

# Phenology of fine roots and shoots using high frequency temporal resolution images in a temperate larch forest

**Azusa Tamura**

Shinshu University

**Hiroyuki Oguma**

National Institute for Environmental Studies

**Roma Fujimoto**

Shinshu University

**Masatoshi Kuribayashi**

Nagano Environmental Conservation Research Institute

**Naoki Makita** (✉ [macky@shinshu-u.ac.jp](mailto:macky@shinshu-u.ac.jp))

Shinshu University <https://orcid.org/0000-0002-8701-766X>

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## Research Article

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# Abstract

## Purpose

Understanding tree phenology reveals the underlying mechanisms through plant functional and productive activities and carbon sinks in forest ecosystems. However, previous research on tree phenology has focused on shoot dynamics rather than tree root dynamics. We aimed to explore seasonal temperature patterns of daily-based root and shoot dynamics by capturing high frequency plant images in a larch forest.

## Methods

We monitored continuous images using an automated digital camera for shoot dynamics and a flatbed scanner for the fine root dynamics in the larch. Using the images, we analyzed the relationship between temperature and plant area index as shoot growth status and total root-area proportion of white and brown roots.

## Results

Larch shoot production had a single mountain-shaped peak with a positive correlation between plant area index and air temperature. Fine root production had two peaks in the bimodal root-growth pattern in early summer and late autumn. Soil temperature was positively correlated with white root proportion and negatively correlated with brown root proportion.

## Conclusion

We found differences between shoots and roots regarding temperature relationships. In particular, the automated flatbed scanner method for the root dynamics allowed the collection of detailed bimodal patterns of root production with shift from whitening to browning color, which had been previously overlooked. Such high frequency temporal resolution analysis can provide an in-depth of mechanisms of fine-root and shoot phenology through different stages of plant development in terms of growth and senescence.

## Introduction

Seasonal variations in forest carbon (C) balance and C allocation have strong phenological components (Baldocchi and Baldocchi 2008; White et al. 2009). The timing of leaf development in spring and leaf abscission in autumn showed variability in C balance and allocation in trees. Previous studies have shown that changes in phenological events can also have a significant impact on ecosystem functions, such as the C cycle (Keeling et al. 1996; Piao et al. 2007; Piao et al. 2008). For example, extended growth

periods have been shown to increase annual gross primary production (GPP) by approximately  $8 \text{ g C m}^{-2} \text{ d}^{-1}$  in temperate and northern deciduous forests, and by approximately  $5 \text{ g C m}^{-2} \text{ d}^{-1}$  in northern policy foliage forests (Falge et al. 2002). In modeling studies, when the growth period of the northern ecosystem extends one day, it has been shown that the annual GPP increases by 0.5–0.6% (Piao et al. 2007; White et al. 2009). Therefore, understanding plant phenology reveals the underlying mechanisms through plant functional and productive activities and C sinks in terrestrial ecosystems.

Phenology is affected by climate zone, vegetation type, and yearly variability in weather. Plant organic matter is produced through photosynthesis; therefore, phenology is adjusted to maximize C assimilation by photosynthesis in the growing environment of the plant shoot (Chabot and Hicks 1982; Kikuzawa 1991). Temperature is generally considered a major driver of shoot phenology (Cleland et al. 2007; Peñuelas et al. 2009; Chuine 2010). Temperature can be the most important environmental factor controlling the timing of spring foliation and autumn senescence phenology in shoots (Wielgolaski 1999; Keenan and Richardson 2015). However, the research on fine root phenology is limited. Tree fine roots, which are defined as  $<2 \text{ mm}$  in diameter, are an important component of ecosystem C, water, and nutrient cycling (Jackson et al. 1997). Fine roots comprise only a small part of the entire tree biomass; however, they constitute a large fraction of annual net primary production and contribute to  $\text{CO}_2$  fluxes from soil and C accumulation in forest soils (Finér et al. 2011; Makita et al. 2021). Thus, knowledge of fine root phenology is important for understanding C dynamics and accumulation in forest ecosystems (Joslin and Henderson 1987; Hendrick and Pregitzer 1993; Zhao et al. 2017).

There are several methods to analyze fine root production and turnover to overcome the poor accessibility of belowground (Vamerali et al. 2012). Conventional methods require destructive soil sampling, such as pinboards, ingrowth mesh bags, and soil coring. In addition, repeated non-destructive observation strategies, such as use of rhizotrons, minirhizotrons, and optical scanners, allow the tracking of the same roots (Milchunas 2009). Minirhizotron method is widely used in a variety of plant species for the study of fine root production, mortality, and longevity (Hendrick and Pregitzer 1996; Johnson et al. 2001; Guo et al. 2008). Recently, automated scanners for root dynamics have been developed and used to provide temporally dense datasets (Dannoura et al. 2012; Ding et al. 2020). While manual observations are well suited for covering spatial variability, the automated scanners can provide an important opportunity to study the temporal variation of growth rates during periods unfavorable for manual measurements, such as night and rainfall. Because of this high temporal resolution, it is also possible to catch the production of new roots and mortality between long observation intervals without being detected (Johnson et al. 2001). In addition, it is possible to observe the response of root dynamics to rapid temporal changes in environmental conditions more effectively without either linear interpolation or models. Here, we used an automated optical scanner method (Dannoura et al. 2008), which uses an A4 flatbed scanner. This allows researchers to non-destructively follow the growth of the same roots at a high temporal resolution.

One crucial question is as follows: How does fine root phenology link with shoot phenology? The answer is important to interpret the integration of whole tree physiology and growth in forest ecosystems. More evidence is needed to validate the temporal patterns of shoots and fine roots in response to plant

phenological attributes based on the non-destructive and high frequency field observation. Forest trees allocate the assimilated C to several parts to reduce any imbalance between C fixation by shoots and soil resource acquisition by roots (Shipley and Meziane 2002). Abramoff and Finzi (2015) reported that the relationship between shoot production and air temperature is positively correlated in temperate forests, and there is a positive correlation between fine root production and soil temperature. Nevertheless, it remains unclear whether root phenological patterns are related to shoot phenology across seasons.

