

Long-term dynamics of fine roots in forest ecosystems

evaluated by scanned image analysis

スキャン画像解析により評価した森林生態系における細根の長期動態

2019

Ryo Nakahata

Division of Forest and Biomaterials Sciences,

Graduate School of Agriculture,

Kyoto University

Abstract

Fine roots have key roles in carbon and nutrient dynamics in forest ecosystems. However, methods for evaluating fine root dynamics have large uncertainties and are developing now. And temporal dynamics of fine roots and their relating factors are poorly understood. Objectives of the present study were to describe long-term dynamics of fine roots, characterize fine root phenology and examine possible factors controlling the fine root dynamics.

To investigate fine root dynamics, flat root scanner or minirhizotron methods were applied for over five years in temperate forests of cypresses (*Chamaecyparis obtusa*) and deciduous oaks (*Quercus serrata*) or a cool temperate forest of beeches (*Fagus crenata*), respectively. And the long-term dynamics of fine root production were analyzed with artificial soil disturbance, environmental conditions (temperature and soil moisture) and physiological factors (leaf and seed dynamics).

Fine roots with large diameter emerged intensively in the immediate year of soil disturbance caused by equipment installation. Therefore, the soil disturbance effects should be taken into account in methods inescapably cutting roots and/or creating root-free soil.

The cypress forest had distinct several patterns of fine root phenology, whereas the phenology was relatively stable in the oak forest. In the beech forest, there were two patterns of summer or autumn dominant phenology. Seasonal variations of fine root production were greatly affected by climate conditions, but the peak root production was controlled by species-specific factors.

In the cypress and oak forests, temperature induced fine root growth initiation in spring, but was not correlated in autumn. Thus, fine root response to temperature varied depending on the season, and there was a hysteresis relationship between fine root phenology and temperature variations. In the beech forest, leaf and seed production significantly affected seasonal variations of fine root production. Especially, fine root production in autumn was related to the proximate factor controlling the masting events of beeches.

Contents	
Abstract.....	1
Chapter 1 General introduction	5
1.1 The roles of fine roots on forest carbon dynamics	5
1.2 Methodology for estimating fine root dynamics	7
1.3 Temporal dynamics of fine roots.....	10
1.4 Objectives	12
Chapter 2 Possible effect of soil disturbance on fine root dynamics	15
2.1 Introduction	15
2.2 Materials and methods.....	20
2.2.1 Study site	20
2.2.2 Scanner preparation for application in forests.....	21
2.2.3 Image acquisition for fine root observation	22
2.2.4 Image analysis.....	23
2.2.5 Calculations	25
2.2.6 Analysis of fine root growth.....	27
2.2.7 Statistical analysis	28
2.3 Results.....	30
2.3.1 Observation periods of each scanner.....	30
2.3.2 Temporal change in standing root area	30
2.3.3 Annual root area production and disappearance	31
2.3.4 Patterns of cumulative root area production	32
2.3.5 Length based fine root production	34
2.3.6 Mean diameter of elongated fine roots.....	35
2.3.7 Diameter distribution of fine roots.....	36
2.4 Discussion.....	38
2.4.1 Applicability of the flat root scanner method in forests.....	38
2.4.2 Distinct two phases in standing fine root area.....	39
2.4.3 Logistic growth of fine root production.....	40
2.4.4 Fine root morphology affected by soil disturbance.....	42
2.4.5 Implication on estimation by the techniques with soil disturbance	44
2.5 Conclusions.....	47
Chapter 3 Temporal dynamics of fine roots in cypress, deciduous oak and beech forests	
76	
3.1 Introduction	76
3.2 Materials and methods.....	78

3.2.1	Study site	78
3.2.2	Fine root observation in the cypress and oak stands.....	79
3.2.3	Fine root observation in the beech stand.....	80
3.2.4	Image analysis.....	81
3.2.5	Calculations	82
3.2.6	Statistical analysis	82
3.3	Results.....	84
3.3.1	Fine root production in a cypress forest	84
3.3.2	Fine root production in a deciduous oak forest	84
3.3.3	Fine root production in beech forests.....	85
3.3.4	Comparisons of seasonal patterns of fine root production	85
3.4	Discussion.....	88
3.4.1	Fine root phenology in three forest types	88
3.4.2	Differences of fine root phenology among dominant species of forests	88
3.5	Conclusions.....	91
Chapter 4	Environmental and physiological factors controlling fine root dynamics	97
4.1	Introduction	97
4.2	Materials and methods.....	99
4.2.1	Study site	99
4.2.2	Soil temperature and moisture.....	99
4.2.3	Fine root observation.....	100
4.2.4	Litterfall observation.....	102
4.2.5	Statistical analysis	102
4.3	Results.....	104
4.3.1	Effects of environmental factors on fine root production in cypress and oak forests 104	
4.3.2	Effects of physiological factors on fine root production in a beech forest	104
4.4	Discussion.....	106
4.4.1	Seasonal plasticity of fine root response to environmental conditions in temperate forests.....	106
4.4.2	Resource allocation mechanisms between above- and below-ground production in the beech forest	107
4.5	Conclusions.....	109
Chapter 5	General conclusions.....	117
Acknowledgments.....		121
References		123

Appendix.....137

Chapter 1 General introduction

1.1 The roles of fine roots on forest carbon dynamics

Elucidation of forest dynamics concerning major sinks and sources of carbon in the ecosystems is required in relation to global climate change (Heimann and Reichstein 2008). Although carbon dynamics of above-ground in forest compartments have been investigated extensively by the conventional methods, those of below-ground production have not been well understood. Net primary production (NPP) in association with below-ground plant organs plays an important role in ecosystem dynamics. Although it has been well established that availability of water, nitrogen and/or phosphorus generally controls carbon allocation and NPP (Nadelhoffer and Raich 1992a), mechanisms regulating soil resources and plant interactions have yet to be clearly understood.

In studies of forest ecology below-ground organs of trees are frequently categorized into coarse roots supporting a plant body and fine roots which have a role of water and nutrient uptake and transport. Definition of the fine roots depends on arbitrary definitions by the researchers considering purpose of a study, target plant and study site (Vogt et al. 1996; Gill and Jackson 2000; Pregitzer et al. 2002). In general, fine roots are frequently defined as roots that are less than 2 mm in diameter (Gill et al. 2002; Noguchi et al. 2007). Since production and mortality of fine roots occur simultaneously and their turnover cycles tend to be shorter than the coarse roots, they also have a role to continuously supply organic matter into soil as plant litter. In previous studies, estimates of fine root turnover have been reported in several biomes. Annual fine root turnover rates were estimated as 0.77 ± 0.70 , 1.21 ± 1.04 and 1.44 ± 0.76 (yr^{-1} ; $\pm\text{SD}$) in the boreal, temperate and tropical forests, respectively (Finér et al. 2011). Although these estimates are based on minirhizotrons or excavation methods (Vogt et al. 1998; Gill et al. 2002; McCormack et al. 2014), mutually agreeable estimates have been rare due to uncertainty in the measurement and calculation methods.

Plant roots are produced by photosynthates produced in leaves. As previous studies indicate,

fine root biomass accounts for only 1-12% of forest total biomass although they consumed 8-69% of NPP (Grier et al. 1981; Vogt et al. 1982; Comeau and Kimmins 1989). Furthermore, recent studies prominently report that fine root production accounts for more than 40% of NPP of forests (Hendrick and Pregitzer 1993; Vogt et al. 1996; Janssens et al. 2002; Tatenno et al. 2004). Therefore, production of fine roots is important in ecosystem carbon dynamics. In the previous studies, estimates of fine root production measured by various approaches have been reported. Fine root production for all plants (trees + understory) was estimated as 311 ± 259 ($n = 39$), 428 ± 375 ($n = 71$) and 596 ± 478 ($n = 32$) ($\text{g m}^{-2} \text{ yr}^{-1}$; $\pm\text{SD}$) in the boreal, temperate and tropical forests, respectively (Finér et al. 2011).

1.2 Methodology for estimating fine root dynamics

While the importance of fine roots is indicated for elucidating forest carbon dynamics, data accumulated with the fine root studies are relatively insufficient. Previous assessments of resource controls on plant dynamics have focused primarily on above-ground processes, while important below-ground processes (such as fine root production and mortality) are understood relatively poorly (Norby et al. 2000; Fitter 2005). That is caused mainly by difficulties of below-ground observation in forest stands. Below-ground organs, not only roots but also soil animals and hyphae, cannot be directly observed under the natural condition.

Various methods have been developed for estimating fine root dynamics in natural ecosystems. Measurement approaches for detecting fine root dynamics can be mainly categorized into excavation and observation methods. The excavation methods include techniques of sequential soil coring (Persson 1980; Vogt and Persson 1991; Ostonen et al. 2005), ingrowth core (Finér et al. 1997; Steingrobe et al. 2000; Ostonen et al. 2005), root mesh (Fahey and Hughes 1994; Jentschke et al. 2001; Montagnoli et al. 2007) and others. The observation methods include rhizotron method (Böhm 1979; Keyes and Grier 1981), minirhizotron method (Hendrick and Pregitzer 1993; Majdi and Öhrvik 2004; Guo et al. 2008a; Vamerali et al. 2012), flat root scanner method (Dannoura et al. 2008; Dannoura et al. 2012) and others. Although these various approaches have been proposed to enhance description of the fine root dynamics, all approaches have advantages and disadvantages in relation to cost, labor and time efficiency, impact on the ecosystems and accuracy (Table 1.1). The observation methods are considered more suitable approaches for the long-term measurement than the excavation methods. Sequential soil coring technique is the most preferred method to quantify the mass-based root abundance and its temporal change among others (Addo-Danso et al. 2016). Although the ingrowth core and root mesh techniques are direct methods quantifying fine root production, those require an assumption that soil disturbance in installing the equipment does not affect negligible on production

of fine roots. On the other hand, observation methods such as using minirhizotrons are based on visually intuitive observation of growth, mortality and decomposition of fine roots emerged on a soil profile through a transparent panel or tube. Minirhizotron methods have been improved significantly since it was first proposed (Bates 1937) and are widely utilized with the development of electric equipment, to investigate the dynamics and functions of fine roots in agricultural and ecological studies (e.g. Bragg et al. 1983; Ruess et al. 2003). Those techniques allow direct measurement of fine root production and mortality, thus avoiding the major limitation that hinders more traditional soil core and budget approaches (Hendrick and Pregitzer 1996). However, the widely used minirhizotron methods generally require expensive equipment such as the specifically designed camera or scanner. Also the observation area tends to be small, rendering acquisition of the root morphology difficult (Table 1.1). Recently proposed flat root scanner method overcomes those weaknesses of the minirhizotron methods (Dannoura et al. 2008), but use of the flat root scanner method in ecological studies is still not common. However, the method has a potential that makes it possible to accomplish high frequency observations and automation of root monitoring, which cannot be accomplished using the other minirhizotron methods. The choice of a method among various approaches in excavation and observation methods tends to be made through consideration of cost, labor requirement, site constraints and individual preferences rather than accuracy and precision (Vogt et al. 1998; Addo-Danso et al. 2016).

Several intensive comparisons (Neill 1992; Majdi 1996; Hertel and Leuschner 2002; Ostonen et al. 2005; Hendricks et al. 2006) and reviews (Vogt et al. 1996; Gill and Jackson 2000; Majdi et al. 2005; Noguchi et al. 2007; Addo-Danso et al. 2016) of those excavation and/or observation methods have been published. Despite these publications, there are still no general consensus among below-ground researchers on what methods most realistically reflect carbon pools or dynamics in the below-ground part of forest ecosystems. This lack of consensus makes it difficult for researchers to

decide which method is most suitable for their study purposes.

1.3 Temporal dynamics of fine roots

It is necessary for understanding seasonal dynamics of carbon and cycling of nutrient resources, and their mechanisms in forest ecosystems to consider below-ground dynamics. Since below-ground organs, especially fine roots, have the key roles on water and nutrient uptake and producing large amount of biomass, several previous studies clarified phenological dynamics of fine roots using mainly the observation methods such as the minirhizotron method, which are able to observe *in situ* dynamics of the roots. So far, remarkable results concerning fine root phenology have been reported in the previous studies.

In general, production of fine roots has seasonality. Peaks of the fine root production have been observed during the growing season, but there is considerable variation, as the main production can occur in spring (Hendrick and Pregitzer 1996; Joslin et al. 2001) or summer (Fahey and Hughes 1994; Burton et al. 2000; Steinaker et al. 2010) depending on the dominant species, climate and probably their annual variation. Soil temperature was considered to be an important factor correlating with root production in forests without a drought condition (Steele et al. 1997). But if there is severe drought during the growing season, soil moisture becomes the restricting factor on fine root production (Joslin et al. 2001). In addition, the possible factors controlling the peaks of fine root production are low temperature damage in winter (Tierney et al. 2001) and the fine root biomass density. On the other hand, photosynthetic activity also possibly affects fine root production (Steinaker et al. 2010). Seasonal patterns of the photosynthetic activity are closely related to forest structure and species composition. Therefore, the seasonal patterns of fine root production may also be associated to the patterns of the photosynthetic activity. However, there have been few studies relating seasonal patterns of fine root production and shoot phenology.

It is therefore still necessary to measure fine-root dynamics and relate them to changes in environmental and endogenous factors in forests with vegetation types and climate conditions

considered. Information on the seasonal variation in fine root production is essential for estimating fine root turnover and annual fine root production. However, further data on the seasonal patterns of fine root production are needed to clarify the factors and mechanisms controlling it in forest ecosystems.

1.4 Objectives

Primary objectives of the present study were to describe long-term dynamics of fine roots and to characterize their patterns by using two types of the observation methods which enable frequent observation of fine roots. Furthermore, in the following two subjects, the fine root dynamics were analyzed in relations to possible mechanisms that created those patterns. Those mechanisms will be presented as hypotheses through the statistical analyses so that they can be tested in the studies that follow.

First subject was about possible effects of artificial soil disturbance on fine root dynamics. Although fine root production has been quantified intensively from necessity of estimation of NPP in forest ecosystems, many estimates of the fine root production have been evaluated with destructive methods such as the sequential soil coring and the ingrowth cores. But, the ingrowth core method measures roots that grow into soil space disturbed by instrument installation. Therefore, effects of soil disturbance can be critical in estimation of fine root production. However, the effects of soil disturbance on fine root dynamics are poorly understood. In Chapter 2, fine root dynamics immediately after soil disturbance were evaluated quantitatively by using the flat root scanner method, and possible effects were suggested.

Second subject of the present study was ecological observation of fine root dynamics in forests. Description of temporal dynamics of fine roots is essential for understanding resource dynamics in relations to plant physiology and ecosystem carbon cycling. So far, there has been a few studies investigating fine root dynamics with long-term monitoring in which seasonal and annual variations can be evaluated. However, the seasonal variations (i.e. phenology) of fine roots may change annually probably due to the annual trends of climate conditions and/or physiological activities of plants. In Chapter 3, temporal changes of fine root production in three typical forests were clarified. Then the fine root phenology was characterized and divided into several specific patterns.

Clarification of seasonal and annual variations of fine root production itself may not elucidate their mechanisms. To understand and predict fine root production, environmental and physiological factors controlling fine root dynamics have to be revealed by the further experiments and analyses. Fine root phenology generally varies depending on years and plant species. These variations may be controlled by environmental conditions and/or physiological mechanisms. Especially in temperate forests having strong seasonality, air temperature and precipitation were deemed primary factors. In addition, it is highly possible that fine root production is related to above-ground dynamics such as leaf expansion and seed formation because of resource supply and allocation. In Chapter 4, how fine root production is controlled by the environmental and physiological factors was analyzed, and possible hypotheses were suggested.

Table 1.1 Comparison of methods for estimating fine root dynamics on type, cost, labor and time efficiency, impact on an ecosystem, unit of measurement, accuracy and targets of the measurement.

The table indicates typical characteristics of each method, and those may not be suitable for all cases in the previous studies

Method	Type	Cost	Labor and time efficiency	Impact on an ecosystem	Unit of measurement	Accuracy	Targets of the measurement
Sequential coring	Excavation	Low	Inefficient	Destructive	Mass based	High	Biomass Production Mortality
Ingrowth core	Excavation	Low	Inefficient	Destructive	Mass based	Low	Production
Root mesh	Excavation	Low	Inefficient	Destructive	Mass based	Low	Production
Rhizotron	Observation	Low	Inefficient	Non-destructive (without set-up)	Area based	High	Biomass Production Decomposition
Minirhizotron	Observation	High	Efficient	Non-destructive (without set-up)	Area based	High	Biomass Production Decomposition
Flat root scanner	Observation	Low	Efficient	Non-destructive (without set-up)	Area based	High	Biomass Production Decomposition

Chapter 2 Possible effect of soil disturbance on fine root dynamics

2.1 Introduction

There is general agreement that fine roots of trees, arbitrarily defined as roots less than 1 or 2 mm in diameter (Pregitzer et al. 2002), have key roles in carbon and nutrient dynamics in forest ecosystems (Nadelhoffer and Raich 1992a; Majdi et al. 2005; Finér et al. 2007). Recent studies estimate that fine root production accounts for more than 40% of net primary production (NPP) in forests (Hendrick and Pregitzer 1993; Vogt et al. 1996; Janssens et al. 2002; Ruess et al. 2003; Tateno et al. 2004), and thus elucidation of fine root dynamics is essential for understanding resource dynamics in terrestrial ecosystems. However, previous methods for estimating fine root production have large uncertainties (Nadelhoffer and Raich 1992b; Hendricks et al. 2006; Finér et al. 2011; Addo-Danso et al. 2016) due to lack of sound methods in assessing fine root dynamics (Fahey et al. 1999; Lauenroth 2000; Norby et al. 2000).

Methods for describing fine root dynamics of ecosystems are divided into direct approaches that can be categorized into two groups: excavation and observation methods and indirect approaches such as use of nitrogen budget (Nadelhoffer et al. 1985) and carbon balance (Raich and Nadelhoffer 1989). Excavation methods such as sequential soil coring (Persson 1980; Vogt and Persson 1991; Ostonen et al. 2005), ingrowth cores (Finér et al. 1997; Steingrobe et al. 2000; Ostonen et al. 2005) and root meshes (Fahey and Hughes 1994; Jentschke et al. 2001; Montagnoli et al. 2007) have been used. The observation methods include rhizotrons (Böhm 1979; Keyes and Grier 1981), minirhizotrons (Hendrick and Pregitzer 1993; Majdi and Öhrvik 2004; Guo et al. 2008a; Vamerali et al. 2012) and more recently flat root scanners (Dannoura et al. 2008; Dannoura et al. 2012). As an *in situ* observation technique, flat root scanners were proposed as an alternative approach (Dannoura et al. 2008; Nakano et al. 2012; Adu et al. 2014; Van Do et al. 2015). They allow observation of fine root

dynamics more quickly and frequently by using multiple scanners and possibly scanning automatization on a larger observation surface (e.g., A4 paper size: approx. 21.0 cm x 29.7 cm) than that of the minirhizotron tubes. Therefore, behavior of fine roots immediately after instrument installation can be directly and frequently observed using the flat root scanner method. In such observation methods, not only the standing root area and length but also production and disappearance rates can be estimated directly by using successive images

The sequential soil coring method is based on sampling soil cores at more or less regular time intervals, and estimates production by calculating differences of fine root biomass and/or necromass between the sampling times (Gower et al. 1992). The ingrowth core method estimates root production by quantifying roots that grew into root-free soil that had been filled in the excavated soil space (Neill 1992). The ingrowth core method is one of the most commonly used approaches to estimate fine root production, and its results are considered to be relatively easy to interpret (Milchunas 2009). The root mesh (also called net sheet) method estimates root production by placing a flat mesh vertically into soil for some time and measure the number and/or mass of roots that protruded the mesh. This method has been applied less frequently than the other excavation methods, but was developed more recently (Hirano et al. 2009; Ohashi et al. 2015). Those methods are based on tenuous assumptions and are subject to additional sampling errors (Fahey et al. 1999; Lauenroth 2000; Epron and Osawa 2017). The ingrowth core method has been considered effective in studying ecosystems with rapid fine root growth and in comparing fine root production among sites or treatments (Neill 1992; Vogt et al. 1998; Oliveira et al. 2000; Godbold et al. 2003; Liu et al. 2014). However, ingrowth core, root mesh and most of the other methods are measuring root production including that of the disturbed roots, because they cause disturbance at the time of device installation by severing living roots that were originally present in the soil. So, the methods involve uncertainties or additional estimation error associated with cutting the existing root systems (Milchunas 2009), requiring their

possible modifications for less disturbance (Laiho et al. 2014). In addition, the ingrowth core method quantifies roots that grow into soil under unnatural conditions in which roots are absent initially and chemical (i.e. water and nutrient availability) and physical (i.e. bulk density) properties have most often been altered. Therefore, I must assume in the estimation of fine root production that the effect of soil disturbance on fine root dynamics are negligible. However, there are few studies that explicitly verify these assumptions.

On the other hand, soil disturbance may be taken into account more easily in the observation methods such as the minirhizotron method (Hendrick and Pregitzer 1992; Hendrick and Pregitzer 1993; Majdi and Öhrvik 2004) and its derivative approaches. In a methodological discussion of the minirhizotron method, Joslin and Wolfe (1999) suggest that minirhizotron data collected immediately after tube installation can be biased by root and soil disturbance during installation, which can result in excessive root proliferation. Their observations suggest that soil disturbance can affect root dynamics. Using the observation methods, it is considered necessary to wait 6-12 month (Johnson et al. 2001) or longer periods (Pritchard et al. 2008) between equipment installation and image collection to allow roots to recolonize the space around the equipment and to permit nutrients to return to pre-disturbance levels.

A critical issue that have to be considered is to clarify if the effects of soil disturbance on fine root dynamics are negligible or substantial, since the assumptions seem different between the excavation and observation methods. It is undesirable if the assumptions contradict between the methods which have been traditionally used as major approaches. Therefore, it must be examined whether the effects of soil disturbance can be negligible on fine root dynamics. However, the examination is not easy because behavior of the roots is hidden under the soil. And only standing values of the roots at a particular location and at a given time can be obtained by any excavation measurement, while observation methods can also yield production and disappearance parameters of

the roots at multiple times at the same location. Although the observation methods yield area and/or length based parameters in a soil profile, not the mass based parameters under the excavation methods, the former methods are more desirable in describing root behavior with a higher time resolution (Majdi et al. 2005). Therefore, the observation methods facilitate collection of data that may be used to infer the effects of soil disturbance through long-term intermittent observations of root dynamics covering a period longer than that of initial root recolonization. However, it should be noted here that the possible effect of soil disturbance on fine root dynamics must await results of controlled field experiments and other examinations.

Moreover, possibility of soil disturbance effects should be considered not only in the amount of fine root production but also in morphology of produced fine roots. It is yet uncertain how the disturbance alter morphology of produced roots such as root diameter (but see Eissenstat 1991) and abundance of mycorrhizal root formation. Morphological traits of fine roots tend to be closely linked to their functional roles such as absorption, transport and turnover. How long fine roots grow can control soil space occupancy, and their diameter determine capacity of nutrient and water transport and physical their strength. And specific root length (SRL) defined as root length per dry root mass is frequently used in the previous studies (Ma et al. 2018) to indicate absorption function of the roots. Thus, morphological analysis, which describe distributions of root length and root diameter within the produced fine roots, can help understand what kinds functions the fine roots develop under different environments. It is necessary to investigate whether morphological traits of fine roots change with soil disturbance.

To infer the possible effect of soil disturbance on fine root dynamics, the present study examined two characteristics of the fine roots: one on their quantity (amount of fine roots) and the other on quality (fine root morphology). The first aim is to describe area based fine root growth quantitatively and model its production patterns following soil disturbance caused by an installation

of the flat root scanner. The second aim is to describe temporal patterns of fine root morphology with their length and diameter for several years after soil disturbance. To test whether the patterns differ in different forest types, the study was conducted in two forests dominated either by *Chamaecyparis obtusa* or by *Quercus serrata*; one is an evergreen conifer and the other is a deciduous broad-leaved species in the typical plantations and the secondary temperate forests, respectively, in Japan. I hypothesize that fine root production and its morphology significantly differ between the year following soil disturbance due to instrument installation and the period thereafter. It is also hypothesized that this difference in root production and morphology is common regardless of forest types. This hypothesis has been supported by previous studies applying the observation methods (Eissenstat 1991; Joslin and Wolfe 1999; Pritchard et al. 2008), which may challenge the implicit assumption of the ingrowth or root mesh methods in which the effect of soil disturbance is usually neglected. By testing this hypothesis, the contradiction of assumptions on soil disturbance between the observation and excavation methods may be highlighted. Then estimation of fine root production affected by soil disturbance is discussed in relation to methodological issues of the excavation methods.

2.2 Materials and methods

2.2.1 Study site

Flat root scanners were set up in two stands of different dominant tree species at Ryukoku Forest, Shiga, Japan at 34°58'N, 135°56'E (Fig. 2.1). One stand was a ca. 80-year-old plantation of *Chamaecyparis obtusa* Endl. (hinoki cypress), an evergreen conifer. The other stand was a secondary forest of *Quercus serrata* Thunb. (konara oak), a deciduous broad-leaved tree. The elevation of the study site is ca. 130 m above sea level. The air temperature averages 14.9 °C and mean annual precipitation is 1530 mm at the Otsu meteorological station (35°00'N, 135°55'E) located at a distance of ca. 4 km from the study site (1981-2010; Japan Meteorological Agency). The soil of the study area is classified as yellowish brown forest soil based on the Japan classification system (Forest Soil Division of Forestry and Forest Product Research Institute 1976) which corresponds to Cambisols in the FAO soil classification (IUSS Working Group 2014; Obara et al. 2015), and is derived from lacustrine sediments of Cenozoic origin that belong to the Kobiwako Group (Ministry of Land Infrastructure Transportation and Tourism 1982; Osawa and Aizawa 2012). The soil bulk densities (\pm SE: standard error) in the cypress and oak stands are 1.23 (\pm 0.02) g cm⁻³ and 1.26 (\pm 0.08) g cm⁻³ respectively with a texture of sandy clay loam. The cypress stand has not been managed for at least 40 years until 2010 (Miyaura 2009). Active self-thinning has been progressing in this stand (Yoda et al. 1963; Osawa and Allen 1993).

The cypress stand is located on a west facing slope (Fig. 2.1) with stand area, stand density and mean stem diameter at breast height (DBH) at ca. 600 m², 1033 ha⁻¹ and 24.4 cm, respectively in 2014 (Table 2.1). The stand also has some canopy and subcanopy trees of *Q. serrata*, *Pinus. densiflora*, and *Ilex pedunculosa* Miq. The relative basal area of *C. obtusa* is 97.2%. The oak stand is located at about 100 m north of the cypress stand across a little valley (Fig. 2.1). The stand area is about 1200 m² with stand density and mean DBH at 3717 ha⁻¹ and 6.5 cm, respectively in 2014 (Table 2.1). The

stand also has some canopy trees of *I. pedunculosa*, *Prunus jamasakura* Sieb. ex Koidz. and *C. obtusa*, and understory trees of *Eurya japonica* Thunb. and *Cleyera japonica* Thunb. The relative basal area of *Q. serrata* is 64.7%.

2.2.2 Scanner preparation for application in forests

In the present study, two types of optical flat-bed scanners were used. One is a charge coupled device (CCD) scanner (GT-S600®, EPSON, Japan). The other is a contact image sensor (CIS) scanner (CanoScan LiDE 210®, Canon, Japan). The CCD-type scanner is thicker than the CIS-type scanner, but the former is suitable for scanning coarse objects clearly by being able to focus on objects of varying depths from the scanning surface. To operate a scanner, the CCD-type needs alternating current (AC) power, while the power of the CIS-type can be supplied from a personal computer (PC) through a universal serial bus (USB) cable (Dannoura et al. 2008).

Each optical flat-bed scanner was covered with a box of acrylic panels of 3 mm thickness for water-tightness (Fig. 2.2), and was inserted vertically in forest soil. Then an image of a soil profile was taken by connecting it to a laptop PC. The CCD-type scanner can be covered completely including the scanning surface within the acrylic box. On the other hand, in the case of the CIS-type, a picture frame-like structure is built with the acrylic panel along the edges of the scanning area because only objects at direct contact with the scanning surface can be focused clearly, and the remaining sides of the scanner are covered entirely with the acrylic panels. An USB cable and, in the case of the CCD-type, an AC power cable are drawn outside for power and data transfer (Fig. 2.2). Then any gaps of the scanner box are filled with silicon filler for waterproofing.

2.2.3 Image acquisition for fine root observation

For observing fine root dynamics, six scanners (Scanner No.: H11, H12, H13a, H13b, H14, H15) and five scanners (Scanner No.: K21, K22, K23, K24, K25) were buried at locations selected haphazardly in the cypress stand and the oak stand, respectively (Fig. 2.3). Although the scanners H11 and H12 are the CCD-type, and the others are the CIS-type, difference in the scanner types was not a problem in the following image analyses.

The flat root scanner was installed into the soil by the following procedures. First, a hole of ca. 50 cm x 20 cm and ca. 30 cm deep was dug on the ground with a shovel. A pair of pruning scissors was used to cut and remove exposed roots. Then the flat root scanner was inserted vertically into the hole with a long side of the scanning surface at a horizontal position. The gaps around the scanner were filled carefully with the removed soil that was free of roots, so that different soil horizons and soil bulk density were approximately reproduced, simulating conditions of the ingrowth cores (i.e. yielding severed roots and creating root-free soil; Fig. 2.4). There was ca. 5 cm thick root-free soil in front of the scanning side. The size of a soil profile scanned by a flat root scanner is 28.8-29.7 cm in width and 20.7-21.6 cm in depth depending on the type of the scanner used. The top edge of the scanning area fits closely to the boundary between organic and mineral soil horizons. Therefore, an area of the soil depth between 0 and 20 cm could be observed. The majority of the vertical distribution of fine roots could be observed on the soil surface, as the previous report indicates that 64% and 51% of fine root biomass between 0-50 cm soil depth were distributed in the upper 20 cm of mineral soil in *C. obtusa* and *Q. serrata* trees, respectively (Karizumi 1979).

The flat root scanners of H11 and H12 were installed in a cypress stand in June 2009, and image acquisition was started. In the same stand, H13a scanner and H14 and H15 scanners were installed additionally in March 2010 and April 2011, respectively. In the oak stand, the scanners of K21, K22, K23 and K24 were established in April 2011, and the K25 scanner was added in March

2012. Because H13a scanner became dysfunctional in May 2012, it was replaced by H13b scanner in August 2012 (The lower-case alphabet “a”, “b”, etc. indicates that the scanner was replaced at that position in the sequence.). The images of soil profiles were taken at a resolution of 600 dpi with 48-bit colors regardless of the scanner type. Frequency of image acquisition was ca. once a week until January 2016; but it was decreased to ca. once in two weeks during the winter season when fine roots were relatively inactive (e.g. Tryon and Chapin III 1983; Aerts et al. 1992). Leaving scanners in the soil during the entire measurement periods facilitated image data collection of the soil profile, since carrying the observation equipment and the maintenance of scanning surface due to dew condensation were not required. Scan image data were saved and accumulated in a laptop PC as still images.

Four scanners (H12, H13a, H13b and K21) out of eleven that were installed were broken by January 2015 (Fig. 2.5; see also Table A1: Appendix). Breakage of the scanners was generally due to submergence of the electrical parts in water or excessive internal water condensation in the scanner box. Overall, long-term observation (> 5 years) of fine roots was possible through sufficiently long life-span of the installed scanners, and by having multiple scanners that continued to operate during the investigation period (Fig. 2.5).

