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ORIGINAL PAPER

Estimating the production and mortality of fine roots using minirhizotrons in a *Pinus densiflora* forest in Gwangneung, Korea

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Abstract The aim of this study was to estimate fine root production (FP) and fine root mortality (FM) at 0–10, 10–20, and 20–30 cm soil depths using minirhizotrons in a 75-year-old *Pinus densiflora* Sieb. et Zucc. forest located in Gwangneung, Korea. We developed the conversion factors (frame cm^{-2}) of three soil depths (0.158 for 0–10 cm, 0.120 for 10–20 cm, and 0.131 for 20–30 cm) based on soil coring and minirhizotron data. FP and FM were estimated using conversion factors from March 26, 2013 to March 2, 2014. The annual FP and FM values at the 0–30 cm soil depth were 3200.2 and 2271.5 kg ha^{-1} yr^{-1}, respectively. The FP estimate accounted for approximately 17 % of the total net primary production at the study site. FP was highest in summer (July 31–September 26), and FM was highest in autumn (September 27–November 29). FP was positively correlated with seasonal change in soil temperature, while FM was not related to that change. The seasonality of FP and FM might be linked to above-ground photosynthetic activity. Both FP and FM at the 0–10 cm depth were significantly higher than at 10–20 and 20–30 cm depths, and this resulted from the decrease in nutrient availability with increasing soil depth. The minirhizotron approach and conversion factors developed in this study will enable fast and accurate estimation of the fine root dynamics in *P. densiflora* forest ecosystems.

Keywords Conversion factor · Fine root · Minirhizotron · Mortality · Production

Introduction

Fine roots are generally defined as roots less than 2 mm in diameter, and they are important for water and nutrient uptake and the storage of organic matter and nutrients in forest ecosystems (Norby and Jackson 2000; Kim 2012). Although fine root biomass accounts for only 1–12 % of total forest biomass, 7–76 % of the net primary production (NPP) of forests is allocated to fine root dynamics (Vogt 1991; Gower et al. 1995). This is because of the fast turnover of the production-death-decomposition cycle (Satomura et al. 2006). Therefore, more accurate estimations of fine root dynamics are needed to clearly understand carbon and nutrient cycling in forests.

The seasonal changes in above-ground plant production are well described, but below-ground plant production, especially in fine roots, is poorly described and is more uncertain (Baddeley and Watson 2004). The seasonality of fine root production (FP) and fine root mortality (FM) could be associated with patterns of above-ground photosynthetic activity (Cote et al. 1998; Block et al. 2006). FP and FM are also affected by seasonal changes in temperature (Steele et al. 1997; Yuan and Chen 2010) and

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precipitation (López et al. 2001; Yuan and Chen 2010). It is important to integrate detailed knowledge of fine root seasonality with quantified site-specific parameters such as soil temperature and soil water content.

Soil coring, ingrowth cores, isotopes, and minirhizotron methods have been used to estimate fine root dynamics in forests (Johnson et al. 2001). Conventional methods such as soil coring and ingrowth cores require intensive labor, and they may alter study site conditions. The minirhizotron technique, a nondestructive and observational method, has allowed the simultaneous measurement of the appearance and disappearance of individual roots (Majdi 1996). Hendricks et al. (2006) conducted comparisons among several methods for the investigation of fine root production, and they reported that the minirhizotron technique proved most reliable for estimating fine root production. Minirhizotron techniques are widely used in fine root studies of life span, production, mortality, and morphology, and the method has been exhaustively reviewed (Majdi 1996; Johnson et al. 2001; Satomura et al. 2007). However, minirhizotrons are less available for the description of fine root biomass because roots can only be observed as images. In order to overcome the limitation of minirhizotrons, several studies have attempted to estimate root biomass using the root length density and specific root length in soil core samples (Johnson et al. 2001; Brown et al. 2009), conversion factors (Noguchi et al. 2004), and regression equations comparing minirhizotron and soil coring data (Box and Ramseur 1993; Jose et al. 2001). Nevertheless, studies converting minirhizotron data to dry weight are still lacking (Jose et al. 2001).

Pinus densiflora Sieb. et Zucc. is the most important coniferous species in Korea, occupying more than 23 % (1,447,000 ha) of Korean forest land (Korea Forest Service 2011). The species is widely distributed in the East Asia region and Korea (Li et al. 2013). Despite its ecological importance, minirhizotron techniques have only been applied twice to *P. densiflora* forests in East Asia (Noh et al. 2012; Han et al. 2014). The objectives of this study were to develop conversion factors suited for *P. densiflora* and to describe seasonal changes in the fine root production and mortality in a *P. densiflora* forest.

