *et al.* (2003) reported that fine roots with shorter longevity disappeared more quickly than did roots with longer longevity. We suggest that the heterorhizy of fine roots in life cycles, that is, the differences among diarch, triarch and tetrarch roots, should affect soil C dynamics through differences in mortality, the anatomical stage at which the roots die, and decomposition rates.

Root-system architecture

Diarch roots are positioned apically relative to triarch or tetrarch roots within the root-system architecture (Fig. 4). Therefore the dynamics, mortality and potential decomposition rates of each protoxylem group should be defined by the position it holds within the fine-root architecture. Our results support the findings of previous studies showing that the individual branching position within the root architecture is important for determining the morphological, physiological and chemical characteristics of individual roots, as well as longevity (Fitter, 1991; Pregitzer *et al.*, 1998; Pregitzer *et al.*, 2002; Guo *et al.*, 2004). Nevertheless, individual root functions within a fine-root system may be determined not only by age differences, but also by ontogenetic traits, as the amount of protoxylem in an individual root is determined ontogenetically (Esau, 1964; Hishi & Takeda, 2005).

There are several possible explanations for the heterogeneous distribution of protoxylem groups within a fine-root system. With regard to material transport, hierarchical distribution may be affected by vascular size. Vascular size differs among protoxylem groups (Esau, 1964). Therefore we suggest that vascular structure, such as protoxylem groups, may constrain fine-root-system architecture. With regard to physiological functions, roots may need both ephemeral primary roots for resource acquisition, and perennial secondary roots with transport ability, like leaves and branches in a shoot system. The absorptive ability of primary roots decreases as they age; therefore apical ephemeral roots are exchanged during the survival of the whole fine-root system, supported by secondary perennial roots. This explanation has also been explored using an ecological concept of a trade-off between longevity and construction cost (Eissenstat *et al.*, 2000). Secondary roots have a high construction cost. In this study, apical ephemeral roots tended to be dynamic and basal perennial roots tended to be static. Our results support the suggested explanations.

Conclusion

This study investigated the dynamics and chemical compositions of different root groups during ephemeral and perennial life cycles. It proved the hypothesis that roots with a higher amount of protoxylem have less fluctuation in length dynamics, a higher proportion of mortality in secondary roots to total mortality, and lower potential decomposition rates, and are more basally positioned within the root-system architecture than are roots with a lesser amount of protoxylem.

Our results suggest that heterorhizic individuals in a fine-root system contribute differently to soil organic dynamics through differences in mortality and potential decomposition rates. Moreover, these mechanisms are defined by the fine-root architecture. Further studies should consider the linkage between the micro-scale level, such as the maintenance of absorptive ability by heterorhizic dynamics in root-system architecture, and the ecosystem level, such as the net primary production or material cycling, to develop an understanding of the function of fine roots in soil organic matter.

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