2.2.4 Image analysis

In the present flat root scanner method, the scanning procedures never misaligned the soil profiles on the original images scanned at the former and the latter measurements, because the scanners were absolutely still in the soil over the entire measurement periods. So, image correction of the scanning position was not necessary before the root tracing analysis.

In the present study, to reduce workloads and enhance operation efficiency of image analysis, a pair of two depth-wise images of 5 cm wide and 20 cm deep was cut out from each scanned original

image of ca. 29 cm wide and ca. 21 cm deep (Fig. 2.6). The depth-wise image was further separated into four small images of 5 cm x 5 cm each. The separated images represent depths of 0-5 cm, 5-10 cm, 10-15 cm and 15-20 cm, respectively (i.e. a total of 200 cm² area of soil surface in each scanner was the target for the following analysis). Cutting and separation of the images were done using the image editing software Adobe Photoshop CS2 (Adobe Systems, San Jose, Calif.). Finally, the set of time series images for several years was prepared for each scanner.

The images from the same position of soil profile taken at different times in sequence were analyzed to evaluate growth and disappearance of individual roots using WinRHIZO Tron MF 2015, a software for root image analysis (Regent Instruments, Canada). After comparison of the previous image and the current image on a dual screen display in a computer by placing both images side by side, only roots that grew in length and/or thickness were traced. The traced individual root is composed of a series of straight line segments called root segments. Whether the root segment ends and a new one begins at a node where the root diameter and/or orientation changes or a new order root starts were defined discretionally by an observer. Then, if the roots disappeared due to decomposition or other reasons, the root disappearance was judged by visual inspection for each root segment.

The following variables were quantified using the data from each segment: segment number, visible root area (VRA), visible root length (VRL), visible root diameter (VRD), cumulative gone root area (GRA), cumulative gone root length (GRL) and cumulative gone root diameter (GRD). Traced root segments were identified by segment number in sequence in each analyzed image. VRA, VRL and VRD are defined as the area (in mm²), length (in mm) and diameter (in mm) of the root segment, respectively, that are projected onto the image at a given time. Not only living roots but also dead roots are included in visible root segments as long as they are visible, because I could not distinguish these two types of roots using only the visible color data on the image. GRA, GRL and GRD are defined as the area (in mm²), length (in mm) and diameter (in mm) of a root segment, respectively, that has

disappeared due to decomposition, disturbance, predation, etc. between the dates of first measurement and the present observation. When a part of the root segment was missing for any reason, the root segment was treated as an area that disappeared. However, a root segment at a given time have either the values of VRA, VRL and VRD or GRA, GRL and GRD, but not both, since whether a root is visible or not is uniquely determined in each root segment. In addition, other properties, e.g. dates of emergence and disappearance, and soil depth were recorded for each root segment through the image analysis.

2.2.5 Calculations

Standing root area (S_{are} ; $\text{mm}^2 \text{ cm}^{-2}$), root area production (P_{are} ; $\text{mm}^2 \text{ cm}^{-2} \text{ d}^{-1}$) and root area disappearance (D_{are} ; $\text{mm}^2 \text{ cm}^{-2} \text{ d}^{-1}$) were calculated on each image (i.e. 5 cm x 20 cm x 2 image) using the values of visible root area (VRA; mm^2) and gone root area (GRA; mm^2) of each segment obtained from a pair of analyzed images. S_{are} ($= \sum \text{VRA} / S$) is the sum of VRA for cm^2 of a given area of a given image. Here, S is defined as a constant that corresponds to the analyzed area of the image and has a value of 200 cm^2 in the present study. P_{are} represents the production on the area of newly-emerged and/or elongated root segments after the previous measurement, and was defined as:

$$P_{are} = \frac{\sum_{i=1}^n (\text{VRA}_{t_{2i}} - \text{VRA}_{t_{1i}} + \text{GRA}_{t_{2i}} - \text{GRA}_{t_{1i}})}{S \cdot \Delta t} \quad \text{Eq. 1}$$

where $\text{VRA}_{t_{1i}}$ and $\text{VRA}_{t_{2i}}$ are the area of a visible root segment (numbered as i) at times t_1 and t_2 , respectively ($t_1 < t_2$), $\text{GRA}_{t_{1i}}$ and $\text{GRA}_{t_{2i}}$ are the area of a gone (disappeared) root segment (numbered as i) at times t_1 and t_2 , and $\Delta t = t_2 - t_1$, respectively. D_{are} represents disappearance of newly-disappeared root segments after the previous measurement, and was calculated as:

$$D_{are} = \frac{\sum_{i=1}^n (GRA_{t_{2i}} - GRA_{t_{1i}})}{S \cdot \Delta t} \quad \text{Eq. 2}$$

From these definitions, S_{are} at time t_n can be calculated alternatively by subtracting cumulative D_{are} from cumulative P_{are} between times t_0 and t_n and adding S_{are_0} (defined as S_{are} at time t_0) to that ($S_{are} = S_{are_0} + \sum P_{are} - \sum D_{are}$). Total root production during a year corresponds to the sum of P_{are} values for all measurement times in the year. Similarly, total root disappearance during a year can be calculated as the sum of D_{are} values for all measurement times in the year. In the present study, roots that newly emerged and rapidly disappeared within Δt could not be measured, but those might be negligible because of the high frequency observations (1-2 week interval) used in the present study.

Fine root length production (P_{len} ; mm cm⁻² d⁻¹) and mean diameter of produced root length (M_{dia} ; mm) were calculated on each scanner using the values of VRL (mm), VRD (mm), GRL (mm) and GRD (mm) obtained from the image analysis. P_{len} represents the production rate on the length of newly emerged or elongated root segments in a given soil surface area between the previous and current measurements, and was defined as:

$$P_{len} = \frac{\sum_{i=1}^n (VRL_{t_{2i}} - VRL_{t_{1i}} + GRL_{t_{2i}} - GRL_{t_{1i}})}{S \cdot \Delta t} \quad \text{Eq. 3}$$

where $VRL_{t_{1i}}$ and $VRL_{t_{2i}}$ are the length of a visible root segment (numbered as i) at times t_1 and t_2 , respectively, $GRL_{t_{1i}}$ and $GRL_{t_{2i}}$ are the length of a gone (disappeared) root segment (numbered as i) at times t_1 and t_2 , respectively. M_{dia} represents the length-weighted mean diameter of newly

emerged or elongated root segments in a given soil surface area between the previous and current measurements, and was calculated as:

$$M_{dia} = \frac{\sum_{i=1}^n VRD_{t_{2i}} (VRL_{t_{2i}} - VRL_{t_{1i}})}{\sum_{i=1}^n (VRL_{t_{2i}} - VRL_{t_{1i}})} \quad \text{Eq. 4}$$

where $VRD_{t_{2i}}$ is the diameter of a visible root segment (numbered as i) at time t_2 . To calculate accurate mean values, the diameter should be weighted with length of each segment because its length has a large variation.

2.2.6 Analysis of fine root growth

Fine root growth that began with root-free soil was analyzed by fitting a logistic curve (Verhulst 1838; Shinozaki and Kira 1956; Hozumi 1973; Hutchinson 1978; Begon et al. 2006) to the estimated cumulative P_{are} values over time after the flat root scanner establishment. A non-linear least-squares method was applied using R statistical software (Version 3.5.0; R Development Core Team 2018) for fitting three parameters of the logistic model. The parameters were estimated by fitting the following logistic equation:

$$a = \frac{A}{1 + ke^{-\lambda t}} \quad \text{Eq. 5}$$

where a is a value of cumulative P_{are} over time, t is time in days, A is the asymptotic upper limit of a , λ is the growth coefficient (both A and λ are constants) and k is an integration constant. Day of the year where the inclination of the logistic curve becomes the greatest (t_{DOY}) can be

calculated by the following equation: $t_{DOY} = \ln k \cdot \lambda^{-1}$. The curve fitting to Eq. 5 was conducted for cumulative P_{are} for each scanner and period. The period used for the curve fitting represents an interval from February in the present year to January in the next year, because fine root production in January and February appeared much lower than the other months.

2.2.7 Statistical analysis

Effects of observation period (e.g. elapsed year of observation: immediate, 2nd, 3rd and 4th year), forest type (cypress stand vs. oak stand) and their interaction on maximum S_{are} , annual P_{are} , annual D_{are} , annual P_{len} and mean M_{dia} , and on estimated parameters of asymptotic upper limit (A) and growth coefficient (λ) for cumulative P_{are} were analyzed according to the two-way analysis of variance (ANOVA) followed by the Tukey-Kramer HSD test. Before the ANOVA, normality of variance of the data were tested according to the Kolmogorov-Smirnov test. If the interactive effect was implicated, the effects of each single factor divided into the levels of the other factor were examined by the t test (for two levels) or the one-way ANOVA (for three or more levels). Before the t test and the ANOVA, normality of variance of the data were tested according to the Kolmogorov-Smirnov test.

To describe frequency distribution of the diameter of produced fine roots, density curves were calculated by the Kernel density estimation that is a non-parametric method to estimate the probability density function and an useful alternative to the histogram for continuous data that comes from an underlying smooth distribution (Wand and Jones 1994). The Kernel density estimation was conducted with the gaussian function using the unbiased cross validation technique for selecting bandwidth (Scott and Terrell 1987). And smooth distribution by the Kernel density estimation is ideal for following comparison of diameter distribution. The differences among diameter distribution were quantified by

comparing D values that are used as test statistic in the Kolmogorov-Smirnov test, instead of another statistical test because sample number of the fine roots cannot be defined in the present analysis.

Fluctuation in overall average of M_{dia} was fitted by using the generalized additive model (GAM) that makes no assumption about the shape of the relationships between response and explanatory variables. This model assumes the gaussian distribution for error of response variables. In the GAM fitting, the thin plate regression splines (Wood 2003) were used with the generalized cross validation criterion for selecting smoothing parameters. The M_{dia} was calculated as the length-weighted mean diameter of newly emerged or elongated root segments (Eq. 4).

These statistical calculations were conducted using the R statistical software.

2.3 Results

2.3.1 Observation periods of each scanner

Duration of the scanner that stayed functional in the soil profile varied between two years and one month and six years and eight months as of January 2016. The mean operational period of the flat root scanners including the broken scanners was estimated to be over 1515 days as of January 2016. However, six scanners out of eleven installed scanners were broken by December 2016. H13a scanner in the cypress stand was virtually disfunctional in May 2012. H12 scanner became unusable in September 2013, and K21 scanner of the oak stand and H13b scanner of the cypress stand were broken in September 2014. H13b scanner succeeded H13a in August 2012. K22 and K23 scanners were disfunctional in December and August 2015, respectively. Operational duration of H12, H13a, H13b, K21, K22 and K23 which were broken were about 1566 days, 793 days, 781 days, 1231 days, 1689 days and 1568 days, respectively (Table A1: Appendix). Breakage of the scanners was generally due to submergence of the electrical parts in water or excessive internal water condensation in the scanner box. Overall, long-term observation (> 5 years) of fine roots was possible through sufficiently long life-span of the installed scanners, and by having multiple scanners that continued to operate during the investigation period.

2.3.2 Temporal change in standing root area

Because observation was started just after installation of scanners, the initial value of standing root area (S_{are}) was zero in each scanner. In general, fine roots emerged in the scanning area during a growing season (Table A1, Appendix), gradually increased, and saturated in quantity over time (Fig. 2.7 and Fig. 2.8), which could be recognized visually across images. Fine roots tended to have light color at the initial stage of their emergence, but it commonly changed gradually into darker colors in

a few to several weeks. In immediate year of scanner installation, S_{are} increased rapidly through single (e.g. H12; Fig. 2.7 and K22; Fig. 2.8) or multiple (e.g. H11; Fig. 2.7 and K24; Fig. 2.8) stages including temporary pauses, the patterns of which were similar to those observed in cumulative root area production (P_{are}) (Fig. 2.9 and Fig. 2.10) due to low root area disappearance in the year (Fig. 2.11). Over time, S_{are} reached saturation phase as more or less stable values with some fluctuations (Fig. 2.7 and Fig. 2.8). S_{are} generally changed over the growing season, and there were various seasonal patterns of S_{are} changes with increase (e.g. H14 in 2012; Fig. 2.7 and K25 in 2013; Fig. 2.8), decrease (e.g. H14 in 2014; Fig. 2.7 and K24 in 2012; Fig. 2.8), convex (e.g. H11 in 2011; Fig. 2.7 and K23 in 2012; Fig. 2.8) or concave (H14 in 2013; Fig. 2.7) patterns. There were no consistent patterns of S_{are} among scanners and years in the two stands.

Fluctuation of S_{are} on each scanner showed its maximum value at varying times during a year (Fig. 2.7 and Fig. 2.8). Means of the maximum S_{are} during a year were not significantly different with the number of elapsed years (immediate, 2nd, 3rd and 4th year) after scanner installation (two-way ANOVA, $P = 0.58$; Table 2.2 and Fig. 2.11), but they tended to differ between the cypress and oak stands (two-way ANOVA, $P = 0.05$), with its value in the cypress stand being higher than that in the oak stand. Thus, the maximum S_{are} in annual variation depended on the forest type, but showed no apparent effect of soil disturbance even in the immediate year.

2.3.3 Annual root area production and disappearance

Annual P_{are} was significantly different with the number of elapsed years after scanner installation (two-way ANOVA, $P < 0.01$; Table 2.2 and Fig. 2.11), but did not differ between the cypress and oak stands (two-way ANOVA, $P = 0.54$). The interactive effect between the factors of elapsed year and forest type was significant (two-way ANOVA, $P = 0.02$), indicating annual patterns of P_{are} following

scanner installation differed between the cypress and oak stands. Estimates of the annual P_{are} among the elapsed years differed significantly in the cypress stand (one-way ANOVA, $P < 0.01$), whereas they were not different in the oak stand (one-way ANOVA, $P = 0.60$), indicating that annual P_{are} in the oak stand was relatively stable. In the cypress stand, annual P_{are} of the immediate year was significantly greater than that of the following years (Tukey-Kramer HSD test). Although P_{are} of the cypress stand was higher than that of the oak stand in the immediate year, the P_{are} values of the following years were lower in contrast.

Annual D_{are} were significantly different with the number of elapsed years after scanner installation (two-way ANOVA, $P < 0.01$; Table 2.2 and Fig. 2.11), but did not differ between the cypress and oak stands (two-way ANOVA, $P = 0.32$). The interactive effect between the factors of elapsed year and forest type was not significant (two-way ANOVA, $P = 0.44$), indicating annual patterns of D_{are} were similar between the stands. Annual D_{are} in the immediate year tended to be lower than that of the following years (Tukey-Kramer HSD test). In the cypress stand, annual D_{are} exceeded annual P_{are} for each elapsed year except for immediate year, illustrated by a decreasing annual trend of S_{are} at the last date of each annual period (data not shown). In contrast, D_{are} was always less than P_{are} values in the oak stand, indicating an increasing annual trend of the S_{are} .

2.3.4 Patterns of cumulative root area production

Fine roots commonly began to grow in early spring, peaked their production during the growing season, and then continued to grow until early winter. There was seasonality in the dynamics of fine root production, as I observed seasonal fluctuation in the cumulative P_{are} . Therefore, the cumulative P_{are} could be described as a logistic curve, though temporary pauses were observed in some cases (Fig. 2.9 and Fig. 2.10).

The asymptotic upper limit (A) of the cumulative P_{are} estimated by fitting of the logistic equation (Eq. 5) varied substantially year by year and among the scanners. There were significant differences in A according to the number of elapsed years since scanner establishment (two-way ANOVA, $P < 0.01$; Table 2.2 and Fig. 2.12). The overall average in the value of A (\pm SE) for each elapsed year was 7.49 (\pm 1.37) mm² cm⁻² in the immediate year, 3.20 (\pm 0.43) mm² cm⁻² in the 2nd year, 3.58 (\pm 0.83) mm² cm⁻² in the 3rd year and 2.92 (\pm 0.78) mm² cm⁻² in the 4th year, respectively (Table A2: Appendix). Estimates of the A did not differ significantly between the cypress and oak stands (two-way ANOVA, $P = 0.97$). The interactive effect between the factors of elapsed year and forest type was significant (two-way ANOVA, $P = 0.05$; Table 2.2 and Fig. 2.12), indicating a difference among the pattern of change in the A value along the elapsed years in the cypress and oak stands. Estimates of the A among the elapsed years differed significantly in the cypress stand (one-way ANOVA, $P < 0.01$), whereas they were not different in the oak stand (one-way ANOVA, $P = 0.67$). In the cypress stand, the A in the immediate year was significantly higher than the other years (Turkey-Kramer HSD test; Table 2.3 and Fig. 2.12). In the immediate year, A in the cypress stand tended to be higher than the oak stand, although the difference was not significant (t test, $P = 0.14$). However, it was significantly lower than the oak stand in the 4th year (t test, $P = 0.04$).

Fine roots tended to be produced more immediately after soil disturbance by the scanner installation (Fig. 2.9 and Fig. 2.10). There were significant differences in the growth coefficient (λ) of the cumulative P_{are} estimated by fitting the logistic curves (Eq. 5) to the observed cumulative P_{are} among the elapsed years (immediate, 2nd, 3rd and 4th year) since scanner installation in both stands (two-way ANOVA, $P = 0.01$; Table 2.2 and Fig. 2.12). The average λ (\pm SE) values in each elapsed year was 0.076 (\pm 0.014) d⁻¹ in the immediate year, 0.035 (\pm 0.006) d⁻¹ in the 2nd year, 0.048 (\pm 0.010) d⁻¹ in the 3rd year and 0.028 (\pm 0.005) d⁻¹ in the 4th year, respectively (Table A2: Appendix). The effect of forest type on λ was not significant (two-way ANOVA, $P = 0.41$; Table 2.2 and Fig.

2.12). The interactive effect between elapsed year and forest type was not significant (two-way ANOVA, $P = 0.24$). However, the λ values among the elapsed years did not differ significantly in the cypress stand (one-way ANOVA, $P = 0.22$), whereas they were significantly different in the oak stand (one-way ANOVA, $P = 0.02$). In the oak stand, the λ value in the immediate year tends to be higher than the other years (Turkey-Kramer HSD test; Table 2.3 and Fig. 2.12).

During the growing season immediately after soil disturbance, the asymptotic upper limit indicating annual production of fine roots (A) was higher in the cypress stand (Table 2.3 and Fig. 2.12) and the growth coefficient indicating instantaneous production rate (λ) was higher in the oak stand (Table 2.3 and Fig. 2.12).

2.3.5 Length based fine root production

P_{len} tended to increase more rapidly in the immediate year of soil disturbance than the following years (Fig. 2.14 and Fig. 2.15). P_{len} in the immediate year was the highest among several years after soil disturbance exclusively in all scanners even in both stands. In the cypress stand, maximum P_{len} of H13a in the immediate year was exceptionally large, while that of H15 was relatively low and has values less than one fifth of those of H13a. On the other hand, the spatial heterogeneity among scanners and inter-annual variation seem to be less distinct in the oak stand. However, significant differences of annual P_{len} were detected in elapsed years since soil disturbance (two-way ANOVA, $P = 0.002$; Table 2.4), not in forest types ($P = 0.68$) and their interaction ($P = 0.13$). The overall average of the value of annual P_{len} (\pm SE) for each elapsed year was 12.51 (\pm 2.45) mm cm⁻² in the immediate year, 6.77 (\pm 0.98) mm cm⁻² in the 2nd year, 4.51 (\pm 0.75) mm cm⁻² in the 3rd year and 4.60 (\pm 1.43) mm cm⁻² in the 4th year, respectively. The annual P_{len} in the immediate year was significantly higher (Turkey-Kramer HSD test) than the following 2nd ($P = 0.05$), 3rd ($P = 0.004$) and 4th ($P = 0.01$) years.

In general, the P_{len} immediately after soil disturbance was obviously higher than that in the same season of the following years (Fig. 2.16). The overall P_{len} peaked at about three or four weeks after initial root emergence in both stands. The P_{len} kept high values for over 100 days in the cypress stand, though that in the oak stand decreased immediately and became stable.

2.3.6 Mean diameter of elongated fine roots

Mean diameter of produced fine roots (M_{dia}) showed temporal changes and had large variations during the growing season (Fig. 2.17 and Fig. 2.18). In the cypress stand, fine roots with relatively high M_{dia} tended to appear intermittently in the middle of the year. On the other hand, changes in M_{dia} were relatively stable through the season except for the immediate year of soil disturbance in the oak stand. Fine roots tended to emerge with high M_{dia} temporarily and immediately after soil disturbance in several scanners (e.g. H11; Fig. 2.17 and K21; Fig. 2.18). And M_{dia} rapidly declined during a few to several weeks after the initial emergence. Although M_{dia} was relatively stable except for the immediate year in several scanners (e.g. H15; Fig. 2.17 and K23; Fig. 2.18), high M_{dia} was intermittently observed also in the following years (e.g. H11; Fig. 2.17 and K22; Fig. 2.18). Especially in the 3rd year (2013) of H14 and the 4th year (2014) of K22, M_{dia} was higher temporarily during the growing season than that in the immediate year.

Although there were various patterns of temporal M_{dia} changes (Fig. 2.17 and Fig. 2.18), overall M_{dia} of the roots at initial emergence immediately after soil disturbance was significantly higher than that in the same season of the following years (Fig. 2.19). High M_{dia} in the initial phase exponentially decreased with time and became stable in about three to four weeks after the first root emergence to a similar level of subsequent M_{dia} values. And the behavior was common in the cypress and oak stands. However, the M_{dia} of initial emergence roots in the cypress stand was higher and its

temporal changes that follows was more variable than that in the oak stand.

2.3.7 Diameter distribution of fine roots

The M_{dia} distribution in each scanner generally concentrated around a common diameter level (c.a. 0.5 mm) regardless of the elapsed years after soil disturbance (Fig. 2.20 and Fig. 2.21), though few scanners showed bimodal patterns (e.g. H12; Fig. 2.20). Small distribution in higher diameter levels was detected in several scanners especially in the immediate year of soil disturbance, indicating high M_{dia} of initial emergence roots (Fig. 2.19). Moreover, few scanners showed exceptional small distribution at high diameter levels even several years after soil disturbance (e.g. H14; Fig. 2.20, K22; Fig. 2.21).

However, mean M_{dia} did not differ among the elapsed year after soil disturbance or forest types. The overall average of the value of M_{dia} (\pm SE) for each elapsed year was 0.52 (\pm 0.0019) mm in the immediate year, 0.48 (\pm 0.029) mm in the 2nd year, 0.50 (\pm 0.044) mm in the 3rd year and 0.46 (\pm 0.027) mm in the 4th year, respectively. And the overall average of M_{dia} (\pm SE) for each forest type was 0.51 (\pm 0.025) mm in the cypress stand and 0.47 (\pm 0.017) mm in the oak stand, respectively. Mean M_{dia} in the immediate year was slightly higher than those of the following years, and the mean M_{dia} in the cypress stand was slightly higher than that in the oak stand. However, effects of elapsed years after soil disturbance and/or forest types were not significant in the mean M_{dia} (two-way ANOVA; Table 2.4).

Although density curves describing distribution patterns of M_{dia} were occasionally unique and irregular (Fig. 2.20 and Fig. 2.21), the overall density curves of M_{dia} for each elapsed year were similar within the forest type (Fig. 2.22). According to pairwise comparisons of M_{dia} distribution among elapsed years of soil disturbance (Table 2.5), distribution of immediate year was relatively

similar to that of the following years in the cypress stand, while that in the oak stand was relatively widely distributed and most different among the elapsed years. In the cypress stand, M_{dia} distribution in the 4th year was the most different among the years.

2.4 Discussion

2.4.1 Applicability of the flat root scanner method in forests

The present investigation is one of few studies that has applied a flat root scanner method to fine root observation in forest stands. There was risk of device failure due to dew condensation and moisture intrusion, since the scanners were left in the soil profile during the entire measurement periods in the present study. Although some scanners broke during the measurement period, the remaining scanners continued to work uneventfully throughout the study (Fig. 2.5). The accidents of scanner failure were probably due to malfunction of the electromechanical components. The mean operational life span in the present study is estimated to be over four years (Table A1: Appendix). Therefore, despite a risk of mechanical failure, the flat root scanner method is a practical approach for field experiments due to its ability in obtaining high quality image data and economical characteristics.

Production dynamics of fine roots immediately after installation of the flat root scanner appeared to be anomalous (Fig. 2.9 and Fig. 2.10) such as was also observed in some previous studies based on the rhizotron (Burke and Raynal 1994) or minirhizotron (Aerts et al. 1989; Price and Hendrick 1998; Joslin and Wolfe 1999) methods. These results clearly support the present hypothesis that soil disturbance alters fine root production. It means that estimates of fine root production by the excavation methods are affected by soil disturbance, and their estimates need to be interpreted carefully. Also, duration between the date of scanner installation and the presumed saturation of standing root area (S_{are}) can be more than one year. Our results are in accordance with previous studies (Hendrick and Pregitzer 1996; Majdi 1996) and a review on minirhizotron methods (Johnson et al. 2001). Therefore, fine root dynamics in the growing season of the immediate year of equipment installation should be interpreted carefully. It is suitable for ecological research in which the equipment is left in the soil for more than one year for observation. However, quantitative determination of the

initial phase being affected by soil disturbance requires field experiments, and has yet to be accomplished.

2.4.2 Distinct two phases in standing fine root area

It was observed that fine roots have reached spatial saturation in standing root area (S_{are}) (therefore probably in biomass also) in less than about one year from the start of their seasonal growth in the cypress and oak stands (Fig. 2.7 and Fig. 2.8). The S_{are} was relatively constant for some duration after reaching a stable state, indicating that the root proliferation was restrained during the immediate year due to spatial capacity. This result can be explained by the observation that root area production (P_{are}) and root area disappearance (D_{are}) after the 2nd year of scanner installation tended to be lower and higher, respectively, than the immediate year (Fig. 2.11), and that fine roots disappear gradually as time advances. Actually, the mass (or area) balance between P_{are} and D_{are} appeared stable after the 2nd year. After reaching spatial saturation, the S_{are} showed various patterns of changes as increase, decrease, convex or concave (Fig. 2.7 and Fig. 2.8). The variation of S_{are} indicates that fine root production and/or disappearance may fluctuate heterogeneously at least in the 5 cm wide horizontal patch scale in the present forests.

Temporal patterns of S_{are} values that begin on a root-free surface indicate an intense proliferation phase in the initial period of observation, and a relatively stable or fluctuating phase in the following periods (Fig. 2.7 and Fig. 2.8). This result is in accordance with above-ground growth patterns of trees in forest stands (Monserud 1984; Hozumi 1985; Hozumi 1987), and also similar to the previous studies focusing on root development of *Cyperus rotundus* L. that characterized the sprouting dynamics by a non-linear sigmoid regression (Shilo et al. 2013) and on root recolonizations in forest fields (Tingey et al. 1996; López et al. 2001; Fukuzawa et al. 2013) observed by

minirhizotrons.

2.4.3 Logistic growth of fine root production

Temporal patterns of cumulative P_{are} in each year could be modeled with a logistic curve with the asymptotic upper limit (A), the growth coefficient (λ) and the integration constant (k) (Fig. 2.9, Fig. 2.10 and Fig. 2.13), as supported by the similarity between the observation value of annual P_{are} and its predicted values by the logistic equation (Fig. 2.11 and Fig. 2.12). The logistic growth of fine roots during a year has two possible causes: phenology of fine root production controlled directly and/or indirectly by environmental factors and a density effect by the present fine roots for acquisition of space, water, nutrient, etc. In particular, the previous studies have clarified the phenology of fine root dynamics being in significant relation to abiotic factors such as soil temperature (Steele et al. 1997; Ruess et al. 2003; Fukuzawa et al. 2013; Makita et al. 2014; Germon et al. 2016), soil water conditions (Teskey and Hinckley 1981; Hendrick and Pregitzer 1997) and precipitation (Hertel et al. 2013), and to biotic factors such as leaf expansion (Joslin et al. 2001; Steinaker et al. 2010; Abramoff and Finzi 2014). While fine root production in the immediate year of scanner installation might involve other effects (e.g. soil disturbance), the production dynamics in the following years were probably controlled mainly by phenology and the density effect of fine roots. Our observations imply that production of fine roots follows a biological rule of essentially exponential growth which is also affected by the presence of the upper limit over time (thus, logistic growth; Verhulst 1838; Shinozaki and Kira 1956; Hozumi 1973; Hutchinson 1978; Begon et al. 2006). I note that fine root production following these basic biological rules has rarely been described quantitatively (but see Shilo et al. 2013 and Adu et al. 2014) probably due to technical difficulties in fine root studies especially in natural communities of plants. I also note that use of flat root scanners and minirhizotrons has made it

possible to describe and analyze quantitative development of fine roots in relation to ecological theories.

We speculate that destructive soil disturbance at the time of scanner installation generates the following effects on fine root dynamics: 1) severing roots that proliferate adventitious fine roots, 2) altering chemical (i.e. water and/or nutrient availability) and physical (i.e. bulk density) properties of the soil, and 3) reducing root competition in the root-free soil (Vogt et al. 1998; Fahey et al. 1999; Lauenroth 2000; Hendricks et al. 2006). Although it is not clear how much effects these conditions may have on fine root dynamics, the present observation seems to suggest that soil disturbance can affect production of fine roots, supporting our hypothesis (see also Joslin and Wolfe 1999). On the other hand, differences in the fine root production patterns may represent annual variations, but their factor was not considered in the present analysis. A more organized field experiment will be desirable to delineate these two factors (soil disturbance and annual environmental effect) on fine root production.

The logistic patterns of cumulative P_{are} immediately after scanner installation differed between cypress and oak stands (Fig. 2.13). In the immediate year of soil disturbance, the A and λ of the logistic curve for cumulative P_{are} were relatively high in the cypress and in the oak stand, respectively. In the cypress stand, A , indicating annual P_{are} , in the immediate year was more than three times higher than that of the following years. The tendency of high production immediately after equipment installation is similar to that observed in a stand of the same species of cypress in a previous study using a minirhizotron method (Noguchi et al. 2011). In the oak stand, λ that represents the rate of production in the immediate year was greater than that in the following years. Also, changes in the value of the A after soil disturbance differed between the two stands, as the result showed significant interactive effects between the elapsed year and forest type (Table 2.2).

Although the A of the oak stand during the immediate year was about the same as that of the later

years in the present study, there is a possibility of underestimating this value. That is because this observation was started in the middle of the growing season in late May or early July (Fig. 2.5) and there was a root-free gap in the front of scanner initially creating a time lapse for roots to grow into the soil profile (Fig. 2.4). The difference of production patterns between the forest types may also indicate different strategies and patterns of development in fine root systems of *C. obtusa* and *Q. serrata*, such as root branching patterns and mycorrhiza formation (Pregitzer et al. 2002; Yamato and Iwase 2005).

2.4.4 Fine root morphology affected by soil disturbance

The present observation was one of few studies that described details of morphological dynamics of fine root production (not standing fine roots) immediately after soil disturbance caused by equipment installation. It is reasonable to focus on fine root production for understanding fine root behavior since fine roots elongate as first-order roots that are the most distal and metabolically active within the branching root system (Ma et al. 2018).