In general, current phenological studies only focus on changes in fine root and shoot production, which do not consider plant growth stages or life events (such as autumn leaf senescence and secondary growth of fine roots). As a result, leaf and root productivity and high productivity periods were overestimated or underestimated. As described below, both photosynthetic capacity of shoots and nutrient absorption ability of fine roots decrease depending on the dormant season and growth stage. To observe the phenology based on the physiological function and growth stage of plants in the present study, we focused on the seasonal changes in the color of shoots and fine roots and. The use of automated digital cameras to monitor the vegetation status is becoming widespread. Digital repeat photographs were used to characterize the development of the leaf area (Garrity et al. 2011). Keenan et al. (2014) show that the observed phenological transitions of bud burst and leaf senescence can be well characterized by digital repeat photography. Changes in shoots related to phenology (i.e., developmental stage) occur in various aspects of leaf color and pigmentation in terms of photosynthetic capacity over the course of the growth season (Ma et al. 2011; McKown et al. 2013). For color of fine roots, the white roots gradually changed to brown. White roots absorb nutrients or water, and the brown roots transport them. Brown roots have a cork layer, which is an effective barrier against external stresses such as drought, disease, or herbivory; however, it also causes a considerable decrease in absorptive ability (Hishi and Takeda 2005). By focusing on the seasonal changes in the color of the plant, it is also possible to understand the growth stage and function of plant growth.

In this study, high temporal resolution images of shoots were captured by the digital camera and those of fine roots by the flatbed scanner for phenological observations. This is useful for identifying the relationships between phenology and driving environmental factors. We used images to follow the phenology of shoots and fine roots of deciduous conifer (*Larix kaempferi*), which is a major species in Japanese forests. Our primary objectives were to: (1) consider the automatic analysis method of root images, (2) elucidate seasonal variations in the production and physiological function of shoots and fine roots, (3) investigate the relationship between phenology patterns and temperature, and (4) determine the relationship between fine root color and soil temperature.

## Materials And Methods

### Study site

The study site was located on Mt. Iizuna, Nagano, central Japan (138 ° 9'13 "E, 36 ° 43'31' N). The annual precipitation is 1,551 mm, and the mean annual temperature is 8.3 °C (Kuribayashi and Hamada

2020). The site is a planted forest of *L. kaempferi*, a deciduous conifer. In November 2018, the forest was 61 years old, and the average diameter at breast height was 30.0 cm (Ozeki and Kuribayashi 2019). The soil contained volcanic ash-derived Andosols. We established a 40 × 40 m plot at the site where *L. kaempferi* was dominant. The tree density of *L. kaempferi* in the plot was 619 trees ha<sup>-1</sup>, and the stand basal area was 45 m<sup>2</sup> ha<sup>-1</sup> (Ozeki and Kuribayashi 2019). At this site, there have been studies conducted on soil C dynamics, especially regarding the contribution of autotrophic and heterotrophic respiration to soil respiration (Makita et al. 2021).

Air temperature was measured at a height of 1.8 m using a temperature probe (HMP155, Vaisala, Finland). Precipitation was measured with a tipping bucket rain gauge (7852-II Davis Instruments, USA). For soil environmental monitoring, soil temperatures and soil moisture (volumetric water content) at 10-cm depth were measured (WD-3-WET-5Y, ARP Co., Japan). All data were logged continuously.

### **Observation of shoot phenology**

To obtain canopy images for shoot phenology, we set a digital camera (D3300, Nikon Corporation, Tokyo, Japan) with a Sigma fish-eye lens at the top of a 2 m high pole, which was installed on the forest floor. It is possible to obtain an image from the upward view. From April 2019, hemispherical photographs were taken automatically at 6 pm in order to minimize effects of direct sunlight. The image resolution was set at 4000 × 6000 pixels with a 24-bit color in TIFF format. The investigation period was from April 21 (before bud burst) to November 15, 2019 (after leaf fall).

### **Analysis of shoots images**

The shoot images, except on rainy days, were calculated for the plant area index (PAI) as the vegetation index value. The PAI is an index of tree growth (Ryu et al. 2012). The PAI was analyzed using the vegetation information analysis software LIA32 (LIA for Win32, Nagoya University, <http://www.agr.nagoya-u.ac.jp/~shinkan/LIA32/index-e.html>). In this software, we used the whole hemispherical photograph and divided plants from backgrounds by binarization using the Intermean algorithm. Green ratio (GR) and red ratio (RR), which are indices of leaf color, were obtained by analyzing the values of red, green, and blue (RGB). We excluded the sky and other non-canopy areas to derive color indices from the images by binarization. Their calculations were performed using R 3.6.0 (The R Foundation, Vienna, Austria). The color indices were calculated as follows:

$$GR = \frac{G}{R + G + B}$$

$$RR = \frac{R}{R + G + B}$$

where R, G, and B are the mean digital number values in the red, green, and blue bands of the image, respectively.

### **Observation of fine-root phenology**

To elucidate the root phenology, the root scanner method was used in the field in reference to Dannoura et al. (2012) and Ding et al. (2020). A transparent box made of acrylic materials (445 mm length, 300 mm height, and 55 mm width) was prepared to protect the scanner from rainwater in the field. To set the acrylic boxes, rectangular solid hole was prepared vertically near the mature larch using a hand shovel in October 2018. A transparent acrylic box was installed at the rectangular solid hole. For root color analysis, a color chart was attached to the surface of box between the box and the soil. We started scanner image acquisition from 7 months after the installation of the acrylic boxes to minimize the disturbance. An A4 flatbed scanner (GT-S640, EPSON, Nagano Prefecture, Japan) was installed in the acrylic box in May 2019. The scanner was always connected to a PC and the power source, and images were taken automatically one time per a day (12 am midnight). The PC automation software, UWSC 5.3.0.2, enabled automatic operation. Images were taken at 3400 × 6480 pixels, 24-bit color, and in TIFF format. A total of 175 images from 26<sup>th</sup> June to 15<sup>th</sup> November in 2019 were used for the analysis.

