Dynamics and Architecture of Fine Root System in a *Cryptomeria japonica* plantation

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Chapter 1

General Introduction

Forest ecosystems are one of the most productive ecosystems on earth and account for about 30-40% of vegetation on land (Waring and Running 2007). Their net primary productivity (NPP) accounts for about 65% of total terrestrial NPP (Whittaker 1975). In these NPP estimations, the NPP of roots has been less studied because of the belowground distribution of roots. NPP of roots includes production of fine and coarse roots. Roots < 2 mm in diameter are traditionally classified as fine roots and all others are classified as coarse roots. Vogt et al. (1986) noticed the importance of fine roots in NPP of forest ecosystems, and showed that fine roots account for 20 to 80 % of NPP in forest ecosystems. Since then, production and biomass studies of fine roots have been conducted to examine the biomass and productivity of fine root systems in various forest ecosystems, from boreal to tropical rain forests (Gower et al. 1996). Fine roots of root systems play an important role in soil nutrient acquisition and water uptake through development of root system (Karizumi 1979; Fitter 2002). Jackson et al. (1997) estimated the global distribution of fine roots biomass, length, and surface area with depth in the soil, and global estimates of nutrient pools in fine roots. Assuming the turnover of fine roots is once per year, NPP of fine roots is estimated to represent 33% of global annual net NPP in terrestrial ecosystems.

Fine root dynamics has been studied to evaluate the contribution of fine roots in carbon and nutrient dynamics of forest ecosystems (Gill and Jackson 2000). Fine root systems are composed of a combination of individual roots of many different diameters (Hishi 2007) and show their architectural characteristics. Carbon investment to the development of fine root systems is related to nutrient absorption and water (Fitter and Stickland 1991; Nielsen et al. 1994; Bouma et al. 2001). Recently, research has focused on the individual roots of root systems, especially the branching properties of individual roots. Individual roots within a fine root system are heterogeneous with respect to morphology, physiology and chemical properties (Hishi 2007).

For example, roots near the apical positions have a high primary to secondary tissue ratio, respiration rate, nitrogen content, and absorption capability, and have a small diameter (Pregitzer et al. 1997, 1998, 2002; Hishi and Takeda 2005a, b; Hishi 2007; Guo et al. 2008a; Valenzuela-Estrada et al. 2008; Hishi et al. 2016). Thus, the branching order of individual roots is needed to better understand the functions of fine root systems (Guo et al. 2008b). Recent studies of fine roots also pointed out that it was important to evaluate at the root system level in order to ascertain the dynamics of the roots (e.g. Hishi 2007; McCormak et al. 2015; Makita et al. 2015). However, the relationship between the size (root diameter) and the functioning of individual roots within fine root systems is not well understood.

Under natural conditions, water and nutrients are heterogeneously distributed in both time and space. Several environmental factors of soil such as moisture, soil bulk density, and nutrient concentration, as well as fungi, affect the architecture and morphology of fine root systems (Eissenstat and Achor 1999; Bengough 2003; Atkinson 2003; Fujimaki et al. 2005; Cheng et al. 2006; Hishi et al. 2006). Plants have a fundamental trait to optimize architecture of root system to maximize nutrient absorptive capacity (Caldwell 1994; Hodge 2004). Studying the development of fine root systems is essential to understand not only the mechanism of acquisition of soil resources, but also the various functional processes of natural ecosystems.

The main objectives of this thesis are as follows. In the chapter two, the distribution patterns of root systems along the soil profile were examined, and root biomass were estimated in two differing soil conditions along a slope in a plantation of *Cryptomeria japonica*. Chapter three describes the relationship between the anatomical characteristics and absorption qualities of individual roots by analyzing the relationship between the diameter, order root and the number of protoxylem. Chapter four examines the dynamics of fine root systems by analyzing the relationship between the mortality and production of fine roots throughout a seasonal cycle, based on the characteristics of fine roots (such as biomass and morphology). Chapter five examines the usage of soil nitrogen resources by plants, based on the development of the root system in relation

to inorganic N in soil. These studies were conducted in a *Cryptomeria japonica* plantation.

Chapter 2

Soil characteristics and above-belowground biomass along a slope in a *Cryptomeria japonica* plantation

Introduction

Forests are composed of various tree species. Tree species can be distinguished by observing their characteristics above-ground. Roots however, have a similar color and form among species. Fine roots show few distinctive external features that permit identification of species. In natural broadleaf forests, fine roots consist of various species, so it is difficult to estimate the fine root biomass for each species. Plantations and coniferous forests are composed of fewer or monotonic species, and as a result allow for the study of fine roots at species level. Thus, most studies of fine roots have been conducted in coniferous and plantation forests in North America, and Japan (Vogt et al, 1987; Noguchi et al. 2007).

In Japan, fine root biomass and morphology of tree species has been primarily studied with the species, i.e., *Cryptomeria japonica* (e.g. Kasuya and Shimada 1996; Noguchi et al. 2004, 2013; Konopka et al. 2006, 2007; Fujimaki et al. 2007; Hishi et al. 2017) and *Chamaecyparis obtusa* (e.g. Sakai and Inoue 1986; Hishi and Takeda 2005a, b; Makita et al. 2015; Miyatani et al. 2016; Doi et al. 2017). Because of the well-established data, *C. japonica* tree species was selected for the present study.

Approximately 70% of the landmass in Japan is occupied by forested areas. (Japan FAO Association 1997). Of This area, *C. japonica* and *C. obtusa* are the dominant species and account for 70 % of the population in plantation forests (Noguchi et al. 2007). The area of plantation forests is 10.29 million hectares, which is about 40% of the forested area. The plantation area of *C. japonica* is 4.48 million hectares, which is 18% of the total forested area and 44% of the plantation forest area (Forest Agency of Japan 2014).

C. japonica is a species that is representative of Japanese plantations. Both *C. japonica*

and *C. obtusa* have been planted for the purpose of timber production. These trees were selected because of their traits and ease of processing, as well as their rapid growth. *C. japonica* and *C. obtusa* have been historically planted on the moist lower or dry mid slope of many plantation areas (Noguchi et al. 2007), and *Pinus densiflora* has been planted in the upper part or ridge of plantation slopes (Katagiri 1996). In this study area, *C. japonica* was also planted on a forest slope. The studies in detailed in chapters two through five were carried out in a *C. japonica* plantation forest.

Study sites were established along a forest slope of a *C. japonica* plantation. Forest slopes provide a natural gradient of soil development from the ridge to bottom parts of the slope. The aim of this chapter is to provide an overview of the site characteristics, particularly the soil characteristics and root biomass. Relationships between above- and below-ground biomass were also analyzed, and fine root biomass of this site were compared with other sites.

Material and methods

Study site

The study was carried out in Oharano Forest Park, Kyoto City, Japan (34°57N, 135°37E, 400 m above sea level, Fig. 2.1). The mean annual temperature was 16.1 °C and the annual precipitation was 1602 mm during the study period (measured at the Kyoto Weather Station, about 12 km from the study site, Fig. 2.2). A 20 m wide × 50 m long plot covering from the lower to the upper part of a forest slope was selected in the plantation (Fig. 2.3). This plot was the main plot of all studies in this thesis described here, and is called “main plot” in this thesis. In the main plot, the organic soil layer was a moder humus with a mean thickness of 2.90 (±0.57) and 4.20 (±0.63) cm in the lower and upper parts of slope, respectively (Table 2.1). Thickness of organic layer was higher in the upper slope than in the lower slope. The soil in the study plot was brown forest soil and is classified as category B_D (Forest Soil Division 1976).

Root characteristics of Cryptomeria japonica D. Don.

The root system of *C. japonica* shows relatively deep distribution (Karizumi 1979) (Fig. 2.4) and the root tips form arbuscular mycorrhiza. Karizumi (1979) also showed that most roots of *C. japonica* usually have five strands of protoxylem (pentarch), but there is little information on anatomical characteristics of individual roots of the fine root systems of *C. japonica*.

Vegetation

Five sub plots measuring 20 m x 10 m each were established along the main plot (20 x 50 m). The plot of lowest slope as P1, and plot of second lower slope position as P2 and so on (Fig. 2.5). A census of the plot's vegetation was carried out in November 2014. For trees larger than 5 cm in diameter at ground level, diameter at breast height (DBH) was measured. DBH of C. japonica was the only tree species with larger diameter than 5 cm in diameter in the plot. A laser distance measuring instrument (Leica DISTO D 3 a) was used to measure tree height. Due to the densely packed canopy of C. japonica, tree height was recorded only for trees that were easily measurable.

Root sampling

Root samples were collected by using soil cores (56 mm inner diameter) in November 2011. The soil columns were separated into the upper organic soil starting from the fermentation layer and divided into organic soil and mineral soil (0-50 cm depth). On sampling occasion, one core was taken from each of the 5 subplots: P1-P5. These samples were transferred to plastic bags and transported them to the laboratory where they were stored in a refrigerator at 4°C until processed. Processing began by rinsing with tap water, after which the fine roots (< 2 mm diameter) were removed. I separated living roots and dead roots by color and texture. Living roots were rigid and lightly colored; dead roots were fragile and darkly colored. Living fine roots were divided into two diameter size; <1 mm and 1-2 mm. After that fine roots were dried

at 70°C to the constant mass and weighed.

Soil sampling

Soil conditions were studied in the lower (P1) and upper part (P5) of the forest slope, and sampling was conducted in November 2014. Six soil cores were collected to measure water content. Soil samples were divided soil samples into organic soil and mineral soil, and then mineral soil samples were divided into 3 soil depths (0-4, 4-8, 8-12 cm). Soil samples were kept in a plastic bag and were transported to the laboratory. After the soil samples were weighed, samples were dried at 70°C weighed to determine water contents.

Six soil cores were sampled in each lower (P1 and P2) and upper (P4 and P5) part of slope to measure soil N and C, inorganic N and amounts of organic layer in October 2013. The soil samples were kept in a plastic bag and transported to the laboratory. The soil cores were separated into 3 parts: organic soil, 0-5 cm and 5-15 cm of mineral soil. The soil samples were sieved using a 2 mm mesh to remove roots and organic matter. To analyze inorganic N concentration, the fine soil samples of 3 g of organic soil and 5 g of mineral soil (measured at fresh weight) were extracted with 50 ml of 2M KCl. The extract was used to measure NH_4^+-N and NO_3^--N pool size. Inorganic N concentration was determined by an auto-analyzer (AutoAnalyzer, BLTEC, Osaka, Japan). The total N and C concentrations were measured by automatic gas chromatography (NC analyzer SUMIGRAPH NC-900, Sumitomo Chemical Co., Osaka, Japan).

Statistical analysis

A Tukey's HSD test was performed for DBH and tree height in relation to its position on the slope. To test the relationships between DBH and tree height with fine root biomass, a correlation analysis was applied A two-way ANOVA was performed to analyze any differences in soil N, C, CN and water along the elevational (slope position) and soil gradients.

Results

Fine root biomass along slope

Fine roots were grouped into two classes, i.e., those with a diameter of 0-1 mm and a diameter of 1-2 mm. Fine root biomass (D <2mm) ranged from 55 - 202 g m^{-2} in the organic layer and 201 - 312 g m^{-2} at 0-50 cm soil depth (Fig.2.6 and Table 2.4). In organic layer, fine root biomass (D <1mm) ranged from 43 - 131 g m^{-2} and fine root biomass (D 1-2mm) ranged from 13 - 90 g m^{-2}. In the organic and mineral soil layer, fine root biomass (D <1 mm) ranged from 151 to 253 g m^{-2} with a mean of 198 g m^{-2} and fine root (D 1-2 mm) ranged from 96 to 245 g m^{-2} with a mean of 171 g m^{-2} (Table 2.4). Total fine root biomass (D <2mm) ranged from 277 to 498 g m^{-2} with a mean of 368 g m^{-2}. Fine root biomass of both diameter class higher in the upper part of slope (P4 and P5) than in the lower part of slope (P1, P2 and P3). Thus, fine root biomass increased along the upper parts of slope in both organic and mineral soil layers.

Soil conditions

Soil water content was significantly lower in the upper part of slope than in the lower parts of slope (Table 2.1). Organic soil depth tended to be higher in the upper parts than in the lower part of slope.

Soil N concentration was significantly higher in the lower parts than in the upper part of slope ($p < 0.01$, Table 2.2) and significantly decreased with increasing soil depth ($p < 0.001$). Soil C concentration significantly varied across soil depth ($p < 0.001$). C concentration of organic soil layers was significantly higher in the upper parts than in the lower parts of slope. While C concentration in the mineral soil layer was rather high around 0-5 and 5-15 cm depth in the lower parts of slope. Soil C/N significantly varied across soil depths ($p < 0.001$), showing that C/N decreased gradually from the surface to the deeper soil layers (Table 2.2). Concentration of NH_4^+-N significantly varied across soil depth ($p < 0.001$), showing that

NH_4^+-N decreased gradually from the surface to the deeper soil layers (Table 2.2). In the upper parts of slope, concentration of NH_4^+-N was significantly higher in organic soil and the 0-5 cm depth ($p < 0.01$ and $p < 0.05$, respectively). Concentration of NO_3^--N also significantly varied across soil depth ($p < 0.001$), showing that NO_3^--N decreased gradually from the surface to deeper soil layers in the lower parts of slope. While this trend was not apparent in the soil of the upper parts of slope. In the lower parts of slope, concentration of NO_3^--N was high at all soil depths.

DBH and tree height along the forest slope

DBH and tree height are both parameters for the estimation of tree biomass above ground (Fig. 2.7), hence this study used DBH and tree height as an index to show aboveground biomass. DBH ranged from 26 - 32 cm with a mean of 27 cm and tree height ranged from 22 - 28 m with a mean of 25 m (Fig. 2.6). There were no differences in DBH along the slope. The tree height however was higher in the lower parts than in the upper parts of slope. Regression between fine root biomass and DBH was no signification, however regression between fine root biomass and tree height was negative correlation ($y = -0.021x + 33$, $r^2 = 0.82$, $p < 0.05$, Fig. 2.8).

Discussion

Soil characteristics at slope position

The topography of forest slopes provides a gradient of soil development by the effect of water availability (Oomasa 1951). In this study site, water contents tended to be lower in the upper parts of slope (Table 2.1). In Japanese forests, soils are frequently drought on the upper slopes, whereas soils usually occur under moisture-rich conditions on the lower slopes, due to drainage characteristics along the slopes (Yanagisawa and Fujita 1999). In this study site, the organic layer was poorly developed in the lower parts of slope, when comparing with those in the upper part of slope (Table 2.1). Thickness of organic layer tended to be higher in the upper part than in

the lower part of the slope. Soil water conditions influence the decomposition rates, resulting in the gradient of organic layer developing along the forest slope (Takeda et al. 1987).

Topographic conditions of slopes also provide a gradient of nutrients. In this study site, N concentration of organic soil layer was no significant differences between the upper parts and the lower parts of slope, whereas, C concentration of organic soil layer was higher in the upper parts of slope (Table 2.2). C and N concentrations in mineral soil layers were significantly higher in the lower parts of slope. Total N and C contents are not directly related to the availability of N for plant growth.