Materials and methods

Study site

The study site was a naturally regenerated *P. densiflora* forest in Gwangneung Experimental Forest (37°47′01″N, 127°10′37″E), Pocheon, central Korea. The stand density (658 trees ha^{-1} in 2010) was naturally maintained without any anthropogenic management since a clear-cut harvest in 1912. The study site was dominated in 2013 by 70- to 80-year-old *P. densiflora*. Elevation, slope, and aspect at the study site were 410–440 m asl, 13°–22°, and SW 250°–260°, respectively. The climate of the study site was characterized by hot, humid summers and cold, dry winters. The average annual precipitation and air temperature at the site were 1518 mm and 11.3 °C, respectively. In 2013, the soil temperature and soil water content at a 10-cm depth were recorded from January 1 to December 10 once every 30 min by data loggers (HOBO Micro Station, Onset Computer Corp., USA) (Fig. 1). Detailed characteristics of the study site are described in Noh et al. (2013).

Minirhizotron

In May 2010, four transparent acrylic tubes, 80 cm in length and 7 cm in inner diameter, were installed in the soil at 45° angles to the ground. In November 2012, two tubes were additionally installed at the site. All six tubes were randomly placed at the study site. The access section of the tube remaining above ground was covered with black tape and aluminum foil to exclude light. From March 2013 to March 2014, images were taken nine times (March 26, May 29, June 25, July 30, August 28, September 26, October 30, and November 29 in 2013, and March 2 in 2014) using a root scanner system (CI-600 Growth Monitoring System, CID, USA). The captured images were separately analyzed at 0–10, 10–20, and 20–30 cm soil depths. The size of a separated image from each soil depth was 19.56 × 14.14 cm. Length and mean diameters of fine roots on the collected images were manually determined using image-analysis software (WinRHIZO Tron MF, Regent, Canada).

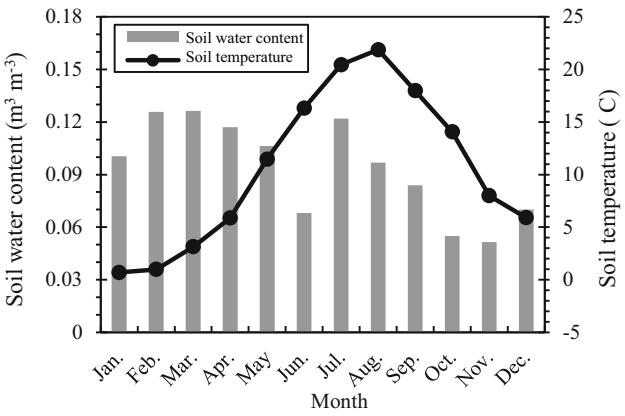

Fig. 1 Mean monthly soil temperature and monthly soil water content in a *P. densiflora* forest from January to December 2013

Soil coring

To estimate fine root biomass from soil cores, we referred to Han et al. (2014) to develop the conversion factors. Soil at the 0–30 cm depth was sampled monthly from May 29 to August 28 in 2013. Soil was separately sampled at 0–10, 10–20, and 20–30 cm depths near the location of the minirhizotron tubes (10 soil cores at each sampling date). Fine roots less than 2 mm in diameter were collected using tweezers and were then washed. To estimate the length and mean diameter of fine roots, 90 fine root samples were scanned and analyzed using image analysis software. The fine root samples were dried at 65 °C, and the dry weights were measured.

Conversion factors

Minirhizotron and soil coring data from May 29 to August 28, 2013 (four times) were used to develop conversion factors. To estimate FP and FM using dry weights, we used the following equations reported by Noguchi et al. (2004) to develop conversion factors suited for *P. densiflora*:

$$W_{UL} = aM_D^2 + b \quad (1)$$

$$B_F = W_{UL} \times L \quad (2)$$

$$C_F = B_S/B_F \quad (3)$$

$$F_P \text{ or } F_M = P_F \text{ or } M_F \times C_F \quad (4)$$

where, L (mm frame^{-1}) is length, W_{UL} (mg mm^{-1}) is weight per unit length, M_D is mean diameter, B_F (mg frame^{-1}) is biomass on the minirhizotron image frame, B_S is biomass per unit stand area measured from soil coring (Han et al. 2014), F_P and F_M (mg cm^{-2}) are fine root production and fine root mortality respectively, P_F and M_F (mg frame^{-1}) are production and mortality on the minirhizotron image frame respectively, and a, b and C_F (frame cm^{-2}, conversion factor) are constants. The C_F was calculated as a ratio of fine root biomass from soil coring (B_S, mg cm^{-2}) to that from the minirhizotron technique (B_F, mg frame^{-1}). To increase accuracy of the estimate, CFs were separately calculated for each soil depth (0–10, 10–20, and 20–30 cm) (Noguchi et al. 2004). The new root lengths (P_F) and dead root lengths (M_F) were multiplied by W_{UL} and the length changes of individual roots. The length changes between sampling dates were calculated by summing all P_F and M_F values. FP and FM were estimated using the CFs of each soil depth, and the FP and FM rates of fine roots were presented as mass per day (kg ha^{-1} day^{-1}).