In the present study, significant differences of produced fine root morphology were found between immediate periods and the following periods after scanner installation with soil disturbance. Length based production of fine roots was obviously high in the immediate year (Fig. 2.14 and Fig. 2.15), and mean diameter of fine roots produced initially after scanner installation tended to be thick (Fig. 2.17 and Fig. 2.18), indicating that soil disturbance can affect morphological traits of fine root length and diameter. This results indicates similar behavior observed by rhizotron (Mason et al. 1970), minirhizotron (Joslin and Wolfe 1999) or ingrowth core (Eissenstat 1991) methods in the previous studies. Especially, Eissenstat (1991) have revealed distinct standing root dynamics in length and diameter between disturbed and undisturbed soil evaluated with excavation techniques. However,

there are a few previous studies that focus on length and diameter of fine roots produced between given time intervals (not standing fine roots at a given time) and conduct continuous long-term observation more than one year. In the present study, long-term fine root dynamics for several years after soil disturbance were covered by *in situ* observation by flat root scanners, and moreover it slightly indicated possibility that the effects of soil disturbance tend to remain even more than one year after the disturbance. For examples, annual P_{len} of some scanners (e.g. H11; Fig. 2.14 and K25; Fig. 2.15) decreased consistently with increasing elapsed years, indicating convergent trend of soil disturbance effects.

On the other hand, M_{dia} was high in initial phase of observation and immediately decreased with time until several weeks after first root emergence (Fig. 2.19). The change of M_{dia} reached a stable phase relatively quickly than the P_{len} change, which indicates the effects of soil disturbance on root diameter are smaller than on root length. And fine roots with thick diameter did not account for substantial fraction of the diameter distribution even in the immediate year of soil disturbance (Fig. 2.22). Thinner roots were mostly detected similarly to the following years. In fact, annual averages of M_{dia} values showed no significant difference among the elapsed years (Table 2.4).

In the present study, temporal changes of fine root dynamics based on projected area in soil profiles were previously indicated (Fig. 2.9 and Fig. 2.10), and it has revealed anomalous behavior of area-based production in the immediate year of soil disturbance. However, it was not mentioned whether the dynamics were caused by their length, diameter or both. Morphological investigation in the present study clarified that the main cause of anomalous fine root area production was caused by high values of root length in the immediate year of soil disturbance, though the root diameter had also high values in periods of initial root emergence. Mean fine root diameter tended to be thick for a limited period of first root emergence, and that is conceptually called as pioneer roots (Eissenstat 1991). The pioneer root is defined as a root with larger diameter which elongate into new unexplored soil

space and develop fibrous roots from itself. Therefore, the soil disturbance can cause the introduction of the pioneer roots. Moreover, the pioneer root invasion indicates that physiological functions of roots also changed temporarily due to soil disturbance since morphological traits such as root diameter are closely related to functional traits (McCormack et al. 2015).

Possible hypothesis can be suggested about why larger values of P_{len} continued longer (Fig. 2.16) than those of M_{dia} (Fig. 2.19) as follows (Fig. 2.23). Plants have to recolonize root systems to recover uptake capacity lost with broken roots after soil disturbance. First, they elongate thick roots as pioneers that can branch absorptive roots into disturbed soil space. Simultaneously or continuously with the pioneer root elongation, absorptive fibrous roots proliferate until they regain uptake capacity or saturate spatial root density, and then fine root dynamics reach a natural state similar with undisturbed conditions.

If this hypothesis is accepted, the difference of period in which disturbance effects were implicated can be reasonably interpreted. However, the soil disturbance with flat root scanner installation causes not only severing roots but also altering chemical and physical soil properties and reducing root competition in the root-free soil (Vogt et al. 1998; Fahey et al. 1999; Lauenroth 2000; Hendricks et al. 2006). So it is considered that those conditions may contribute simultaneously to inducing pioneer root invasion.

2.4.5 Implication on estimation by the techniques with soil disturbance

Distinct phases of intense proliferation and stable fluctuation in S_{are} changes (Fig. 2.7 and Fig. 2.8) were similar to the root recolonization of *Chamaecyparis obtusa* (Hishi and Takeda 2005) and other species (Fahey and Hughes 1994; Jiménez et al. 2009) measured by using ingrowth cores. Therefore, it can be suggested that changes in S_{are} observed in the present study may represent changes in

standing fine root mass that have been measured by ingrowth cores that have commonly been regarded as fine root production without consideration of fine root decomposition (but see Osawa and Aizawa 2012; Li et al. 2013). However, S_{are} is a result of fine root production minus disappearance, and cannot be automatically equated with production. Therefore, caution must be exercised in its interpretation.

The annual changes in logistic patterns for cumulative P_{are} , especially A indicating annual P_{are} , were different between the cypress and oak stands (Fig. 2.13), as the interactive effect of elapsed year after soil disturbance and forest type has shown significant (Table 2.2). This pattern raises a question to an assumption that the ingrowth core technique (and other analogous methods) tolerates comparison of fine root production among forest types or treatments (Neill 1992; Steele et al. 1997; Godbold et al. 2003; Mei et al. 2010; Liu et al. 2014). Actually, the relationships in annual P_{are} between the cypress and oak stands indicated opposing effects between the period immediately after soil disturbance and the following period (Fig. 2.11).

Our results suggest a possibility that total production and/or instantaneous production rate during a growing season were accelerated by soil disturbance in evergreen coniferous (*C. obtusa*) and deciduous broad-leaved (*Q. serrata*) forests. These results are supporting our hypothesis that fine root dynamics is significantly affected by the instrument installation with soil disturbance. They also suggest that the assumption of no disturbance effects on fine root production should not be accepted without careful examination. Therefore, if our suggestion of overestimation in fine root production during the year immediately following soil disturbance is real, the fine root production estimates obtained with techniques involving disturbance effects (e.g. ingrowth core, minirhizotron, and flat root scanner methods) are recommended to be viewed with caution if the data contained those during a period immediately following the instrument installation. In particular, ordinary use of the ingrowth core and root mesh methods cannot avoid this possibility (but see Osawa and Aizawa 2012), and extra

caution is required when interpreting data. Such a possibility needs to be examined carefully through field experiments and other techniques in future studies. This study demonstrates the need for longer study periods, wherein the data from the first year may be rejected as anomalous due to the effects of soil disturbance.

Although excavation methods as typified by an ingrowth core inescapably cause soil disturbance, the influence of disturbance on fine root dynamics was basically ignored except for a few studies (Steingrobe et al. 2001). However, it is necessary for evaluating fine root behavior in natural conditions to quantify the effects of soil disturbance and take account of that in the estimation. The current analysis indicated possible effects of soil disturbance on area-based fine root production, implying those on mass-based production also. Furthermore, this study revealed that soil disturbance affects not only root length but also root diameter. Therefore, root morphological traits should be carefully interpreted with caution in the destructive techniques such as an ingrowth core and the analogous methods. And researchers who investigate fine root dynamics have to embrace common sense of disturbance effects on fine root behavior regardless of whether the excavation or observation methods are used.

2.5 Conclusions

The present study showed applicability and potential of the flat root scanner method for detailed description of fine root production dynamics.

The seasonal patterns of standing root area indicated distinctive patterns of intense proliferation phase and relatively stable or fluctuating phase in the initial period and the following periods of observation, respectively. Cumulative root area production during a year followed a logistic growth curve in both species examined and for both immediate years after the scanner installation and the later years. Also, the asymptotic upper limit and/or root growth coefficient in the logistic curve varied between the growing season immediately following scanner installation (and soil disturbance) and the later years. Variation of the logistic growth also depended on the forest type.

Fine roots may require an initial period of growth to occupy the soil space in front of the scanning surface which causes a different pattern of apparent root production during the first growing season. Duration of at least one year after soil disturbance may be required before the data to show more or less natural patterns of fine root dynamics. Our results suggest possible effects of soil disturbance on fine root dynamics and recommend rejection of an assumption that disturbance effects are negligible in the excavation methods. Therefore, I consider that estimates of fine root production with ingrowth cores and root meshes should be interpreted with caution.

The present study succeeded in describing morphological traits of produced fine roots (i.e. first-order roots) by a flat scanner method. Fine root dynamics immediately after scanner installation were more active than in the following periods. Length-based fine root production was drastically high in the immediate year and gradually decreased with time. On the other hand, produced fine root diameter was thick temporally for several weeks after first root emergence into disturbed soil space, which indicates soil disturbance induced pioneer roots that can develop absorptive fibrous roots. It was clarified that soil disturbance can affect not only amount of fine root production but also their

morphological traits. The soil disturbance effects should be taken into account in methods inescapably cutting roots and/or creating root-free soil.

Table 2.1 Stand description of the cypress and oak stands. Values in parentheses indicate those of canopy tree species (*Chamaecyparis obtusa* in the cypress stand; *Quercus serrata* in the oak stand). Basal area means sum of cross-sectional areas of tree stems at breast height (1.3 m). Tree census was conducted in November 2014. Understory vegetation with DBH < 2 cm was ignored. Dead trees were excluded

	Plot size (ha)	Mean DBH (cm)	Mean height (m)	Stem density (stems ha ⁻¹)	Basal area (m ² ha ⁻¹)
Cypress stand	0.06	24.4 (24.9)	20.2 (20.6)	1033 (983)	53.4 (52.0)
Oak stand	0.12	6.5 (22.0)	6.8 (17.8)	3717 (392)	24.1 (15.6)

Table 2.2 Two-way ANOVA for the effects of elapsed year (immediate, 2nd, 3rd and 4th year), forest type (cypress stand vs. oak stand) and their interaction on maximum standing root area (maximum S_{are}), annual root area production (annual P_{are}) and annual root area disappearance (annual D_{are}), and on estimated parameters of asymptotic upper limit (A) and growth coefficient (λ). The maximum S_{are} , annual P_{are} and annual D_{are} were observed values; the A and λ were estimated values of the parameters of the logistic curve fitted it to the cumulative P_{are}

	<i>F</i> value	<i>P</i> value
Maximum standing root area		
Elapsed year	0.67	0.579
Forest type	4.09	0.051
Elapsed year x Forest type	0.48	0.699
Annual root area production		
Elapsed year	9.36	< 0.001
Forest type	0.38	0.543
Elapsed year x Forest type	3.93	0.017
Annual root area disappearance		
Elapsed year	4.91	0.007
Forest type	1.04	0.315
Elapsed year x Forest type	0.93	0.439
Asymptotic upper limit (<i>A</i>)		
Elapsed year	6.07	0.002
Forest type	0.001	0.971
Elapsed year x Forest type	2.95	0.048
Growth coefficient (λ)		
Elapsed year	4.56	0.009
Forest type	0.71	0.406
Elapsed year x Forest type	1.41	0.259

Table 2.3 Means (\pm SE) of asymptotic upper limit (A) and growth coefficient (λ) of cumulative root area production (P_{are}) for each elapsed year and forest type. Different letters indicate significant differences ($P < 0.05$) among elapsed years in each forest type in multiple comparisons by the Tukey-Kramer HSD test

Forest type	Elapsed year	Asymptotic upper limit (A ; mm ² cm ⁻²)			Growth coefficient (λ ; d ⁻¹)		
Cypress stand							
	Immediate	9.38	± 2.01	a	0.040	± 0.016	a
	2nd	2.76	± 0.59	b	0.019	± 0.008	a
	3rd	3.05	± 1.37	b	0.040	± 0.016	a
	4th	1.62	± 0.66	b	0.015	± 0.009	a
Oak stand							
	Immediate	5.22	± 1.35	a	0.049	± 0.022	a
	2nd	3.73	± 0.59	a	0.020	± 0.009	b
	3rd	4.38	± 0.35	a	0.014	± 0.007	b
	4th	4.64	± 0.98	a	0.014	± 0.008	ab

Table 2.4 Two-way ANOVA for the effects of elapsed year (immediate, 2nd, 3rd and 4th year) after soil disturbance, forest type (cypress vs. oak stand) and their interaction on annual fine root length production (P_{len}) and mean diameter of produced fine roots (M_{dia}). Annual P_{len} was determined by sum of root length produced from January to December in each year. M_{dia} was calculated as the weighted average based on root length

Effect	Annual length production (mm cm ⁻²)		Mean diameter (mm)	
	F value	P value	F value	P value
Elapsed year	6.26	0.002	0.66	0.582
Forest type	0.23	0.637	1.56	0.221
Elapsed year x Forest type	2.06	0.125	0.74	0.537

Table 2.5 D values on pairwise comparisons among elapsed years of soil disturbance for density curves of fine root diameter distribution in the cypress and oak stands. The D values are generally calculated as a test statistic in the Kolmogorov-Smirnov test and indicate the degree of difference between sample distributions

Cypress stand		Immediate	2nd	3rd
	2nd	0.03		
	3rd	0.08	0.10	
	4th	0.19	0.16	0.24
Oak stand		Immediate	2nd	3rd
	2nd	0.11		
	3rd	0.13	0.03	
	4th	0.12	0.06	0.09

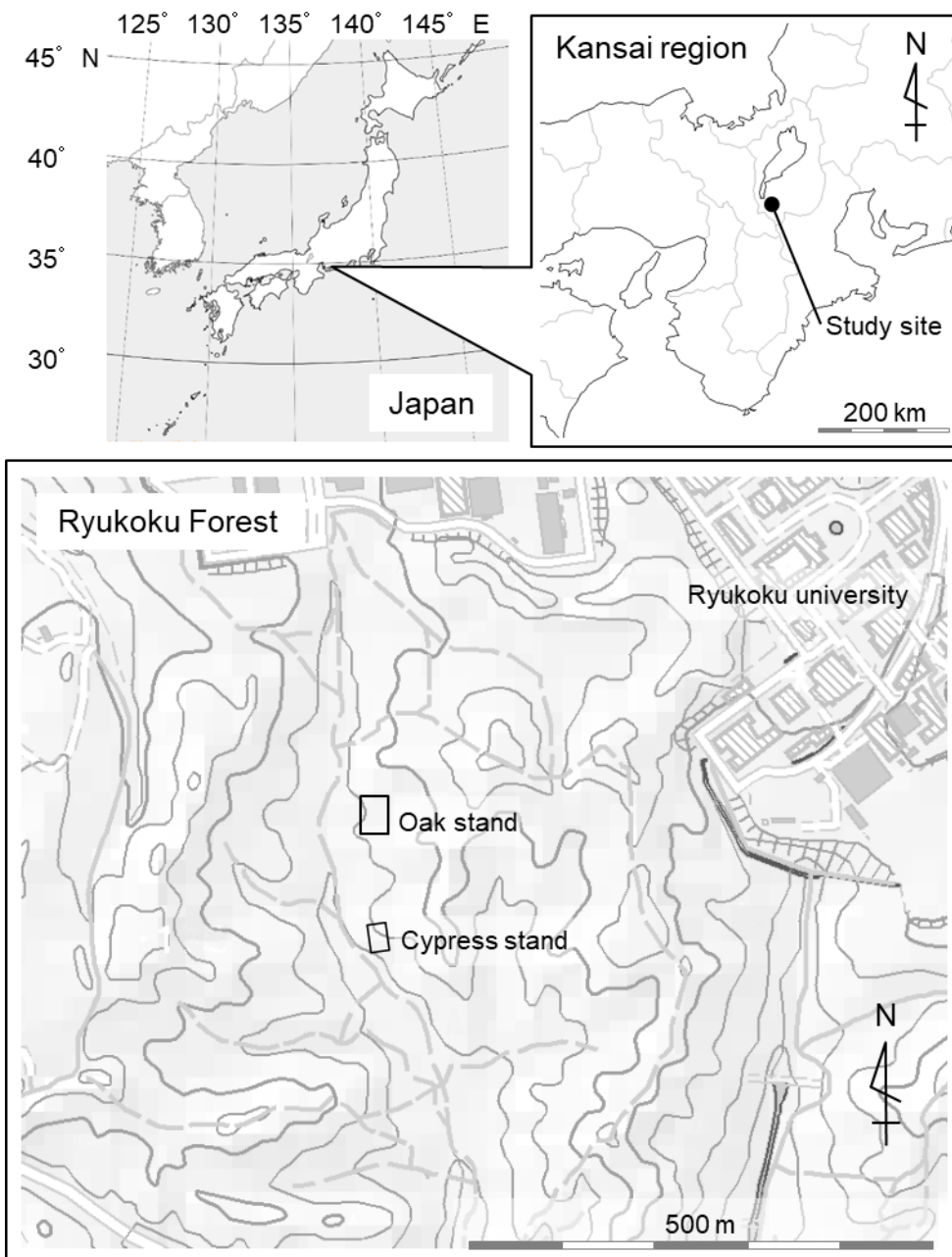


Fig. 2.1 Location of the study site at $34^{\circ}58'N$, $135^{\circ}56'E$ and a topographic map of the Ryukoku Forest showing plot locations of the cypress and oak stands. The cypress stand is located on a west facing slope; the oak stand is located across a little valley. The distance between both stands is about 100 m. The contour lines were drawn with 10 m interval in elevation

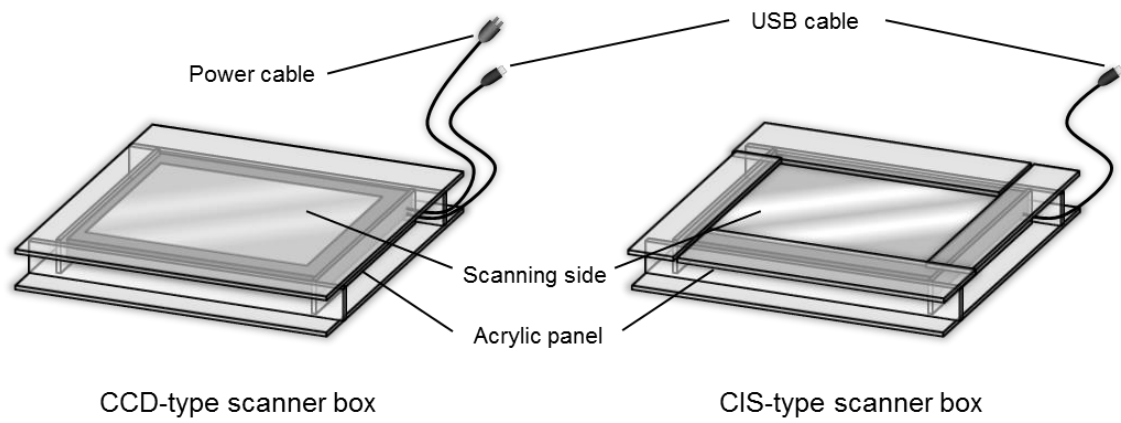


Fig. 2.2 Schematic diagrams of two types of scanner boxes. For the CCD-type scanner, the scanning surface was covered with a transparent acrylic panel. On the other hand, the scanning surface was exposed without a transparent acrylic panel on the CIS-type scanner. USB cable (and power cable for the CCD-type) protrudes from the scanner box for connection to the laptop computer (and the battery for the CCD-type)

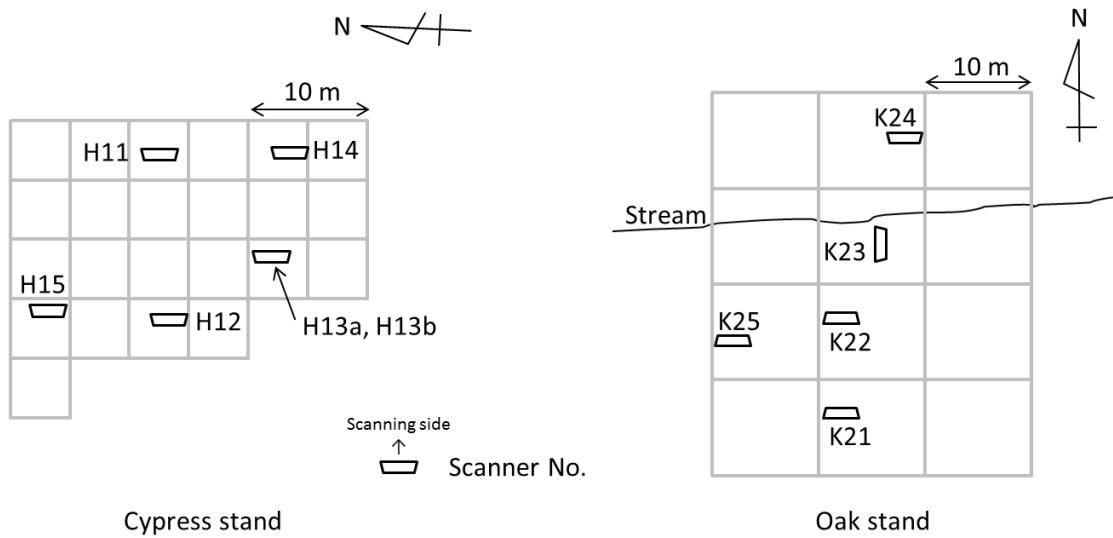


Fig. 2.3 Locations of flat root scanners in the cypress and oak stands. Flat root scanners were installed at haphazard locations. The longest side of the square figure of scanner no. represents scanning side, which generally faced an uphill direction because of ease in installation and image acquisition

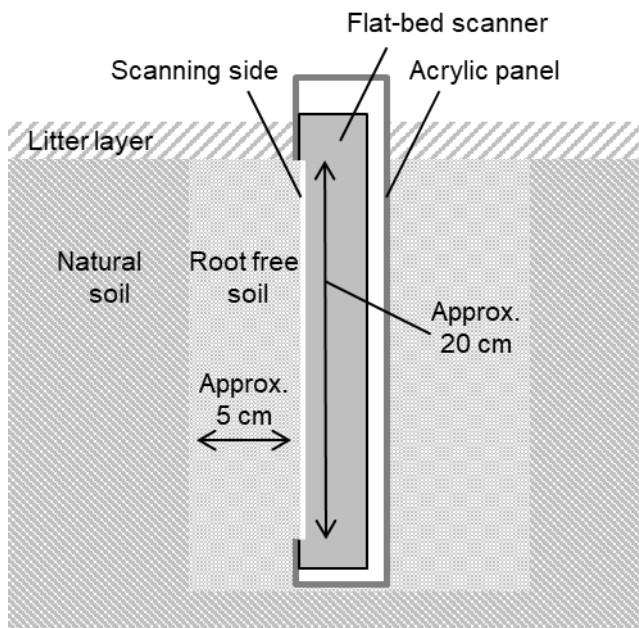


Fig. 2.4 Schematic representation of the flat root scanner set-up by a CIS type scanner as an example.

Root free soil filled the gaps around the scanner, so that soil layers and bulk density are approximately recreated, simulating conditions (i.e. yielding severed roots and creating root-free soil) of the ingrowth cores

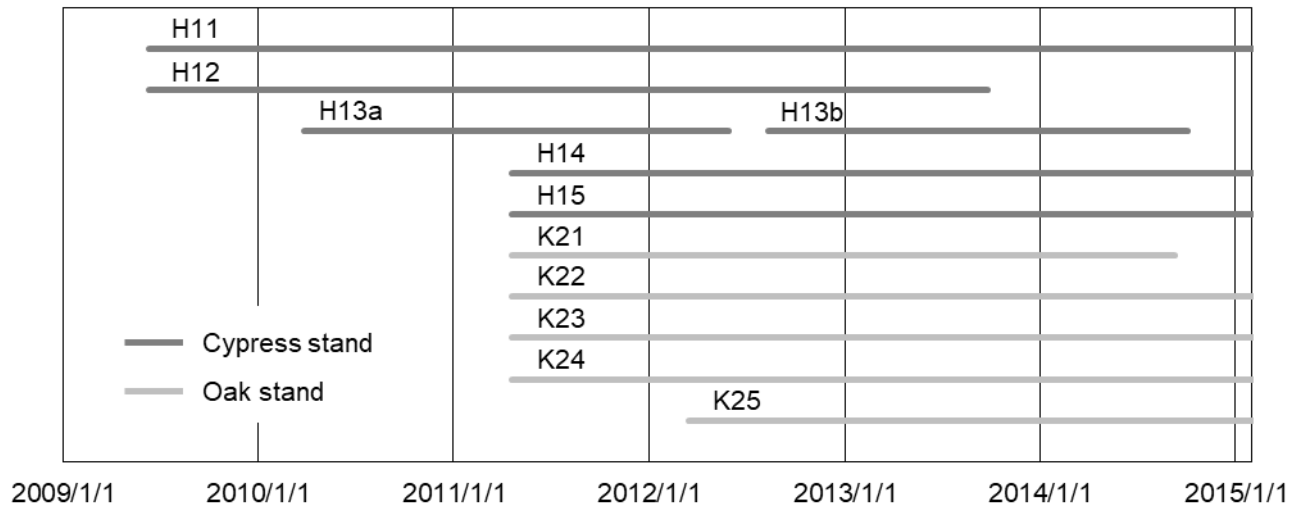


Fig. 2.5 Observation periods of the flat root scanners installed in the cypress and oak stands. The lines indicate ranges of observation periods until January of 2015. The scanners that did not reach January of 2015 indicate that monitoring could not be continued due to malfunction. Since H13a was broken and was replaced by H13b in 2012, soil disturbance was created again for this scanner and the observation was restarted



Fig. 2.6 The original image (28.8 cm in width x 20.7 cm in depth) of scanner No. H11 at November 27, 2009 as an example. Red squares represent area for the target of image analysis. Tips of growing fine roots tend to have light color. Vertical and horizontal straight lines were drawn at 5 cm intervals on the scanning surface as a rough guide

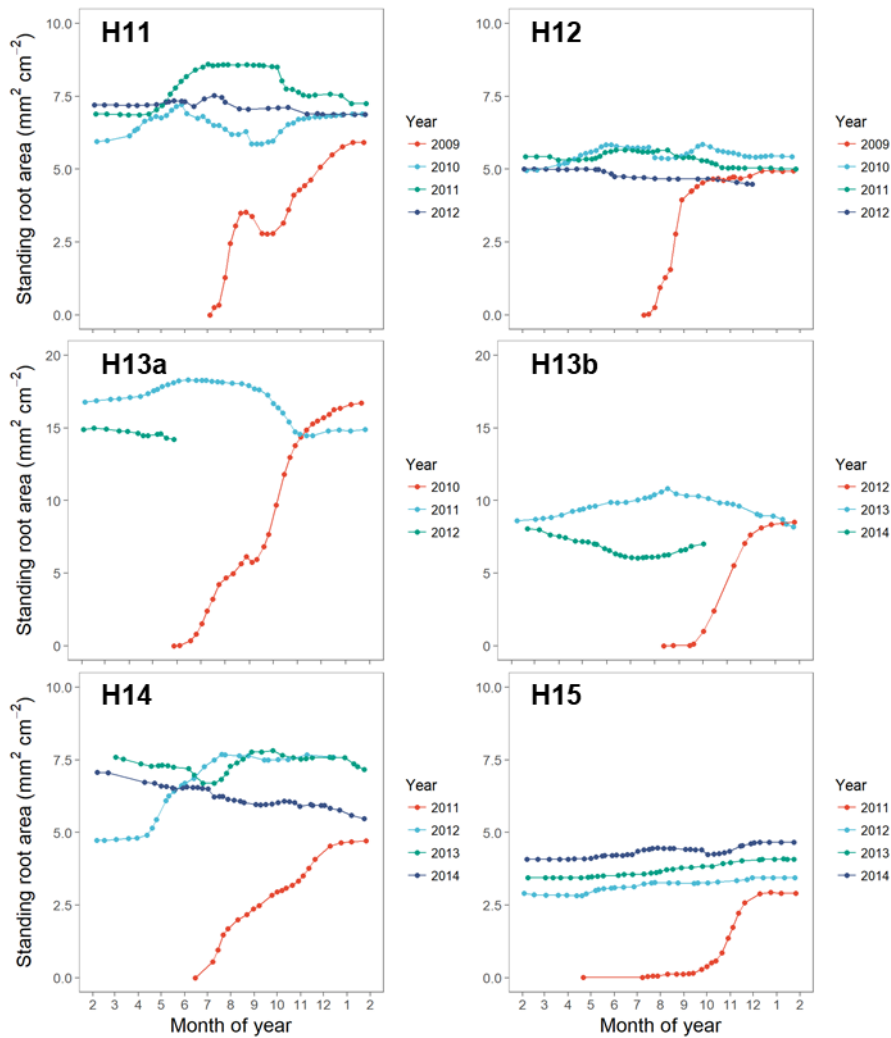


Fig. 2.7 Temporal changes of standing root area (S_{are}) within the observation periods of each scanner in the cypress stand. Each color indicates the elapsed year of observation from February of the present year to January of the next year. Note that the range of vertical axis in scanner H13a and H13b is twice as high as that of the other panels

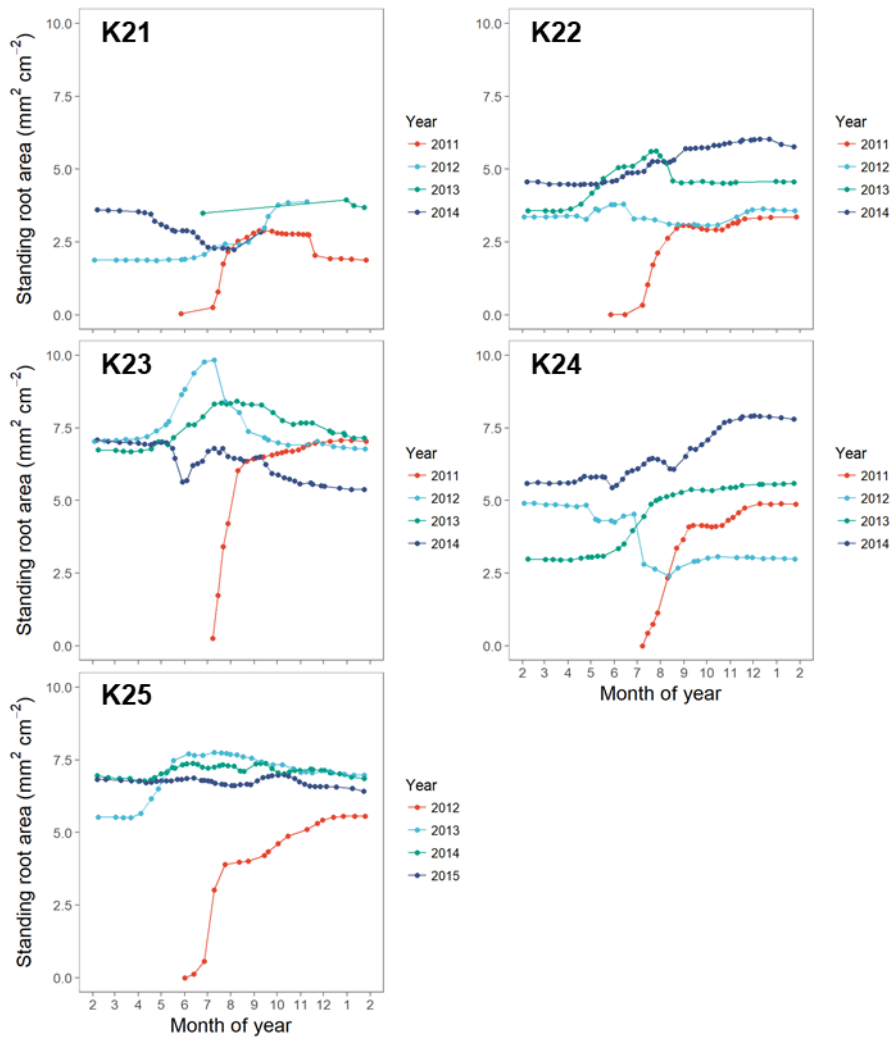


Fig. 2.8 Temporal changes of standing root area (S_{are}) within the observation periods of each scanner in the oak stand. Each color indicates the elapsed year of observation from February of the present year to January of the next year.