Total mineral nitrogen was higher in the lower parts than in the upper parts of slope (Table 2.2). Mineral nitrogen amounts are a good index of soil fertility. In the upper parts of slope, soil N in the form of NH_4^+-N was dominant, whereas NO_3^--N was dominant in the lower parts of slope. This result suggested a shift in dominance of inorganic N sources along a topographical gradient (Table 2.1). Similar trends have been reported in the forest slope of the cool temperate forests in Ashiu. Mineral nitrogen composition shifted from NH_4^+-N, dominance in the ridge to NO_3^--N dominance in the bottom parts of forest slope (Hirobe et al. 2003; Tateno and Takeda 2003; Fujimaki et al. 2004).

In this study site, nitrification rates were higher in the lower parts of slope. A similar trend was reported in Tateno and Takeda (2003). Soil NO_3^--N has a high mobility and tends to be lost through leaching, but soil NH_4^+-N has a lower mobility because forest soil has a negative charge (Binkley and Vitousek 1989; Miller et al. 2007; Lima et al. 2010), therefore, higher NO_3^--N concentration in lower slope may favor uptake of N in forest soil (Binkley and Hart 1989). These results suggested that the lower parts of slope provided more available mineral nitrogen for plants than the upper parts of slope. Whereas in the upper parts of slope, soils provide NH_4^+-N form for tree growth.

Above- and below-ground biomass along slope elevation

Abundance of fine root biomass was studied along the forest slopes, from the ridge to bottom. Abundance of fine roots have been explained by various environmental factors such as water contents (Sakai and Inoue 1986; Enoki et al. 1996; Kasuya and Shimada 1996), soil texture (Borken et al. 2007) and nutrient availability along the slope (Tateno et al. 2004; Fujimaki et al. 2004). Amounts and composition of mineral nitrogen also influences fine root biomass (Noguchi et al. 2007).

Abundance of fine root biomass should be explained in the context of the interaction of plant allocation and resource availability. Plants adjust their resource acquisition to maximize the capture of the most limiting resources. Temperate forests are considered the nitrogen limited ecosystems (Chapin 1980; McGroddy et al. 2004; Reich & Olesksyn 2004). Soil conditions along forest slopes provide a gradient of nitrogen availability. Root biomass could be explained by the allocation pattern between above- and below-ground NPP.

In this study site, nitrogen availability was higher in the lower parts of slope (Table 2.1). Correlating to this fertility, tree height was higher in the lower parts of the slope (Fig. 2.5). Aboveground biomass and NPP were positively related to soil N availability (Reich et al. 1997; Tateno et al. 2004, 2010). Whereas fine root biomass increased along the slope (Fig. 2.6). A regression analysis between fine root biomass and tree height showed a negative correlation (Fig. 2.7). In this study, the correlation analysis suggests that soil N availability also influenced above- and belowground biomass allocation patterns at this site. Tateno et al. (2004) found that aboveground NPP was negatively correlated to belowground NPP, suggesting that decreasing aboveground NPP along a topographical sequence was a result of a shift in allocation to belowground NPP (Tateno et al. 2004).

Abundance of fine roots in the organic layer accounted for the differences in abundances of fine root between the upper and lower slope. In study site, fine root biomass of organic layer was about 4 times higher in the upper plot (P5) than in the lower plot (P1). Total N

concentration and mineral nitrogen concentrations were higher in the lower parts than in the upper parts of the slope. Further, mineral nitrogen composition was different between the upper and the lower parts of slope. Previous studies suggest that inorganic N influence fine root biomass (Fujimaki et al. 2004; Noguchi et al. 2013). Fujimaki et al. (2004) found that fine root biomass was high at upper slope, where mineral nitrogen was mainly represented by a high NH_4^+-N concentration in soil. Low nutrient supply limited plant growth, leading plants to allocate resource to fine root biomass (Fujimaki et al. 2003; Tateno et al. 2004). It was suggested that in the upper parts of slope, dominance of NH_4^+-N in the organic layers leaded to a higher fine root biomass. These results suggest that *C. japonica* changed allocate patterns of NPP to increase fine root biomass along a topographical sequence, especially in organic soil as a result of increasing the dominance of soil NH_4^+-N.

Fine root biomass of C. japonica in this study site comparing with other C. japonica site

In this study, the average fine root biomass of <1mm and <2mm in diameter was 171 g m^{-2} and 368 g m^{-2}, respectively (Table 2.4). Fine root biomass of needle litter in Japan was very difference between species (Noguchi et al. 2007). Fine root biomass (D <2mm) of *P. densiflora, C. japonica* and *C. obtusa* were 49 g m^{-2}, 421 g m^{-2} and 638 g m^{-2}, respectively (Table 2.3). Fine root biomass (D <2mm) of *C. japonica* ranged from 56 - 1128g m^{-2} (average, 421g m^{-2}, Table 2.4). Thus, fine root biomass of *C. japonica* at this site is comparable with those in the other study sites of Japan. Vogt et al. (1996) reviewed ranges for fine root biomass (D < 2mm), and found they were 75 - 1633g m^{-2} (average, 526 g m^{-2}) in temperate forests (Table 2.3). Jackson et al. (1997) estimated fine root biomass on a global scale, and reported that fine root biomass was 440 g m^{-2} for temperate coniferous forests. These data were mostly obtained from studies of North American and European forests. This study shows that fine root biomass in Japanese forests is generally comparable with or smaller than those in temperate forests of North America and Europe (Noguchi et al. 2007).

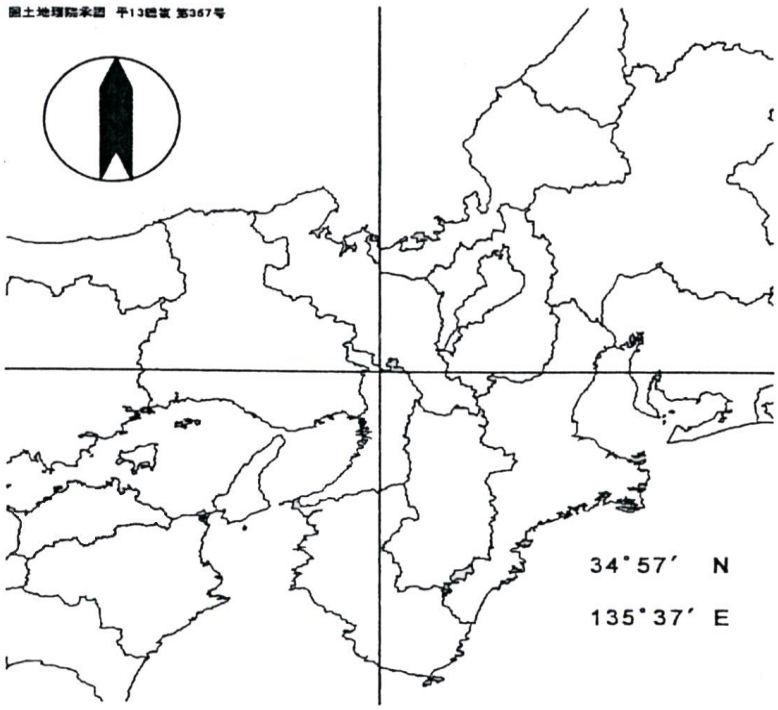

Fig. 2.1 The location of Oharano Forest Park study site

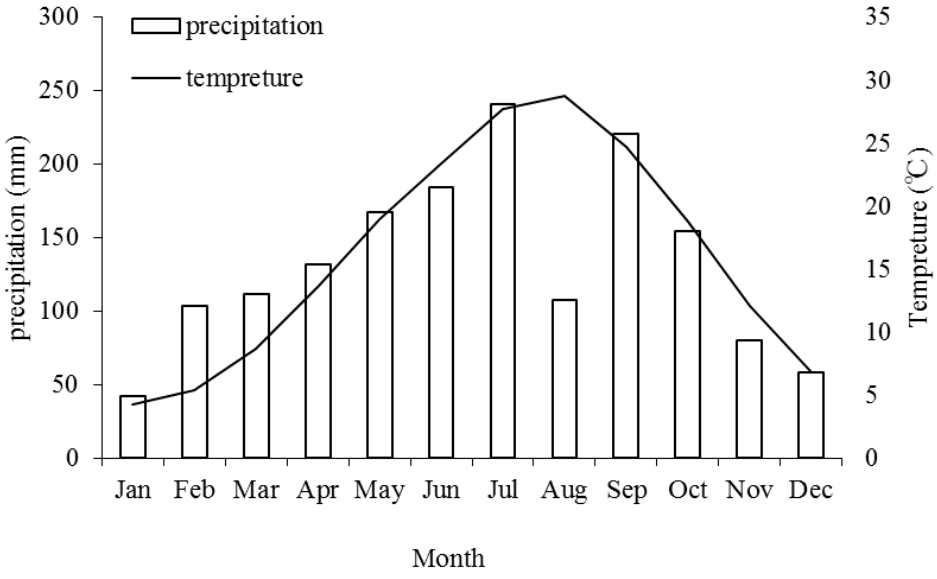

Fig. 2.2 Temperature and precipitation during the study periods (2008-2014) measured at the Kyoto Weather Station, about 12 km from the study site.

Fig. 2.3 The main study plot along a slope of a *Cryptomeria japonica* plantation in Oharano Forest Park. The photograph was taken from the top of the slope.

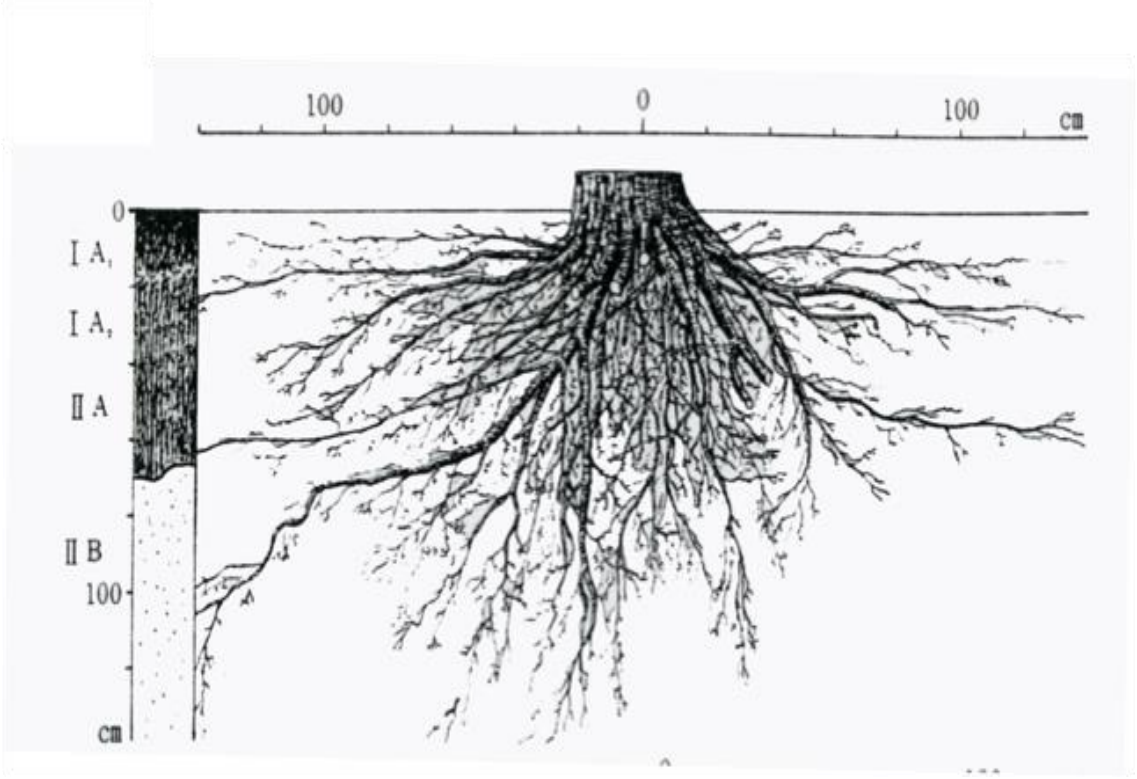

Fig. 2.4 An illustration of a whole root system of *Cryptomeria japonica* (Karizumi 1979)

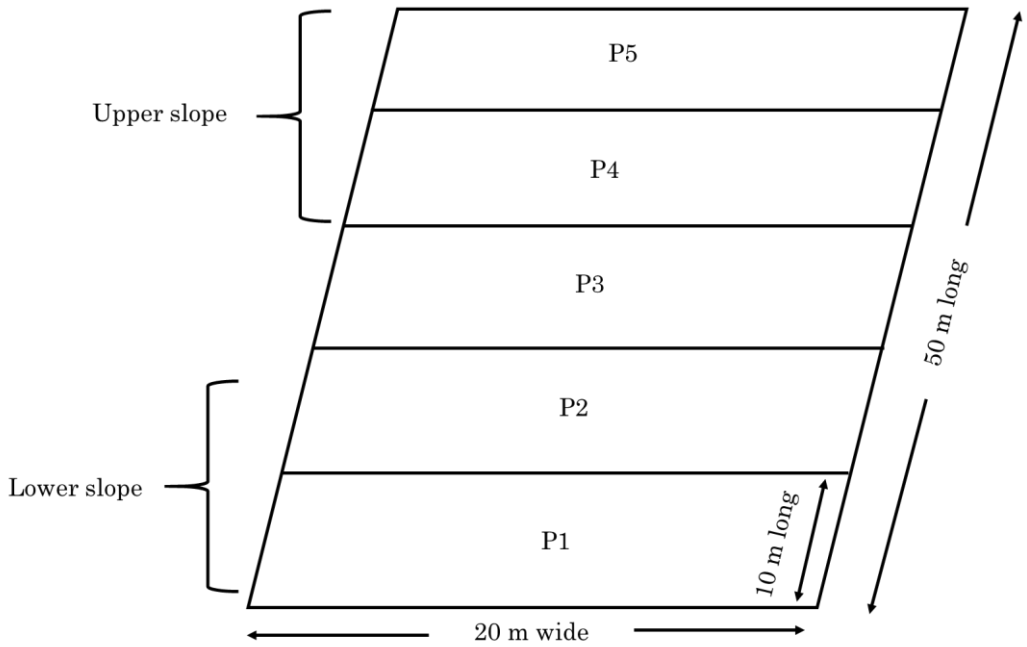

Fig. 2.5 Layout of the main plot (20 × 50 m long) for this thesis and the study plots noted in chapter 2 (P1 to P5)

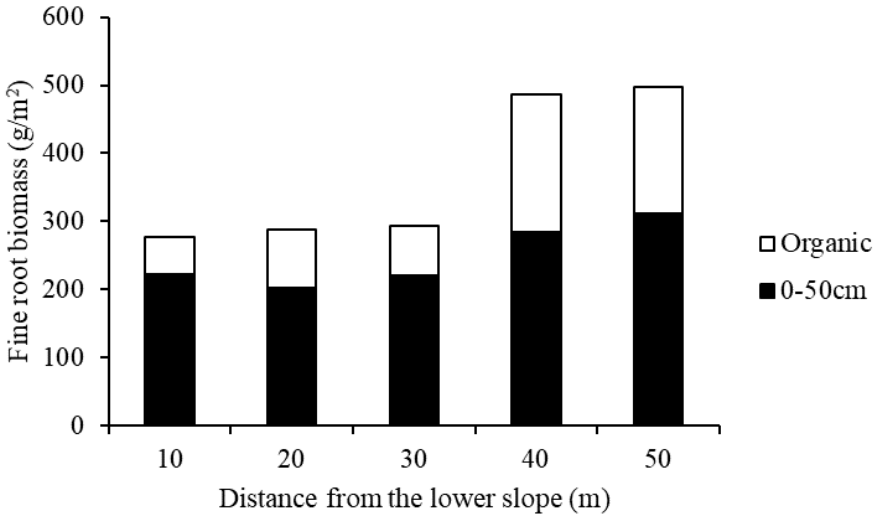

Fig. 2.6 Fine root biomass (organic soil and 0-50cm depths) along the topographical sequence.