### **Analysis of fine roots images**

To extract only the root part of the captured image, living roots had to be distinguished from the background in the entire area of the scanner images. Before measuring the sample images, we manually extracted the root system from ten test-images in reference to Kume et al. (2018) and defined the threshold that distinguished living roots from other dead roots and soils based on root and soil color, and status of the structure (Fig. S1). Then, the scanner images were binarized by the predetermined threshold, and each image was automatically extracted. We calculated the percentages of the projected area of roots per the area of scanner frame (root area index) in all images.

To determine the root function of living roots in the image, the extracted root parts were separated as white and brown roots. Firstly, we manually extracted the white and brown roots from the test-image and defined the threshold for white and brown roots, which was based on the RGB color (Fig. S2). Then, the extracted root images were automatically binarized by the predetermined threshold. Finally, we calculated the proportion of the projected area of white or brown roots per area of total roots in all images and used proportion of white- or brown-root are per the area of scanner frame as an evaluation index.

All image analysis for fine roots were used by a software, ImageJ (Rasband, W.S., ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA, <http://rsb.info.nih.gov/ij/>, 1997-2012). This software is possible to create macros and automated repeated tasks for above analysis, such as the binarization using the predetermined threshold and their calculations.

### **Collection and analysis of roots and soil**

Root sampling was conducted in July 2019. We randomly selected 12 points in the plot and excavated at four soil depths (0–10, 10–20, 20–30, and 30–40 cm) using a hand auger ( $\Phi = 6.0$  cm). In the laboratory, all roots contained in each soil core were carefully isolated from the soil and organic matter using a 0.5-mm mesh. These roots were separated between living and dead roots by visually inspecting resilience, brittleness, and color of the bark and xylem, as described by Vogt and Persson (1991). All living roots of

larix were categorized by diameter into <0.5, 0.5–2.0, and >2.0 mm classes and were separated from the roots of other species. The samples were dried at 70 °C for 48 h and then weighed.

Soil sampling was conducted simultaneously with root sampling at each sampling point next to the location of root-core excavation. Each soil sample was air-dried, sieved through a 2-mm mesh, ground, and used to determine soil properties. Soil samples were analyzed for pH, electric conductivity (EC), and C/N. The pH and EC were measured with a pH meter (LAQUA twin pH, Horiba, Kyoto, Japan) and an EC meter (LAQUA twin pH, Horiba, Kyoto, Japan) after mixing the soil sample with distilled water at 1:10. Soil C/N was measured using an elemental analyzer (Flash EA 1112, Thermo Fisher Scientific, Cambridge, MA, USA).

## Results

### Environmental data

The maximum daily average temperature at the survey site was 24.7 °C (August 2019), and the minimum daily average temperature was -2.7 °C (November 2019) (Fig. 1). There was no seasonal pattern of precipitation; however, there were days when the daily precipitation exceeded 100 mm. The soil temperature tended to be similar to the air temperature, with a maximum daily average soil temperature of 24.8 °C (August 2019) and minimum daily average soil temperature of 3.6 °C (April 2019). Soil moisture fluctuated between 20–60%.

Regarding soil conditions, the pH fluctuated between 4.37 and 4.68, and the C/N fluctuated between 17.0 and 18.3 (Table S1). The C/N increased toward the surface layer. The value of EC was larger toward the surface layer; the maximum and minimum values were 77.7 mS m<sup>-1</sup> at 0–10 cm soil depth and 44.4 mS m<sup>-1</sup> at 20–30 cm soil depth, respectively.

In August 2019, when the fine root biomass was investigated using a soil core, most of the roots at the depth where the scanner was buried (0–40 cm) were larch (Table S2). Fine root biomass tended to increase in the upper layers of the soil, regardless of root diameter class.

### Seasonal patterns of shoot and fine roots

Regarding larch shoots, time-series changes in PAI, GR, and RR were detected from hemispherical photos, and the timings of shoot production and color changes were clarified. The PAI before the leafing stage was 0.5 m<sup>2</sup> m<sup>-2</sup>, and it increased from April to early June to 1.4 m<sup>2</sup> m<sup>-2</sup> (Fig. 2a). Subsequently, it gradually increased until the end of August. After September, the PAI continued to decrease until the end of the survey. The GR of shoots was ~0.33 before leafing, and it increased and peaked in May (Fig. 2b). The RR remained almost unchanged until the end of September but increased significantly to 0.37 in early October, and then decreased to ~0.33 in late October (Fig. 2b).

For the time-series changes in the percentages of the projected area of roots per the area of scanner frame (root area index), a bimodal growth pattern was observed (Fig. 3a). Almost no roots were present at the start of the survey; however, the root area index had its first peak in early August and a second small peak in September. Most of the roots disappeared in October. Regarding the proportion of white to total roots, white roots often accounted for  $\geq 50\%$  from the start of the survey to August; however, the proportion of brown roots subsequently increased to 70% (Fig. 3b).

## Temperature effects

In the analysis of the relationship between phenology and temperature factors, the aboveground PAI was positively correlated with air temperature (Fig. 4a, b). However, the tendency of change differs depending on the season, and PAI was divided into spring/summer (leafing) and autumn/winter (leaf senescence), even though the air temperature was the same. The percentages of the projected area of roots per the area of scanner frame (root area index) was positively correlated with soil temperature (Fig. 4c, d); however, when the roots were divided into white and brown roots, the relationship with soil temperature was different (Fig. 5). The proportion of white roots in the images increased in spring and summer when the soil temperature was high. On the other hand, the proportion of brown roots increased in autumn and winter when the soil temperature was low.