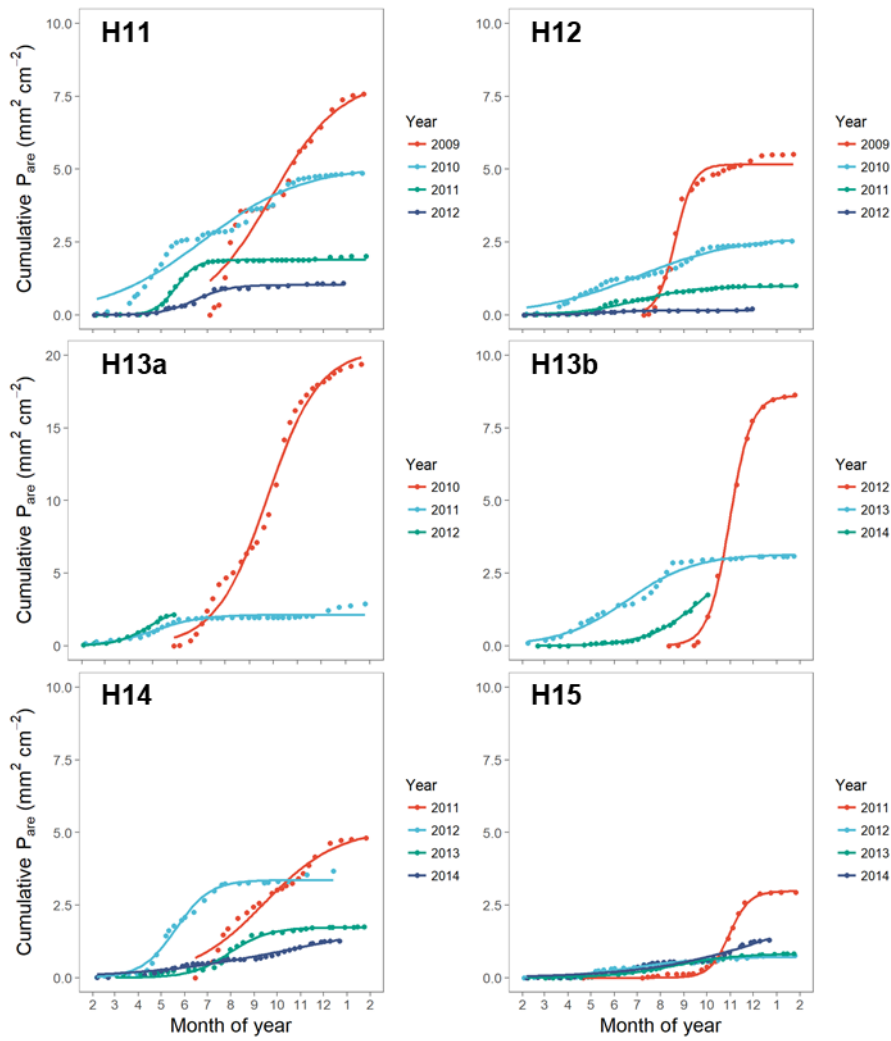


Fig. 2.9 Cumulative root area production (P_{are}) within the observation periods of each scanner in the cypress stand. Curves indicate the fitted logistic relationships with colors representing the elapsed years after soil disturbance. Note that the range of vertical axis in scanner H13a is twice as high as that of the other figures

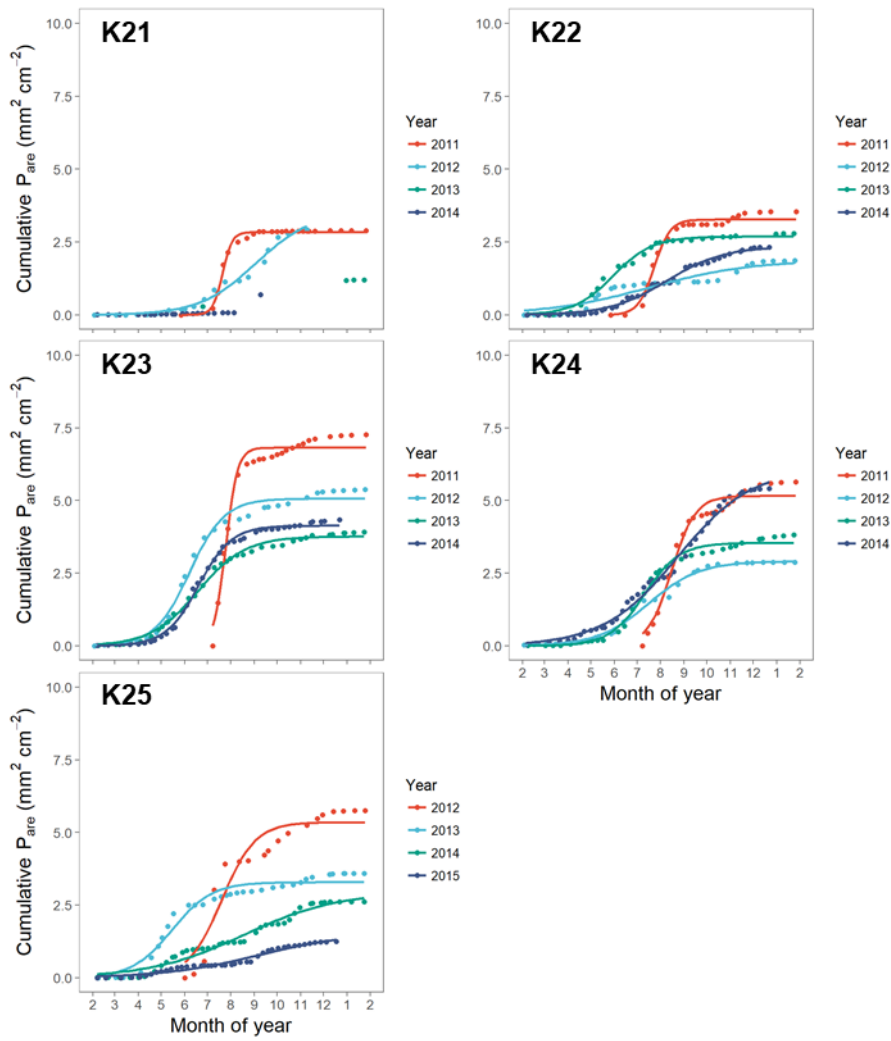


Fig. 2.10 Cumulative root area production (P_{are}) within the observation periods of each scanner in the oak stand. Curves indicate the fitted logistic relationships with colors representing the elapsed years after soil disturbance.

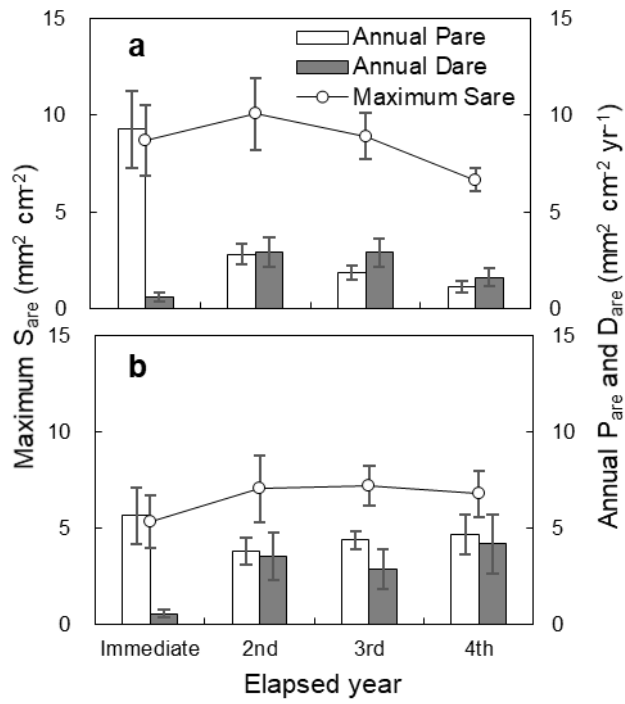


Fig. 2.11 Means (\pm SE) of maximum standing root area (S_{ave}), annual root area production (P_{ave}) and root area disappearance (D_{ave}) in each elapsed year following soil disturbance caused by scanner installation in (a) cypress and (b) oak stands. Each value was calculated for the period from February of the present year to January of the next year

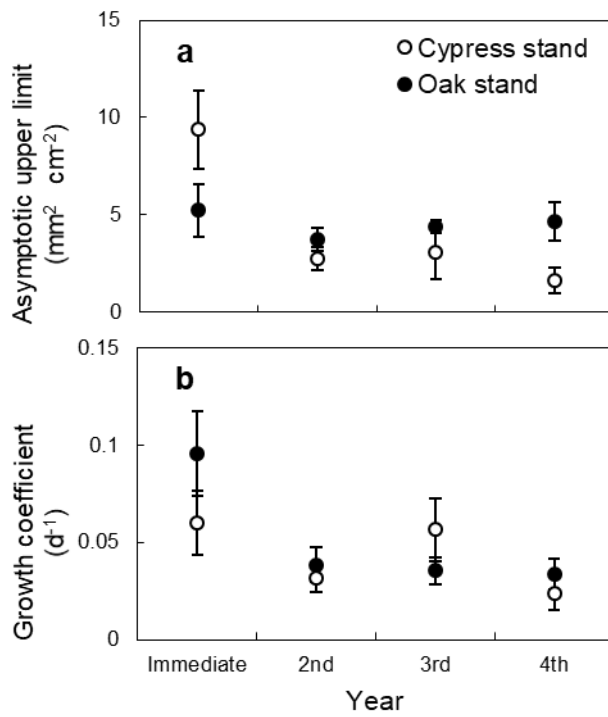


Fig. 2.12 Means (\pm SE) of the estimated (a) asymptotic upper limit (A) and (b) growth coefficient (λ) for the cumulative root area production (P_{are}) in relation to difference in the elapsed year after installation of each scanner in the cypress and oak stands

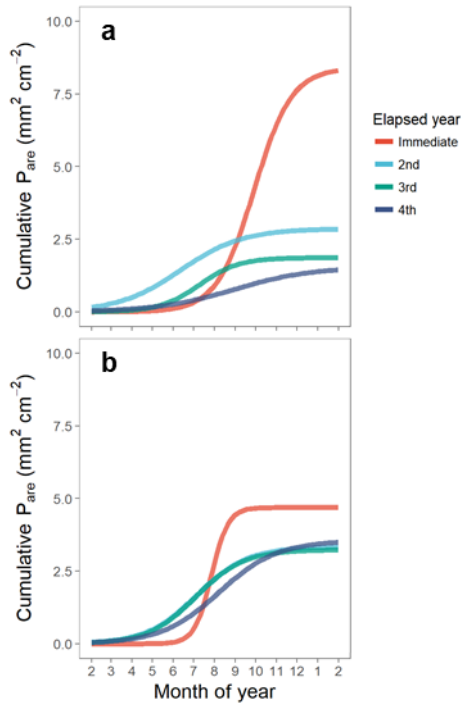


Fig. 2.13 Annual and seasonal production patterns of fine roots after soil disturbance in (a) cypress and (b) oak stands. The curves were modeled by the logistic equation (Eq. 5) using average values of asymptotic upper limit in production (A), growth coefficient (λ) and integration constant (k) for each elapsed year and stand (Table A2: Appendix)

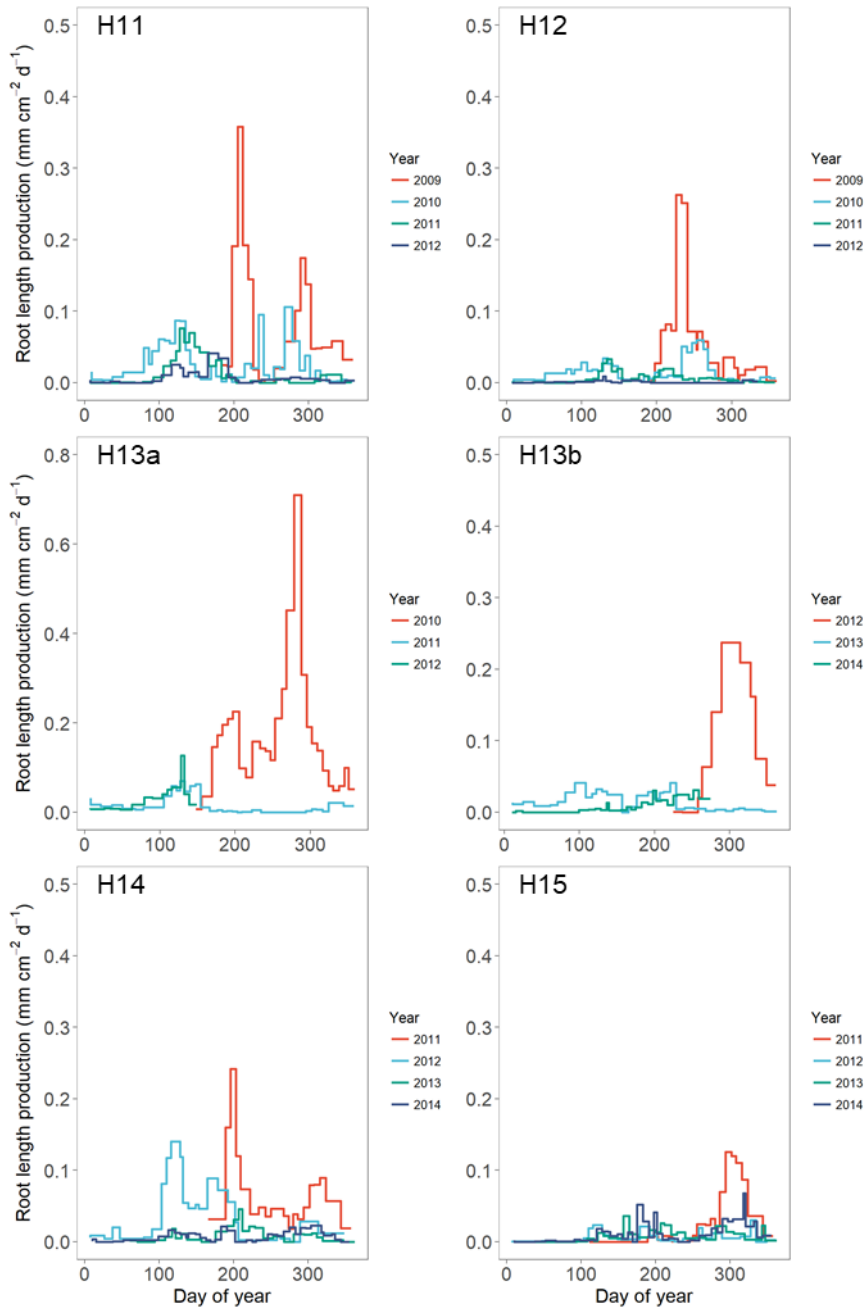


Fig. 2.14 Root length production (P_{len}) within the observation years of each scanner in the cypress stand. Each color indicates the year of observation. Note that the range of vertical axis in scanner H13a is different than the other figures

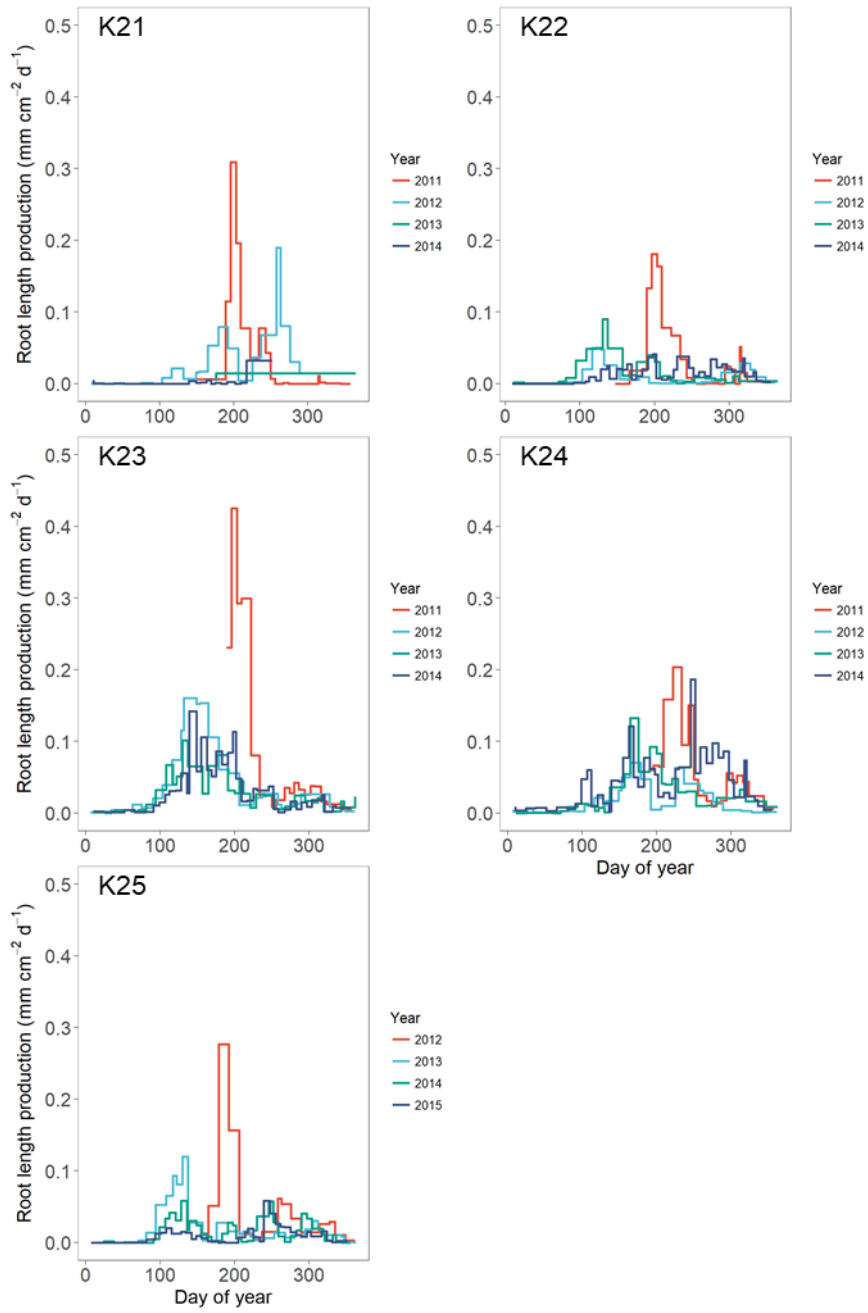


Fig. 2.15 Root length production (P_{len}) within the observation years of each scanner in the oak stand. Each color indicates the year of observation

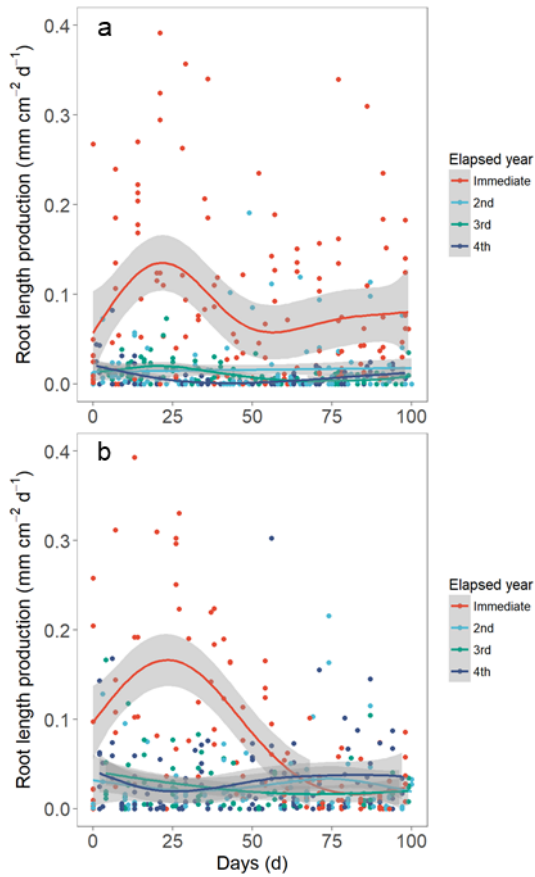


Fig. 2.16 Overall fluctuations in root length production (P_{len}) within one hundred days after first root appearance in the immediate year of soil disturbance and those within the corresponding periods in the following years in the (a) cypress and (b) oak stands. Each color indicates the elapsed years after soil disturbance. Curve fitting for each elapsed year was conducted by the generalized additive model with shaded bands showing $\pm 95\%$ confidence intervals

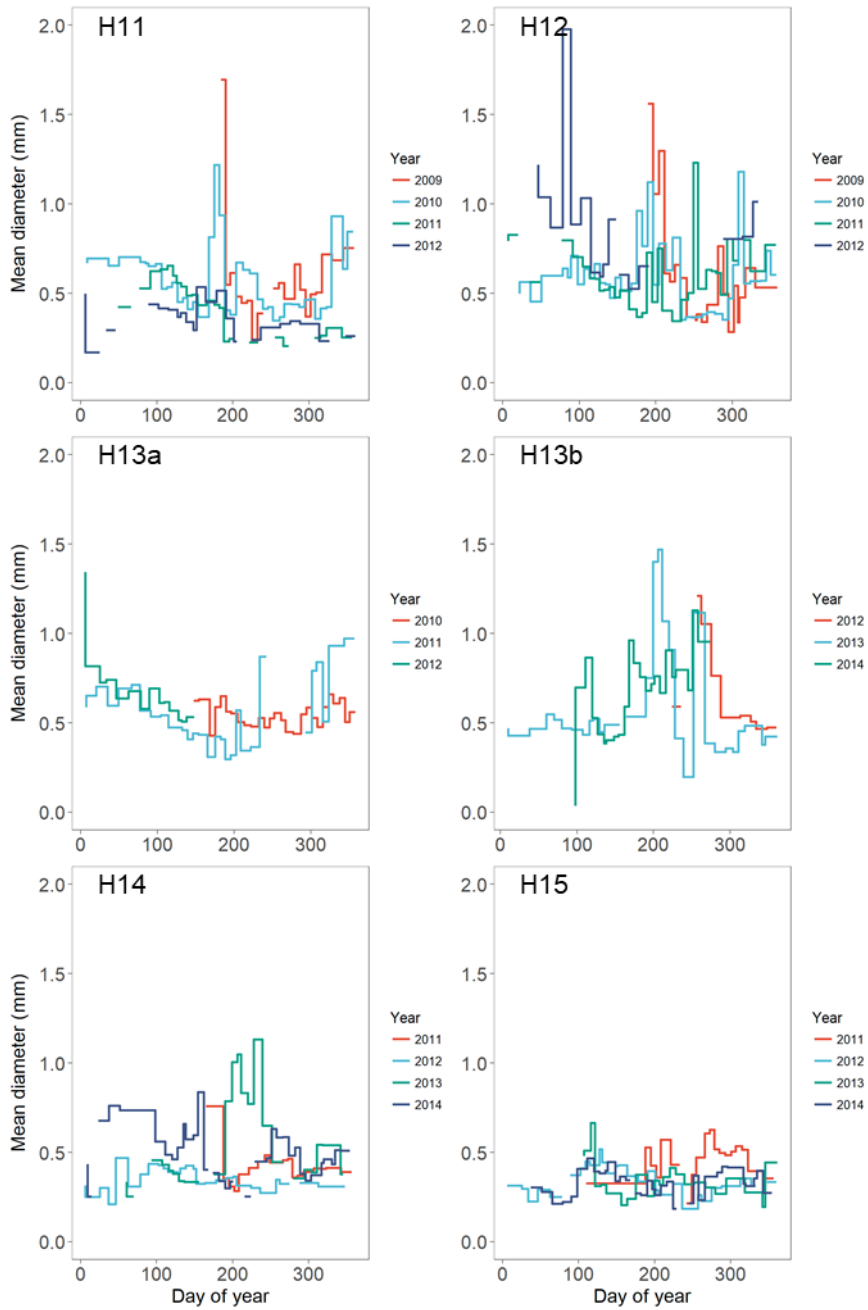


Fig. 2.17 Mean diameter of produced fine roots (M_{dia}) within the observation years of each scanner in the cypress stand. The diameter was calculated by the weighted average of the root length. Each color indicates the year of observation. The breaks of a continuous line mean that there were no fine roots produced during the period

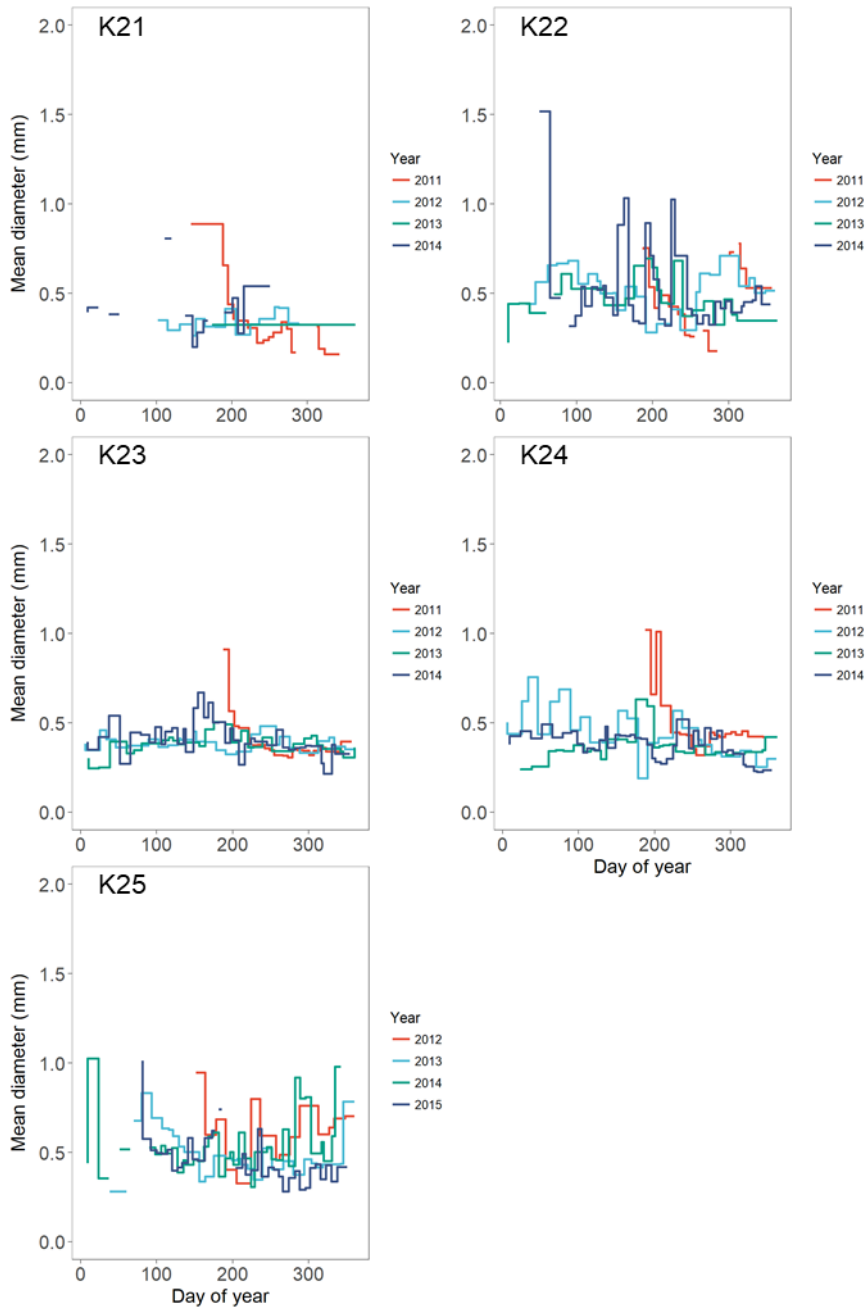


Fig. 2.18 Mean diameter of produced fine roots (M_{dia}) within the observation years of each scanner in the oak stand. The diameter was calculated by the weighted average of the root length. Each color indicates the year of observation. The breaks of a continuous line mean that there were no fine roots produced during the period

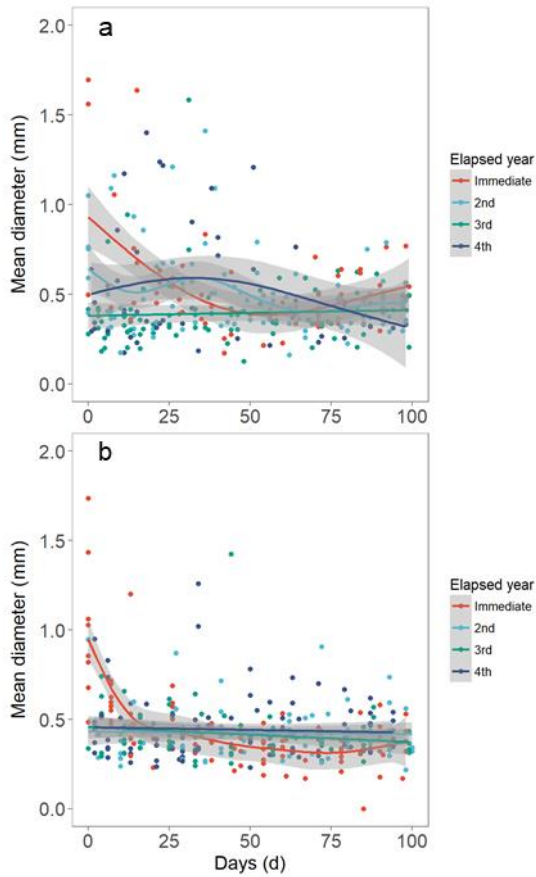


Fig. 2.19 Overall fluctuations in mean diameter of produced fine roots (M_{dia}) within one hundred days after first root appearance in the immediate year of soil disturbance and those within the corresponding periods in the following years in the (a) cypress and (b) oak stands. Each color indicates the elapsed years after soil disturbance. Curve fitting for each elapsed year was conducted by the generalized additive model with shaded bands showing $\pm 95\%$ confidence intervals

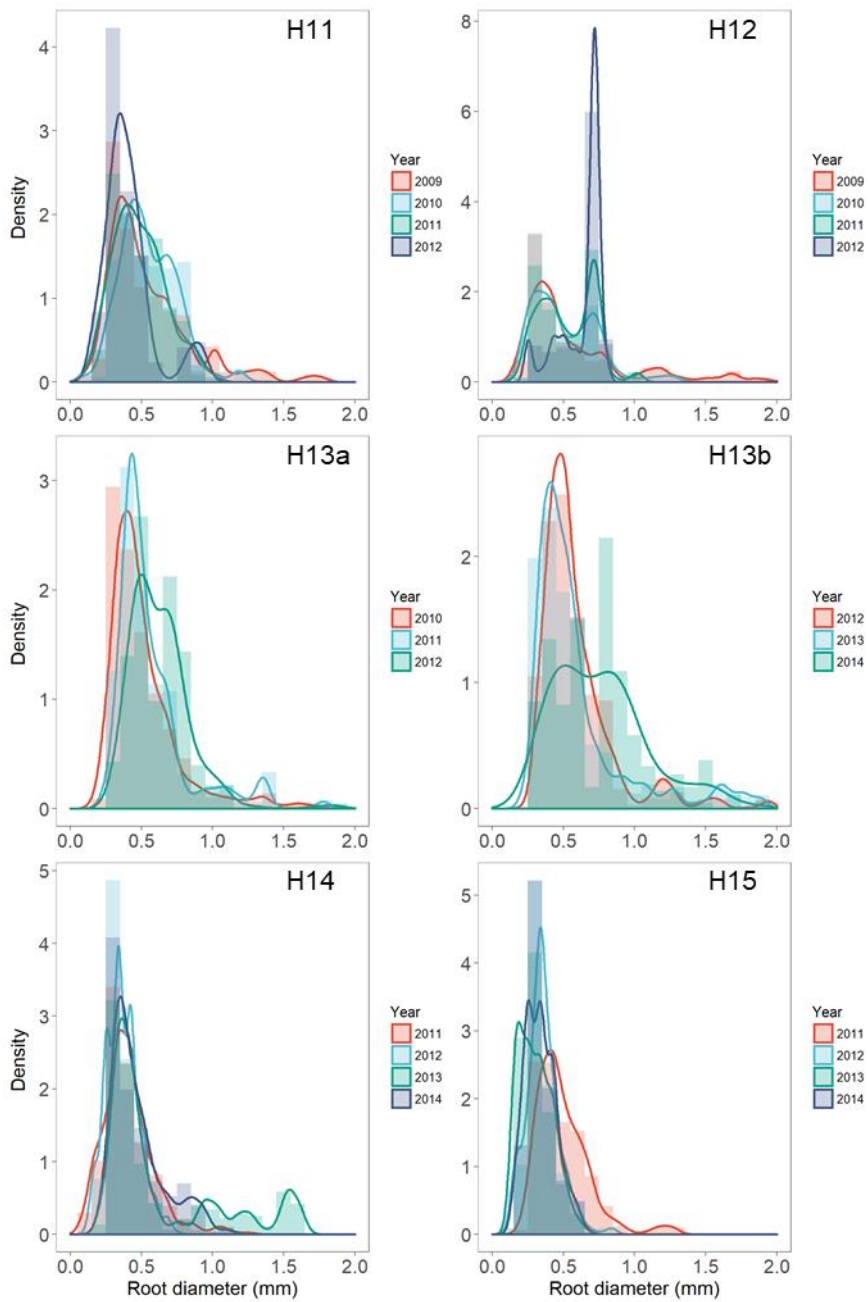


Fig. 2.20 Density curves and histograms of fine root diameter distribution within the observation years of each scanner in the cypress stand. The diameter used for calculating the density was weighted with the root length. Each color indicates the year of observation. The value of integral on root diameter (i.e. area of the density curve) is equal to one theoretically