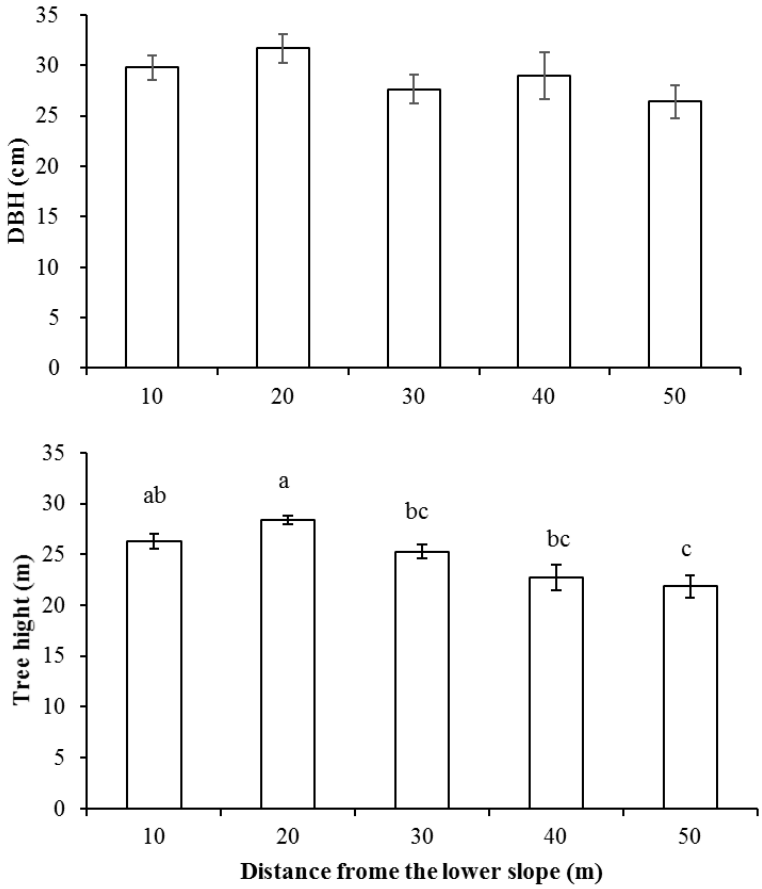

Fig. 2.7 DBH and Tree height along the topographical sequence (Values are mean ± SE). Different letters are significant differences ($p < 0.05$)

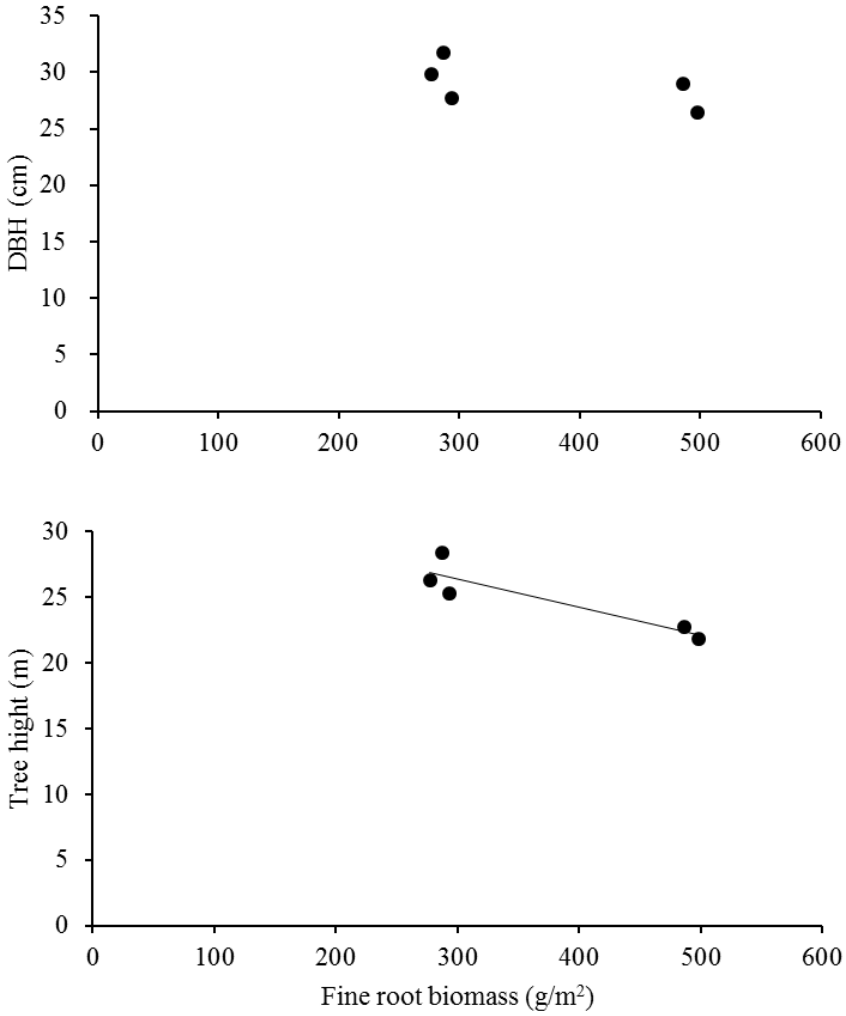

Fig. 2.8 DBH and tree height in relation to fine root biomass. The regression was y = -0.0213x + 32.7 ($R^2 = 0.82$, $P < 0.05$) for tree height.

Table 2.1 Water contents at upper and lower slope positions (Values are mean ± SE)

Depth	Slope	Water content (w/w %)	organic layer depth (cm)
Organic	Upper	33 (8) ns	4.20 (0.63) ns
	Lower	48 (5)	2.90 (0.57)
0-4 cm	Upper	23 (3) *	-
	Lower	34 (4)	-
4-8 cm	Upper	19 (3) ns	-
	Lower	26 (3)	-
8-12 cm	Upper	17 (1) **	-
	Lower	25 (1)	-
slope		**	-
depth		***	-
slope*depth		ns	-

Upper of slope : P4 and P5 in this chapter, Lower of slope : P1 and P2 in this chapter

Probabilities from two-way ANOVA of soil properties between soil position and depths. Student's t-test was conducted to detect differences between slope positions within each soil depth.

ns Not significant, Significance: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Table 2.2 Soil properties in sampling plots (Values are mean ± SE)

Depth	Slope	N (mg g^{-1})	C (mg g^{-1})	C/N	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	NH_4^+-N + NO_3^--N (mg kg^{-1})
Organic	Upper	12.5 (0.3) ns	380.5 (15.1) *	30.5 (1.4) **	44.2 (2.9) **	1.2 (0.2)***	45.4 (3.0) **
	Lower	13.3 (1.1)	318.9 (31.1)	23.9 (0.9)	25.1 (3.0)	47.2 (7.0)	72.3 (5.7)
0-5 cm	Upper	1.5 (0.3) **	24.9 (4.7) **	16.3 (0.5) ns	12.5 (0.6) *	2.4 (1.8)**	14.9 (1.6) ns
	Lower	3.6 (0.3)	54.1 (4.7)	15.1 (0.5)	8.6 (1.3)	12.5 (1.3)	21.1 (2.4)
5-15 cm	Upper	1.2 (0.1) **	19.0 (2.3) **	15.9 (0.9) ns	7.1 (0.3) ns	0.5 (0.1)***	7.6 (0.3) ns
	Lower	2.3 (0.3)	32.1 (3.3)	14.3 (0.6)	8.6 (1.1)	7.3 (1.0)	15.8 (1.2)
depth		***	***	***	***	***	***
position		**	ns	***	***	***	***
position × depth		ns	**	**	***	***	**

Probabilities from two-way ANOVA of soil properties between soil position and depths. Student's t-test was conducted to detect differences between slope positions within each soil depth.

ns Not significant, Significance: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Table 2.3 Fine root biomass of *Cryptomeria japonica*, other main Japanese tree species and other temperate forests

Tree species of forest type	Fine root biomass (g m^{-2})		Reference
	D < 1mm	D < 2 mm	
Japanese forest			
Cryptomeria japonica	140	421	Table 2.4
Chamaecyparis obtusa	519	638	Noguchi et al. (2007)
Pinus densiflora	21	49	Noguchi et al. (2007)
Other temprate forests			
Needleleaf evergreen[a]	896	513	Vogt et al. (1996)
Temprate coniferous forest	-	500	Jackson et al. (1997)

Data presented are means with number of study sites in parentheses

[a]Data in Appendix B of Vogt et al. (1996) were reanalyzed by Noguchi et al. (2007)

Table 2.4 Fine root biomass of *Cryptomeria japonica* in Japanese forest

Prefecture	Sites	Subsites	Latitude	Longitude	Stand age	Tree density (tree ha^{-1})	DBH (cm)	Tree height (m)	FRB (g m^{-2})	Mean FRB (g m^{-2})	FRD (mm)	Soil depth (cm)	Method	Reference
Ibaraki			36°10'	140°13'	23	-	16.5	12.4	-	108[b]	<1	40	Coring	Noguchi et al. 2004
	Chiyoda Exp Sta (FFPRI)		36°10'	140°13'	28	4350	14.5	-	-	116[c]	<1	35	Coring	Konôpka et al. 2006
		Control	36°10'	140°13'	28	4000	15.9	16.9	-	43[d]	<1	20	Coring	Noguchi et al. 2013
Hyogo	Kasumi[a]	-	35°37'	134°39'	41	700	-	-	-	48	<1	20	Coring	Hirano et al. 2017
Hyogo	Takarazuka[a]	-	34°49'	135°16'	50	1100	-	-	-	62	<1	20	Coring	Hirano et al. 2017
Kyoto	Wachi[a]	-	35°19'	135°19'	52	1200	-	-	-	35	<1	20	Coring	Hirano et al. 2017
Hyogo	Yamasaki[a]	-	35°02'	134°32'	45	1500	-	-	-	135	<1	20	Coring	Hirano et al. 2017
Shiga	Aibano[a]	-	35°20'	135°55'	42	1200	-	-	-	170	<1	20	Coring	Hirano et al. 2017
Hyogo	Anji[a]	-	34°58'	134°36'	43	700	-	-	-	142	<1	20	Coring	Hirano et al. 2017
Shiga	Hyakusaiji[a]	-	35°07'	136°19'	61	1700	-	-	-	285	<1	20	Coring	Hirano et al. 2017
Shiga	Kuta[a]	-	35°19'	135°49'	42	1800	-	-	-	187	<1	20	Coring	Hirano et al. 2017
Kyoto	Oharano Forest Park (Kyoto City)	P1	34°05'	135°37'	43	1020	26.4	21.8	181	198	<1	50	Coring	This study
		P2					-31.7	-28.4	151					
		P3							160					
		P4							242					
		P5							253					
Kyoto	Ohno Exp Forest (Kyoto Pref Univ)	OH1	35°30'	135°30'	26	1240	20.0	13.6	171	453	<2	50	Block	Kasuya & Shimada 1996
		OH2					-25.7	-19.5	462					
		OH3							231					
		OH4							285					
		OH5							379					
		OH6							419					
		OH7							736					
		OH8							942					
Shiga	Mt Ryuo	RY1	35°01'	136°20'	40	1200	15	9	428	744	<2	50	Block	Kasuya & Shimada 1996
		RY2					-25	-20	654					
		RY3							985					
		RY4							796					
		RY5							856					

Shiga	Mt Hieizan	-	35°05'	136°50'	40	1200	24.6	19.3	-	1128	<2	12	Coring	Yamashita et al. 2004
Nara	Mt Gomadan	15 year	34°04'	135°34'	15	3250	6.9	-	-	639	<2	50	Coring	Fujimaki et al. 2007
		30 year			30	3013	14.2	-	-	422				
		41 year			41	2300	18.5	-	-	453				
		88 year			88	1725	23.1	-	-	291				
Ibaraki	Chiyoda Exp Sta (FFPRI)	Cntrol	36°10'	140°13'	28	4000	15.9	16.9	-	56[d]	<2	20		Noguchi et al. 2013
Hyogo	Kasumi[a]	-	35°37'	134°39'	41	700	-	-	-	82	<2	20	Coring	Hirano et al. 2017
Hyogo	Takarazuka[a]	-	34°49'	135°16'	50	1100	-	-	-	108	<2	20	Coring	Hirano et al. 2017
Kyoto	Wachi[a]	-	35°19'	135°19'	52	1200	-	-	-	80	<2	20	Coring	Hirano et al. 2017
Hyogo	Yamasaki[a]	-	35°02'	134°32'	45	1500	-	-	-	205	<2	20	Coring	Hirano et al. 2017
Shiga	Aibano[a]	-	35°20'	135°55'	42	1200	-	-	-	236	<2	20	Coring	Hirano et al. 2017
Hyogo	Anji[a]	-	34°58'	134°36'	43	700	-	-	-	225	<2	20	Coring	Hirano et al. 2017
Shiga	Hyakusaiji[a]	-	35°07'	136°19'	61	1700	-	-	-	423	<2	20	Coring	Hirano et al. 2017
Shiga	Kuta[a]	-	35°19'	135°49'	42	1800	-	-	-	284	<2	20	Coring	Hirano et al. 2017
Kyoto	Oharano Forest Park (Kyoto City)	P1	34°05'	135°37'	43	1020	26.4	21.8	277	368	<2	50	Coring	This study
		P2					-31.7	-28.4	287					
		P3							294					
		P4							486					
		P5							498					

DBH, stem diameter at breast height; FRB, fine root biomass; FRD, fine root diameter

[a]Site location data from Tanikawa et al. 2014

[b]Date on mean annual fine root biomass using core sampling data from Noguchi et al. 2004

[c]Data on mean annual fine root biomass from Konopka et al. 2006

[d]Data on control site from Noguchi et al. 2013

Chapter 3

Characteristics of fine root system in anatomy, morphology and diameter

Introduction

Individual roots in fine root systems consist of two functional groups, roots with primary development (primary root) and roots with secondary developments (secondary root) (Pregitzer 2002; Guo et al. 2008a). Primary roots have a living cortex, develop symbiotic associations with soil fungi, and are responsible for water and nutrient absorption (Hishi 2007; Guo et al. 2008a). Secondary roots have a cork layer and secondary xylem that provides protection from environmental stresses and carries out transport, anchorage, and storage functions (Brundrett 2002; Guo et al. 2008a, b; Valenzuela-Estrada et al. 2008). Primary and secondary roots have been distinguished based on their branching order, number of protoxylem groups, and diameter within the fine root system (e.g., Hishi and Takeda 2005a, b; Guo et al. 2008a).