## Discussion

We assessed the seasonal pattern of daily-based root and shoot production by capturing high-frequency plant images in a larch forest. For the larch shoot, we captured the seasonal changes in shoot biomass and the photosynthetic period from the hemispherical images (Fig. 2). Fluctuations in canopy color indices (such as GR and RR) are caused by seasonal variation in foliage pigments (Sims and Gamon 2002; Wingate et al. 2015; Brown et al. 2017). In our study, the spring peaks of PAI and GR showed that the leafing stage occurred from the start of observation period to May. The time series of PAI, a measure of canopy leaf content, was characterized by increased symmetry, with a peak in May showing bud burst and subsequent shoot growth during the summer (Fig. 2a). On the other hand, the time series of GR was characterized by pronounced asymmetry (Fig. 2b). A spring peak was observed in May, while a subsequent decline in GR values occurred after the start of June. This result is consistent with several previous studies, including time-series data from Brown et al. (2020), which used hemispherical photographs collected from under the canopy of deciduous forests in southern England (Ryu et al. 2012; Nagai et al. 2013). The time-series variation of RR, characterized by the peak of autumn, is consistent with results of study by Liu et al. (2020) in deciduous forests in Canada. The RR peak indicates advances in leaf senescence, which will subsequently be followed by leaf out. Seasonal changes in canopy color indices, extracted from repeat canopy photography, were closely related to seasonal changes in the phenology of deciduous forests, such as bud-break, leaf expansion, and the start/end of the photosynthetic period (Richardson et al. 2009; Sakamoto et al. 2012; Ide and Oguma 2013; Keenan et al. 2014; Toomey et al. 2015; Wingate et al. 2015; Moore et al. 2016; Nagai et al. 2016). In other words, by combining the time series data of PAI and color indices, it is possible to more accurately identify

fluctuations in shoot biomass and the period of photosynthesis and estimate the seasonal effects of phenological fluctuations on C budgets. Consequently, the PAI and daily mean temperature were positively correlated (Fig. 4), which corresponded to shoot phenology across air temperature (Hänninen et al. 2007; Palacio and Montserrat-Martí 2007; Slaney et al. 2007).

For the fine root, we used the flatbed-scanner method to capture fine root dynamics in a larch forest during the growing period. We obtained diurnal and seasonal patterns of root growth, mortality, and color change for each growth stage. In our study, root production peaked twice (bimodal root-growth pattern): early summer and late autumn (Fig. 4). Root production peaked in summer and autumn, which is consistent with several previous studies (Burke and Raynal 1994; Ruess et al. 1998; Tierney et al. 2003; Fukuzawa et al. 2007). Soil temperature is an important factor in quantifying root production, including the initiation and cessation of root growth, cell elongation, root length and diameter extension, and initiation of new lateral roots, which regulate other vital functions of a tree (Pregitzer et al. 2000). Our findings agree with the general pattern of fine root growth observed in northern temperate forests (Brassard et al. 2011) and in Italy for *Quercus cerris* (Montagnoli et al. 2012) and *F. sylvatica* (Montagnoli et al. 2014). Warmer soil temperatures often result in higher rates of root respiration and nutrient uptake, which do not inhibit the physiological activity of trees (Atkin et al. 2000) but have potential significant consequences on root growth (Pregitzer et al. 2000). Indeed, in our study, there was a positive correlation between the fine root area index and soil temperature (Fig. 4). This suggests that the warmer summer soil temperature seemed to trigger fine root growth during the growth season.

However, our results did not always show high root production in summer and autumn; these two seasons had bimodal patterns of root growth despite higher soil temperatures. In our study, fine root mortality was the highest in late summer, which is consistent with the results of previous studies (Tierney et al. 2003; Tingey et al. 2005). The other possible reason for the variability in root production may be the C substrate from plant photosynthesis activity. Shoot C assimilation rates were strongly correlated with belowground C allocation to the roots (Litton and Giardina 2008). Additionally, root growth in deciduous species is assumed to peak early in the growth season and is correlated with shoot growth (Newman et al. 2006). In other words, the decline in shoot physiological activity might lead to low root production and high root mortality, even at warmer soil temperatures. Under forest field conditions, trees can change the prevailing irradiance to protect and optimize photosynthesis. Photooxidative damage to leaf thylakoid membranes leads to photoinhibition and stomatal closure in shoots. The shoot protects the photosynthetic apparatus by downregulating it at higher temperatures under high photon flux (Franco and Lüttge 2002). Photoinhibitory damage and stomatal closure contribute significantly to photosynthetic depression and indirectly to the decline in C supply to the root system. Makita et al. (2014) showed that weather conditions under high temperature stress cause a depression in CO<sub>2</sub> assimilation in deciduous trees and then a sharp reduction in root respiration rate. Indeed, it seems that the PAI and GR ratio in our study slightly decreased from August (Fig. 2). Thus, the root production and mortality in the growth season might reflect a combination of soil temperature and phenological timing of shoot development, senescence, and abscission, resulting in the bimodal patterns in our study.

To help address these knowledge gaps regarding the factors affecting root growth, information on root color is an important indicator of functions across different stages of tissue growth (Lipp and Andersen 2003). We tested the color-based classification that allows distinguishing living roots between white and brown roots using the adaptive color threshold method for image binarization. Our results indicated that the proportion of white root production was higher in summer and gradually decreased in autumn (Fig. 3). In contrast, the brown root proportion was lower in summer and tended to increase in autumn. Root pigmentation or browning is commonly used as an index of root age or decreased root absorptive ability and shift of root function from absorption to transportation (Wells and Eissenstat 2002; Hishi 2007). Newborn roots are commonly white and become brown several weeks to months after production (Wells and Eissenstat 2001). Brown roots are frequently recognized as secondary roots with the formation of cork cells (McKenzie and Peterson 1995). This means that some of the white roots produced in the summer could shift from primary to secondary root growth by changing their color to brown. In addition, some of the white roots produced in autumn did not represent a major shift in root growth, which means that most of the white roots in autumn may disappear and decompose without turning into brown roots. Consequently, the different behaviors of white and brown roots in season were attributed to differences in the relationship of soil temperature with positive white root proportion and negative brown root proportion (Fig. 5). By analyzing root color, we suggested that bimodal patterns of root production in our study would exhibit a distinct functional proportion between the first and second peaks of the root area index and reflect the phenology of root functions through different stages of root tissue growth. We believe that future work on root production to improve root dynamics by considering fine root function, particularly root color, will lead to a better understanding of fine root phenology.