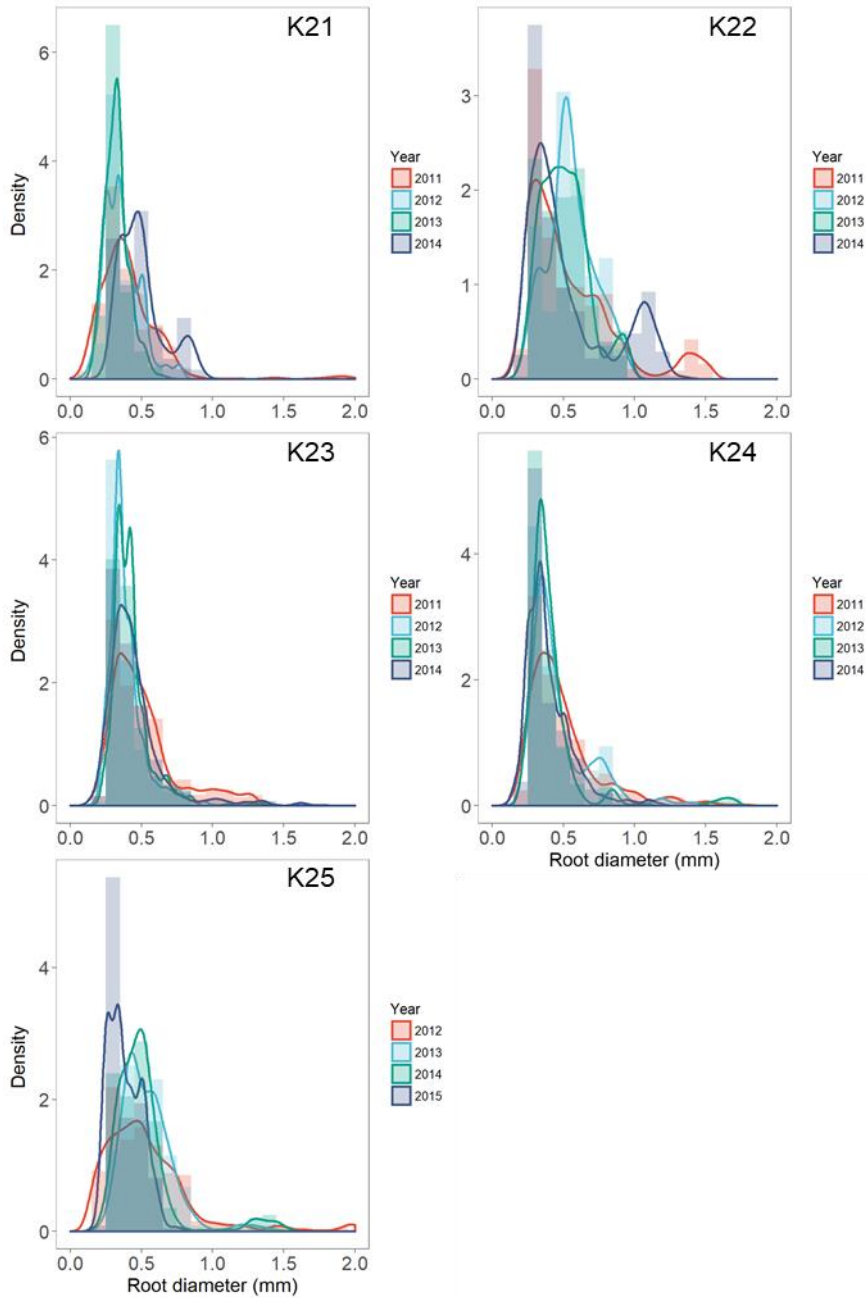


Fig. 2.21 Density curves and histograms of fine root diameter distribution within the observation years of each scanner in the oak stand. The diameter used for calculating the density was weighted with the root length. Each color indicates the year of observation. The value of integral on root diameter (i.e. area of the density curve) is equal to one theoretically

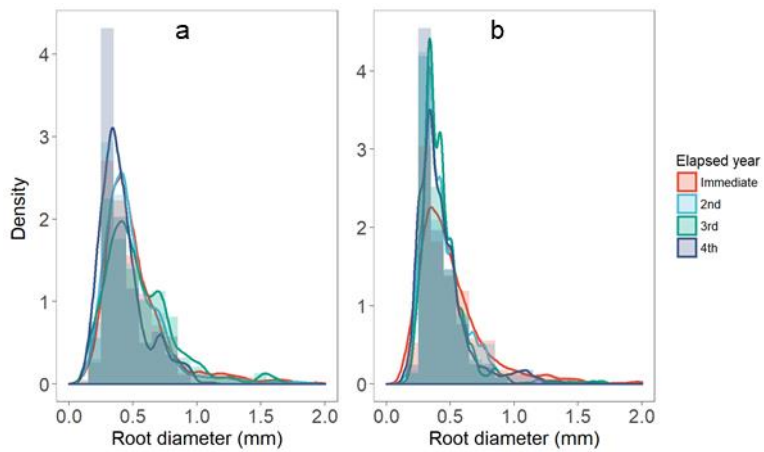


Fig. 2.22 Overall density curves and histograms of fine root diameter distribution within the elapsed years after scanner installation in the (a) cypress and (b) oak stands. Each curve contains data of all scanner in each stand.

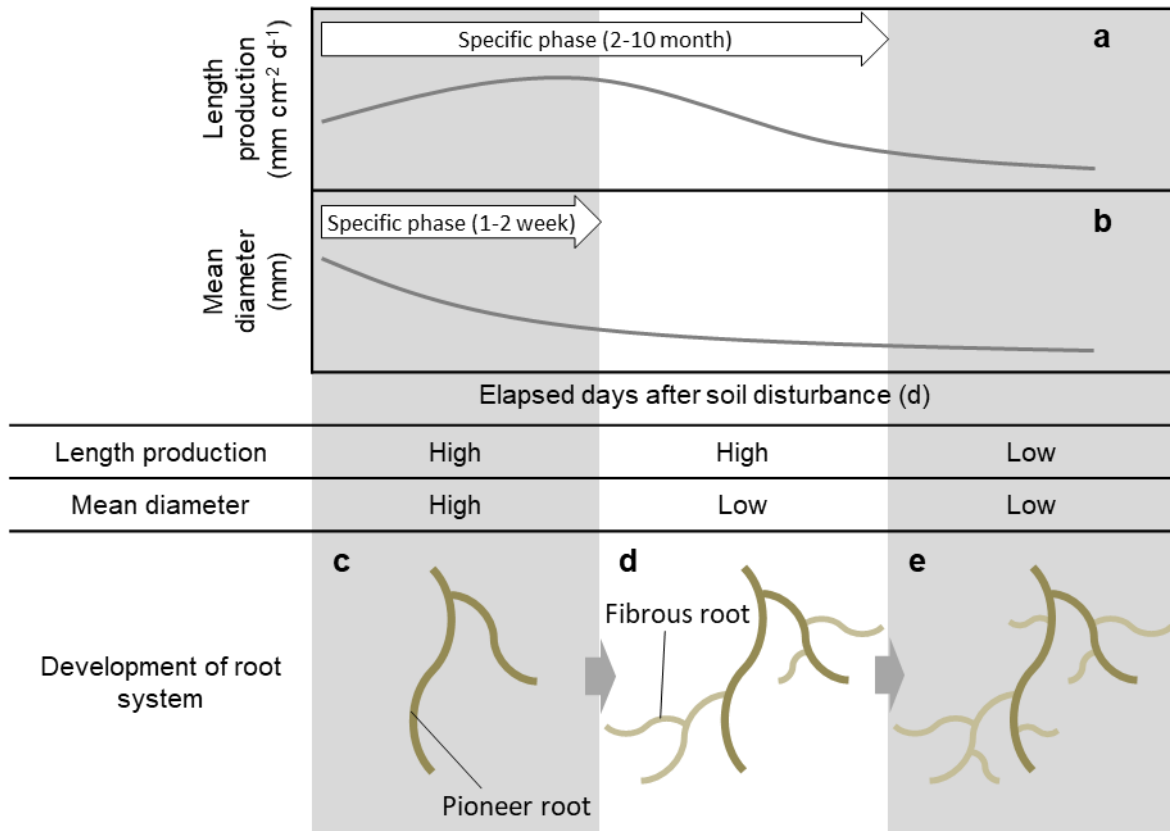


Fig. 2.23 Schematic diagram of development of the root system after soil disturbance. Figures above were described by reference to Fig. 2.16 and Fig. 2.19, and illustrate the typical relationships between days after soil disturbance and root length production (a; red line) or mean diameter of elongated roots (b; blue line). Figures below explain distinct three phases of root development; (c) intensive production of the pioneer roots, (d) intensive production of the fibrous roots and (e) the stable phase

Chapter 3 Temporal dynamics of fine roots in cypress, deciduous oak and beech forests

3.1 Introduction

Fine roots are the most physiologically active component of below-ground biomass. They have relatively high turnover rate (Hendrick and Pregitzer 1993) due to their short life span (Guo et al. 2008b). New fine roots are produced and old fine roots die simultaneously each year. Therefore, fine root production has obvious phenology during a growing season. The phenology of fine roots has been characterized among several species (McCormack et al. 2014) and biomes (Abramoff and Finzi 2014). It is indicated that the seasonal patterns of root production have a primary peak between late spring and summer (Burke and Raynal 1994; Burton et al. 2000; Joslin et al. 2001; Satomura et al. 2006; Steinaker et al. 2010). However, those seasonal patterns may not be typical for the production of fine roots in all species and can be further complicated as patterns change among years and with varying climatic conditions (McCormack et al. 2014). Although temporal variations within a year have been described in various forest types, interannual variations of fine root phenology are poorly understood. In order to evaluate fine root phenology with intra- and interannual variations at a stand level, it is necessary to conduct a long-term and high frequency investigation for several years preferably by observation approaches which are less destructive.

However, there are few studies investigating fine root phenology in stands of *Chamaecyparis obtusa* Endl., *Quercus serrata* Thunb. and *Fagus crenata* Blume, which are the typical species in a plantation, a temperate secondary forest and a cool temperate natural forest in Japan, respectively. The previous study indicates that fine root elongation peaked in late summer for three growing seasons in a young cypress (*C. obtusa*) stand (Noguchi et al. 2011). Cypress may have fine

root dynamics that correspond to their indeciduate dynamics of leaves. In contrast, deciduous oaks (*Q. serrata*) may have clear patterns between foliation and defoliation. Beeches (*F. crenata*) are also deciduous, but generally have a large annual variation in reproduction which may induce variations of phenology in below-ground dynamics.

The aims of this study are to describe production phenology of fine roots for several years in three forest types dominated either by cypresses, deciduous oaks or beeches, and to characterize the patterns of fine root phenology by comparing each other. This study evaluates a difference of the phenology among dominant species especially by comparison of the cypress and oak stands in a common site. Additionally, an effect of climate conditions on fine root phenology can be implicated by comparison between the oak and beech stands which have the same life form but are differently located either in temperate or cool temperate regions.

3.2 Materials and methods

3.2.1 Study site

The present study was conducted in three forest stands dominated either by cypresses, oaks or beeches. The cypress and oak stands were located at Ryukoku Forest, Shiga, Japan at 34°58'N, 135°56'E. The cypress stand was a plantation of *C. obtusa*, an evergreen conifer. The oak stand was a secondary forest of *Q. serrata*, a deciduous broad-leaved tree. The Ryukoku Forest is characterized by a temperate climate, with a mean air temperature and mean annual precipitation of 14.9 °C and 1530 mm, respectively. Further details of the site description in the Ryukoku Forest are found in Chapter 2.

On the other hand, the beech stand was located in the Naeba Mountains, Niigata, Japan at 36°51'N, 138°46'E, where mature natural forests of *F. crenata* are widely distributed. The elevation of the study site is ca. 550 m above sea level. This site was one of several permanent plots established along the altitudinal gradient in 1970 for long-term ecological monitoring within the framework of the International Biological Program (Kakubari 1977). The Naeba Mountains belongs to a cool temperate region, with mean air temperature and mean annual precipitation of 11.5 °C and 2222 mm, respectively (1979-2011; Japan Meteorological Agency). The ground is covered by snow approximately from November to April and snow accumulation reaches around 3-4 m. The bedrock is predominantly andesite and basalt, on which moderately water-retentive brown forest soil has formed (Han et al. 2008). The plot of the beech stand is situated beside a river with stand area, stand density and mean stem diameter at breast height (DBH) at 2000 m², 430 ha⁻¹ and 25.7 cm, respectively in 2015. The forest canopy is dominated by *F. crenata*, whereas understory vegetation is composed of *Acer rufinerve* Siebold et Zucc. and *Eleutherococcus sciadophylloides* H. Ohashi etc. The relative basal area of *F. crenata* is 96.8%. Further details of the site description were presented in Kakubari (1977) and Kubota et al. (2005).

3.2.2 Fine root observation in the cypress and oak stands

In the cypress and oak stands at the Ryukoku Forest, a flat root scanner method was applied to observe fine root dynamics. This method is based on the scanned images of fine roots taken by a flat-bed scanner inserted into the soil profile. In comparison with a more traditional minirhizotron method, the flat root scanner method has some advantages in economy and observation area. The scanner boxes were hand-made within which the flat root scanners were inserted to be used for specialized field application. Techniques of the scanner preparation and installation are the same as those explained in Chapter 2.

For the cypress and oak stands, ten (Scanner No.: H11a, H12a, H13a, H14a, H15a, K21a, K22a, K23a, K24a, K25a) flat root scanners were prepared. At first, two flat root scanners numbered as H11a and H12a were established in a cypress stand in June 2009. Additionally, H13a scanner and H14a and H15a scanners were buried in March 2010 and April 2011, respectively. In the oak stand, the flat root scanners of K21a, K22a, K23a and K24a were installed in April 2011, and K25a scanner was added in March 2012. Scanning soil profiles by each scanner was started soon after their installation and continuously conducted almost weekly (biweekly in the dormant season from December to March) until the end of 2017. However, several scanners became dysfunctional probably due to submergence of the electrical parts in water or excessive internal water condensation inside the scanner box (Table 3.1). Therefore, seven new scanners (Scanner No.: H12b, H13b, H13c, H13d, K21b, K22b, K23b) were alternatively established at the same location where the scanners were broken, as the H12a was replaced by H12b (The lower-case alphabet “a”, “b”, etc. indicates that the scanner was replaced at that position in the sequence.). For the following image analysis, a pair of two depth-wise images of 5 cm wide and 20 cm deep was cut out from each scanned original image, and then further separated into four small images of 5 cm x 5 cm each, which represents soil depths of 0–5cm, 5–10

cm, 10–15 cm and 15–20 cm, respectively (i.e. a total of 200 cm² area of soil surface in each scanner was the target for the following analysis.). The image processing of cut and separation were executed using the image editing software Adobe Photoshop CS2 (Adobe Systems, San Jose, Calif.). Finally, the set of time series images for several years was prepared for each scanner.

3.2.3 Fine root observation in the beech stand

In the beech stand located in the Naeba Mountains, fine root dynamics were observed by the minirhizotron method which is traditional one of the observation techniques. In general, the minirhizotron method is based on observation of roots projected on a transparent tube by the specialized camera or scanner, and estimates production, decomposition, turnover or lifespan of roots. The minirhizotrons provide a nondestructive, *in situ* method for viewing roots and are one of the best tools available for directly studying roots (Johnson et al. 2001). In the present study, transparent acrylic tubes with 70 mm in diameter were established at four locations selected haphazardly beside the study plot in August 2008. Bottom end of the tube was covered with a cone-shaped cap for water-tightness, and grid points were marked on outside surface of the tube by a fluorescent paint for adjusting positions of area in image analysis. In installation of the tube, first, a hole having 62 mm in diameter, which was slightly less than the tube in diameter, was dug by a soil core sampler to a depth of more than 40 cm. Then the minirhizotron tube was inserted vertically into the hole by tapping gently with a rubber hammer. Exposed head of the tube were covered with aluminum tapes and a polyvinyl chloride cap for protection from light and water.

Soil profiles were scanned from April 2009 to May 2017 with monthly intervals (but semimonthly for 2009 and 2010) except for the period when the ground was covered by snow. The scanning was conducted as follows. First, inside surface of the tube was wiped with a towel for

removing water condensation. Before scanning, the CI-600 In-Situ Root Imager, a specialized cylindrical scanner for minirhizotrons (CID Inc., WA, USA), was calibrated for adjusting optical intensity by using a white tube. Then the calibrated scanner was inserted into the tube in the ground, and a 21.6 cm x 19.6 cm image of the soil profile was taken by connecting it a laptop computer. Two or three images per tube were scanned by changing a vertical position of the scanner for covering the entire depth of the soil profile between 0 and 30 cm. The soil profiles were scanned with a resolution of 400 dpi and 48 bit colors, and they were saved as still images in TIF format.

Entire tube image was made by combining the acquired two or three images in each tube. To reduce workloads and enhance operation efficiency of the image analysis, a pair of two depth-wise images of 5 cm wide and 30 cm deep was cut out from each tube image of ca. 20 cm wide and more than 30 cm deep . The depth-wise image was further separated into six small images of 5 cm x 5 cm each. The separated images represent depths of 0-5 cm, 5-10 cm, 10-15 cm, 15-20 cm, 20-25 cm and 25-30 cm, respectively (i.e. a total of 300 cm² area of soil surface in each tube was the target for the following analysis). Cutting and separation of the images were done using the image editing software Adobe Photoshop CS2. Finally, the set of time series images for several years was prepared for each tube.

3.2.4 Image analysis

Image analyses for the cypress, oak and beech stands were the same and basically followed the methods explained in Chapter 2. The images of soil profile taken at different times in sequence were analyzed to evaluate fine root production ($\text{mm}^2 \text{cm}^{-2} \text{d}^{-1}$) using WinRHIZO Tron MF 2015, a software for root image analysis (Regent Instruments, Canada). Only roots that grew in length and/or thickness were traced. The traced individual root is composed of a series of straight line segments having

thickness called root segment in WinRHIZO Tron. After image analysis, the following variables were quantified using the data from each segment: segment number, visible root area (VRA) and cumulative gone root area (GRA). Traced root segments were identified by segment number in sequence in each analyzed image. VRA is defined as the area of a visible root segment (in mm²) that appears onto the image at a given time. GRA is defined as the area of a root segment (in mm²) that has disappeared due to decomposition, disturbance, predation, etc. between the dates of first measurement and the present observation. However, a root segment at a given time have either the values of VRA or GRA, but not both, since whether a root is visible or not is uniquely determined in each root segment.

3.2.5 Calculations

Fine root production (mm² cm⁻² d⁻¹), which is the same as P_{are} in the Chapter 2, was calculated on each depth-wise image using the values of visible root area (VRA; mm²) and gone root area (GRA; mm²) of each segment obtained from a pair of analyzed images. Fine root production represents the production on the area of newly-emerged and/or elongated root segments after the previous measurement, and was defined as Eq. 1 in Chapter 2. In the Eq. 1, S is defined as the constant that corresponds to the analyzed area (cm²) and has a value of 200 cm² for the flat root scanners or 300 cm² for the minirhizotrons.

3.2.6 Statistical analysis

Similarity of the seasonal patterns of fine root production among the years in each forest stand was analyzed using the Pearson product-moment correlation coefficient. Using the correlation matrix, the cluster analysis was conducted to classify the seasonal patterns into groups characterized significantly. Data were normalized using the Box-Cox transformation (Box and Cox 1964) prior to the correlation

analysis. This is a useful data transformation technique used to stabilize variance, make the data more Gaussian distribution-like and improve the validity of measures of association such as the Pearson product-moment correlation between variables.

These statistical calculations were conducted using the R statistical software (Version 3.5.0; R Development Core Team 2018).

3.3 Results

3.3.1 Fine root production in a cypress forest

Flat root scanner measurements revealed temporal variation in the area-based production of fine roots from the beginning of 2011 to the end of 2017 in the cypress stand, which had peaks intermittently in the growing season every year (Fig. 3.1). Fine root production was high in the growing season but low during the dormant season. Generally, fine roots began to grow from mid-March to early April, though the timing was slightly different among the years. The beginning date of the seasonal growth in 2017 tended to be earlier than in the other years. Maximum fine root production occurred each on May 13th, May 11th, July 31st, September 16th, September 12th, June 23rd and May 9th during seven years from 2011 to 2017, respectively, indicating that the phenology varied across the years. However, distinct several peaks within a year were found with temporary pauses in 2011, 2016 and 2017. On the other hand, fine roots grew continuously during the growing season in 2014 and 2015.

3.3.2 Fine root production in a deciduous oak forest

Fine root production was clarified for several years from the beginning of 2012 to the end of 2016 in the oak stand (Fig. 3.2). The oak stand had obvious seasonal variations of fine root production every year. In common with the cypress stand, fine roots were inactive during winter and began to grow in spring from mid-March to early April. Beginning date of the seasonal growth in 2013 tended to be earlier than that of the other years. The measured short-term fine root production reached the maximum on September 19th, May 17th, September 9th, November 20th and May 12th in five years from 2012 to 2016, respectively. Although the maximum values varied among the years, similar peaks occurred in the other seasons. For an example, in 2015 the maximum value appeared on November 20th, whereas a similar peak was found on May 1st. There were distinct several peaks within a year in the oak stand, and temporal peaks of fine root production was separated clearly with temporary pauses

during the growing seasons. In 2012 and 2014, the temporary pauses were observed among the scanners during the same periods in early summer.

3.3.3 Fine root production in beech forests

Long-term dynamics of fine roots in the beech stand were observed using minirhizotrons from 2010 to 2016 with monthly intervals (Fig. 3.3). Although the minirhizotron observation was less frequent compared to that of the flat root scanners in the present study, they provided data on temporal variations in which phenology of the fine roots could be evaluated. In the beech stand, fine roots began to grow in early to mid-summer probably from June to July. However, there was possibility that fine roots were produced before the first observation in the late spring in 2013 and 2016, because fine root production before the first observation in the years (i.e. in the snow covering season) was slightly high than that in the other years. Maximum values of the short-term fine root production were found on September 25th, July 18th, October 22nd, August 26th, September 21st, September 16th, July 21st and June 16th in seven years from 2010 to 2016, respectively. In the beech stand, fine root production generally peaked in late summer or autumn without temporary pauses during the growing season.

3.3.4 Comparisons of seasonal patterns of fine root production

The long-term observation revealed seasonal variations of fine root production for seven, five and seven years in the cypress, oak and beech stands, respectively (Fig. 3.1, Fig. 3.2 and Fig. 3.3). Production phenology of fine roots varied among the forest types and across the observation years in the given study sites. All seasonal patterns tended to follow a concentrated pattern with the peak typically occurring between mid-April and late November. However, patterns of bimodal phenology were observed mainly in the oak stand. The timing of the peak production varied across the years even

in the same stand. For example, the cypress stand shifted the timing of peak production by more than 2 months between 2011 and 2013. In contrast, the oak stand had nearly identical timings of peak production between 2012 and 2014, and the beech stand suggested unimodal phenology in fine root production though total annual production varied across the years. A greater variability in the seasonal patterns of fine root production within a forest type appeared in the cypress stand.

As shown in the results of the Pearson product-moment correlation analyses (Table A3), seasonal patterns of fine root production tended to vary among the years in the cypress stand. The correlation coefficients were not significant in more than half of combinations among the seasonal patterns. On the other hand, all seasonal patterns had strongly positive correlations in the oak stand. The seasonal patterns of 2014, 2015 and 2017 in the cypress stand had significant positive correlations with those of all years in the oak stand. And, most correlation coefficients were significantly positive within the beech stand. The maximum correlation coefficient was found between 2010 and 2016 in the beech stand ($r = 0.97$; Table A3), whereas the minimum coefficient appeared between 2011 in the cypress stand and 2014 in the beech stand ($r = -0.60$; Table A3).

Using the correlation matrix of fine root phenology (Table A3), the cluster analysis was conducted to classify the seasonal patterns (Fig. 3.4). Since a correlation coefficient of 0.42 corresponds to a significant level at $P = 0.05$, the seasonal patterns could be categorized into five groups which could be characterized conceptually as follows: a spring-autumn type, a spring dominant type, a continuous modal type, an autumn unimodal type and a summer unimodal type (Fig. 3.5). The spring-autumn and spring dominant types were found in 2011 and 2016 or 2012 and 2017 in the cypress stand, respectively. Both types have similar phenology in spring and summer but were slightly different in autumn. The continuous modal type was characterized by continuous fine root production from April to November. Several patterns in the cypress stand and all patterns in the oak stand were involved in this phenology type. Those observations indicate that fine root phenology in the oak stand

was relatively stable across the years, whereas the cypress stand had a greater seasonal variability. The autumn unimodal and summer unimodal types were found in the beech stand and could be distinguished from the other types by the timing of growth initiation within the year.

3.4 Discussion

3.4.1 Fine root phenology in three forest types

In the cypress stand, fine root production occurred between May and December and showed various seasonal patterns (Fig. 3.1). The growing season of fine roots was similar to the results in the previous study (Noguchi et al. 2011) investigating root elongation phenology in young stands of the same cypress species (*C. obtusa*). Although seasonal patterns of fine root production described in Noguchi et al. (2011) were similar to those of the present study in 2013 and 2014, my results revealed that the cypress stand shows various patterns of fine root phenology.

In the oak stand, fine root production was temporarily low during the summer every year (Fig. 2.2), suggesting bimodal phenology especially in 2011 and 2013. These patterns were similar to seasonal variations of fine root elongation observed by using minirhizotrons in a deciduous oak forest (Joslin et al. 2001). However, the results of the bimodal root phenology in deciduous oak forests were poorly reported in the previous studies probably due to low time resolution with a use of monthly observation intervals. High frequency observations in the present study contributed to clarify the specific patterns of fine root phenology in the deciduous oak forest.

In the beech stand, unimodal phenology of fine root production was observed for several growing seasons (Fig. 3.3), though fine root observations were less frequent than the cypress and oak stands. And fine root production showed large interannual variations among the years. Those unimodal phenology and a large interannual variation can also be found in a cool temperate forest dominated by deciduous broad-leaved trees (e.g. Fukuzawa et al. 2013).

3.4.2 Differences of fine root phenology among dominant species of forests

Fine root production in three forest stands had clear seasonal variations (Fig. 3.1, Fig. 3.2 and Fig.

3.3), as being suggested by the previous studies in temperate and cool temperate forests (e.g. Satomura et al. 2006; Fukuzawa et al. 2013). The timing of root growth initiation in spring was not different between the cypress and oak stands, whereas that tended to be later in the beech stand. The difference of the timings can be explained by climate conditions. In the beech stand located in a cool temperate region, the ground was covered by snow usually from November to April, leading to a longer dormant season than the temperate forests. In the temperate forests, the date of growth initiation was early March in 2012 in both stands, but that in 2014 was early April regardless of the stand types. This indicates that the timing of fine root growth initiation depends on the annual tendency of environmental conditions rather than on species characteristics of the dominants.

The dates of peak root production tended to vary among forest types and across measurement years, as previous studies reported wide variation of root production across several temperate tree species and years (e.g. McCormack et al. 2014). While it is currently unclear what specific processes caused this variation, it is possible that this variation represents different strategies of tree species to utilize temporally variable resources and to respond to seasonal or annual changes in local climate. However, production of fine roots was commonly low in early June in the cypress stand and in early August in the oak stand (Fig. 3.4), indicating that there may be potential factors that limit fine root growth at different times over the season. In contrast, there was no pause of fine root production during the growing season in the beech stand although it belongs to the same family with the deciduous oaks. This may be related to the shorter growing season limited by cool temperature climate and heavy snow in the dormant season. In the further studies, it will be important to separate the roles of peak root production and their relative importance to whole-plant resource acquisition and carbon allocation.

The seasonal patterns of fine root production were different between the cypress and oak stand, indicating that phenology of fine root production depends on the dominant tree species. Also,

the beech stand had phenology distinctly different from both temperate tree species examined, implicating that climate conditions may alter the fine root phenology. In the present study sites, production phenology of fine roots was divided into five types which can be characterized as a spring-autumn type, a spring dominant type, a continuous modal type, an autumn unimodal type and a summer unimodal type (Fig. 3.5). Production phenology of the oak stand was categorized consistently into the continuous modal type, and there was not significant interannual variation across the five-year period. In contrast, the cypress stand had a large variation of fine root phenology, showing distinct three types. In the beech stand, seasonal patterns of fine root production could be separated into the summer unimodal and autumn unimodal types. It is therefore considered that the effects of dominant species on the patterns of seasonal fine root production are likely to be species-specific, and are generally different. And, the variability of fine root phenology may represent sensitivity to annual changes of environmental conditions. In addition, field measurements of a short periods such as covering only one or two growing seasons are not enough to clarify and generalize fine root phenology in forest ecosystems.

3.5 Conclusions

Long-term dynamics of fine roots in cypress, deciduous oak and beech forest stands were evaluated by scanned image analysis using flat root scanners or minirhizotrons. In the cypress stand, fine root production was observed for seven years and there were several variations of fine root phenology such as spring dominant types or a continuous modal type. Although the beginning dates of growth were concentrated on early April, the peak root production occurred at various periods within a year. On the other hand, multiple peaks were found in fine root phenology of the oak stand, but the phenology was relatively stable across those five years of observation. In contrast, the beech stand tended to follow a concentrated pattern of root production with the peak typically occurring in summer or autumn. Fine roots in the beech stand began to grow usually between May and July, and that was obviously later than the cypress and oak stands probably due to a longer period of snow covering. Seasonal variations of fine root production were greatly affected by climate conditions, whereas the peak root production can be controlled by species-specific factors.