The branching order of individual roots is important for understanding root functions in fine root systems (Guo et al. 2008a). First-order roots have relatively smaller diameter, higher specific root length (Pregitzer et al. 2002), higher nitrogen concentrations, higher respiration rates (Pregitzer et al. 1998, 2002), and a shorter lifespan (Wells et al. 2002). Therefore, branching order has been reported as a useful indicator to characterize individual roots as primary or secondary development (Pregitzer 2002; Guo et al. 2008a). Individual roots in the fine root system also show different anatomical characteristics. The number of protoxylem groups, first formed in the xylem, often reflects life-cycle differences of individual roots within the same root system (Hishi and Takeda 2005a, b). Therefore, the number of protoxylem groups within the fine root system is an indicator of whether the fine root will progress to secondary growth or not, because the number of protoxylem groups does not change throughout the life cycle of individual roots (Noelle 1910; Hishi and Takeda 2005a). Hishi and Takeda (2005a) studied root anatomy in *C. obtusa* and found that roots with two strands of protoxylem (diarch

roots) tend to die before secondary development, while roots with four strands of protoxylem (tetrarch roots) usually advance to secondary development before they die; thus, diarch roots tend to be ephemeral and have nutrient uptake capacity, whereas tetrarch roots tend to be perennial and have transport capacity. Therefore, the number of protoxylem groups can be an indicator to characterize individual roots as primary or secondary roots. Diameter is also a useful index to distinguish primary or secondary roots. Roots with a high number of protoxylem groups are larger in diameter and usually become secondary development, while roots with a low number of protoxylem groups are smaller in diameter and few roots become secondary development (Hishi and Takeda 2005a; Zadworny and Eissenstat 2011).

In Japan, research on the morphological features of fine root of *C. obtusa* has been conducted, but it has not been clarified on *C. japonica* which is the same Hinoki genus. In this chapter, individual roots of *C. japonica* were characterized based on their diameter, branching order, and number of protoxylem groups. The objectives of this study were (1) to describe the anatomy of primary and secondary roots and (2) to determine an indicator (diameter, branching order, and/or number of protoxylem groups) for classifying individual roots as primary or secondary roots.

Materials and Methods

Anatomical characteristics of fine root systems

Samples of fine root systems were collected in May, August, and November 2012. The study plot of 10 × 15 m long was created in the lowest pert of slope on main plot (main plot size: 20 × 50 m long, see chapter 2, Fig. 2.5). The plot was divided 6 subplots of 5 × 5 m long that were used for root sampling. Six soil blocks (15 × 15 cm) were excavated at a 10-cm depth on each sampling occasion. Four root systems were carefully gathered from each soil block, and a total of 72 fine root systems (6 subplots × 4 root systems × 3 sampling times) were collected. Root samples were placed in plastic bags and transported to the laboratory. Soil samples were stored

in a refrigerator at 4°C. All root samples were gently washed with tap water to remove organic matter and soil minerals, and each root sample was stored in 70% ethanol.

In the analysis, I included only fine roots from the first three branching orders because most of the fourth-order root segments were incompletely excavated in the samples. Individual roots of fine root systems were separated into different branching orders following the procedure described by Pregitzer et al. (2002). The most distal root tips were labeled as first-order roots; roots with two first-order roots joined together were labeled as second-order roots; and two second-order roots joined together were labeled as third-order roots. Sixty root segments were randomly selected from the first and second order, and 24 root segments were selected from third order at each sampling time. A total of 432 root segments were selected for anatomical observation. Root segments were kept in a Petri dish in 70% ethanol, and cross-sections of individual roots were dissected manually at the center of each root segment under a dissecting microscope. The root diameter, the number of protoxylem groups, and the presence of secondary xylem were recorded. These observation methods are detailed in McKenzie and Peterson (1995a, b) and Hishi and Takeda (2005a). Secondary roots have secondary xylem, however primary roots have no secondary xylem but have passage cells. All anatomical observations were performed with a Nikon Eclipse 80i microscope equipped with a 130-W mercury light. UV illumination was achieved with a UV-1A filter.

Calculation of overlap degree for primary and secondary roots

The overlap degree between primary and secondary roots was calculated in diameter, branching order, and protoxylem group categories for using Pianka overlap index (Pianka 1973). This index has been used for estimating niche overlap for two species to one resource category. In this study I used this index for estimating the overlap degree for primary and secondary roots on each category. Overlap degree, $\alpha_{ps,}$ was calculated by following equation:

$$\alpha_{ps} = \frac{\sum_{i=1} p_{pi} p_{si}}{\sqrt{\sum_{i=1} (p_{pi})^2} \sqrt{\sum_{i=1} (p_{si})^2}}$$

where p_{pi} and p_{si} are the proportions of the category *i* of *p*, primary root, and *s*, secondary root, respectively. The index is symmetrical and assumes values between 0 and 1. Zero indicates that primary and secondary roots shows exact correspondence to the categories, one indicates complete overlap, and intermediate values show partial overlap in the categories.

Calculation of relative error for primary and secondary roots

The relative error was calculated to set the boundary diameter, branching order, and number of protoxylem groups for distinguishing primary from secondary roots. Relative error was calculated by following equation:

$$\text{Relative error} = | Op - Vp | / Oa,$$

where Op is the total number of individual roots with primary development identified by morphology, Vp is the number of estimated primary roots identified by diameter, branching order, and number of protoxylem groups, Oa is the total number of examined roots.

Statistical analysis

One-way analysis of variance was applied to test the differences in root diameter of roots with different branching orders and numbers of protoxylem groups. The Bonferroni correction test was applied for multiple comparisons among root branching orders and number of protoxylem groups.

Results and Discussion

Anatomical characteristics of fine roots in C. japonica

Among of the observed roots, 251 were primary roots and 181 were secondary roots. Primary and secondary roots had different anatomical traits. Passage cells, which are epidermal cells that

lack secondary walls (Hishi and Takeda 2005a), were found in primary roots (Fig. 3.1a, b, and d). These cells have low resistance to water (Peterson and Enstone 1996) and therefore absorb water and nutrients (Taylor and Peterson 2000). Secondary roots formed mature central metaxylem in a convex curve and developed secondary xylem around metaxylem (Fig. 3.1c, e, and f). Intact cortical cells with phi-thickenings were observed mainly in primary roots and in early stages of secondary development (Fig. 3.1a, b, d, and e). Phi-thickenings are found on the radial and tangential walls of root cortical cells (Gerrath et al. 2002, 2005) and are considered to be supportive tissues (Weerdenburg and Peterson 1983). In secondary roots, the vascular system expanded as the cortical cells shrunk (Fig. 3.1c and f). These results showed that cortical cells and phi-thickenings collapsed and endodermis was compressed as the secondary vascular system developed in the secondary roots. Hishi and Takeda (2005a) and Hishi (2007) also suggested that the diameters of most primary and secondary roots showed no clear increments during their growth periods. The results suggested that the diameter does not change during the transition from primary to secondary growth in the fine roots because secondary vascular tissues expand into the cortical layer.

Individual roots were separated into six size categories based on 0.10-mm diameter-intervals (Fig. 3.2a). The mode of primary roots was 0.51-0.60 mm and that of secondary roots was 0.60 - 0.70 mm. The mean diameters were significantly different between the primary (0.52 mm) and secondary roots (0.65 mm; $P < 0.001$). In the branching order category, the proportion of primary to secondary roots was highest in first-order roots, and third-order roots consisted entirely of secondary roots (Fig. 3.2b). Second-order roots consisted of both primary and secondary roots. These trends are similar to those found in previous studies (Hishi and Takeda 2005a; Guo et al. 2008a). Diarch roots were mainly primary roots, while pentarch roots were mainly secondary roots (Fig. 3.1a and f, Fig. 3.2c). Triarch and tetrarch roots were either primary or secondary (Fig. 3.1b, c, d, and e), but the proportions of primary roots were higher in the triarch than in the tetrarch roots (Fig. 3.2c). Roots with a high number

of protoxylem groups have large diameters at early developmental stages and proceed to secondary growth with diameter growth (Zadworny and Eissenstat 2011). These big roots (e.g., >1.0 mm) were rarely found in first-third order root system in this study.

Indicator for separating primary from secondary roots by relative error

The proportion of primary to secondary roots decreased from <0.4 mm to 0.8< mm diameter size class. In 0.8< mm diameter class, all roots were secondary roots. In this study, the total number of roots that examined was 432, from which 251 were primary roots and 181 were secondary roots. The boundary diameter for separating primary from secondary roots was first set at 0.40 mm (Vp = 19) with a relative error of 0.537 (Table. 3.1). As the diameter increased, the relative error decreased and the Vp increased. It was identified that the relative error was minimum at 0.60 mm (Vp = 281), which was set as the boundary diameter for separating primary from secondary roots. Individual roots were separated into first-, second-, and third-order roots (Table. 3.2). The mean diameter of first (0.52 mm), second (0.58 mm), and third-order roots (0.71 mm) differed significantly between all three categories ($P < 0.001$). When the first-order roots were grouped as primary and all others as secondary (Vp = 180), the relative error was 0.164. When the first- and second-order roots were grouped as primary (Vp = 360), the relative error increased. Therefore, only the first-order roots were grouped as primary, while all others were grouped as secondary. Individual roots were separated into diarch, triarch, tetrarch and pentarch roots based on the number of protoxylem groups (Table. 3.3). The mean diameter of diarch (0.48 mm), triarch (0.54 mm), tetrarch (0.65 mm), and pentarch (0.80 mm) roots differed significantly ($P < 0.001$). When diarch roots were grouped as primary and all others as secondary roots (Vp = 51), the relative error was 0.463. When both diarch and triarch roots were grouped as primary (Vp = 293), the relative error was 0.097. Therefore, diarch and triarch were considered as primary roots and grouped as ephemeral, while tetrarch and pentarch were considered as secondary roots and grouped as perennial.

The minimum relative error for separating individual roots into primary and secondary using diameter, branching order and protoxylem group was 0.069, 0.164, and 0.097, respectively (Table 1, 2, and 3). The lowest value of relative error was obtained when diameter was used to separate primary from secondary roots. The diameter is continuous data, while the branching order and the number of protoxylem groups are categorical data. Based on diameter, data could be separated into objective categories corresponding to different interval sizes. The relative error decreased with interval size; therefore, diameter is probably better indicator for separating primary from secondary roots in this relative error method.

Indicator for separating primary from secondary roots by overlap degree

The overlap degree of primary and secondary root groups, α_{ps}, was 0.635 in diameter category. Then the overlap degree, α_{ps}, was 0.577 in branching order category. The proportion of primary to secondary roots decreased from diarch to pentarch roots. Then, the overlap degree, α_{ps}, was 0.719 in protoxylem group category. In this comparison, the overlap degree of primary and secondary roots along the category was over 50% in three categories. Therefore, present results suggest that it is difficult to correctly separate fine roots into primary or secondary roots by using diameter, branching order and protoxylem group category in this overlap degree method.

Which is the best indicator for separating primary and secondary fine roots?

Different results were shown for relative error and overlap degree. In the relative error I focused only on numbers of roots. In each category, I researched how close the number was to the total number of primary roots. On the other hand, in each category, the overlap degree focused on the degree of overlap between the distribution zones of the primary and secondary roots. Therefore, it was inferred that different results were obtained. Considering both results, the diameter of individual roots was identified as effective indicator for separating primary from secondary roots. Size dependent anatomical traits have been demonstrated in *C. obtusa* (e.g. Hishi and

Takeda 2005a, b). Hishi and Takeda (2005a) showed that mean diameter of diarch, triarch and tetrarch roots ranged from 0.42-0.44 mm, 0.47-0.49 mm and 0.57-0.80 mm, respectively, in orange, red and brown colored roots, and these diameters significantly differed between protoxylem groups. It was also shown that the proportion of secondary roots increased with the number of protoxylem groups (Hishi and Takeda 2005b), results that were in agreements with this study. The mean diameter of diarch and triarch roots in *C. obtusa* was lower their roots in *C. japonica* (Table. 3.2). The boundary diameter for separating fine roots with primary and secondary development in *C. obtusa* might be between 0.50 and 0.60 mm. In this study, diameter was also selected as a good indicator because its measurement simpler and more convenient than the evaluation of branching order or the of protoxylem groups. In this study, I concluded that diameter (< 0.60 mm) was a simple and effective indicator for separating ephemeral from perennial roots in *C. japonica*.

From this and previous researches (Hishi and Takeda 2005a, b), the root diameter of *C. japonica* was higher than that of *C. obtusa*. It is known that the diameter of individual roots differs greatly among species (Guo et al. 2008a; Comas and Eissenstat 2009; Chen et al. 2013; Gu et al. 2014). Chen et al. (2013) investigated the diameter of the first order root of the 35 types of angiosperm subtropical spices, as a result, the CV (coefficient of variation) was 56.39 %. Gu et al. (2014) also reported that root diameter in first order varied nearly nine-fold between the thinnest (*Cratoxylum cochinchinense Lour.*:135 ± 4 μm) and the thickest (*Cryptocarya chinensis Hemsl.*:1113 ± 17 μm) for 50 tropical and temperate trees. Therefore, because root morphology varies greatly among species, applying a single diameter cutoff across species can be problematic (McCormack et al. 2015). In order to determine the diameter for separating primary and secondary root, there is a need to investigate the relationship between species diameter and anatomical characteristics.

Table 3.1 Total number of primary and secondary roots separated into 6 categories corresponding to 0.10 mm diameter intervals. The relative error shows the relation of actual and estimated numbers in each category.

Diameter class (mm)	n	Primary	Secondary	Relative error
<0.40	19	18	1	0.537
0.41-0.50	112	96	16	0.278
0.51-0.60	150	102	48	0.069
0.61-0.70	110	31	79	0.324
0.71-0.80	29	4	25	0.391
0.81<	12	0	12	0.419

Table 3.2 Diameter and total number of primary and secondary roots separated into 3 categories based on the branching order. The relative error shows the relation of actual and estimated numbers in each category.

Order	n	Diameter (mm)	Primary	Secondary	Realtive error
First	180	0.52 (0.01) c	155	25	0.164
Second	180	0.58 (0.01) b	96	84	0.252
Third	72	0.71 (0.02) a	0	72	0.419

Different letters in the same column denote significant differences ($p < 0.05$).

Table 3.3 Diameter and total number of primary and secondary roots separated into 4 categories based on the number of protoxylem groups. The relative error shows the relation of actual and estimated numbers in each category.