It has been suggested that root growth is largely linked to shoot growth and asynchronous growth (Medvigy et al. 2009; Abramoff and Finzi 2015). In our study, the root biomass distribution peaks lagged those of the shoots (Fig. 2-3). Our results also showed that the main root growth in summer was asynchronous with the shoot defoliation period, which may be due to the use of C assimilated in the shoot after proliferation for fine root production. This is consistent with the results of temperate forest species (Hendrick and Pregitzer 1996; Iivonen et al. 2001; Joslin et al. 2001; McCormack et al. 2014; Abramoff and Finzi 2015). Possible reasons for asynchrony in shoot and fine root phenology include competition for photosynthates and/or the relationship between growth phenology and temperature (Radville et al. 2016). Regarding the C source for root growth in the fall, it is likely that the C stored during photosynthesis was allocated to the growth of roots because the shoot had already begun to senesce in the fall. Furthermore, it has been shown that shoot phenology is affected by air temperature (Delpierre et al. 2016) and roots are affected by soil temperature (Tierney et al. 2003). At the study site, the atmosphere warmed earlier than the soil in the spring; however, air temperatures were lower than soil temperatures and below freezing in winter (Fig. 1). It has been reported that root production is higher than shoot production because of soil temperature retention during the fall (Blume-Werry et al. 2016; Makoto et al. 2020). Therefore, the timing of the start and end of shoot growth was considered to be earlier than that for the roots (Makoto et al. 2020).

While it remains difficult to draw firm conclusions about the causes and effects of root and shoot phenology from the specific species and limited period data, our study indicates more important implications for daily root and shoot phenology. Particularly for fine roots, the automatic flatbed scanner method which connected the scanner to a power supply and PC allowed us to obtain detailed root production patterns that had been overlooked in previous studies. Johnson et al. (2001) reported that fine root production from manual observations was underestimated by 28% and 54%, respectively, when 4- and 8-week sampling intervals were compared with 2-week intervals. Because the turnover of fine roots is usually rapid (Finér et al. 2011), a shorter sampling period can capture the timing and duration of growth. Manual observations effectively cover spatial variability; however, automated observations enable the analysis of temporal variations in plant growth, especially during nighttime and rainfall period when manual measurements are impractical. This high temporal resolution also makes it possible to observe the response of root dynamics to rapid temporal changes in environmental conditions effectively without the use of linear interpolation or models. Such frequency observation for plant phenology will enable the data to be contrasted with terrestrial phenology, which has already been observed frequently with the development of remote sensing.

In addition, many root images in this study were analyzed more quickly and objectively by automatic analysis using the digital number of the image as a threshold. Nakano et al. (2012) developed an automatic addition to track root growth dynamics with results similar to those of manual calculations. With the development of tracking analysis, root studies using scanners can be even less laborious. However, we suspect that this automatic method tends to generate noise caused by soil particles compared to manual trace analysis. Although rough noise can be removed by image filtering, the analysis method should be improved, particularly to enable discrimination of fine roots by considering root morphology and tracking of emerging fine roots. By developing an analysis method for the acquired image, it will be possible to understand the patterns in production and mortality of fine roots using an objective-time-scale season and the biological and environmental factors which control plant dynamics and function.

## **Declarations**

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## Author Contributions

N.M and A.T designed the study. N.M, A.T, R.F. and M.K. collected the data. N.M, A.T and H.O analyzed the data. N.M and A.T wrote the manuscript. All authors approved the final manuscript.

## Data availability

Data are available on request from the corresponding author

## Conflict of Interest

The authors declare that they have no conflict of interest.

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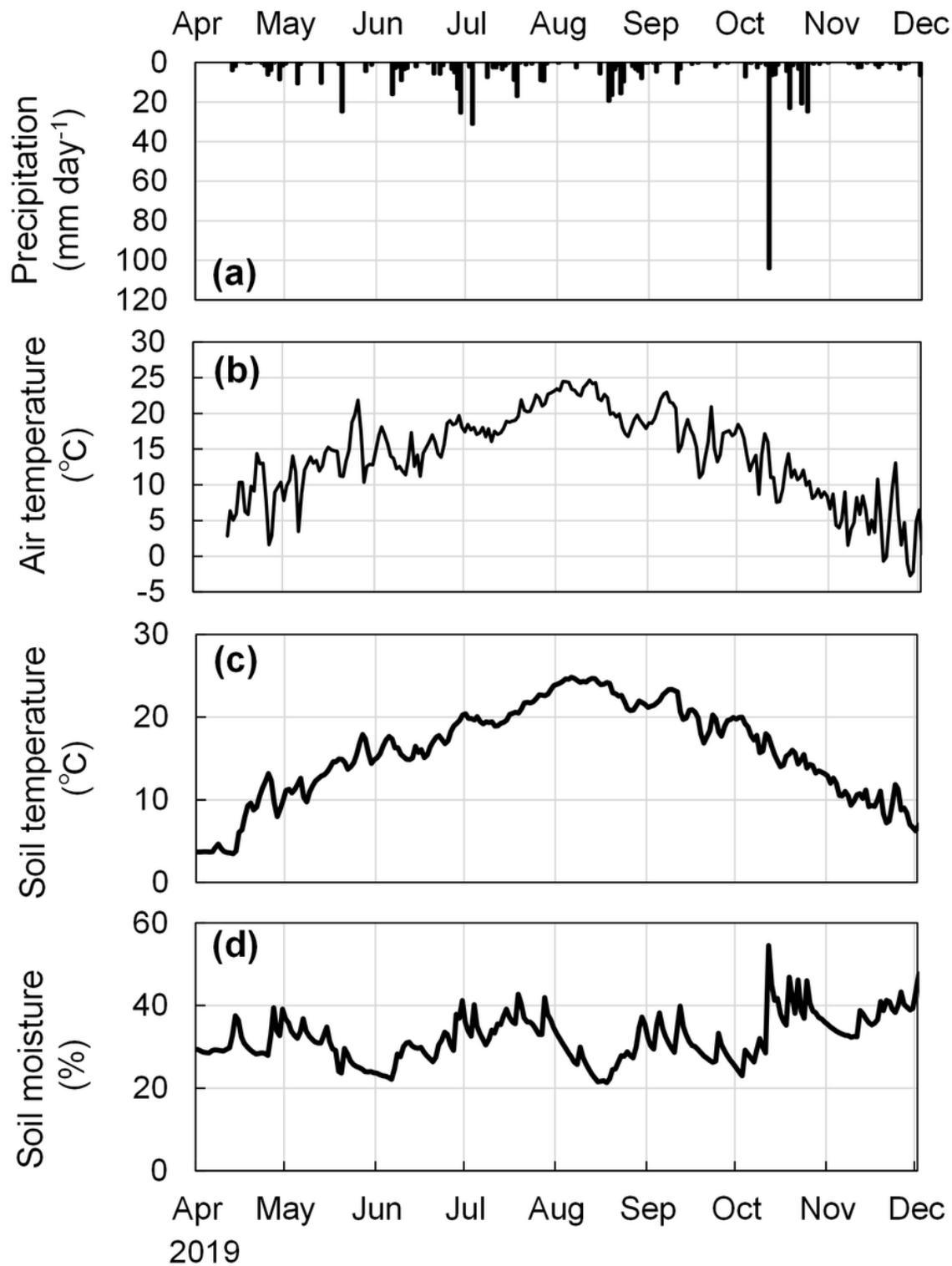
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## Figures