Table 3.1 Dates of scanner installation and final scanning for each scanner no. The scanners were replaced by new one in each discontinuation of the scanning due to instrument fault. The lower-case alphabet “a”, “b”, etc. indicates that the scanner was replaced at that position in the sequence

Forest stand	Scanner no.	Installation date	Final date of scanning
Cypress stand			
	H11a	2009/6/12	
	H12a	2009/6/12	2013/9/25
	H12b	2015/6/6	2016/12/21
	H13a	2010/3/27	2012/5/28
	H13b	2012/8/12	2014/10/2
	H13c	2014/10/31	2015/10/16
	H13d	2016/2/21	
	H14a	2011/4/21	2016/6/3
	H15a	2011/4/21	2016/6/10
Oak stand			
	K21a	2011/4/21	2014/9/9
	K21b	2014/10/31	2017/3/17
	K22a	2011/4/21	2015/12/5
	K22b	2016/2/21	2017/8/23
	K23a	2011/4/21	2015/8/6
	K23b	2015/10/16	
	K24a	2011/4/21	2016/7/21
	K25a	2012/3/15	2016/5/7

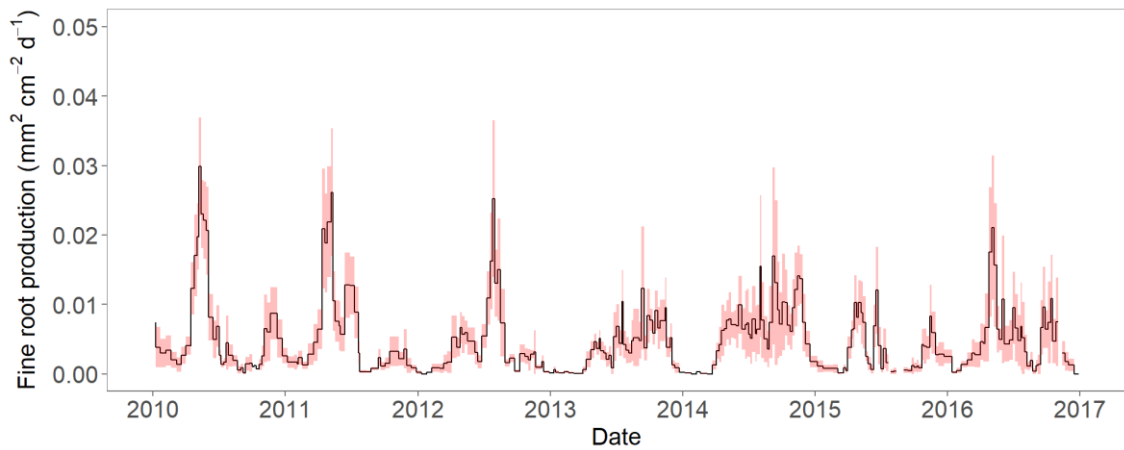


Fig. 3.1 Long-term fluctuation of mean (\pm SE) of fine root production in the cypress stand. This shows daily-fractionated values of the root area newly produced during each measurement interval. The initial data of each scanner were excluded in consideration of soil disturbance

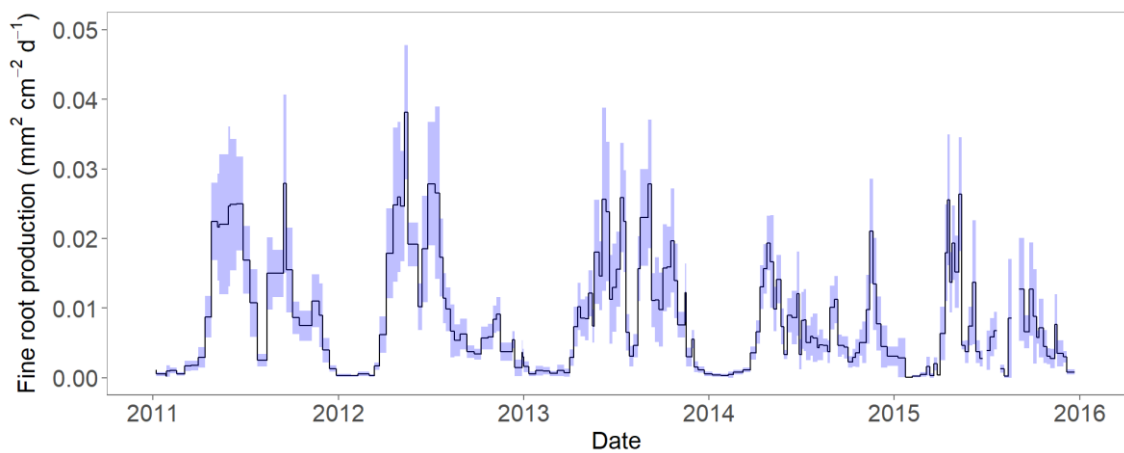


Fig. 3.2 Long-term fluctuation of mean (\pm SE) of fine root production in the oak stand. This shows daily-fractionated values of the root area newly produced during each measurement interval. The initial data of each scanner were excluded in consideration of soil disturbance

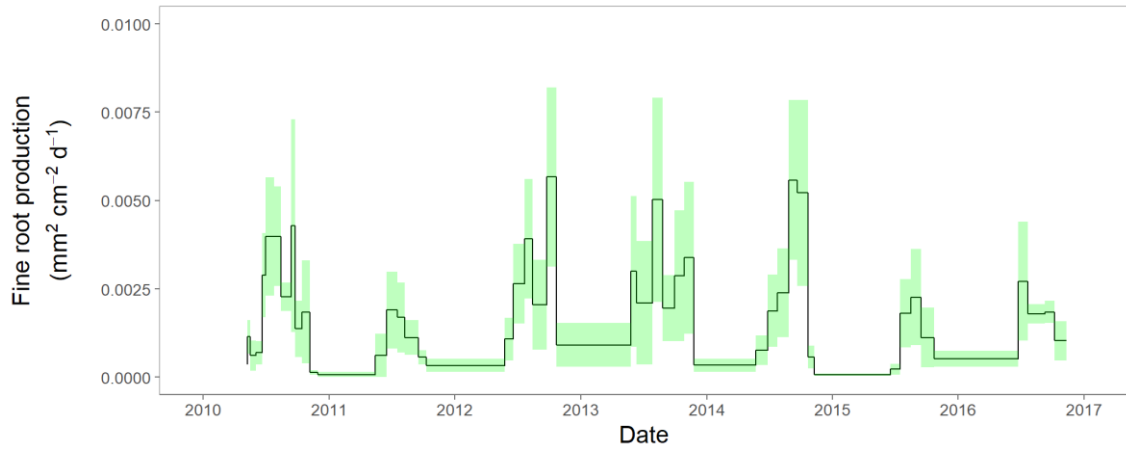


Fig. 3.3 Long-term fluctuation of mean (\pm SE) of fine root production in the cypress stand. This shows daily-fractionated values of the root area newly produced during each measurement interval

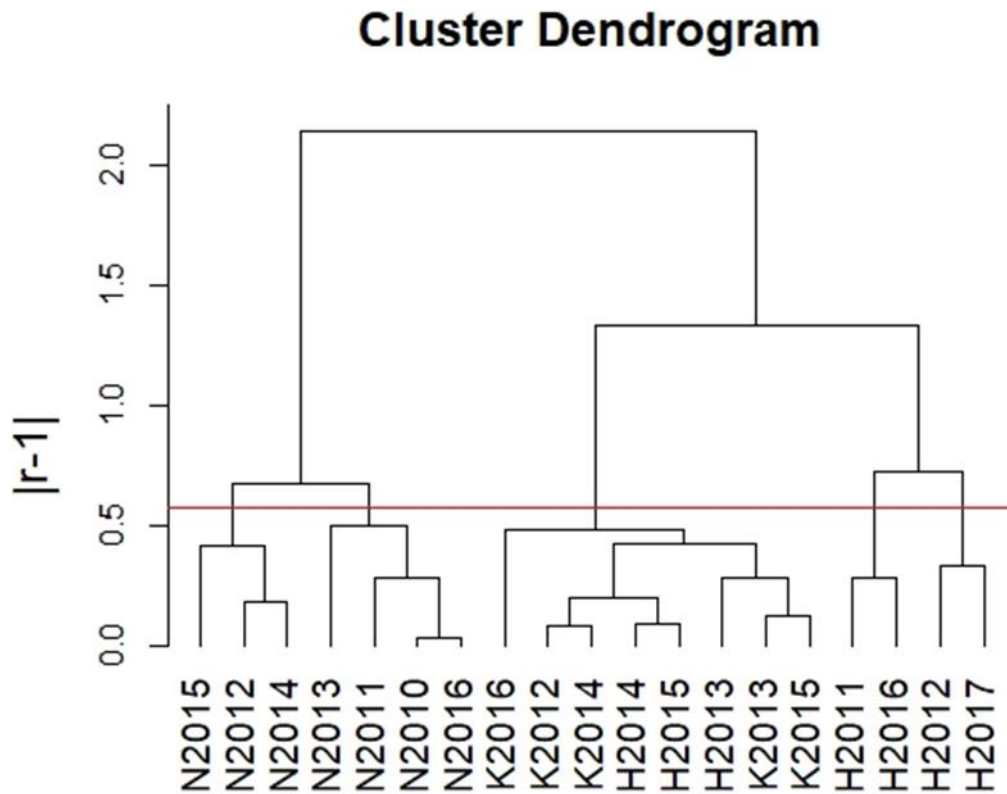


Fig. 3.4 Cluster dendrogram yielded by the cluster analysis using the correlation matrix. The correlation coefficients in the matrix were calculated for all combinations among seasonal patterns of fine root production in each year within each stand. Vertical axis means the absolute value of correlation coefficients (r) minus one. The horizontal line represents 0.42 of r indicating a significance level for 0.05

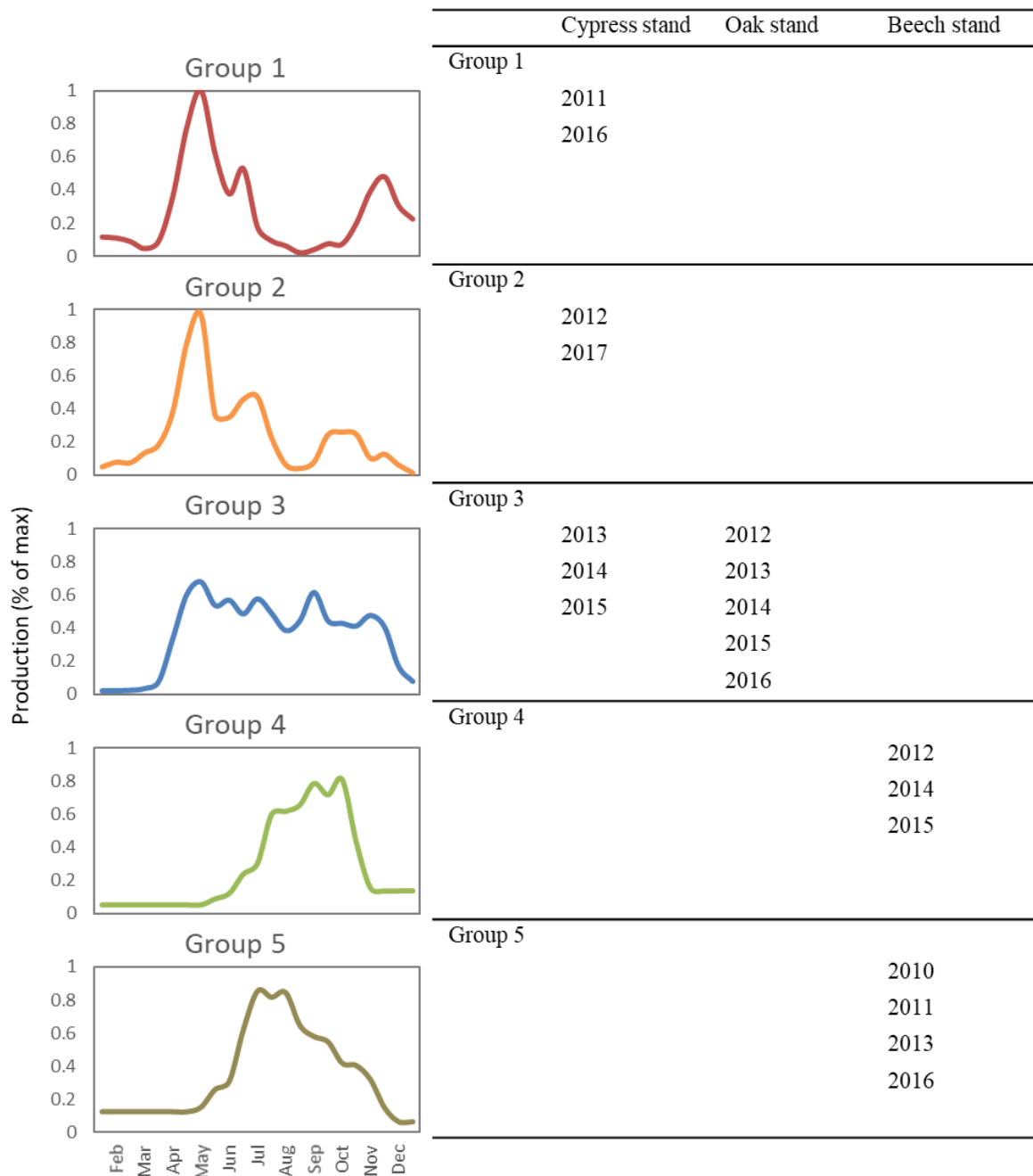


Fig. 3.5 Five groups of seasonal patterns in fine root production classified by the cluster analysis. A table shows the years of each phenology corresponding to the five groups. Left figures indicate averaged patterns among the years in each group which could be characterized as follows: a spring-autumn type, a spring dominant type, a continuous modal type, an autumn unimodal type and a summer unimodal type, respectively. Vertical axis means relative values of production (% of max) that do not necessarily equal 1, because several years with differently timed maximum production were averaged

Chapter 4 Environmental and physiological factors controlling fine root dynamics

4.1 Introduction

Identifying primary factors controlling fine root production is critical for predicting how plants respond to changing environment and allocate limited resources to growth and reproduction. A few studies investigating phenology of both fine roots and shoots of trees indicate positive relationships between above- and below-ground productivity (Steinaker et al. 2010; Abramoff and Finzi 2014). Moreover, these dynamics are greatly affected by environment factors such as temperature and precipitation (Steele et al. 1997; Joslin et al. 2001). Thus the factors controlling fine root dynamics can be separated into physiological and environmental factors. The physiological factors such as foliage and reproduction may affect fine roots directly. In the case of the environmental factors, soil moisture has a direct effect, but temperature may affect fine roots directly in soil and/or indirectly through shoot response. In addition, peak root production may be restricted by cold damage in winter (Tierney et al. 2001) and strong prolonged summer drought (Joslin et al. 2001).

However, much more data on temporal changes of fine root production are needed to elucidate the factors and mechanisms determining fine root productivity. Ideally, measurement interval of fine roots should be highly frequent in order to compare with rapidly changing environment such as soil moisture. Additionally, the long measurement period is preferable to evaluate interannual variations of fine root dynamics and compare them with interannual trends of climate conditions and plant physiological dynamics. But, there are few studies that investigated fine root dynamics with frequent observation for several years evaluating annual variations.

The aims of the present study are to examine possible effects of environmental factors on fine root production and to suggest possible physiological relationships between above- and below-ground production. The environmental effects of soil temperature and moisture were evaluated in two temperate forests dominated either by *Chamaecyparis obtusa* or by *Quercus serrata* in the nearby sites

where fine root dynamics were frequently observed. I hypothesize that fine root production is strongly correlated with temperature and is restricted by occasional drought conditions in the soil. But the correlations between fine root production and environmental factors may vary depending on the season because behavior of the fine roots may be related to above-ground phenology such as foliation and defoliation.

On the other hand, physiological relationships between the patterns of above- and below-ground productions were analyzed in a cool temperate beech forest of *Fagus crenata* where the masting phenomenon (seed production occurring with large annual variation with spatial synchrony) appears. Heavy reproduction has been observed at the cost of woody growth due to limited resources (Yasumura et al. 2006; Han et al. 2011), and the masting phenomenon is often explained under the assumption of resource budget models (Isagi et al. 1997; Satake and Iwasa 2000). Here, I hypothesize that seasonal variations of fine root production vary year by year with interannual variations of seed and leaf production.

4.2 Materials and methods

4.2.1 Study site

To achieve the objectives of the present study, fine root observation was conducted and was compared with environmental conditions in a cypress plantation and a deciduous oak secondary forest at Ryukoku Forest, Shiga, Japan at 34°58'N, 135°56'E. Fine root production and leaf and seed production were estimated in a natural forest of beeches at the Naeba Mountains, Niigata, Japan at 36°51'N, 138°46'E. Further details of the site description are given in Chapter 2 and Chapter 3.

At Ryukoku Forest, meteorological data of air temperature, precipitation and others have been monitored at the observation tower by a research team of Dr. T. Yokota at Ryukoku University. Soil environmental data of soil temperature and moisture were measured within each stand. Annual mean air temperature from 2010 to 2017 averages 15.5 °C and ranges from 15.1 to 16.0 °C (Fig. 4.1). Annual precipitation from 2010 to 2017 averages 1681 mm and ranges from 1465 to 1940 mm (Japan Meteorological Agency). Monthly mean soil volumetric water content (%; soil moisture) was the lowest mostly in summer, indicating that there was a drought condition.

At Naeba Mountains, annual mean air temperature from 2010 to 2017 averages 11.6 °C and ranges from 11.2 to 12.5 °C at a nearby meteorological weather station to the Naeba Mountains (Japan Meteorological Agency). And annual precipitation from 2010 to 2017 averages 2448 mm and ranges from 2014 to 2912 mm (Fig. 4.1).

4.2.2 Soil temperature and moisture

Soil temperature and soil moisture (volumetric water content) were monitored nearby the observation tower in the study site. Soil temperature and moisture were automatically measured using a thermistor thermometer and a sensor using time domain reflectometry (TDR) method, respectively, and were

recorded in data logger systems with 10 minute intervals for the period from 2010 to 2017. Sensors of the thermistor and the TDR have been buried at 10 cm depth of soil. In the following statistical analysis in comparison with fine root dynamics, the recorded values were averaged for the corresponding periods of fine root observation intervals.

At the Ryukoku Forest, soil temperature showed consistent seasonal variations each year (Fig. 4.2). Soil temperature in 2016 was slightly high and that in 2017 was relatively low among the study periods from 2010 to 2017. On the other hand, soil moisture tended to be low from May to September within seasonal variations (Fig. 4.2). In 2011, soil moisture in May and June was clearly higher than that in the other years, corresponding to high precipitation in May 2011 (Fig. 4.1). Soil moisture in 2014 was relatively low among the other years.

4.2.3 Fine root observation

Fine root dynamics in the Ryukoku Forest and the Naeba Mountains were observed either by flat root scanners or by minirhizotrons. The methods and measurement procedures are the same as those of Chapter 2 and Chapter 3.

In the Ryukoku Forest, fine root observation using flat root scanners started in the cypress and oak stands from June 2009 and April 2011, respectively. The flat root scanners were buried at five locations selected haphazardly in each stand. Scanning soil profiles was conducted weekly (biweekly in the dormant season from December to March) until the end of 2017. However, there was a lack of data in 2017 in the oak stand due to dysfunction of the scanners. For following image analysis, a pair of two depth-wise images of 5 cm wide and 20 cm deep was cut out from each scanned image. The image processing of cut and separation were executed using the image editing software Adobe Photoshop CS2 (Adobe Systems, San Jose, Calif.). Finally, the set of time series images for several years was prepared for each scanner.

In the beech stand of the Naeba Mountains, minirhizotron tubes were established at four locations selected haphazardly beside the study plot in August 2009. Soil profiles were scanned by the CI-600 In-Situ Root Imager, a specialized cylindrical scanner for minirhizotrons (CID Inc., WA, USA) from April 2010 to May 2017 with monthly intervals (but semimonthly for 2010) except for the period when the ground was covered by snow. Two or three images per tube were scanned with changing a vertical position of the scanner for covering entire depth of the soil profile between 0 and 30 cm. Then entire tube image was made by combining the two or three images in each tube. A pair of two depth-wise images of 5 cm wide and 30 cm deep was cut out from each tube image for the following image analysis.

Analysis of the images taken by flat root scanners or minirhizotrons were the same and basically followed the methods explained in Chapter 2. The soil profile images were analyzed using WinRHIZO Tron MF 2015, a software for root image analysis (Regent Instruments, Canada) for estimating area-based fine root production ($\text{mm}^2 \text{cm}^{-2} \text{d}^{-1}$). After image analysis, the following variables were quantified using the data from each root segment: visible root area (VRA) and cumulative gone root area (GRA). VRA is defined as the area of a visible root segment (in mm^2) that appears onto the image at a given time. GRA is defined as the area of a root segment (in mm^2) that has disappeared between the dates of first measurement and the present observation.

Fine root production ($\text{mm}^2 \text{cm}^{-2} \text{d}^{-1}$), which is the same as P_{are} in Chapter 2, was calculated for each depth-wise image using the values of visible root area (VRA; mm^2) and gone root area (GRA; mm^2) of each root segment obtained from a pair of analyzed images. Fine root production represents the production of the area of newly-emerged and/or elongated root segments after the previous measurement, and was defined as Eq. 1 in Chapter 2. In the Eq. 1, S is defined as the analyzed area and has a value of 200 cm^2 for the flat root scanners or 300 cm^2 for the minirhizotrons.

4.2.4 Litterfall observation

Ten litter traps having area of 0.25 m² were placed in the plot and fixed about 1 m above the ground except for the snow cover season (from late May to late November) from 2010 to 2015 at the beech forest. The litter falling into the litter traps was collected monthly. Samples of the litterfall were sorted into leaves of dominant species (*F. crenata*), leaves of other species, seeds (cupules and nuts) of dominant species, woody tissues and others. Then the samples were oven-dried to a constant mass at 75 °C and weighted. Annual production of leaves and seeds (Mg ha⁻¹ yr⁻¹) was estimated as the sum of litterfall sampled within the year.

4.2.5 Statistical analysis

Effects of explanatory variables of environmental factors (soil temperature and moisture) or physiological factors (leaf and seed production) on response variables (fine root production) were evaluated by fitting the generalized linear mixed model (GLMM) with maximum likelihood estimation method. The GLMM is able to model variables that do not satisfy the assumptions of a standard linear model, allowing fixed and random factors to be distinguished in the model. The model use in the present case was justified by the Gamma distribution of response variables under consideration and the inclusion of a random source of variation (year). Temporal variations of fine root production in the cypress and oak stand across five or three years were treated as response variables. The fixed effects of the environmental and physiological factors on those parameters were tested assuming a normal error distribution of explanatory variables with a log link function. The fixed effects included potentiality of main effects of each factors and two-factor interaction effects in all combinations. The variance of year was included in the GLMM as a random factor. The factors included in the model were selected based on Akaike's Information Criterion (AIC), and multicollinearity among

explanatory variables were excluded using a reference index of Variance Information Factor (VIF).

These analyses were performed by using R software, the statistical analysis software.

4.3 Results

4.3.1 Effects of environmental factors on fine root production in cypress and oak forests

In both cypress and oak stands, possible effects of environmental factors on fine root production were significantly suggested in spring and summer (Table 4.1). Temperature had significant positive and negative effects in May and April, and in July, respectively (GLMM, $P < 0.05$; Table 4.1). In the oak stand, regression coefficients in May and April were higher than those in the cypress stand, indicating rapid reaction to the increasing temperature (Fig. 4.3).

The effects of soil moisture differed between both stands. A negative effect was found only in August in the cypress stand (GLMM, $P < 0.01$; Table 4.1). Whereas positive and negative correlations were suggested in May and in July and September, respectively, in the oak stand (GLMM, $P < 0.05$; Table 4.1). The positive correlation in May indicates that fine root production is accelerated with increasing soil moisture during late spring. However, interactive effects between temperature and moisture also appeared during summer, indicating that both factors were dependent on each other.

The seasonal relationship between monthly temperature and fine root production was not linearly controlled but exhibited a hysteresis pattern (Fig. 4.4). Temperature effects were significant in spring but insignificant in autumn even though the conditions were similar (Fig. 4.3). This result positively suggests that fine root response to environmental conditions varies depending on the season. On the other hand, soil moisture did not show a clear seasonal relationship with fine roots (Fig. 4.4).

4.3.2 Effects of physiological factors on fine root production in a beech forest

In the beech stand, the biennial masting dynamics were observed, as great seed production occurred in 2011, 2013 and 2015 (Fig. 4.5). Estimates of seed production were $1.85 \text{ Mg ha}^{-1} \text{ yr}^{-1}$, 1.53 Mg ha^{-1}

yr⁻¹ and 2.10 Mg ha⁻¹ yr⁻¹ in 2011, 2013 and 2015, respectively. The seed production had negative correlations with fine root production in September and October, but in contrast that showed a positive correlation with fine root production in September and October of the previous year (Table 4.2, Fig. 4.6 and Fig. 4.7). On the other hand, leaf production ranged from 1.84 in 2014 to 3.37 in 2010, indicating its interannual variations. Significant correlations between annual leaf production and monthly fine root production were detected only in June and July positively. Whereas there were no significant correlations between leaf production in the current year and monthly root production in the previous year.

4.4 Discussion

4.4.1 Seasonal plasticity of fine root response to environmental conditions in temperate forests

Temperature has been considered as a dominant factor controlling fine root elongation of several species in temperate, cool temperate and boreal forests (Tryon and Chapin III 1983; Burke and Raynal 1994; Steele et al. 1997; Steinaker et al. 2010; Germon et al. 2016). In the present study, there were possible effects of temperature on fine root dynamics in spring but that did not appear in autumn (Fig. 4.3), indicating that fine root response to environmental factors varied depending on the season. Fine roots responded strongly to temperature increase in spring, but were not affected by its decrease in autumn even under similar temperate conditions. This pattern can be characterized by the seasonal hysteresis dynamics (Fig. 4.4) (Tierney et al. 2003), and indicated a physiological relationship between fine roots and leaves which was clearly controlled by foliation and defoliation. Generally in temperate and boreal forests, root production follows shoot or leaf production within the season (Abramoff and Finzi 2014). Thus it is difficult to elucidate mechanisms of fine root phenology only using environmental factors. There may be physiological relationships between fine root production and leaf dynamics and/or carbon storage. Furthermore, this hysteresis dynamics may be a possible factor to explain hysteresis dynamics of soil respiration (Kominami et al. 2012). Production of fine roots is linked more or less to their respiration, and eventually that may affect the seasonal pattern of soil respiration. During the spring, fine roots responded to temperature more rapidly in the oak stand than in the cypress stand (Fig. 4.3). This may reflect the above-ground phenology of both dominant species because leaf expansion of deciduous oaks also occurs rapidly than cypresses.

On the other hand, soil moisture positively affected fine root production of the oak stand in May (Table 4.1), indicating that fine root growth was facilitated in rich soil water conditions. Whereas, that had negative correlations in summer in both stands. A possible interpretation on the negative correlations is that trees were exploring soil environments having favorable water by elongating roots

under drying conditions. However, the effect of soil moisture cannot be understood simply, because that varies depending on temperature conditions, as the interaction has shown significant.

4.4.2 Resource allocation mechanisms between above- and below-ground production in the beech forest

Annual leaf production was correlated with monthly fine root production in June and July in the current year (Table 4.2), indicating that fine root production during early growing season increased with increasing amount of leaf expansion occurred probably from May to June (Han et al. 2016). This interpretation is supported by the previous study showing that fine root growth is delayed later than leaf out generally in boreal and temperate forests (Abramoff and Finzi 2014). It can be suggested that fine roots are produced during spring to ensure water supply for transpiration in leaves. Because leaf expansion probably occurs before root elongation, leaf production may irreversibly affect fine root dynamics in early summer.

Seed production was correlated negatively with fine root production in autumn in the current year, but positively with that in September in the previous year (Table 4.2, Fig. 4.6 and Fig. 4.7). To understand these correlation relationships, two possible hypotheses can be suggested. One is that fine root production in the current autumn is prevented by carbon deprivation due to a lot of seed formation in the current year. And the other is that fine root production in autumn is facilitated in order to ensure nutrient resources for seed production in the following year. The second hypothesis can be explained from a process determining a number of produced seeds (Kon and Noda 2007). Generally, amount of seed production depends on a number of flowers in the current year, and the flower number is determined in the previous spring or summer probably due to climate cues. This means that the amount of seed production is predetermined in the early season of the previous year (given that increase in insect predating the seeds is minimal), and therefore seed production consuming a large amount of

nutrient resources could be correlated with fine root production in the previous autumn. However, it is not clear whether fine root production in autumn is controlled by carbon deprivation or nutrient resource demand or both. The biennial masting behavior observed in the present study site is not typical and at high frequency compared with other beech forests (Masaki et al. 2008). In the beech forest having biennial masting events, it is difficult to separate different mechanisms. To differentiate the two hypotheses distinctively, detailed physiological dynamics such as nitrogen storage (Han et al. 2014) and carbon allocation (Han et al. 2016) have to be examined within individual trees in which masting events occur irregularly. Further observation and/or experiments are required to elucidate resource allocation mechanisms between above- and below-ground production.

4.5 Conclusions

The analyses of the present study revealed possible effects of environmental and physiological factors controlling fine root dynamics in forest ecosystems. In the temperate forests dominated by cypresses or deciduous oaks, temperature was a primary factor to control general trends of fine root phenology, and induced fine root growth initiation in spring, but was not correlated in autumn. Therefore, fine root response to temperature varied depending on the season, and there was a hysteresis relationship between fine root phenology and temperature variations. On the other hand, soil moisture affected fine root production negatively in summer, though the effects varied depending on temperature conditions.

In the beech forest with biennial masting phenomenon, fine root production in early growing season increased with increasing annual leaf production. The masting events can affect resource allocation patterns between above- and below-ground production, as seed production was correlated with fine roots produced mainly in autumn. The results suggest a possibility that fine root production is accelerated for gathering nutrient resources before producing a large amount of seeds, and/or is prevented after that due to carbon deprivation.