Protoxylem group	n	Diameter (mm)	Primary	Secondary	Realtive error
Diarch	51	0.48 (0.01) d	51	0	0.463
Triarch	242	0.54 (0.01) c	168	74	0.097
Tetrarch	127	0.65 (0.01) b	32	95	0.391
Pentarch	12	0.80 (0.06) a	0	12	0.419

Different letters in the same column denote significant differences ($p < 0.05$)

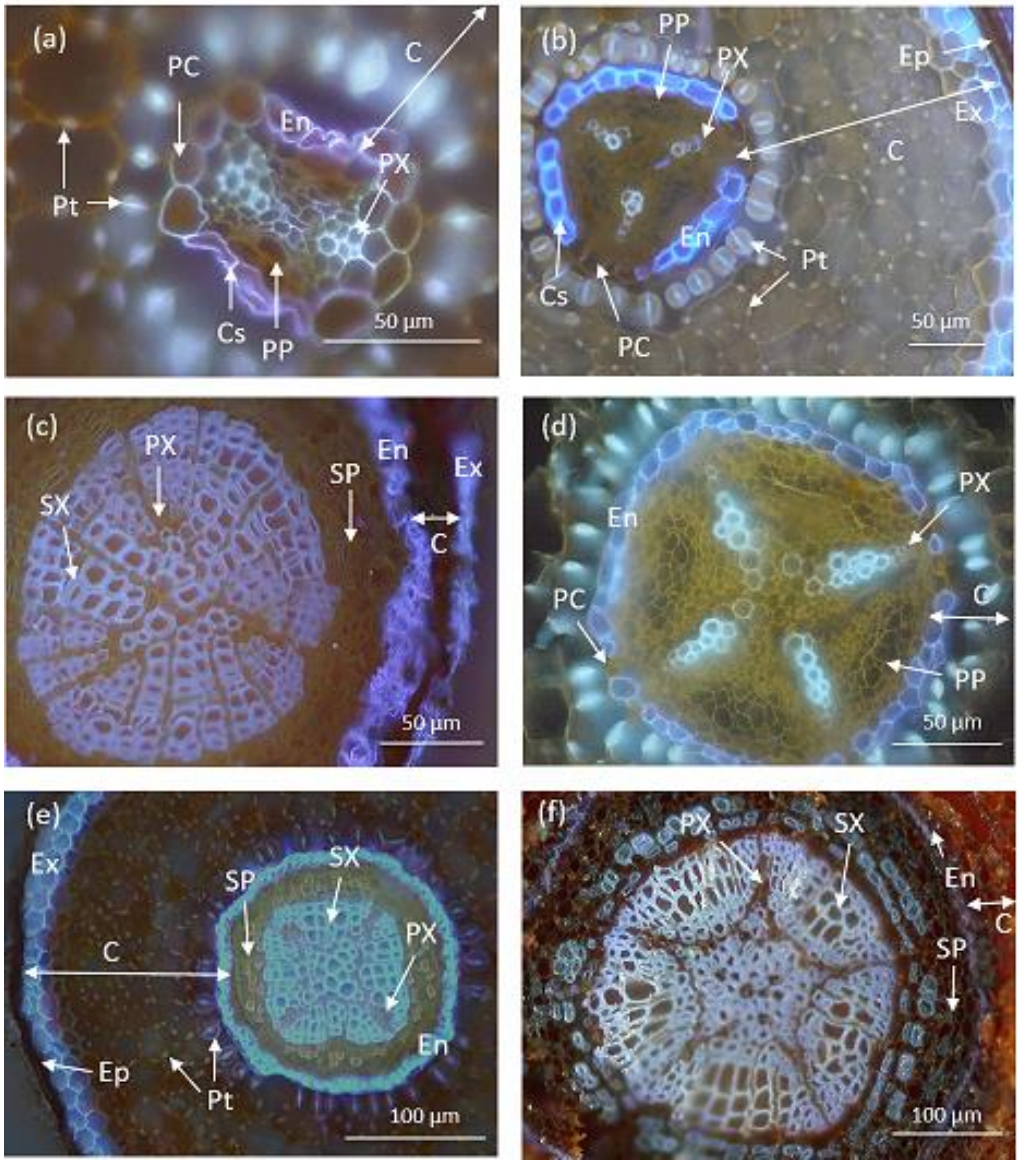

Fig. 3.1 Light micrograph of root cross sections. (a) Diarch root with primary development. (b) Triarch root with primary development. (c) Triarch root with secondary development. (d) Tetrarch root with primary development. (e) Tetrarch root with secondary development. (f) Pentarch root with secondary development. PX, protoxylem; SX, secondary xylem; PP, protophloem; SP, secondary phloem; En, endodermis; Ex, exodermis; Ep, epidermis; PC, passage cell; C, cortex; Cs, casparian strip; Pt, phi-thickening

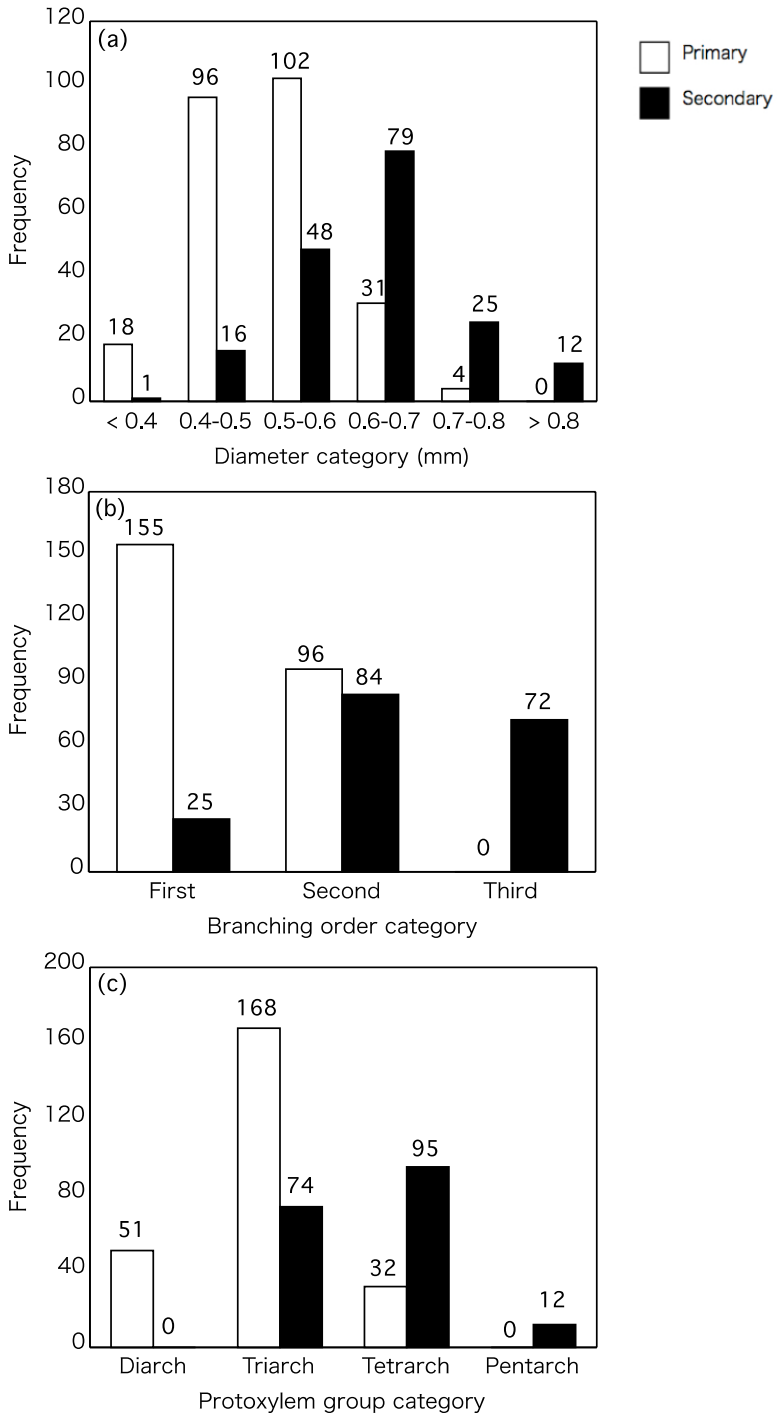

Fig. 3.2 Distribution of primary and secondary roots at (a) diameter category, (b) branching order category and (c) protoxylem group category. Number in this figure means the number of roots in each category.

Chapter 4

Seasonal dynamics of fine root biomass and root tips

Introduction

Fine root dynamics reflect the production and mortality processes of individual roots in root systems. Root systems are characterized by root biomass and architecture. Root tips are first-order roots and more ephemeral roots than the higher order roots in root systems (Guo et al. 2008a). Dynamics of individual roots are represented by root tip dynamics (Hishi and Takeda 2005b), and results in architectural change of root systems (Hishi 2007). Konôpka et al. (2006) showed that the seasonal trends in root tip dynamics track those of fine root biomass. The dynamics of root tips relate to that of fine root biomass.

Fine root dynamics has been studied in many forests, and also studied in *C. japonica* forest, for instant, seasonal root biomass dynamics (Konôpka et al. 2006, 2007), root biomass distribution along soil profiles (Noguchi et al. 2004), changes of root biomass to nitrogen condition (Noguchi et al. 2013) and so forth. Among plant organs, fine roots have the closest relationship with the soil environment (Konôpka et al. 2007). Fine roots are readily able to change their morphology and developmental patterns in response to soil environment (Cheng et al. 2006). Therefore, fine root dynamics are influenced by soil environmental conditions. However, root behavior and life-cycle in different soil environment, such as organic and mineral soil layer, are unclear in *C. japonica*.

C. japonica plantations were well established in B_D with moder type humus (Kasuya and Shimada 1996). Roots are distributed in the organic and mineral soil layers in these plantations. In this chapter fine root biomass and root tip dynamics in the organic and mineral soil layers were studied. I also studied relationship between fine root dynamics and inorganic nitrogen concentration.

Materials and methods

Root sampling

I established the study plot located on the lowest part of main plot (main plot size: 20 × 50m long, see chapter 2, Fig. 2.5). The plot measured 20 ×10 m long: I subdivided it into 10 subplots (10 × 2 m long). Root samples were collected from soil cores (56 mm inner diameter, 4 cm depth) in June, September, and December of 2008, and in March and June of 2009. On each sampling occasion, a core was taken from each of the 10 subplots at 8 cm depth. I divided soil samples into organic soil and mineral soil, and then mineral soil samples (0-4 cm depth) were collected. I transferred samples to plastic bags and transported them to the laboratory where they were stored in a refrigerator at 4°C until processed. Processing began by rinsing with tap water, after which the fine roots were removed. I separated living roots and dead roots by color and texture. Living roots were rigid and lightly colored; dead roots were fragile and darkly colored. I grouped living roots into two diameter classes: <1 and 1–2 mm diameter. All fine root samples were digitally scanned; I counted the numbers of root tips in the scans and measured their lengths using an image analysis system (WinRHIZO 2007d; Regent Instruments, Quebec City, PQ, Canada). I selected 10 root tips randomly from each of the root sample scanner images and measured tip diameter. Root tips were grouped into three diameter classes: <0.5, 0.5–1 and 1-2 mm. Root tip number of each diameter class was estimated by proportion of root tips of each diameter class in each root sample scanner image. Root tip density of each diameter class was calculated from the number of root tips per soil volume. After the scanning procedure, fine root samples were oven dried (70°C for 48 h) and weighed. Branching intensity was calculated from the number of root tips divided by total root length. This simple measure is used to quantify branching frequency (Comas and Eissenstat 2009).

Soil sampling for measuring inorganic nitrogen

I established the study plot located on the lowest part of main plot (main plot size: 20 × 50m

long, see chapter 2, Fig. 2.4). The plot measured 20 ×10 m long. Soil coring was conducted in April, June, August and November of 2010 by using 100-mL soil cores (10 cm depth). Five cores were sampled in study on each sampling occasion. I divided soil samples into organic soil and mineral soil, and then mineral soil samples (0-5cm depth) were collected. Soil samples were kept in a plastic bag and were transported to the laboratory. Soil samples were sieved through a 2 mm mesh and roots and organic matter were removed. The fine soil was used to analyze inorganic N concentration. Soil samples of 3 g (organic soil) and 5g (mineral soil) were extracted with 50 ml of 2M KCl and the extract was used to measure NH_4^+-N and NO_3^--N pool size. Subsamples of fine soils were oven dried at 105˚C and weighed to determine their water content. Inorganic N concentration was determined by an auto-analyzer (AutoAnalyzer Ⅲ, BLTEC, Osaka, Japan).

Statistical analysis

All study parameters were compared between months by one-way ANOVA and between organic and mineral soil layers by t-test at each month. Thukey's HSD multiple comparisons test was used to detect significant ($p < 0.05$), when differences were considered significance ($p < 0.05$).

Results

Seasonal changes in inorganic nitrogen concentration

NO_3^--N concentrations increased from April to August, and decreased towards November in both soil layers, whereas NH_4^+-N concentrations also increased from April to August, and decreased towards November in both layers but there were no significant differences between months in organic soil layer (Table. 4.1). Concentrations of NO_3^--N and NH_4^+-N were both higher in organic than in mineral soil layer.

Fine root biomass, root tip density and branching intensity

Fine root biomass was separated into two diameter classes: <1 mm: FRB1 and 1-2 mm: FRB2 in this chapter. Mean annual FRB1 and FRB2 were 39.1 g m^{-2} and 20.2 g m^{-2} in the organic soil layer, and 18.8 g m^{-2} and 14.9 g m^{-2} in the mineral soil layer, respectively. Mean annual total fine root biomass (<2 mm diameter within organic and mineral soil layers) was 92.8 g m^{-2}, ranging from 47.7 to 133.2 g m^{-2} through the seasons. FRB2 remained more or less stable across months in both soil layers (Fig. 4.1). FRB2 showed no significant differences between organic and mineral soil layers. While, FRB1 decreased from June and September to December and March in both soil layers (Fig. 4.1). There were clear seasonal changes in FRB1 in both organic and mineral soil layers (one-way ANOVA, $p < 0.01$ and $p < 0.05$, respectively). FRB1 was higher in the organic than in the mineral soil layer, except to March.

Root tip density was separated into three classes: <0.5 mm: RTD0.5, 0.5-1 mm: RTD1 and 1-2 mm: RTD2 in this chapter. RTD2 remained more or less stable across months in both soil layers (Fig. 4.2). In addition, RTD2 showed no differences with respect to the organic and mineral soil layers at each month. While, RT0.5 and RTD1 decreased from June and September to December and March in both soil layers (Fig. 4.2). There were clear seasonal changes in RTD0.5 in both organic and mineral soil layers (one-way ANOVA, $p < 0.01$ and $p < 0.001$, respectively), and also in RTD1 in organic and mineral soil layers (one-way ANOVA, $p < 0.05$ and $p < 0.01$, respectively). RTD0.5 was significantly higher in the organic than in the mineral soil layer, except to March, and RTD1 also tended to be higher in the organic than in the mineral soil layer. Fig. 4.3 showed ratio of root tip diameter in organic and mineral soil layers in each season. Ratio of <0.5mm diameter was higher in organic soil layer than mineral soil layer in each season (except to March). Ratio of <0.5mm diameter decrease from Jun to March, whereas, ratio of 0.5-1mm diameter increased in winter period.

Branching intensity (BI) decreased from June and September to December and March in both layers (Fig. 4.4). There were clear seasonal changes in BI in organic and mineral soil layers (one-way ANOVA, $p < 0.01$ and $p < 0.01$, respectively)**.** BI tended to be higher in organic

soil layer than in mineral soil layer (only significant difference in September) (Fig. 4.3).

Discussion

Fine root system architecture is composed of individual roots (Hishi 2007). In the root systems, individual root diameter decreased from the base to apical fine roots (Pregitzer 2002; Guo et al. 2008a; Valenzuela-Estrada et al. 2008). The variability of FRB1 was higher in the organic than in the mineral soil layer (Fig. 4.1). While, the variability in FRB2 was rather stable over the season. These results of fine root dynamics suggest that basic root sized 1-2 mm produced the fine root seized less than 1 mm during summer period. Therefore, dynamics of fine root production (<1 mm) accounted for those of total fine roots in this study site.