**Figure 1**

Environmental conditions of the study site in a *Larix kaempfer* forest in Nagano, Japan. (a) Daily precipitation, (b) daily mean temperature at 1.8 m high, (c) daily mean value of soil temperature, and (d) daily mean value of soil moisture (volumetric water content). The soil temperature and soil moisture were measured at the soil surface layer (0–10 cm).

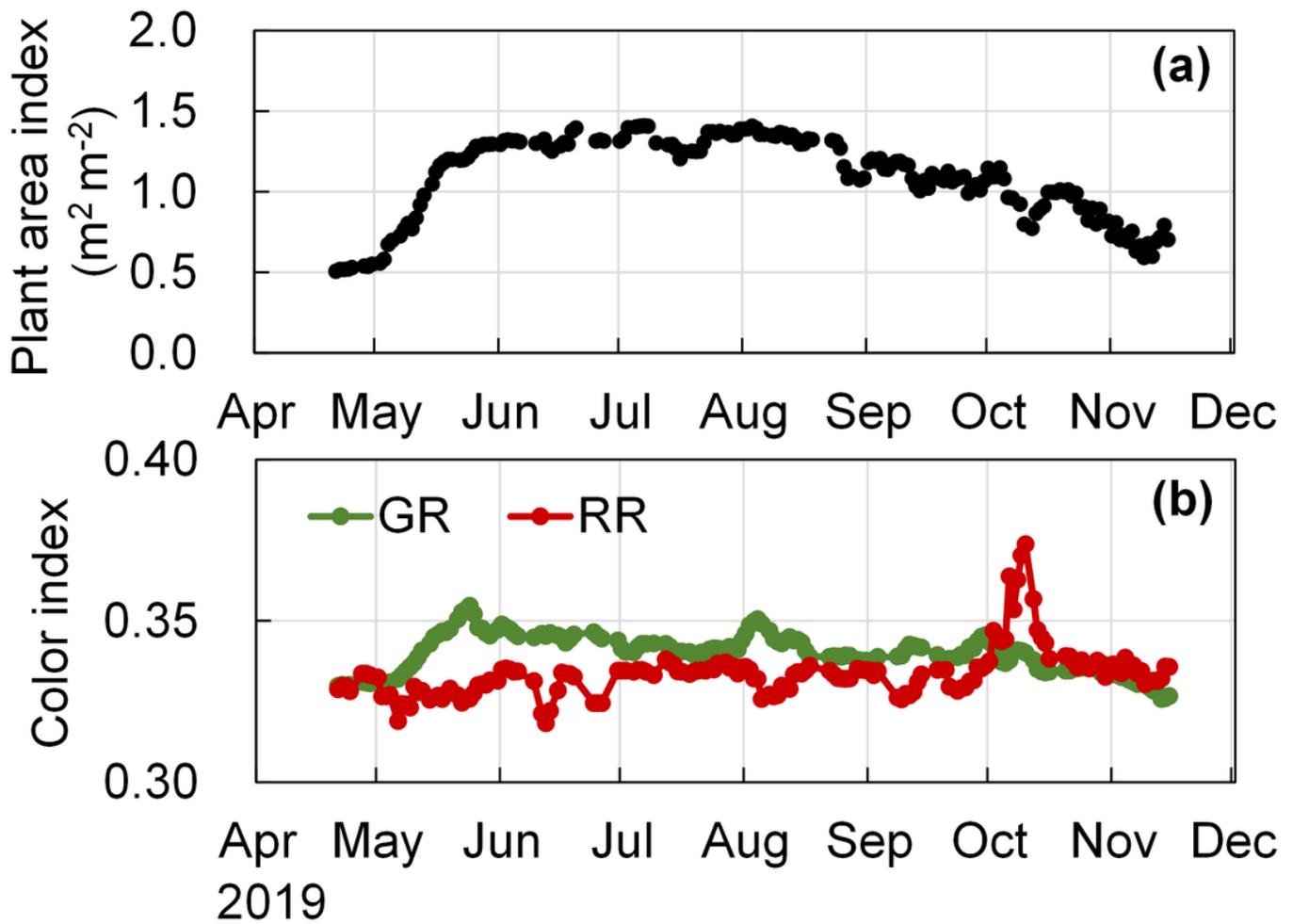
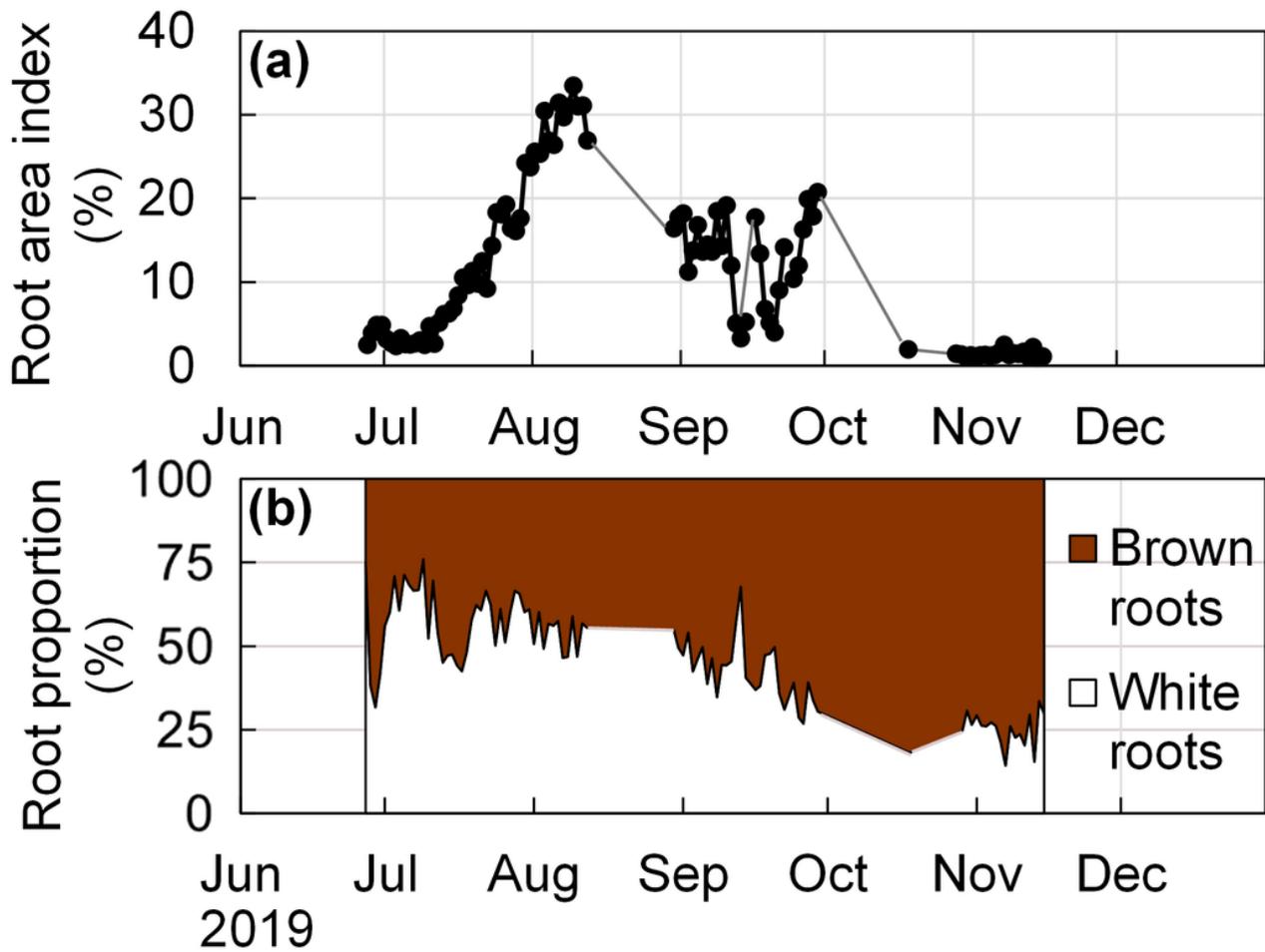