Table 4.1 Summary statistics for the test of fixed effects (soil temperature and moisture of the periods corresponding to fine root observation) in the generalized linear mixed model (GLMM), with response variables of area based fine root production ($\text{mm}^2 \text{ cm}^{-2} \text{ d}^{-1}$) of each measurement intervals within month in the cypress and oak stands from 2011 to 2017 (from 2012 to 2016 for the oak stand). Values represent the estimates of regression coefficients

	March	April	May	June	July	August	September	October	November
Cypress stand									
Soil temp	0.16 *	0.29 ***			-0.18 *				
Soil water						-1.03 **			
Interaction					0.01 *	0.05 **	0.01 *		
Oak stand									
Soil temp	0.34 ***	0.34 ***			-1.30 **				
Soil water			0.19 **		-2.43 **		-0.37 *		
Interaction					0.12 **	0.01 ***	0.02 **		0.02 ***

* : $P < 0.05$, ** : $P < 0.01$, *** : $P < 0.001$

Table 4.2 Summary statistics for the test of fixed effects (annual seed and leaf production) in the generalized linear mixed model (GLMM), with response variables of monthly fine root production ($\text{mm}^2 \text{ cm}^{-2} \text{ d}^{-1}$) in the beech stand from 2010 to 2016. Values represent the estimates of regression coefficients

	Fine root production in previous year				
	June	July	August	September	October
Seed production				0.55 **	0.66 *
Leaf production					
	Fine root production in current year				
	June	July	August	September	October
Seed production				-0.51 ***	-0.87 **
Leaf production	1.49 *	1.14 *			

*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$

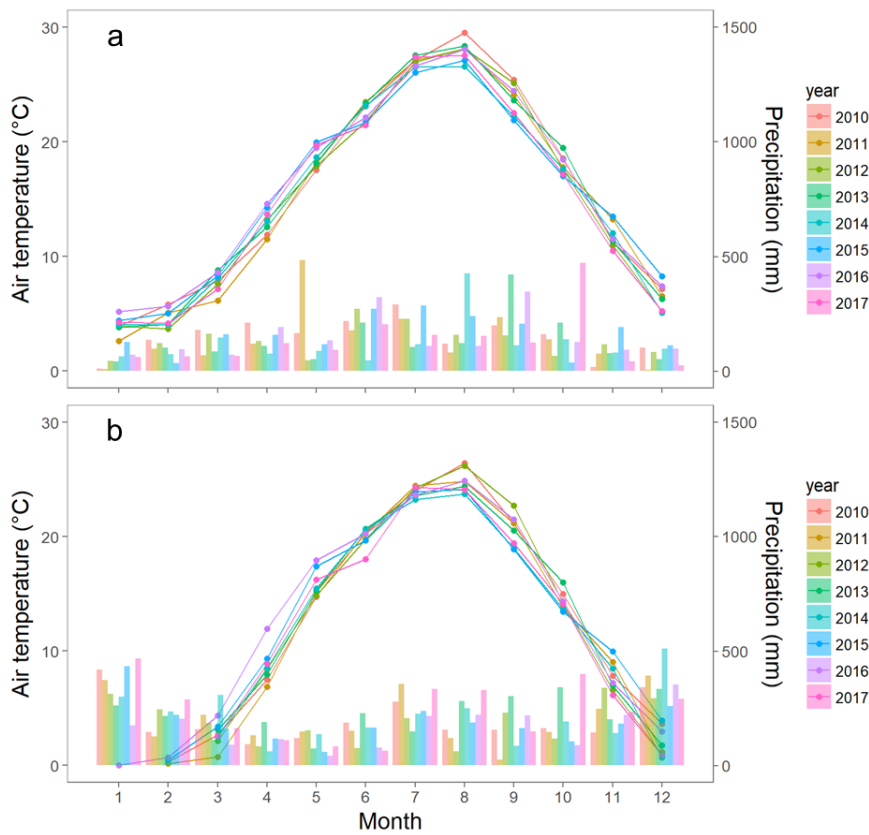


Fig. 4.1 Monthly values of mean air temperature and cumulative precipitation from 2010 to 2017 in (a) the Ryukoku Forest and (b) the Naeba Mountains. The observation values were measured at the weather stations closest to both sites by Japan Meteorological Agency

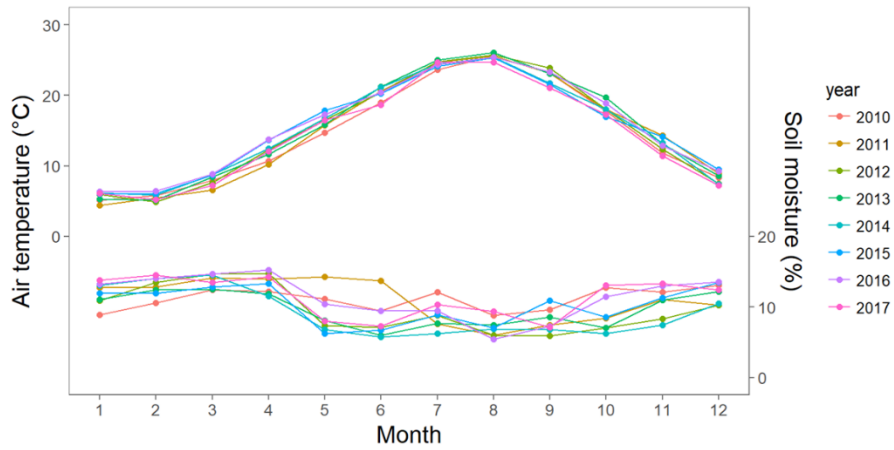


Fig. 4.2 Monthly values of mean soil temperature and mean soil moisture (volumetric water content) from 2010 to 2017 in the Ryukoku Forest. The observation values were monitored nearby the observation tower in the study site. Daily values are found at Fig. A1 (Appendix)

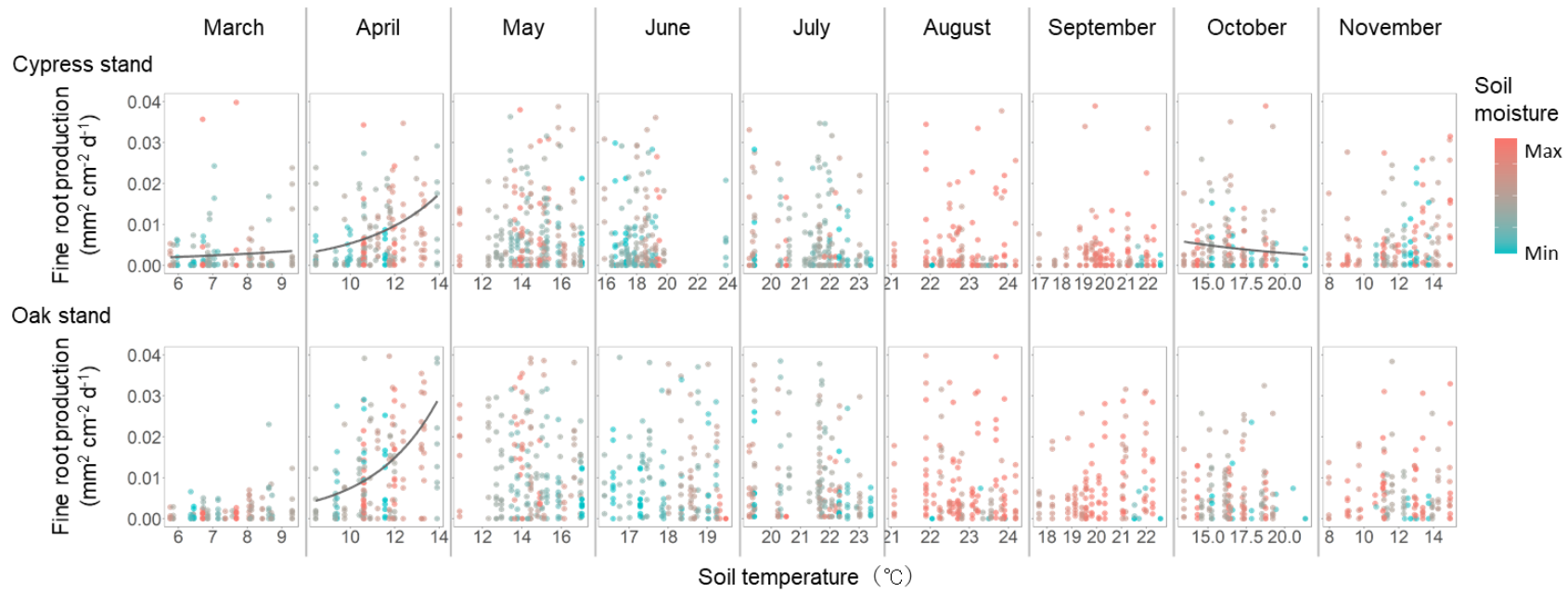


Fig. 4.3 Relationships between fine root production of each measurement intervals and soil temperature or soil moisture of the corresponding periods. Regression curves were fitted by the GLMM. Black solid lines represent significant correlations between vertical (fine root production) and horizontal (soil temperature) axes

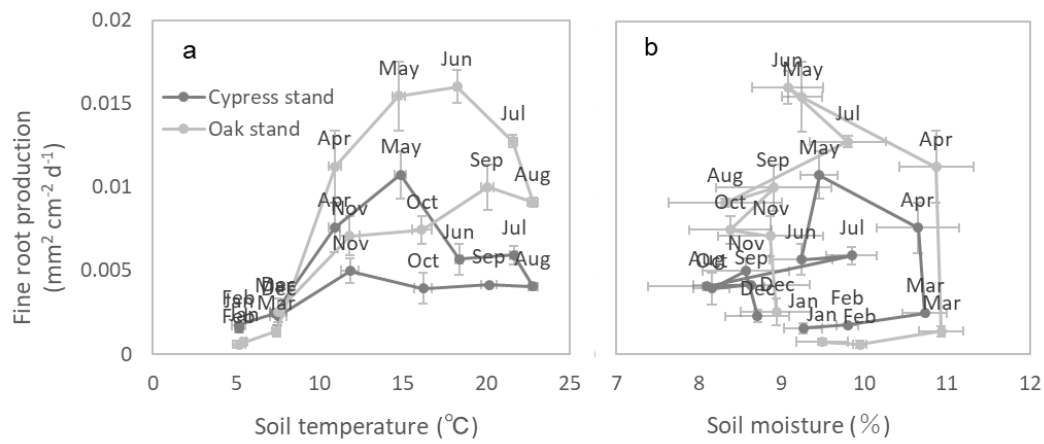


Fig. 4.4 Relationships between monthly averaged values of (a) soil temperature or (b) soil moisture and fine root production in the cypress and oak stands. Bars show the standard error of mean

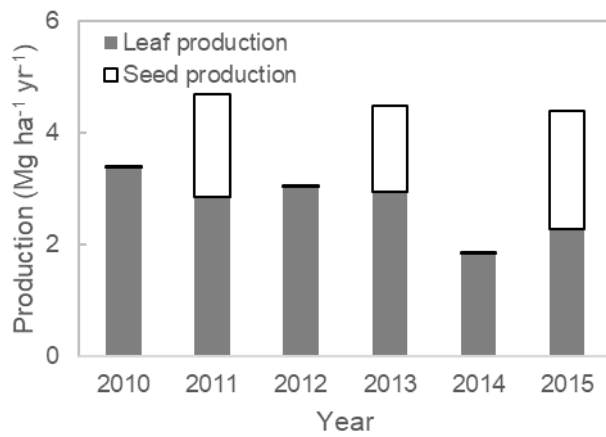


Fig. 4.5 Leaf and seed production in the beech stand from 2010 to 2015, indicating biennial masting phenomenon.

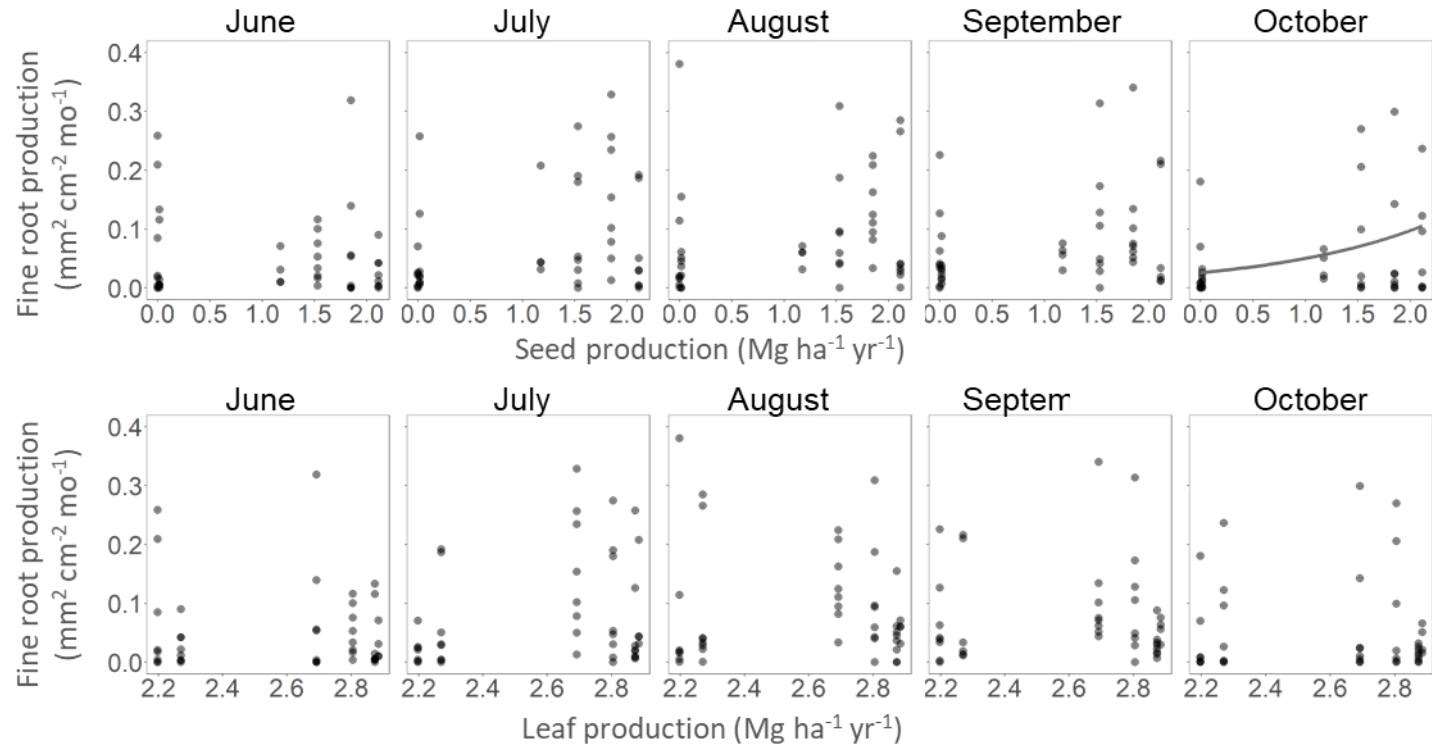


Fig. 4.6 Relationships between annual leaf or seed production in the current year and monthly fine root production in the previous year. Regression curves were fitted by the GLMM. Black solid lines represent significant correlations between vertical (fine root production) and horizontal (leaf or seed production) axes. Values of horizontal axis were the same among horizontal panels

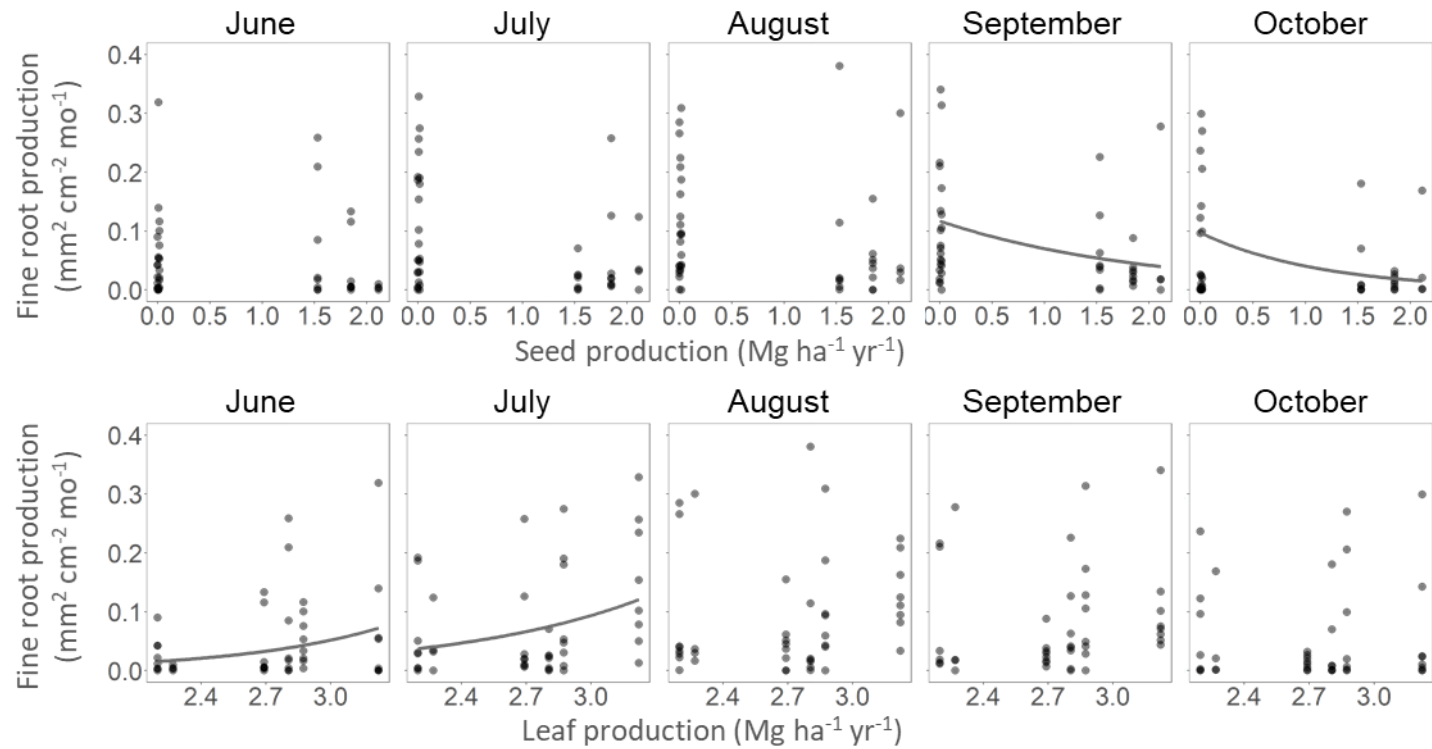


Fig. 4.7 Relationships between annual leaf or seed production in the current year and monthly fine root production in the same year. Regression curves were fitted by the GLMM. Black solid lines represent significant correlations between vertical (fine root production) and horizontal (leaf or seed production) axes. Values of horizontal axis were the same among horizontal panels

Chapter 5 General conclusions

The present study tried to describe long-term dynamics of fine roots, characterize fine root phenology and examine possible factors controlling fine root dynamics. Fine root dynamics were investigated by using two types of the observation methods. One is a flat root scanner method and the other is a minirhizotron method. While the minirhizotron method has been used traditionally in the previous studies, the flat root scanner was poorly applied especially in investigation at the ecosystem level. The present study showed applicability and potential of the flat root scanner method for detailed description of fine root production dynamics.

In Chapter 2, area-based fine root production projected on soil profiles after soil disturbance was evaluated quantitatively by a flat scanner method in the cypress and oak stands. Cumulative root area production within a year followed a logistic growth curve providing both an asymptotic upper limit and a growth coefficient. The asymptotic upper limit and/or root growth coefficient in the logistic curve varied between the growing seasons immediately after soil disturbance and the later years. Also this study described morphological traits of produced fine roots (i.e. first-order roots). Length-based fine root production was drastically high in the immediate year and gradually decreased with time. Diameter of produced fine roots tended to be thick temporally for several weeks after first root emergence into disturbed soil. This indicates that soil disturbance induced pioneer roots that develop absorptive fibrous roots from themselves. Thus, the results suggest that soil disturbance can affect not only amount of fine root production but also their morphological traits. In use of the observation methods, duration of more than three month after soil disturbance may be required before the data to show more or less natural patterns of fine root dynamics. Therefore, the soil disturbance effects should be taken into account in methods inescapably cutting roots and/or creating root-free soil.

In Chapter 3, seasonal variations of fine root production were clarified for several years in three forests dominated either by cypresses, deciduous oaks or beeches. In the cypress forest,

phenology of fine root production showed several variations such as spring dominant types or a continuous modal type. Although the beginning dates of root growth were concentrated on early April, the peak root production occurred in various periods within a year. In the oak stand, several peaks could be found in seasonal patterns of fine root production. However, the fine root phenology tended to be relatively stable across several years. In contrast, fine root phenology of the beech forest tended to follow a concentrated pattern with the peak typically occurring in summer or autumn. Fine roots in the beech stand began to grow usually between May and July, and that was obviously later than the cypress and oak forests probably due to a longer period of snow covering. Seasonal variations of fine root production were greatly affected by climate conditions, whereas the peak root production can be controlled by species-specific factors.

In Chapter 4, I focused on relationships between fine root dynamics and environmental and/or physiological factors in the cypress, oak and beech forests. The effects of environmental factors of temperature and soil moisture were examined mainly in the cypress and oak forests. On the other hand, the effects of physiological factors of leaf and seed production dynamics were evaluated in the beech forest in relation to the masting phenomenon. By statistical analysis using multivariate regressions, possible effects of those factors were detected for each forest. In the cypress and oak forests, temperature was a primary factor to control general trends of fine root phenology, and that induced fine root growth initiation in spring. Temperature had strong correlations with fine root production in spring, but that was not significant in autumn. This result suggests that fine root response to temperature varied depending on the season, and there was a hysteresis relationship between fine root phenology and temperature variations. On the other hand, soil moisture had negative effects on fine root production in summer. In the beech forest, a large amount of seed production was observed biennially. Fine root production in early growing season was facilitated with increasing annual leaf production. Also annual seed production was correlated with fine root produced in autumn. This

indicates that large interannual variations of seed production can affect resource allocation patterns between above- and below-ground production in the beech forest.

The possible relationships between fine root dynamics and environmental or physiological factors which were revealed in Chapter 4 may explain some characteristics of fine root phenology suggested in Chapter 3. In the cypress and oak forests, growth initiation of fine roots during spring (March and April) was controlled significantly by increase in temperature. Actually, the characterized phenology described similar patterns of growth initiation among the spring-autumn type, spring dominant type and continuous modal type. Those phenology patterns might be characterized by differences of fine root dynamics after the spring season. In the cypress forest, the negative correlation of soil moisture at August indicates low production during summer in the spring-autumn type and spring dominant type. Whereas fine root phenology in the oak forest characterized by the continuous modal type did not show clear trend of low production during summer, negative effects of soil moisture were detected. Those results imply that soil moisture affects fine root dynamics lightly and/or shortly in the oak forest than those in the cypress forest. However, the relationships between fine root dynamics and environmental factors should be interpreted carefully because the statistical results also indicate interactive effects between temperature and soil moisture on fine root production.

In the beech forest, there was a possible relationship between fine root dynamics and masting phenomena. However, the biennial masting behavior did not correspond interannual changes of characterized fine root phenology (autumn unimodal type and summer unimodal type). Because seed production was significantly related to fine root production in a limited period (September and October) not in entire seasonal variations. On the other hand, fine root production in early summer (June and July) had significant relationships with leaf production. Those results indicate that fine root phenology can be affected by leaf expansion in the earlier period and masting behavior in later period of the growing season.

In the present study, various patterns of fine root dynamics were observed by the *in situ* observation methods. These methods allowed description of detailed temporal variations of fine root dynamics which cannot be evaluated easily by the excavation methods. Long-term dynamics of fine roots were evaluated for over five years in the cypress, deciduous oak and beech forests. And the data of the fine root dynamics obtained by long-term and high frequency observations permit advanced statistical analyses with various factors such as soil disturbance, environmental and physiological conditions. Therefore, I could suggest a critical consideration on methodology for estimating fine root production in relation to soil disturbance, and reveal characteristics of fine root phenology among species and years. Furthermore, I could find many considerable effects of environmental and physiological factors on fine root production. Fine root response to changing environment should not be interpreted without consideration of its variability depending on the season. And fine roots have key roles in resource allocation mechanisms within a tree and their dynamics may be the possible proximate factor relating to the masting events in the beech forest. Although these mechanisms can be reasonably interpreted, the discussion cannot go beyond the level of presenting hypotheses. To achieve a comprehensive understanding of the factors controlling fine root dynamics, further experimental study is required, and environmental and physiological variables should be analyzed together in the same study site.

Acknowledgments

The present study was supported by Laboratory of Forest Utilization, Division of Forest and Biomaterials Sciences, Graduate school of Agriculture, Kyoto University, and by Laboratories of Silviculture and Macroecology, Department of Bioresources, Department of Agriculture, Shizuoka University. Professor Akira Osawa, Associate professor Naoki Okada, Assistant professor Masako Dannoura, and my laboratory members helped me a lot to conduct this study and they gave me valuable comments whenever. Especially, I'd like to appreciate Prof. Osawa, who is my supervisor for allowing use of accumulated scanning data of a long-term. My study of doctor course could not be conducted without his large amount of scanning data and his special supports. He supported most of my study in various ways. He induced me to break through problems about calculation and methodology using scanner data. And I'd like to thank Ms. Jiyoung An who is an alumna in Laboratory of Forest Utilization. She had established the stand plots and taken several series of data previously at the Ryukoku Forest. So I was able to work smoothly from the beginning of the present study. I also thank Mr. Yuto Kameda who is an alumnus in Laboratory of Forest Utilization for helping the field work and the data analysis.

Field works at the Naeba Mountains were accomplished by much support from research collaborators of Shizuoka University. I'd like to appreciate Prof. Mizunaga and associate Prof. Naramoto for allowing the work at permanent plots and use of research data in the study site. I also thank Ms. Sato. who is an alumna of Laboratory of Silviculture. She had established minirhizotron systems and taken several series of data previously in the study site.

I'd like to appreciate Prof. Miyaura of Ryukoku University for allowing the work at Ryukoku Forest. In addition, I really appreciate Dr. Nahoko Kurachi of Ryukoku University for suggesting me valuable comments and hints, helping the field work, and giving me the tree census data set. And I appreciate to Associate Prof. Taketo Yokota of Ryukoku University for allowing use of

meteorological data of Ryukoku Forest. The data helped me to conduct advanced analysis. I also thank Mr. Ishii, Mr. Shintani and Ms. Nakamura. They are alumni of Ryukoku University, and they often helped me to conduct the field works.

References

- Abramoff RZ, Finzi AC (2014) Are above-and below-ground phenology in sync? *New Phytol* 205:1054–1061. doi: 10.1111/nph.13111
- Addo-Danso SD, Prescott CE, Smith AR (2016) Methods for estimating root biomass and production in forest and woodland ecosystem carbon studies: A review. *For Ecol Manage* 359:332–351. doi: 10.1016/j.foreco.2015.08.015
- Adu MO, Chatot A, Wiesel L, Bennett MJ, Broadley MR, White PJ, Dupuy LX (2014) A scanner system for high-resolution quantification of variation in root growth dynamics of Brassica. *J Exp Bot* 65:2039–2048. doi: 10.1093/jxb/eru048
- Aerts R, Bakker C, Caluwe H De (1992) Root turnover as determinant of the cycling of C, N, and P in a dry heathland ecosystem. *Biogeochemistry* 15:175–190.
- Aerts R, Berendse F, Klerk NM, Bakker C (1989) Root production and root turnover in two dominant species of wet heathlands. *Oecologia* 81:374–378. doi: 10.1007/BF00377087
- Bates GH (1937) A device for the observation of root growth in the soil. *Nature* 139:966–967.
- Begon M, Colin RT, Harper JL (2006) *Ecology: From Individuals to Ecosystems*. Blackwell Publishing, Oxford
- Böhm W (1979) *Methods of studying root systems*. Springer Science & Business Media
- Box GEP, Cox DR (1964) An analysis of transformations. *J R Stat Soc Ser B* 211–252.
- Bragg PL, Govi G, Cannell RQ (1983) A comparison of methods, including angled and vertical minirhizotrons, for studying root growth and distribution in a spring oat crop. *Plant Soil* 73:435–440. doi: 10.1007/BF02184322
- Burke MK, Raynal DJ (1994) Fine root growth phenology, production, and turnover in a northern hardwood forest ecosystem. *Plant Soil* 162:135–146. doi: 10.1007/BF01416099
- Burton AJ, Pregitzer KS, Hendrick RL (2000) Relationships between fine root dynamics and nitrogen

- availability in Michigan northern hardwood forests. *Oecologia* 125:389–399. doi:
10.1007/s004420000455
- Comeau PG, Kimmins JP (1989) Above- and below-ground biomass and production of lodgepole pine on sites with differing soil moisture regimes. *Can J For Res* 19:447–454. doi: 10.1139/x89-070
- Dannoura M, Kominami Y, Makita N, Oguma H (2012) Flat optical scanner method and root dynamics. In: Mancuso S (ed) *Measuring Roots*. Springer, pp 127–133
- Dannoura M, Kominami Y, Oguma H, Kanazawa Y (2008) The development of an optical scanner method for observation of plant root dynamics. *Plant Root* 2:14–18. doi: 10.3117/plantroot.2.14
- Eissenstat DM (1991) On the relationship between specific root length and the rate of root proliferation: a field study using citrus rootstocks. *New Phytol* 118:63–68. doi: 10.1111/j.1469-8137.1991.tb00565.x
- Epron D, Osawa A (2017) Fine roots: when anisotropy matters. *Tree Physiol* 37:693–696. doi: 10.1093/treephys/tpx063
- Fahey TJ, Bledsoe CS, Day FP, Ruess RW, Smucker AJM (1999) Fine root production and demography. Stand soil methods long-term *Ecol Res Oxford Univ Press New York* 437–455.
- Fahey TJ, Hughes JW (1994) Fine root dynamics in a northern hardwood forest ecosystem, Hubbard Brook Experimental Forest, NH. *J Ecol* 82:533–548.
- Finér L, Helmisaari H-S, Lõhmus K, Majdi H, Brunner I, Børja I, Eldhuset T, Godbold D, Grebenc T, Konôpka B (2007) Variation in fine root biomass of three European tree species: Beech (*Fagus sylvatica* L.), Norway spruce (*Picea abies* L. Karst.), and Scots pine (*Pinus sylvestris* L.). *Plant Biosyst* 141:394–405.
- Finér L, Messier C, De Grandpré L (1997) Fine-root dynamics in mixed boreal conifer - broad-leafed forest stands at different successional stages after fire. *Can J For Res* 27:304–314. doi: 10.1139/x96-170

- Finér L, Ohashi M, Noguchi K, Hirano Y (2011) Fine root production and turnover in forest ecosystems in relation to stand and environmental characteristics. *For Ecol Manage* 262:2008–2023. doi: 10.1016/j.foreco.2011.08.042
- Fitter AH (2005) Darkness visible: Reflections on underground ecology. *J Ecol* 93:231–243. doi: 10.1111/j.1365-2745.2005.00990.x
- Forest Soil Division of Forestry and Forest Product Research Institute (1976) Classification of Forest Soil in Japan (1975). *Bull. Gov. For. Exp. Stn.* 280:1–28 (in Japanese).
- Fukuzawa K, Shibata H, Takagi K, Satoh F, Koike T, Sasa K (2013) Temporal variation in fine-root biomass, production and mortality in a cool temperate forest covered with dense understory vegetation in northern Japan. *For Ecol Manage* 310:700–710. doi: 10.1016/j.foreco.2013.09.015
- Germon A, Cardinael R, Prieto I, Mao Z, Kim J, Stokes A, Dupraz C, Laclau JP, Jourdan C (2016) Unexpected phenology and lifespan of shallow and deep fine roots of walnut trees grown in a silvoarable Mediterranean agroforestry system. *Plant Soil* 401:409–426. doi: 10.1007/s11104-015-2753-5
- Gill R a., Burke IC, Lauenroth WK, Milchunas DG (2002) Longevity and turnover of roots in the shortgrass steppe: Influence of diameter and depth. *Plant Ecol* 159:241–251. doi: 10.1023/A:1015529507670
- Gill R a., Jackson RB (2000) Global patterns of root turnover for terrestrial ecosystems. *New Phytol* 147:13–31. doi: 10.1046/j.1469-8137.2000.00681.x
- Godbold DL, Fritz H-W, Jentschke G, Meesenburg H, Rademacher P (2003) Root turnover and root necromass accumulation of Norway spruce (*Picea abies*) are affected by soil acidity. *Tree Physiol* 23:915–921. doi: 10.1093/treephys/23.13.915
- Gower ST, Vogt K a, Grier CC (1992) Carbon dynamics of Rocky Mountain Douglas-Fir: Influence of water and nutrient availability. *Ecol Monogr* 62:43–65.