Dynamics of root tips are influenced by birth and death processes of apical roots in the root systems (Hishi and Takeda 2005b). FRB1, RTD0.5 and RTD1 showed similar seasonal patterns in both organic and mineral soil layers (Fig. 4.1 and 2). Seasonal changes in RTD0.5 and RTD1 showed that apical roots were produced in summer and disappeared in winter. These seasonal patterns of root tip abundances were similar to the previous study (Konôpka et al. 2006). In the organic soil layer both RTD0.5 and RTD1 were variable, while in the mineral soil layer RTD1 mainly showed dynamics of root tip density (Fig. 4.2).

In this study site, ratio of <0.5mm diameter was larger in organic soil layer than mineral soil layer (Fig. 4.3). The diameter of root tips constituting root systems were smaller in organic soil layer than in mineral soil layer (in this study, Wang et al. 2007). Stresses are usually greater in organic soil layer than in mineral soil layer (Takeda 1987; Hishi et al. 2006). Drought stress (Bryla et al. 2001; Robinson et al. 2003), low pH, high aluminum toxicity (Tomioka and Takenaka 2001; Godbold et al. 2003), and biological stress from competition with soil organisms (Wells et al. 2002) can induce root suberization, pigmentation, stunting, or shortened longevity (Hishi et al. 2006). The diameter of individual roots is generally important index of turnover or longevity (Eissenstat and Yanai 1997; Wells and Eissenstat 2001). Small diameter

roots have shorter life span than big diameter roots (Wells and Eissenstat 2001; Joslin et al. 2006). Primary root of *C. japonica* can be separated from secondary root at the boundary size of 0.5-0.6mm, as shown in chapter 3. Therefore <0.5 mm diameter roots correspond to the primary roots. So, in this study site, primary roots were abundant in the organic layer.

The birth and death processes of root tip abundances also resulted in the architecture of root systems, which may be summarized by branching intensity. BI was higher in summer than in winter period (Fig. 4.4), and the dynamics of BI is similar to those of RTD0.5 and RTD1 dynamics. These results suggest that highly active fine root tips (RTD0.5 in this study) were produced in summer and became the apical roots (first order) of the root system. The BI increased by the production of new very fine roots (<0.5 mm diameter in this study) in summer and these very fine root growth resulted in the increments of the root systems in summer. Then, in winter, the very fine root tips composing the apical roots of root system died, and the roots of the higher order became apical roots. The death of the very fine root tips resulted in the increments in the size of root tips and decline of the root system in winter season. These seasonal patterns of root tip abundances were similar to the previous study (Wang et al. 2007). First order root with small diameter is more short-lived than higher order root with larger diameter (Eissenstat et al. 2000). Guo et al. (2008b) reported that across all 36 tree spices, first-order roots consistently accounted for > 50% of total root number and > 60% of total root number mortality of the fine root pool. Longevity of first-order root of temperate tree species was assumed 0.7 year, whereas that of fifth-order root was 3.56 year (Guo et al. 2008b). Thus, individual roots, especially roots of <0.5 mm size class were ephemeral and contributed to nutrient absorption in the organic soil layer.

Very fine roots were mainly produced in the organic soil layer during the summer period in this site. This suggested that the organic soil layer provided temporal habitat for root colonization of *C. japonica* together with the mineral soil layer. In this site, both NO_3^--N and NH_4^+-N were abundant in summer period (Table. 4.1). Inorganic form nitrogen was higher in

the organic than in the mineral soil layer (Table. 4.1). Dynamics of fine root biomass, root tip abundances and branching intensity well corresponded to those of mineral nitrogen, especially $NH4^+$-N form. In root systems, apical roots of small diameter are fibrous roots with high nutrient absorptive ability (Zadworny and Eissenstat 2011). In addition, a high abundance of ephemeral apical roots increases exploitation of nutrient with their absorptive ability in fine root systems (Hishi et al. 2006). Individual roots in root system had a great plasticity in soil with a high heterogeneity of resources availability (Eissenstat and Caldwell 1988; Eissenstat 1992; Wang et al. 2007). These results might imply that N uptake capacity of root systems was likely enhanced by very fine roots within the organic soil layer, especially during the growth season of summer period. Thus ephemeral root tip production enable *C. japonica* to exploit effectively the nitrogen in organic soil together with the nitrogen uptake in mineral soil.

Table 4.1 Seasonal changes in inorganic N concentration (Values are mean ± SE).

Month	NO_3^--N (mg kg^{-1})			NH_4^+-N (mg kg^{-1})		
	Organic	Mineral		Organic	Mineral	
April	13.04 (3.07) b	2.13 (0.59) b	**	4.94 (2.59)	2.01 (0.62) b	ns
June	19.37 (2.79) ab	7.39 (1.27) ab	**	6.81 (1.77)	1.28 (0.15) b	*
Agust	33.13 (8.06) a	9.83 (2.43) a	*	12.18 (2.09)	6.04 (1.78) a	*
November	8.49 (2.63) b	4.08 (0.74) ab	ns	5.78 (0.95)	1.21 (0.36) b	**

Different letters indicate significant differences between sampling dates.

Significant differences in a given date between organic and mineral soils are marked with an asterisks (*$p < 0.05$, **$p < 0.01$, ns not significant)

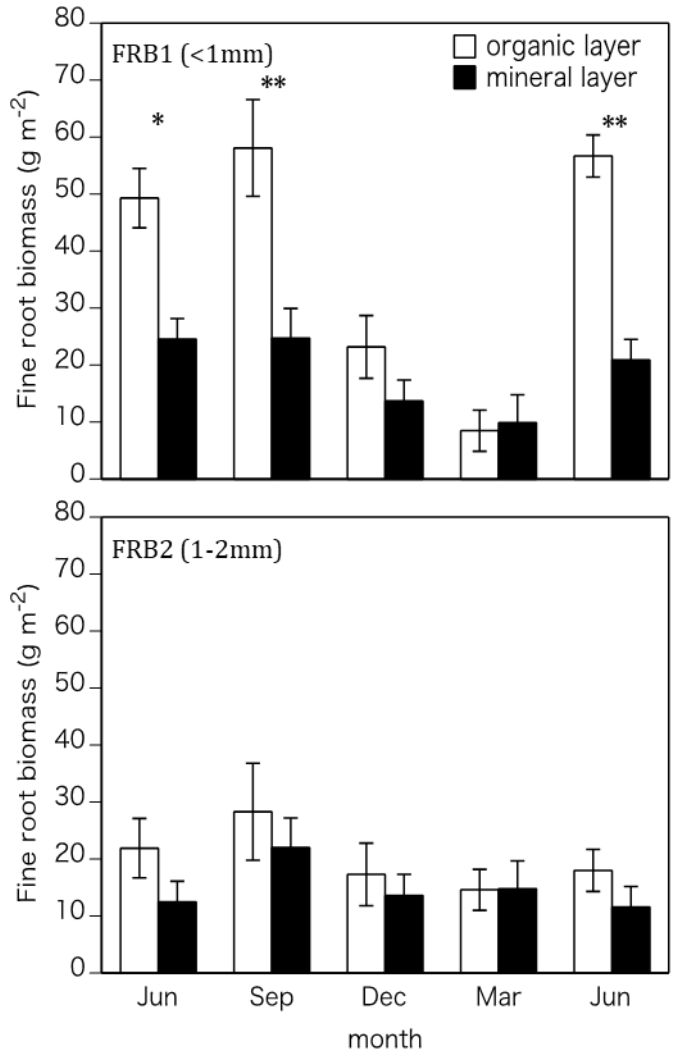

Fig. 4.1 Fine root diameter was separated into two classes: <1 mm: FRB1 and 1-2 mm: FRB2 in this chapter. FRB1 and FRB2 in organic and mineral soil layer in each season. Values are means ± SE. Significant differences in a given date between roots in organic and mineral soil layers are marked with an asterisks (* $p < 0.05$, ** $p < 0.01$).

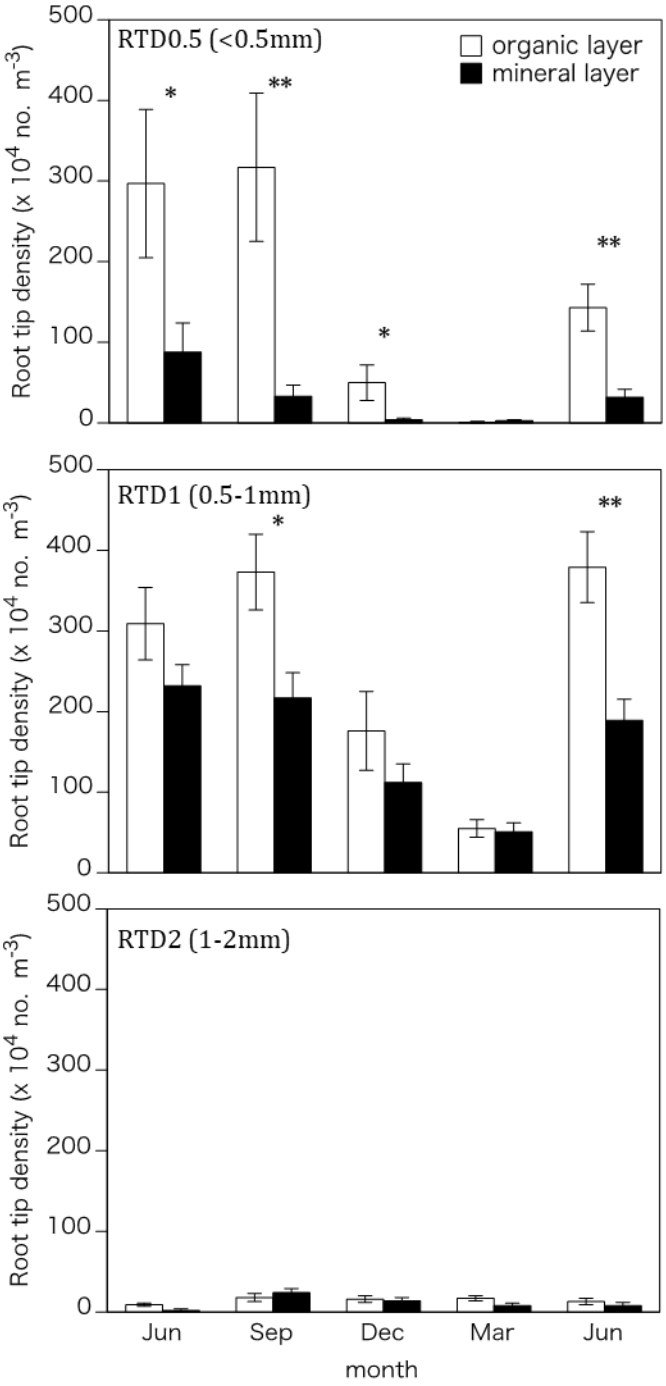

Fig. 4.2 Root tip density was separated into three classes: <0.5 mm: RTD0.5, 0.5-1 mm: RTD1 and 1-2 mm: RTD2 in this chapter. RTD0.5, RTD1 and RTD2 in organic and mineral soil layers in each season. Values are means ± SE. Significant differences in a given date between roots in organic and mineral soil layers are marked with an asterisks (* $p < 0.05$, ** $p < 0.01$).

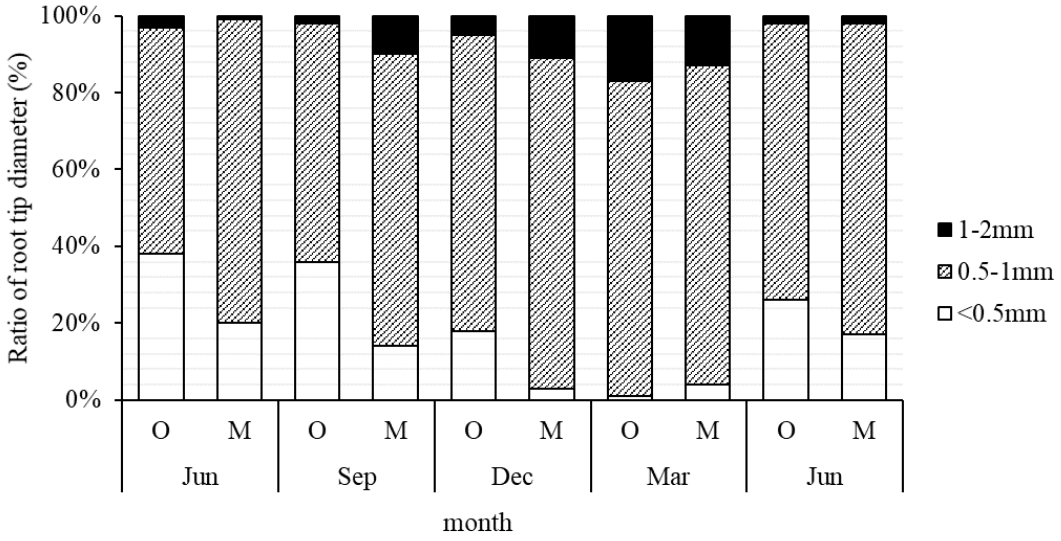

Fig. 4.3 Ratio of root tip diameter in organic and mineral soil layers in each season.

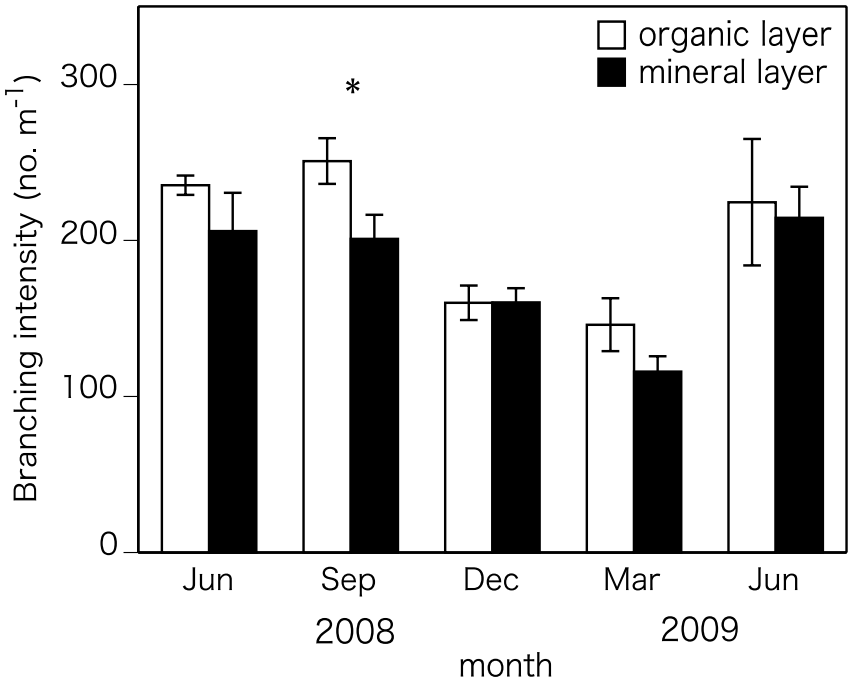

Fig. 4.4 Branching intensity (BI) in organic and mineral soil layers in each season. Values are means ± SE. Significant differences in a given date between roots in organic and mineral soil layers are marked with an asterisk (* $p < 0.05$).