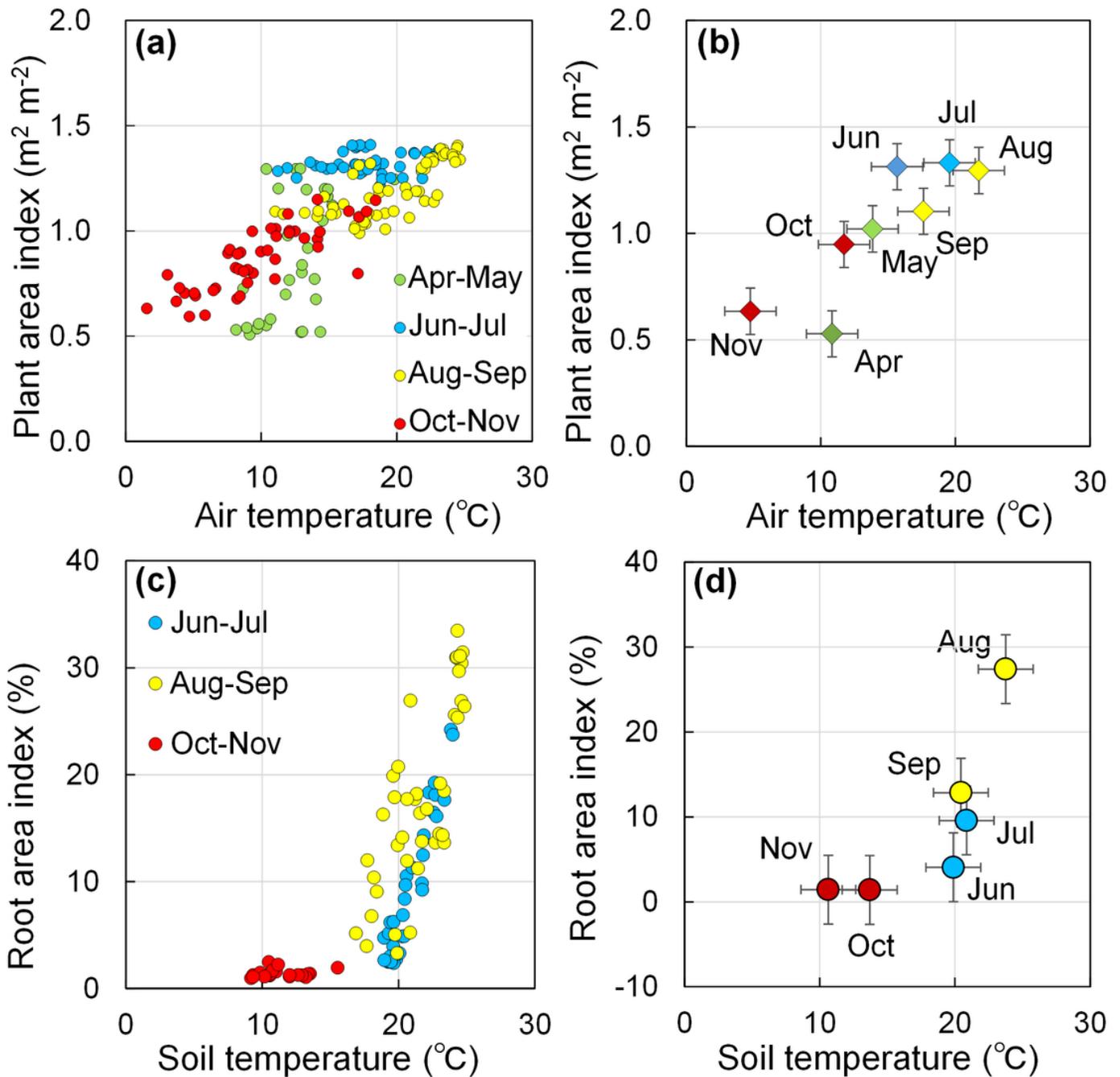
Figure 2

Seasonal variation of (a) Plant area index (PAI) and (b) Green ratio (GR) and Red ratio (RR) from June 2019 to November 2019.



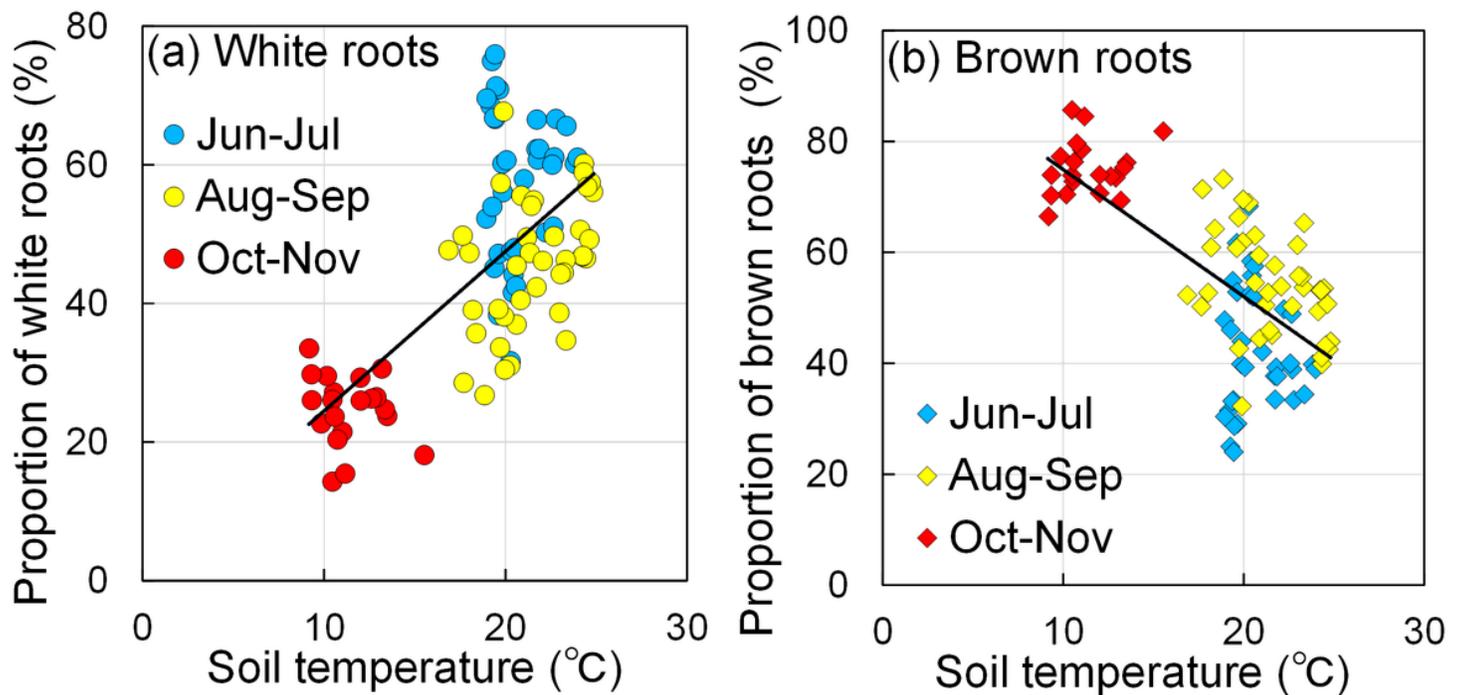
**Figure 3**

Seasonal dynamics of (a) the percentages of the projected area of roots per the area of scanner frame (root area index) and (b) proportion of two types of roots measured using the scanner method from June 2019 to November 2019. Gray lines show missing data.



**Figure 4**

Relationships between (a-b) air temperature and PAI and (c-d) soil temperature and root area index. Each dot reflects the month in which the image was taken.



**Figure 5**

Relationships between soil temperature and (a) proportion of white roots and (b) proportion of brown roots. Each dot reflects the month in which the image was taken.

## Supplementary Files

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