- Grier CC, Vogt KA, Keyes MR, Edmonds RL (1981) Biomass distribution and above- and below-ground production in young and mature *Abies amabilis* zone ecosystems of the Washington Cascades. *Can J For Res* 11:155–167. doi: 10.1139/x81-021
- Guo D, Li H, Mitchell RJ, Han W, Hendricks JJ, Fahey TJ, Hendrick RL (2008a) Fine root heterogeneity by branch order : resolving the discrepancy in root longevity and turnover estimates between minirhizotron and C isotopic methods. *New Phytol* 177:443–456. doi: 10.1111/j.1469-8137.2007.02242.x
- Guo D, Mitchell RJ, Withington JM, Fan PP, Hendricks JJ (2008b) Endogenous and exogenous controls of root life span, mortality and nitrogen flux in a longleaf pine forest: Root branch order predominates. *J Ecol* 96:737–745. doi: 10.1111/j.1365-2745.2008.01385.x
- Han Q, Kabeya D, Hoch G (2011) Leaf traits, shoot growth and seed production in mature *Fagus sylvatica* trees after 8 years of CO₂ enrichment. *Ann Bot* 107:1405–1411. doi: 10.1093/aob/mcr082
- Han Q, Kabeya D, Iio A, Inagaki Y, Kakubari Y (2014) Nitrogen storage dynamics are affected by masting events in *Fagus crenata*. *Oecologia* 174:679–687. doi: 10.1007/s00442-013-2824-3
- Han Q, Kabeya D, Iio A, Kakubari Y (2008) Masting in *Fagus crenata* and its influence on the nitrogen content and dry mass of winter buds. *Tree Physiol* 28:1269–1276.
- Han Q, Kagawa A, Kabeya D, Inagaki Y (2016) Reproduction-related variation in carbon allocation to woody tissues in *Fagus crenata* using a natural ¹³C approach. *Tree Physiol* 00:1–10. doi: 10.1093/treephys/tpw074
- Heimann M, Reichstein M (2008) Terrestrial ecosystem carbon dynamics and climate feedbacks. *Nature* 451:289–292. doi: 10.1038/nature06591
- Hendrick RL, Pregitzer KS (1992) The demography of fine roots in a northern hardwood forest. *Ecology* 73:1094–1104. doi: 10.2307/1940183
- Hendrick RL, Pregitzer KS (1993) The dynamics of fine root length, biomass, and nitrogen content in two

- northern hardwood ecosystems. *Can J For Res* 23:2507–2520. doi: 10.1139/x93-312
- Hendrick RL, Pregitzer KS (1996) Applications of minirhizotrons to understand root function in forests and other natural ecosystems. *Plant Soil* 185:293–304. doi: 10.1007/BF02257535
- Hendrick RL, Pregitzer KS (1997) The relationship between fine root demography and the soil environment in northern hardwood forests. *Ecoscience* 4:99–105. doi: 10.1080/11956860.1997.11682383
- Hendricks JJ, Hendrick RL, Wilson C a., Mitchell RJ, Pecot SD, Guo D (2006) Assessing the patterns and controls of fine root dynamics: An empirical test and methodological review. *J Ecol* 94:40–57. doi: 10.1111/j.1365-2745.2005.01067.x
- Hertel D, Leuschner C (2002) A comparison of four different fine root production estimates with ecosystem carbon balance data in a *Fagus-Quercus* mixed forest. *Plant Soil* 239:237–251. doi: 10.1023/A:1015030320845
- Hertel D, Strecker T, Müller-Haubold H, Leuschner C (2013) Fine root biomass and dynamics in beech forests across a precipitation gradient - Is optimal resource partitioning theory applicable to water-limited mature trees? *J Ecol* 101:1183–1200. doi: 10.1111/1365-2745.12124
- Hirano Y, Noguchi K, Ohashi M, Hishi T, Makita N, Fujii S, Finér L (2009) A new method for placing and lifting root meshes for estimating fine root production in forest ecosystems. *Plant Root* 3:26–31. doi: 10.3117/plantroot.3.26
- Hishi T, Takeda H (2005) Dynamics of heterorhizic root systems: Protoxylem groups within the fine-root system of *Chamaecyparis obtusa*. *New Phytol* 167:509–521. doi: 10.1111/j.1469-8137.2005.01418.x
- Hozumi K (1973) Interaction among plant individuals. Ecology Series 10. Kyoritsu Shuppan, Tokyo (in Japanese)
- Hozumi K (1985) Phase diagrammatic approach to the analysis of growth curve using theu-w diagram—

- Basic aspects. *J Plant Res* 98:239–250.
- Hozumi K (1987) Analysis of growth curve of stem volume in some woody species using the u-w diagram. *Bot Mag* 100:87–97. doi: 10.1007/BF02488422
- Hutchinson GE (1978) An introduction to population ecology. Hew Haven Connecticut Yale University Press 1978.
- Isagi Y, Sugimura K, Sumida a, Ito H (1997) How does masting happen and synchronize? *J Theor Biol* 187:231–239. doi: 10.1006/jtbi.1997.0442
- IUSS Working Group (2014) World reference base for soil resources 2014 international soil classification system for naming soils and creating legends for soil maps.
- Janssens IA, Sampson DA, Curiel-Yuste J, Carrara A, Ceulemans R (2002) The carbon cost of fine root turnover in a Scots pine forest. *For Ecol Manage* 168:231–240. doi: 10.1016/S0378-1127(01)00755-1
- Jentschke G, Drexhage M, Fritz HW, Fritz E, Schella B, Lee DH, Gruber F, Heimann J, Kuhr M, Schmidt J, Schmidt S, Zimmermann R, Godbold DL (2001) Does soil acidity reduce subsoil rooting in norway spruce (*picea abies*)? *Plant Soil* 237:91–108. doi: 10.1023/A:1013305712465
- Jiménez EM, Moreno FH, Lloyd J, Peñuela MC, Patiño S (2009) Fine root dynamics for forests on contrasting soils in the colombian Amazon. *Biogeosciences Discuss* 6:3415–3453. doi: 10.5194/bgd-6-3415-2009
- Johnson MG, Tingey DT, Phillips DL, Storm MJ (2001) Advancing fine root research with minirhizotrons. *Environ Exp Bot* 45:263–289.
- Joslin JD, Wolfe MH (1999) Disturbances during minirhizotron installation can affect root observation data. *Soil Sci Soc Am J* 63:218–221. doi: 10.2136/sssaj1999.03615995006300010031x
- Joslin JD, Wolfe MH, Hanson PJ (2001) Factors controlling the timing of root elongation intensity in a mature upland oak stand. *Plant Soil* 228:201–212. doi: 10.1023/A:1004866705021

- Kakubari Y (1977) Beech forests in the Naeba Mountains I. Distribution of primary productivity along the altitudinal gradients.
- Karizumi N (1979) Illustrations of tree roots. Seibundo Shinkosha Publishing Co. Ltd. (in Japanese)
- Keyes MR, Grier CC (1981) Above- and below-ground net production in 40-year-old Douglas-fir stands on low and high productivity sites. *Can J For Res* 11:599–605. doi: 10.1139/x81-082
- Kominami Y, Jomura M, Ataka M, Tamai K, Miyama T, Dannoura M, Makita N, Yoshimura K (2012) Heterotrophic respiration causes seasonal hysteresis in soil respiration in a warm-temperate forest. *J For Res* 17:296–304. doi: 10.1007/s10310-011-0315-0
- Kon H, Noda T (2007) Experimental investigation on weather cues for mast seeding of *Fagus crenata*. *Ecol Res* 22:802–806. doi: 10.1007/s11284-006-0320-5
- Kubota M, Tenhunen J, Zimmermann R, Schmidt M, Kakubari Y (2005) Influence of environmental conditions on radial patterns of sap flux density of a 70-year *Fagus crenata* trees in the Naeba Mountains, Japan. *Ann For Sci* 62:289–296. doi: 10.1051/forest.2005023
- Laiho R, Bhuiyan R, Straková P, Mäkiranta P, Badorek T, Penttilä T (2014) Modified ingrowth core method plus infrared calibration models for estimating fine root production in peatlands. *Plant Soil* 385:311–327. doi: 10.1007/s11104-014-2225-3
- Lauenroth W (2000) Methods of Estimating Belowground Net Primary Production. In: Sala O, Jackson R, Mooney H, Howarth R (eds) *Methods in Ecosystem Science* SE - 5. Springer New York, pp 58–71
- Li X, Zhu J, Lange H, Han S (2012) A modified ingrowth core method for measuring fine root production, mortality and decomposition in forests. *Tree Physiol* 33:18–25. doi: 10.1093/treephys/tps124
- Liu C, Xiang W, Lei P, Deng X, Tian D, Fang X, Peng C (2014) Standing fine root mass and production in four Chinese subtropical forests along a succession and species diversity gradient. *Plant Soil*

376:445–459. doi: 10.1007/s11104-013-1998-0

López B, Sabaté S, Gracia CA (2001) Annual and seasonal changes in fine root biomass of a *Quercus ilex* L. forest. *Plant Soil* 230:125–134.

Ma Z, Guo D, Xu X, Lu M, Bardgett RD, Eissenstat DM, McCormack ML, Hedin LO (2018) Evolutionary history resolves global organization of root functional traits. *Nature* 555:94–97. doi: 10.1038/nature25783

Majdi H (1996) Root sampling methods - Applications and limitations of the minirhizotron technique. *Plant Soil* 185:255–258. doi: 10.1007/BF02257530

Majdi H, Öhrvik J (2004) Interactive effects of soil warming and fertilization root production, mortality, and longevity in a Norway spruce stand in Northern Sweden. *Glob Chang Biol* 10:182–188. doi: 10.1111/j.1365-2486.2004.00733.x

Majdi H, Pregitzer K, Morén AS, Nylund JE, I. Ågren G (2005) Measuring fine root turnover in forest ecosystems. *Plant Soil* 276:1–8. doi: 10.1007/s11104-005-3104-8

Makita N, Kosugi Y, Kawamura M (2014) Linkages between diurnal patterns of root respiration and leaf photosynthesis in *Quercus crispula* and *Fagus crenata* seedlings. *J Agric Meteorol* 70:151–162. doi: 10.2480/agrmet.D-14-00006

Masaki T, Oka T, Osumi K, Suzuki W (2008) Geographical variation in climatic cues for mast seeding of *Fagus crenata*. *Popul Ecol* 50:357–366. doi: 10.1007/s10144-008-0104-6

Mason GF, Bhar DS, Hilton RJ (1970) Root growth studies on Mugho pine. *Can J Bot* 48:43–47.

McCormack ML, Adams TS, Smithwick E a H, Eissenstat DM (2014) Variability in root production, phenology, and turnover rate among 12 temperate tree species. *Ecology* 95:2224–2235. doi: 10.1890/13-1942.1

McCormack ML, Dickie IA, Eissenstat DM, Fahey TJ, Fernandez CW, Guo D, Helmisaari HS, Hobbie EA, Iversen CM, Jackson RB, Leppälammii-Kujansuu J, Norby RJ, Phillips RP, Pregitzer KS,

- Pritchard SG, Rewald B, Zadworny M (2015) Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New Phytol* 207:505–518. doi: 10.1111/nph.13363
- Mei L, Gu J, Zhang Z, Wang Z (2010) Responses of fine root mass, length, production and turnover to soil nitrogen fertilization in *Larix gmelinii* and *Fraxinus mandshurica* forests in Northeastern China. *J For Res* 15:194–201. doi: 10.1007/s10310-009-0176-y
- Milchunas DG (2009) Estimating root production: Comparison of 11 methods in shortgrass steppe and review of biases. *Ecosystems* 12:1381–1402. doi: 10.1007/s10021-009-9295-8
- Ministry of Land Infrastructure Transportation and Tourism (1982) Land and real property in Japan. In: 1/50,000 L. Classif. base Surv. Soil map. Kyoto Tohokubu Kyoto Tonanbu (in Japanese).
- Miyaura T (2009) Forest changes at Seta Hill after the World War II. A 2008 Report of the Satoyama Regional Symbiosis Studies Open Research Center, Ryukoku University (in Japanese)
- Monserud RA (1984) Height growth and site index curves for inland Douglas fir based on stem analysis data and forest habitat type. *For Sci* 30 (4):943–965.
- Montagnoli A, Di Iorio A, Scippa GS, Chiatante D (2007) Turnover in fine root is affected by forest management of beech stands (*Fagus sylvatica* L.). In: 4th International Symposium on Physiological Processes in Roots of Woody Plants. Book of Abstracts.
- Nadelhoffer KJ, Aber JD, Melillo JM (1985) Fine roots, net primary production, and soil nitrogen availability: a new hypothesis. *Ecology* 66:1377–1390.
- Nadelhoffer KJ, Raich JW (1992a) Fine root production estimates and belowground carbon allocation in forest ecosystems. *Ecology* 73:1139–1147.
- Nadelhoffer KJ, Raich JW (1992b) Fine Root Production Estimates and Belowground Carbon Allocation in Forest Ecosystems. 73:1139–1147.
- Nakano A, Ikeno H, Kimura T, Sakamoto H, Dannoura M, Hirano Y, Makita N, Finér L, Ohashi M

- (2012) Automated analysis of fine-root dynamics using a series of digital images. *J Plant Nutr Soil Sci* 175:775–783. doi: 10.1002/jpln.201100316
- Neill C (1992) Comparison of Soil Coring and Ingrowth Methods for Measuring Belowground Production. *Ecology* 73:1918–1921.
- Noguchi K, Han Q, Araki MG, Kawasaki T, Kaneko S, Takahashi M, Chiba Y (2011) Fine-root dynamics in a young hinoki cypress (*Chamaecyparis obtusa*) stand for 3 years following thinning. *J For Res* 16:284–291. doi: 10.1007/s10310-010-0221-x
- Noguchi K, Konôpka B, Satomura T, Kaneko S, Takahashi M (2007) Biomass and production of fine roots in Japanese forests. *J For Res* 12:83–95. doi: 10.1007/s10310-006-0262-3
- Norby R, Fitter A, Jackson R (2000) Root dynamics and global change: An ecosystem perspective. *New Phytol* 147:3–12.
- Obara H, Maejima Y, Kohyama K, Ohkura T, Takata Y (2015) Outline of the Comprehensive Soil Classification System of Japan—First Approximation. *Japan Agric Res Q* 49:217–226.
- Ohashi M, Nakano A, Hirano Y, Noguchi K, Ikeno H, Fukae R, Yamase K, Makita N, Finér L (2015) Applicability of the net sheet method for estimating fine root production in forest ecosystems. *Trees* 30:571–578. doi: 10.1007/s00468-015-1308-y
- Oliveira M do RG, Noordwijk M van, Gaze SR, Brouwer G, Bonas S, Moscas G, Hairiah K (2000) Auger Sampling, Ingrowth Cores and Pinboard Methods. In: Smit AL, Bengough AG, Engels C, et al. (eds) *Root Methods*. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 175–210
- Osawa A, Aizawa R (2012) A new approach to estimate fine root production, mortality, and decomposition using litter bag experiments and soil core techniques. *Plant Soil* 355:167–181. doi: 10.1007/s11104-011-1090-6
- Osawa A, Allen RB (1993) Allometric theory theory explains self-thinning relationships of mountain beech and red pine. *Ecology* 74:1020–1032.

- Ostonen I, Lõhmus K, Pajuste K (2005) Fine root biomass, production and its proportion of NPP in a fertile middle-aged Norway spruce forest: Comparison of soil core and ingrowth core methods. *For Ecol Manage* 212:264–277. doi: 10.1016/j.foreco.2005.03.064
- Persson H (1980) Spatial Distribution of Fine-Root Growth, Mortality and Decomposition in a Young Scots Pine Stand in Central Sweden. *Oikos* 34:77–87. doi: 10.2307/3544552
- Pregitzer K, DeForest J, Burton AJ, Allen MF, Ruess RW, Hendrick RL (2002) Fine root architecture of nine North American trees. *Ecol Monogr* 72:293–309. doi: 10.1890/0012-9615(2002)072[0293:FRAONN]2.0.CO;2
- Price JS, Hendrick RL (1998) Fine root length production, mortality and standing root crop dynamics in an intensively managed sweetgum (*Liquidambar styraciflua* L.) coppice. *Plant Soil* 205:193–201. doi: 10.1023/A:1004324714371
- Pritchard SG, Strand AE, McCormack ML, Davis MA, Oren R (2008) Mycorrhizal and rhizomorph dynamics in a loblolly pine forest during 5 years of free-air-CO₂-enrichment. *Glob Chang Biol* 14:1252–1264.
- Raich JW, Nadelhoffer KJ (1989) Belowground carbon allocation in forest ecosystems: global trends. *Ecology* 70:1346–1354.
- Ruess RW, Hendrick RL, Burton AJ, Pregitzer KS, Sveinbjornsson B, Allen ME, Maurer GE (2003) Coupling fine root dynamics with ecosystem carbon cycling in black spruce forests of interior Alaska. *Ecol Monogr* 73:643–662. doi: 10.1890/02-4032
- Satake a, Iwasa Y (2000) Pollen coupling of forest trees: forming synchronized and periodic reproduction out of chaos. *J Theor Biol* 203:63–84. doi: 10.1006/jtbi.1999.1066
- Satomura T, Hashimoto Y, Koizumi H, Nakane K, Horikoshi T (2006) Seasonal patterns of fine root demography in a cool-temperate deciduous forest in central Japan. *Ecol Res* 21:741–753. doi: 10.1007/s11284-006-0182-x

- Scott DW, Terrell GR (1987) Biased and unbiased cross-validation in density estimation. *J Am Stat Assoc* 82:1131–1146.
- Shilo T, Rubin B, Ephrath JE, Eizenberg H (2013) Continuous non-destructive monitoring of *Cyperus rotundus* development using a minirhizotron. *Weed Res* 53:164–168. doi: 10.1111/wre.12015
- Shinozaki K, Kira T (1956) Intraspecific competition among higher plants VII. Logistic theory of the C-D effect. *J Inst Polytechnics, Osaka City Univ Series D7*:35–72.
- Steele SJ, Gower ST, Vogel JG, Norman JM (1997) Root mass, net primary production and turnover in aspen, jack pine and black spruce forests in Saskatchewan and Manitoba, Canada. *Tree Physiol* 17:577–587.
- Steinaker DF, Wilson SD, Peltzer DA (2010) Asynchronicity in root and shoot phenology in grasses and woody plants. *Glob Chang Biol* 16:2241–2251. doi: 10.1111/j.1365-2486.2009.02065.x
- Steingrobe B, Schmid H, Claassen N (2000) The use of the ingrowth core method for measuring root production of arable crops - influence of soil conditions inside the ingrowth core on root growth. *J Plant Nutr Soil Sci* 163:617–622. doi: 10.1002/1522-2624(200012)163:6<617::AID-JPLN617>3.0.CO;2-0
- Steingrobe B, Schmid H, Claassen N (2001) The use of the ingrowth core method for measuring root production of arable crops - Influence of soil and root disturbance during installation of the bags on root ingrowth into the cores. *Eur J Agron* 15:143–151. doi: 10.1016/S1161-0301(01)00100-9
- Tateno R, Hishi T, Takeda H (2004) Above- and belowground biomass and net primary production in a cool-temperate deciduous forest in relation to topographical changes in soil nitrogen. *For Ecol Manage* 193:297–306. doi: 10.1016/j.foreco.2003.11.011
- Teskey RO, Hinckley TM (1981) Influence of temperature and water potential on root growth of white oak. *Physiol Plant* 52:363–369. doi: 10.1111/j.1399-3054.1981.tb06055.x
- Tierney GL, Fahey TJ, Groffman PM, Hardy JP, Fitzhugh RD, Driscoll CT, Yavitt JB (2003)

- Environmental control of fine root dynamics in a northern hardwood forest. *Glob Chang Biol* 9:670–679. doi: 10.1046/j.1365-2486.2003.00622.x
- Tierney GL, Fahey TJ, Peter M, Hardy JP, Fitzhugh RD, Driscoll CT (2001) Soil freezing alters fine root dynamics in a northern hardwood forest. 175–190.
- Tingey DT, Johnson MG, Phillips DL, Johnson DW, Ball JT (1996) Effects of elevated CO₂ and nitrogen on the synchrony of shoot and root growth in ponderosa pine. *Tree Physiol* 16:905–914.
- Tryon PR, Chapin III FS (1983) Temperature control over root growth and root biomass in taiga forest trees. *Can J For Res* 13:827–833. doi: 10.1139/x83-112
- Vamerli T, Bandiera M, Mosca G (2012) Minirhizotrons in Modern Root Studies. In: Mancuso S (ed) *Measuring Roots: An Updated Approach*. Springer Berlin Heidelberg, Berlin, Heidelberg,
- Van Do T, Sato T, Saito S, Kozan O (2015) Fine-root production and litterfall: main contributions to net primary production in an old-growth evergreen broad-leaved forest in southwestern Japan. *Ecol Res* 30:921–930. doi: 10.1007/s11284-015-1295-x
- Verhulst PF (1838) Notice sur la loi que la population poursuit dans son accroissement. In: *Correspondance mathématique et physique* 10. pp 113–121
- Vogt KA, Grier CC, Meier CE, Edmonds RL, Vogt A, Grier CC, Meier CE (1982) Mycorrhizal Role in Net Primary Production and Nutrient Cycling in *Abies Amabilis* Ecosystems in Western Washington Linked references are available on JSTOR for this article : MYCORRHIZAL ROLE IN NET PRIMARY PRODUCTION AND NUTRIENT CYCLING IN *ABIES AMABILIS*. 63:370–380.
- Vogt KA, Persson H (1991) Measuring growth and development of roots. *Tech approaches For tree Ecophysiol* 477–501.
- Vogt KA, Vogt DJ, Bloomfield J (1998) Analysis of some direct and indirect methods for estimating root biomass and production of forests at an ecosystem level. In: Box Jr. J (ed) *Root Demographics*

- and Their Efficiencies in Sustainable Agriculture, Grasslands and Forest Ecosystems SE - 61.
Springer Netherlands, pp 687–720
- Vogt KA, Vogt DJ, Palmiotto PA, Boon P, O'Hara J, Asbjornsen H (1996) Review of root dynamics in forest ecosystems grouped by climate, climatic forest type and species. *Plant Soil An Int. J. Plant-Soil Relationships* 187:159–219.
- Wand MP, Jones MC (1994) Kernel smoothing. Chapman and Hall/CRC
- Wood SN (2003) Thin plate regression splines. *J R Stat Soc Ser B Stat Methodol* 65:95–114. doi: 10.1111/1467-9868.00374
- Yamato M, Iwase K (2005) Community analysis of arbuscular mycorrhizal fungi in a warm-temperate deciduous broad-leaved forest and introduction of the fungal community into the seedlings of indigenous woody plants. *Mycoscience* 46:334–342. doi: 10.1007/s10267-005-0256-6
- Yasumura Y, Hikosaka K, Hirose T (2006) Resource allocation to vegetative and reproductive growth in relation to mast seeding in *Fagus crenata*. *For Ecol Manage* 229:228–233. doi: 10.1016/j.foreco.2006.04.003
- Yoda K, Kira T, Ogawa H, Hozumi K (1963) Self-thinning in overcrowded pure stands under cultivated and natural conditions. *J Biol Osaka City Univ* 14:107–129.

Appendix

Table A1 Dates of scanner installation (SI), first root emergence (FE), and last scanning (LS) in each flat root scanner. Duration between dates of scanner installation and last scanning, and between dates of scanner installation and first root emergence were calculated using those dates

Plot	Scanner No.	Date of scanner installation (SI)	First date of root emergence (FE)	Last date of scanning (LS)	Duration between SI and LS (d)	Duration between SI and FE (d)
Cypress stand						
	H11	2009/6/12	2009/7/10	2016/1/23	2416	28
	H12	2009/6/12	2009/7/16	2013/9/25	1566	34
	H13a	2010/3/27	2010/6/25	2012/5/28	793	90
	H13b	2012/8/12	2012/8/24	2014/10/2	781	12
	H14	2011/4/21	2011/7/8	2016/1/23	1738	78
	H15	2011/4/21	2011/7/15	2016/1/23	1738	85
	Averages				1505	55
Oak stand						
	K21	2011/4/21	2011/7/8	2014/9/9	1237	78
	K22	2011/4/21	2011/5/27	2015/12/5	1689	36
	K23	2011/4/21	2011/7/8	2015/8/6	1568	78
	K24	2011/4/21	2011/7/15	2016/1/23	1738	85
	K25	2012/3/15	2012/6/13	2016/1/23	1409	90
	Averages				1528	73
Overall averages					1515	63

Table A2 Estimated values of asymptotic upper limit (A ; mm² cm⁻²), growth coefficient (λ ; d⁻¹) and day of the year of the maximum value of inclination (t_{DOY} ; d) by fitting the logistic curve (Eq. 5) for cumulative root area production (P_{are}) in each scanner and elapsed year from scanner installation. The t_{DOY} was calculated by the following equation: $t_{DOY} = \ln k \cdot \lambda^{-1}$, where k is an integration constant of the logistic equation

Forest type	Scanner No.	Asymptotic upper limit (A ; mm ² cm ⁻²)						Growth coefficient (λ ; d ⁻¹)						Day of year on maximum inclination (t_{DOY} ; d)					
		Immediate	2nd	3rd	4th	5th	6th	Immediate	2nd	3rd	4th	5th	6th	Immediate	2nd	3rd	4th	5th	6th
Cypress stand																			
	H11	8.78	4.22	2.24	1.36	0.28	0.82	0.024	0.016	0.072	0.042	0.048	0.019	256	171	140	160	153	274
	H12	7.92	3.39	0.80	0.20			0.109	0.021	0.028	0.031			232	220	175	123		
	H13a	17.55	1.58	3.03				0.029	0.019	0.057				251	144	115			
	H13b	12.25	2.57	9.67				0.076	0.025	0.024				298	169	311			
	H14	6.25	4.15	1.76	2.01			0.022	0.057	0.128	0.011			264	132	206	254		
	H15	3.51	0.63	0.82	2.92			0.100	0.054	0.030	0.012			305	134	228	382		
	Average	9.38	2.76	3.05	1.62	0.28	0.82	0.060	0.032	0.056	0.024	0.048	0.019	268	162	196	230	153	274
Oak stand																			
	K21	1.69	3.86					0.109	0.029					200	218				
	K22	2.22	2.06	3.33	2.73			0.156	0.013	0.038	0.029			201	232	156	251		
	K23	7.89	5.36	4.72	5.20			0.120	0.053	0.039	0.049			206	151	175	170		
	K24	7.02	2.80	4.85	5.99			0.062	0.033	0.049	0.023			223	183	200	206		
	K25	7.31	4.57	4.60				0.033	0.064	0.016				215	122	244			
	Average	5.22	3.73	4.38	4.64			0.096	0.038	0.035	0.034			209	181	194	209		
Overall average		7.49	3.20	3.58	2.92	0.28	0.82	0.076	0.035	0.048	0.028	0.048	0.019	241	171	195	221	153	274

Table A3 The correlation matrix of the seasonal patterns of fine root production. Characters of “H”, “K” and “N” represent the forest stands of cypresses, oaks and beeches, respectively, and the following numbers correspond to the years

	H2011	H2012	H2013	H2014	H2015	H2016	H2017	K2012	K2013	K2014	K2015	K2016	N2010	N2011	N2012	N2013	N2014	N2015
H2012	0.63*	1.00																
H2013	0.11	0.32	1.00															
H2014	0.00	0.12	0.63*	1.00														
H2015	0.15	0.17	0.65*	0.91*	1.00													
H2016	0.71*	0.71*	0.01	0.18	0.31	1.00												
H2017	0.26	0.67*	0.59*	0.46*	0.40	0.31	1.00											
K2012	0.25	0.44*	0.67*	0.83*	0.85*	0.28	0.65*	1.00										
K2013	0.41	0.64*	0.83*	0.67*	0.77*	0.45*	0.63*	0.83*	1.00									
K2014	0.02	0.30	0.70*	0.90*	0.82*	0.10	0.61*	0.92*	0.76*	1.00								
K2015	0.39	0.45*	0.68*	0.74*	0.88*	0.47*	0.45*	0.84*	0.87*	0.70*	1.00							
K2016	0.30	0.48*	0.39	0.62*	0.66*	0.46*	0.60*	0.68*	0.65*	0.63*	0.69*	1.00						
N2010	-0.43*	-0.02	0.63*	0.62*	0.44*	-0.33	0.45*	0.53*	0.43*	0.71*	0.22	0.24	1.00					
N2011	-0.23	-0.14	0.59*	0.70*	0.67*	-0.17	0.13	0.62*	0.51*	0.70*	0.46*	0.14	0.77*	1.00				
N2012	-0.48*	-0.29	0.46*	0.73*	0.65*	-0.24	0.20	0.51*	0.33	0.66*	0.30	0.20	0.73*	0.52*	1.00			
N2013	-0.29	-0.11	0.62*	0.68*	0.55*	-0.27	0.37	0.56*	0.38	0.73*	0.32	0.21	0.65*	0.45*	0.54*	1.00		
N2014	-0.60*	-0.19	0.43*	0.59*	0.41	-0.45*	0.40	0.48*	0.25	0.68*	0.12	0.29	0.77*	0.42*	0.81*	0.54*	1.00	
N2015	-0.56*	-0.59*	0.35	0.64*	0.58*	-0.43*	-0.12	0.31	0.13	0.45*	0.28	0.10	0.51*	0.34	0.59*	0.44*	0.66*	1.00
N2016	-0.41	0.12	0.47*	0.35	0.13	-0.36	0.50*	0.35	0.29	0.54*	-0.01	0.18	0.97*	0.74*	0.71*	0.65*	0.81*	0.44*

*: $P < 0.05$,

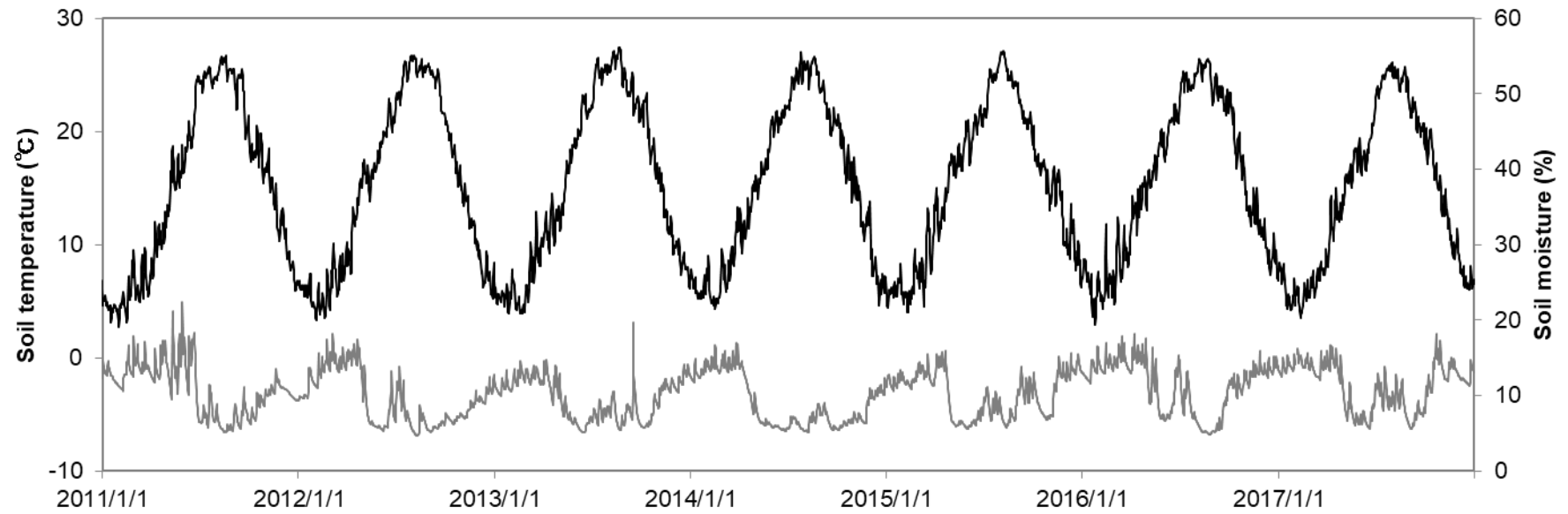


Fig. A1 Daily values of mean soil temperature (black line) and mean soil moisture (volumetric water content) (gray line) from 2010 to 2017 in the Ryukoku Forest. The observation values were monitored nearby the observation tower in the study site