Chapter5

Fine root plasticity for adaptation to localized soil enrichment

Introduction

Fine roots play an important role in the nutrient absorption by plants. Plants allocate biomass to absorptive root and change architecture of root system in response to soil nutrient availability (Hutchings and de Kroon 1994; Hodge 2004). Availability of nitrogen in the soil is important for the NPP in many forest ecosystems. Plants optimize their uptake nitrogen resources from soil by their plasticity of root growth (Caldwell et al. 1992; Cahill et al. 2010; Chen et al. 2018; Wang et al. 2018a). Changes in fine root biomass and morphology across the soil nutrient conditions have been demonstrated in the previous fine root studies (e.g. Makita et al. 2015; Miyatani et al. 2016; Doi et al. 2017).

The root system is a combination of individual roots which has a different morphology, chemical composition and physical property (Pregitzer et al. 2002; Hishi and Takeda 2005a, b; Guo et al. 2008a; Chapter 3). The soil profile changes from the organic layer to the mineral layer and creates environmental gradient along a soil vertical profile. Nutrient availability generally decreases and bulk density increases with soil depth (Schenk 2005; Ugawa et al. 2010). Soil environment changes with the soil depth. Then the structure and function of fine root also changes accordingly (Nepstad et al. 1994; Schulze et al. 1996; Cheng et al. 2006). Roots tend to have thick diameter (Pregitzer et al. 1998; Wang et al. 2006), low N concentration (Makita et al. 2011; Burton et al. 2012) in the deep soil. According to the availability of nitrogen, fine roots show modification in shape such as specific root length (SRL) (Makita et al. 2015; Wang et al. 2016).

In the chapter 2, I only investigated fine root biomass in <2 mm diameter along topographic sequence. Root systems are composed by individual roots of different order in the architecture of root systems. So traits of individual roots of first, second, and third order are

examined for their morphology and physiological properties. The architecture of root systems is described by the bifurcation ratio. Then I analyzed the plasticity of fine root systems for the adaptation to the different nitrogen availability provided along a soil profile in both the lower and upper parts of the forest slope. The lower part of slope showed the dominance of nitrate, whereas the upper parts of slope show the dominance of ammonium. Then I discussed the plasticity of fine root systems in response to the different nitrogen conditions provided along the forest slope.

Materials and Methods

I established two study plots (10 × 20 m long) at lowest and uppermost position of slope in the main plot (main plot size: 20 × 50 m long, see chapter 2, Fig. 2.5). In this chapter, study plots of lower part and upper parts of slope were defined as “lower plot” and “upper plot”, respectively. Here, I adapted two sampling methods for the fine root study. Soil core sampling were carried out for the estimation of root system abundances. Besides the sampling, soil block sampling was carried out for the analysis of root systems.

Core sampling for the estimation of root system abundances

For the estimation of root system abundances, six soil cores (20 mm inner diameter, 25 cm depth) were sampled in the upper and lower plots in October 2013. A total 12 soil cores were sampled. Fine root growth is peak in summer - autumn season in *C. japonica* stand as shown in the previous studies (Konôpka et al. 2006; Chapter 4). Roots were removed from the soil and washed with tap water. Fine root systems with the first three branching orders were counted in this study. Among these individual roots, first- and second- order roots are primary and absorptive roots as shown in the chapter 3. Root systems composed of first order and of first and second order were omitted for the counting of root systems. The root system size was defined 1-3 order roots in this study. Root systems were counted by visual inspection in each sample

and abundances of root system (SND) was expressed by the total number of root systems per core volume.

Block sampling for analysis of root systems

Besides the core sampling, soil block samples were collected for the analysis of intact root systems. A soil block (15 × 15 cm) was excavated at 3 soil depths (organic, 0-5cm and 5-15cm) in the upper and lower plots. In each plot, six soil blocks were collected in October 2013. Block samples were collected near the sampling point, where I conducted soil core sampling for the estimation of root system abundances. Two samples with large intact root systems were carefully selected from each soil block. A total of 72 root samples (6 sampling point × 2 root systems × 2 slope positions x 3 soil layers) without any disturbance were collected. All samples were placed in a plastic bag and transported to the laboratory, then kept at 4°C until further analysis.

Measurements of root systems collected by the block sampling

In the laboratory, the samples of root systems were gently washed with tap water to remove organic matter and mineral soil. Basing on the criteria of Vogt and Persson (1991), fragments of dead roots were carefully isolated using forceps from the root systems. After the cleaning, the root systems were kept moist conditions with water and were dissected into branching orders following the protocols described in chapter 3. The root order samples were scanned by scanner. The average root diameter and total length of each order were measured using an image analysis system WinRHIZO 2007d.

For each order, number of individual roots were counted under a binocular microscope. After scanning, individual roots of each order were lumped together and were dried at 70°C for 48h. Then total weight of each order was measured. Next, specific root length (SRL) and root tissue density (RTD) of individual roots of each root system were calculated. SRL is the ratio of

length to biomass of root and is used as indicator of root benefit to root cost, assuming that resource acquisition is proportional to root length and root biomass is proportional to root cost (construction and maintenance) (Eissenstat and Yanai 1997, Ostonen et al. 2007). RTD is defined as root biomass per root volume and indicator of C cost of constructing and maintenance root. Total N and C concentrations of individual roots were measured by NC Analyzer. Because the amounts of root samples were limited, the N and C concentrations of each branching order root were pooled in each sampling point.

Mass and length of individual root system were measured and then were defined the weight (System W) and root system length (System L). Branching order and bifurcation ratio (Br) of root system were calculated for each root system. The Br of root system was calculated by taking the anti-logarithm of the absolute value of the slope of log (number of segments of a given order) versus order (Berntson 1995).

Biomass and RLD estimation using the properties of individual roots.

Abundances of root systems were used to estimate the biomass (mg cm^{-3}) and root length density (RLD: cm cm^{-3}) of root system. For the block soil samples, weights and length were measured for the two root systems and the average weight (System W) and length (System L) of the root systems were calculated for six samples in each of two plots. Abundances of root systems were estimated by the core sampling. Then total biomass and RLD were calculated for the 6 samples of each plot by the following formula;

$$\text{Biomass} = (\text{System W}) \times (\text{SND})$$

$$\text{RLD} = (\text{System L}) \times (\text{SND})$$

where SND was expressed by the total number of root systems per core volume. Average of biomass and RLD were calculated for the 6 samples for each plot. Coefficient variations (CV) of biomass and RLD of root system and SND were calculated.

Statistics analysis

I performed a two-way ANOVA in order to detect significant differences in soil properties and root traits of each root category with slope position and soil depth considered main effects. Student's *t*-test was conducted to detect differences between slope positions within each soil depth.

Results

Traits of individual fine roots composing the root systems

Traits of individual roots and root system were expressed by the diameter, the size (weight and length), the shape parameter (RTD and SRL) and physiological parameter (N, C and C/N). Individual roots were grouped into the first, second, and third order groups. Table 5.1 and 5.2 showed the traits of individual roots across the study site and soil layers. The traits showed significant differences along the soil profile from the organic to mineral soil layers. Root diameter of each branching order significantly increased with increasing soil depth (except to the second order). Weight of each branching order per 1 root system was also significantly increased with increasing soil depth, while length of branching order per 1 root system was no differences between soil depths except to the third order. RTD of second order significantly increased with soil depth. SRL of each individual root category significantly decreased with soil depth. These results show the changes of traits along the soil profile.

Next, I examined the changes in the traits of individual roots between lower and upper plots. Root diameter of first and second orders was significantly smaller in organic soil layer of upper plot than those of lower plot ($p < 0.05$ and $p < 0.05$, respectively). In the mineral soil layers, diameter of individual roots showed no significant differences between lower and upper plots. Weight and length of second order was significantly higher in upper slope than in lower slope ($p < 0.05$ and $p < 0.05$, respectively). In organic soil layer, RTD of second order was significantly higher in upper plot than lower plot ($p < 0.05$). SRL of each branching order

showed no differences between upper and lower plots.

Table 5.3 showed nutrient concentration of individual roots. Root N concentration of each individual root category significantly decreased with increasing soil depth and was higher in lower plot than upper plot. Root C concentration of each category significantly decreased with increasing soil depth. While there were no significant differences in C concentration of individual roots between lower and upper plots. Root C/N of each category significantly increased with increasing soil depth (except to third order) and was higher in upper plot than lower plot.

Properties of root systems

Architecture of root systems was expressed by the branching ratio (Br). Br was higher in upper than lower plot ($p < 0.001$, Table 5.4). In both plots, Br significantly decreased with increasing soil depth ($p < 0.001$). System W and System L significantly increased with increasing soil depth ($p < 0.001$ and $p < 0.05$, respectively, Table 5.4). While there were no significant differences between upper and lower plots.

Abundances and biomass of root systems in the soils

I have estimated the abundances of root systems per organic or soil volumes by the soil core sampling (Table 5.5). Density of root system number (SND) significantly varied across soil depth ($p < 0.05$). SND was significantly higher in the organic layer than in the mineral soil layers in both plots. In the organic layer, SND was 0.42 and 0.22 per $1cm^3$ in upper and lower plots, respectively and show a significant difference between upper and lower plots ($p < 0.05$). In the mineral soil layers, SND was rather low and was 0.03-0.06, and 0.03-0.06 in upper and lower plots respectively. Root biomass per soil volume significantly decreased with increasing soil depth. Biomass was tended to be high in upper plot, although there were no significant differences between upper and lower plots. RLD of each category significantly varied across

soil depth ($p < 0.05$), suggesting that RLD was significantly higher at upper slope in organic soil but there were no differences between slopes in 0-5 and 5-15 cm. In organic soil layer, RLD was significantly higher in upper than lower plot ($p < 0.05$).

Table 5.6 showed that CV of biomass, RLD and SND. CV of SND was similar in both plots and each soil depth. CV of biomass and RLD were different between plot and soil depth. CV of biomass and RLD in organic layer was 1.6-2.0 times higher in upper plot than in lower plot, while that in mineral layer was 0.8-1.2 times higher in upper plot than in lower plot.

Discussions

Forest soils provide different environmental conditions in vertical and horizontal scales. Forest soils show vertical profiles reflecting the environmental conditions in the habitat. I have already demonstrated the differences of fine root abundances along the soil profile (see Chapter 2 and 4). In the horizontal scale, I selected study plots along the forest slope. Fine roots were abundant in organic soil layer of the upper sites. Trees allocate NPP in both below and above ground and allocation patterns are depending the soil conditions. Allocation patterns of NPP were already shown in Chapter 2, below-ground allocations were high in the upper parts of the slope.

Biomass of fine roots was composed by individual roots with their architecture. Here I have analyzed allocation pattern of belowground NPP into the individual roots of three different orders, the root systems, and the total root biomass and total root length. Biomass allocation patterns were examined in the three levels, i.e., traits of individual roots, properties of root systems, and abundances of root systems in soils.

Traits of individual roots were expressed by the size (diameter, weight and length), the shape parameter (RTD and SRL) and physiological parameter (N, C and C/N). Diameter of each category of individual roots was smaller in the organic than in the deeper soils (Table 5.1). Also, diameter of fine roots was smaller in upper than lower plots in organic layer. Morphological traits (diameter, weight, RTD, and SRL) changed with soil depth (Table 5.1 and 2). Soil layer

composed of organic and mineral soil layer, and each layer showed apparently different properties. Mechanical impedance, such as soil bulk density, which is high in deeper soil (Bengough 2003; Wang et al. 2016; Wang et al. 2018a). Previous studies reported that roots tented to have thicker diameter with deep soil than that with shallow soil (Fahey and Hughes 1994; Wells et al. 2002; Wang et al. 2006; Makita et al. 2011; Wang et al. 2018a). Plants may have benefit to growth deep roots with thick diameter and high RTD to penetrate into soil easily and improve transport capacity of water and nutrients in soil (Bengough 2003; Hutchings et al. 2003). It is benefit for roots to increase tissue density to increase strength, but roots are required to have high costs such as slower growth and higher maintenance costs (Kramer-Walter et al. 2016). These results suggested that soil texture modified morphological traits of individual roots, and high soil hardness and bulk density induced thick diameter, high RTD and low SRL in this study.

Mineral nitrogen form had effects on the abundances of root biomass in the upper and lower plots (Fujimaki et al. 2004; Chapter 2). But morphological traits of individual roots showed no significant differences between the upper and lower plots. Doi et al. (2017) reported that NH_4^+-N concentration was negative relation to diameter, and positive relation to SRL of lower order root. In contrast, Miyatani et al. (2016) observed that very fine roots (D <0.5 mm) was no relationships between NH_4^+-N concentration and SRL, whereas SRL was positive relation to NO_3^--N concentration. Individual roots are constrained by their morphological properties. The traits of individual roots showed modification of size and shape parameters. Therefore, mineral nitrogen form has no significant effects on the traits of individual roots in this study.

N concentrations of each root category was higher in lower plot than in upper plot (Table 5.3). Soil N fertility induces root functionality such as RTD and root N concentration (Makita et al. 2015). High soil N availability leads higher root N concentration, which means that roots cells contain more sored N and proteins (Makita et al. 2015). Nitrogen and proteins need replacement and repair root cells and are linked with cell activities such as respiration and

nutrient assimilation (Ryan et al. 1996; Pregitzer et al. 1998; Burton et al. 2012; Makita et al. 2015). Jia et al. (2010) suggested that increased soil N availability enhanced root N concentration by increasing soil N uptake. Root have morphological and physiological plasticity in response to soil environment, for instant soil N, soil bulk density and water contents, by increasing root potential capacity (de Kroon and Visser 2003; Hodge 2004). Compared with upper and lower plots, potential of nutrient absorptive capacity of individual roots may be higher at upper slope in sight of root physiology. In this study sites, mineral nitrogen form may affect the physiological properties of individual roots.

Br was higher in upper plot than in lower plot (Table 5.4). It is known that NH_4^+-N promote root branching (Lima et al. 2010; Giel and von Wiren 2014; Doi et al. 2017). High Br indicated that root system high frequently branched. High branching induced a high amount root tips and large foraging area of root systems in soil. Apical root tips have higher nutrient absorptive capacity than low order roots in root system (Pregitzer et al. 2002; Hishi and Takeda 2005; Guo et al. 2008a; Chapter 3). Therefore, root system of upper plot had high nutrient absorptive capacity by changing root system architecture through the increments of branching ratio and the enlargements of foraging area by root systems. *C. japonica* might adopt strategies of changing root system architecture and increasing biomass to increase nutrient absorbed amount at upper slope in which inorganic N form was dominance of NH_4^+-N.

SND was higher in organic soil at upper slope than lower slope (Table 5.5). System W and System L showed no differences between upper and lower plots, although both System W and L were tended to be higher in upper plot than in lower plot (Table 5.4). This result suggested that whole root system was bigger at upper slope than lower slope in organic layer. Because high SND, System W and System L induced high biomass and RLD, biomass and RLD were also tended to be higher in organic soil in upper slope than lower slope (Table 5.5). Previous studies found that fine root biomass was greater in upper slope, which had high NH_4^+-N concentration in soil, than at lower slope (Fujimaki et al. 2004; Chapter 2). These results supported the present study. It was suggested that upper slope site was low nutrient supply and limited plant growth. Fine root is concentrated in the surface layers of the soil in which plant growth is limited by soil N availability (Fujimaki et al. 2004). In this study sites, trees allocated high NPP into the fine root systems with the high branching of absorptive roots

in the upper plots where ammonium form was dominant mineral nitrogen. Whereas in the lower plots, trees allocated small amount of NPP into the fine root systems with low branching of absorptive roots. Thus, the mineral nitrogen availability effects on the fine root biomass through the changes of architecture and abundance of root systems in soils.

CV of SND was similar in plots and soil depths, whereas, CV of biomass and RLD was different in plots and soil depths (Table 5.6). These results suggested that variations of biomass and length of root tips were high. In organic layer CV of biomass and RLD was high especially in upper plot. This result suggested that roots response to soil heterogeneity, such as bulk density, nutrient and pH. Previous studies and chapter 2 showed that fine root biomass was higher in position of upper slope than lower slope.

In conclusion, *C. japonica* may change strategies of NPP allocation in response to inorganic N forms. It was suggested that individual roots mainly changed physiological traits to increase potential absorptive capacity in soil with high NO_3^--N concentration at lower slope, whereas *C. japonica* increased mass of nutrient absorption by changing root system architecture and increasing biomass in soil with high NH_4^+-N concentration at upper slope.

Table 5.1 Branching order properties in average diameter, and total weight and length of each branching order per 1 root system at various soil positions and depths (Values are mean ± SE)

	Depth	Slope	First	Second	Third
Diameter	Organic	Upper	0.49 (0.02)*	0.49 (0.03)*	0.54 (0.03) ns
mm		Lower	0.55 (0.01)	0.58 (0.02)	0.61 (0.02)
	0-5 cm	Upper	0.58 (0.03) ns	0.56 (0.02) ns	0.68 (0.03) ns
		Lower	0.57 (0.02)	0.60 (0.03)	0.75 (0.06)
	5-15 cm	Upper	0.59 (0.02) ns	0.59 (0.03) ns	0.81 (0.07) ns
		Lower	0.57 (0.02)	0.57 (0.03)	0.80 (0.05)
	depth		*	ns	***
	slope		ns	ns	ns
	depth*slope		ns	ns	ns
Weight	Organic	Upper	2.26 (0.60) ns	2.17 (0.57) ns	1.09 (0.29) ns
mg / system		Lower	1.33 (0.16)	1.44 (0.20)	0.80 (0.09)
	0-5 cm	Upper	3.11 (0.46) ns	3.61 (0.56) ns	3.56 (0.60) ns
		Lower	2.94 (0.56)	2.67 (0.49)	4.20 (0.90)
	5-15 cm	Upper	3.47 (0.64) ns	3.89 (0.82) ns	6.09 (1.85) ns
		Lower	2.22 (0.30)	2.42 (0.35)	4.35 (0.68)
	depth		*	*	***
	slope		ns	*	ns
	depth*slope		ns	ns	ns
Length	Organic	Upper	5.86 (1.33) ns	5.24 (1.05) ns	2.24 (0.32) ns
cm /system		Lower	3.24 (0.35)	3.62 (0.46)	1.72 (0.18)
	0-5 cm	Upper	5.77 (0.85) ns	6.98 (0.97) ns	4.81 (0.76) ns
		Lower	5.96 (1.18)	5.05 (0.82)	4.98 (0.90)
	5-15 cm	Upper	5.92 (1.25) ns	6.63 (1.46) ns	4.78 (0.75) ns
		Lower	4.59 (0.92)	4.84 (0.82)	4.42 (0.58)
	depth		ns	ns	***
	slope		ns	*	ns
	depth*slope		ns	ns	ns

Probabilities from two-way ANOVA of root traits in each root category between soil position and depths. Student's t-test was conducted to detect differences between slope positions within each soil depth.

ns Not significant, Significance: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Table 5.2 Root tissue density (RTD) and specific root length (SRL) at various soil positions and depths (Values are mean ± SE)

	Depth	Slope	First	Second	Third
RTD	Organic	Upper	0.20 (0.02) ns	0.20 (0.01)*	0.19 (0.01) ns
$g\ cm^{-3}$		Lower	0.17 (0.01)	0.15 (0.01)	0.16 (0.01)
	0-5 cm	Upper	0.23 (0.02) ns	0.22 (0.01) ns	0.24 (0.05) ns
		Lower	0.21 (0.03)	0.19 (0.01)	0.18 (0.02)
	5-15 cm	Upper	0.23 (0.01) ns	0.23 (0.02) ns	0.22 (0.01) ns
		Lower	0.23 (0.02)	0.24 (0.03)	0.21 (0.02)
	depth		ns	**	ns
	slope		ns	ns	ns
	depth*slope		ns	ns	ns
SRL	Organic	Upper	28.7 (1.3) ns	28.6 (2.1) ns	26.6 (2.9) ns
$m\ g^{-1}$		Lower	25.1 (1.2)	26.6 (1.7)	21.9 (1.2)
	0-5 cm	Upper	18.9 (1.1) ns	20.2 (1.3) ns	14.1 (1.4) ns
		Lower	20.0 (1.1)	20.5 (1.4)	16.1 (2.8)
	5-15 cm	Upper	17.3 (1.1) ns	17.4 (1.4) ns	12.1 (2.1) ns
		Lower	19.4 (2.1)	20.2 (2.4)	11.0 (0.9)
	depth		***	***	***
	slope		ns	ns	ns
	depth*slope		ns	ns	ns

Probabilities from two-way ANOVA of root traits in each root category between soil position and depths. Student's t-test was conducted to detect differences between slope positions within each soil depth.

ns Not significant, Significance: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Table 5.3 Branching root properties in nutrient contents of different root categories at various soil positions and depths (Values are mean ± SE)

	Depth	Slope	First	Second	Third
N	Organic	Upper	13.3 (0.5)***	10.5 (0.6)***	7.1 (0.5)**
mg kg^{-1}		Lower	17.3 (0.7)	15.2 (0.7)	11.3 (0.7)
	0-5 cm	Upper	11.5 (0.5)*	9.4 (0.8)*	6.5 (0.6) ns
		Lower	13.4 (0.4)	11.4 (0.2)	8.2 (0.6)
	5-15 cm	Upper	9.6 (0.5)**	7.6 (0.5)***	5.7 (0.5)***
		Lower	12.4 (0.5)	10.8 (0.5)	8.5 (0.4)
	depth		***	***	**
	slope		***	***	***
	depth*slope		ns	ns	ns
C	Organic	Upper	483.8 (9.1) ns	489.6 (7.5) ns	487.0 (6.1) ns
mg kg^{-1}		Lower	474.9 (12.6)	489.8 (10.3)	488.7 (4.4)
	0-5 cm	Upper	408.6 (5.5)*	457.3 (5.3) ns	464.8 (7.9) ns
		Lower	386.4 (7.4)	439.0 (10.3)	471.3 (4.0)
	5-15 cm	Upper	391.4 (14.0) ns	446.9 (9.7) ns	452.3 (13.2) ns
		Lower	380.2 (12.0)	438.8 (13.4)	474.8 (12.2)
	depth		***	***	*
	slope		ns	ns	ns
	depth*slope		ns	ns	ns
C/N	Organic	Upper	36.8 (2.0)**	47.6 (3.3)**	70.4 (5.7)**
		Lower	27.5 (1.1)	32.5 (1.5)	44.1 (3.1)
	0-5 cm	Upper	36.1 (1.9)**	50.8 (4.7)*	74.1 (6.6) ns
		Lower	28.8 (0.9)	38.8 (1.7)	60.0 (6.1)
	5-15 cm	Upper	40.8 (1.2)***	60.2 (4.3)**	81.5 (6.4)**
		Lower	30.8 (1.1)	41.1 (2.6)	56.9 (3.8)
	depth		*	*	ns
	slope		***	***	***
	depth*slope		ns	ns	ns

Probabilities from two-way ANOVA of nutrient concentrations in each root category between soil position and depths. Student's t-test was conducted to detect differences between slope positions within each soil depth.

ns Not significant, Significance: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Table 5.4 Root system properties in branching ratio (Br), root system mass (System W) and root system length (System L) at various soil positions and depths (Values are mean ± SE)

Depth	Slope	Br	System W (mg)	System L (cm)
Organic	Upper	4.00 (0.13)***	5.52 (1.40) ns	13.34 (2.54) ns
	Lower	3.28 (0.11)	3.57 (0.32)	8.59 (0.63)
0-5 cm	Upper	3.71 (0.13)**	10.28 (1.45) ns	17.56 (2.13) ns
	Lower	3.13 (0.12)	9.82 (1.75)	16.00 (2.54)
5-15 cm	Upper	2.68 (0.24) ns	13.45 (2.93) ns	17.33 (2.89) ns
	Lower	2.91 (0.14)	8.99 (1.22)	13.85 (2.01)
depth		***	***	*
slope		***	ns	ns
depth*slope		ns	ns	ns

Probabilities from two-way ANOVA of root architectural traits in each root category between soil position and depths. Student's t-test was conducted to detect differences between slope positions within each soil depth.

ns Not significant, Significance: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Table 5.5 Root system number density (SND), root biomass per soil volume and root length density (RLD) in different root categories at various soil positions and depths (Values are mean ± SE)

Depth	Slope	SND (no. cm^{-3})	Biomass (mg cm^{-3})	RLD (cm cm^{-3})
Organic	Upper	0.42 (0.08)*	2.50 (0.94) ns	5.76 (1.70) *
	Lower	0.22 (0.04)	0.79 (0.12)	1.89 (0.28)
0-5 cm	Upper	0.06 (0.01) ns	0.69 (0.14) ns	1.19 (0.22) ns
	Lower	0.06 (0.01)	0.59 (0.15)	0.98 (0.20)
5-15 cm	Upper	0.03 (0.01) ns	0.34 (0.07) ns	0.46 (0.09) ns
	Lower	0.03 (0.01)	0.29 (0.06)	0.43 (0.08)
depth		***	**	***
slope		*	ns	*
depth*slope		*	ns	*

Probabilities from two-way ANOVA of root abundance in each root category between soil position and depths. Student's t-test was conducted to detect differences between slope positions within each soil depth.

ns Not significant, Significance: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Table 5.6 Coefficient variation of root biomass per soil volume and root length density (RLD) and SND in different root categories at various soil position and depths

Depth	Slope	Coefficient variation		
		Biomass	RLD	SND
Organic	Upper	0.86	0.68	0.42
	Lower	0.42	0.42	0.39
0-5 cm	Upper	0.61	0.58	0.40
	Lower	0.58	0.47	0.38
5-15 cm	Upper	0.51	0.51	0.31
	Lower	0.64	0.56	0.34

要旨

細根研究はこれまで、養分吸収能力が高いとされる細根を直径で分類することにより、種内・種間比較が行われてきた。しかし、直径の大きさは種に依存することより、吸収能力などを比較することが難しかった。それに対し、次数根という根系に占める位置による分類方法が提案された。個根に着目することで直径に依存せず、より詳細に吸収能力などを比較できるようになり、研究が進んできた。しかし根は個根で存在しているのではなく、様々な性質を持つ個根で構成された根系で成り立っている。そのため、近年、細根の森林生態系における物質循環を理解するには、個根だけでなく、根系単位でその動態や応答に着目することが重要であると指摘されている。本研究では日本主要樹木であるスギを材料とし、個根と根系の関係性について明らかにするとともに、根系が土壌立地条件によってどのように応答するかを明らかにすることを目的とした。

2 章では研究調査地である大原野森林公園のスギ人工林の特徴を把握するため、土壌条件と地上部－地下部現存量との関係の調査を行った。スギは日本の歴史的経緯から、斜面に植えられていることが多く、本調査地も斜面に実験区を設定した。斜面での土壌特性としては、斜面上部では乾燥しており、無機態窒素形態としては NH_4^+-N 濃度が高く、土壌養分の加給性が低くなっており、一方、斜面下部は湿潤で、無機態窒素形態としては NO_3^--N が高く、土壌養分の加給性が高くなっていることが示唆された。また、斜面株から上部にかけて地上部現存量は小さく、地下部現存量は大きくなり、負の相関を示した。斜面に沿った土壌養分、水分量、有機物層の厚さなどの土壌特性の変化に伴って、地上部と地下部の現存量は相補完的に存在していることが示唆された。

3 章では、個根の特性について調べるため、1-3 次数根を対象として、その解剖学的特徴の記述と、直径、次数根、原生木部数との関係性について調べた。養分吸収能力のある二次肥大していない根（吸収根：primary root）は 1,2 次数根に多く、直径は小さく、原生木部数は二原基, 三原基が多くなっていた。一方、養分吸収能力のない二次肥大した根（通導根：secondary root）は 3 次根に多く、直径は大きく、原生木部数は四原基が多くなっていた。以上から、吸収根は 1,2 次根に多く、また原生木部数が二次肥大と関連していることをスギにおいて明らかにした。また、吸収能力に着目した根系の研究においては、3 次数根までを対象とすれば十分であることが示唆された。吸収根と通導根を分けるには直径、次数根、原生木部数のどれを使えば良いか検討した結果、作

業効率なども勘案すると直径を用いるのが簡便でいいと考えられ、スギでは直径の境界を 0.5~0.6mm に設定することで、吸収根と通導根を分けられることを提案した。

4 章では、土壌基質を考慮した根系の動態を明らかにするため、有機物層と鉱物質層での細根量と根端密度の季節動態の調査をした。直径 2mm 以下を対象とした。根端は根系の先端にあり養分吸収能力が高いが、寿命が短いため回転率が高く、根系構造に変動に大きく関わっている。吸収根の直径を<0.5mm で区別した。植物にとって重要な無機態窒素濃度も合わせて調べることにより、土壌養分との関係性についても調べた。細根量、根端密度ともに季節動態は主に直径<1 mm の変動によって起こっており、夏場に高く冬場に低くなる傾向にあった。特に有機物層において顕著に変動をしていた。このような細根量と根端密度の変動に伴い、細根の分枝強度が変動していた。以上から、根端による根系の分枝により細根量と根端密度の変動が生じていることが示唆された。この変動は無機態窒素濃度とも関連しており、土壌養分が豊富である夏場に、有機物層において根系を拡大することで、窒素の吸収を最適化させていることが示唆された。

以上の調査過程から、根系は土壌基質や無機態窒素濃度により応答が異なることが示唆された。さらなる検討のため、5 章では土壌深度による気質の変化と斜面立地による養分変化に対して、個根、根系、根量のどのレベルで細根の分布が応答しているかを検討した。個根においては、土壌深度による基質の変化に対してその形態的特徴が変化し、深度が大きくなると直径や根密度は高くなるが、一方、比根長は小さくなった。これは、土壌は表層よりも、深度が深いほうが土壌は硬くなり、空隙が小さくなることによるという土壌組成の変化によって起こると考えられた。一方、斜面上部と下部での形態的特徴の差は小さく、土壌基質ほどの違いは見られなかった。しかし、生理特性は異なり、NO_3^--N 濃度の高い斜面下部では，個根の窒素濃度は高く，少ない炭素投資により窒素を利用していることが示唆された。根系単位では、サイズは斜面上部で大きい傾向にあり、分枝率も高くなっていた。また、現存量を示す根量や根長密度も斜面上部で高くなっていた。NH_4^+-N の優占する斜面上部では，有機物層での根系の分枝率を高め，細根密度を高めることで空間あたりの根量を増やすことにより養分吸収量を高めていることが示唆された。以上から、土壌有機物層，鉱物質層の無機態窒素濃度に対応し、斜面下部の NO_3^--N が豊富な条件では、個根の生理特性を変化させることにより養分吸収能力を高めており、斜面上部の NH_4^+-N が優占する条件では、根系構造と根量を変化させ根の養分吸収の空間量を増大させることで窒素吸収量を上げていることが示唆された。

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