Statistical analysis

Non-linear regression analysis was performed to fit the M_D and W_{UL} of fine roots. Linear regression analysis was performed to predict fine root biomass from minirhizotron data and measured fine root biomass from soil coring. The effect of the observation period (time interval between two sampling dates) and soil depth (0–10, 10–20, and 20–30 cm) on FP and FM were analyzed using the split-plot design analysis of variance (ANOVA). For the split-plot design ANOVA analysis, the observed data in each minirhizotron tube were set as a block, while the observation period and soil depth were treated as the two primary factors. Furthermore, linear regression was used to clarify the relationship between FP and FM and soil temperature and soil water content. The results were considered significant when the *P*-values were less than 0.05. All statistical analyses were conducted using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA).

Results and discussion

Using the results of the regression analysis between W_{UL} and M_D, an Eq. (1) was obtained ($W_{UL} = 0.170 \times M_D^2 + 0.035$; $P < 0.0001$). The CFs were 0.158, 0.120, and 0.131 at 0–10, 10–20, and 20–30 cm soil depths, respectively (Table 1). A significant linear relationship was observed between the fine root biomass from minirhizotrons and the measured fine root biomass from soil coring ($R^2 = 0.80$) (Fig. 2). These relationships have been observed in other studies. For instance, Noguchi et al. (2004) and Jose et al. (2001) reported a significant correlation between the predicted and measured fine root biomass using conversion factors for *Cryptomeria japonica* ($R^2 = 0.83$) and the linear regression equations for *Juglans nigra* ($R^2 = 0.87$) and *Quercus rubra* ($R^2 = 0.70$), respectively.

The FP and FM rates (kg ha^{-1} day^{-1}) at the 0–30 cm depth ranged from 8.15 to 16.31 and from 3.48 to 12.37 during the study period, respectively (Fig. 3). The seasonal changes in the FP rate were not significant ($P = 0.10$), but marked seasonal variation was observed (Fig. 3a). The seasonal changes in the FM rate were significant. The FP and FM rates showed different seasonal patterns (i.e., the FM peaked after the peak of FP). The seasonal patterns of the FP rate at the 0–30 cm soil depth increased until September 26 in 2013 and then decreased until March 2 in 2014 (Fig. 3a). In contrast, the FM rate increased until November 29 in 2013 and then decreased until March 2 in 2014 (Fig. 3b). In other words, FP was high in the growing season (spring and summer), and FM was high after the growing season (autumn). These patterns of FP and FM were similar to those reported by Noh et al. (2012) from a study at the same site. These results agreed with those of previous studies, which reported the relationship between the seasonality of FP and the leaf expansion period (spring and summer) and between the seasonality of FM and the

Table 1 Conversion factors at each soil depth based on the fine root biomass estimates from minirhizotron (B_F) and soil coring (B_S) data

Soil depth (cm)	Fine root biomass		Conversion factors
	Minirhizotron B_F (mg frame^{-1})	Soil coring* B_S (mg cm^{-2})	B_S/B_F (frame cm^{-2})
0–10	120.235	19.002	0.158
10–20	85.959	10.331	0.120
20–30	56.203	7.386	0.131

* Han et al. 2014; B_F, biomass on the minirhizotron image frame; B_S, biomass per unit stand area

Fine root biomass from B_F and B_S values are the means of eight samples

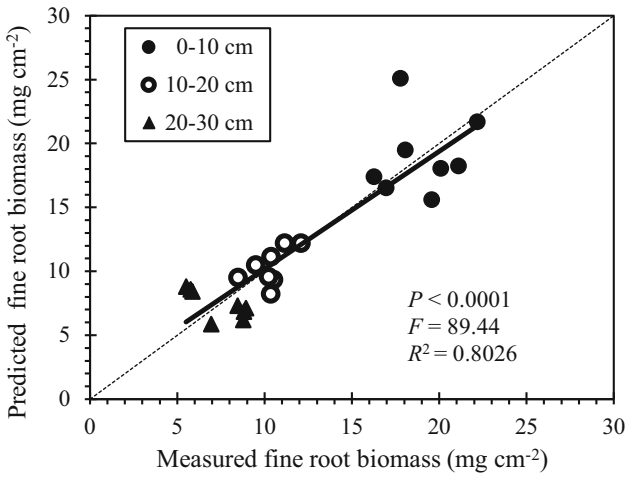

Fig. 2 Linear regression analysis between the measured fine root biomass from soil coring and the predicted fine root biomass from minirhizotron data. The predicted fine root biomass was calculated using conversion factors (Table 1). Each *point* represents the mean fine root biomass at each soil depth

leaf-fall period (autumn) (Block et al. 2006; Satomura et al. 2006). It is believed that the growth of fine roots may be promoted to supply large amounts of nutrients and water for the synthesis of photosynthates during the growing season. After the growing season, fine roots die concurrently with the shedding of leaves in autumn (Satomura et al. 2006).

Both FP and FM rates at the 0–10 cm depth were significantly higher than those at the 10–20 and 20–30 cm depths ($P = 0.0008$ and $P = 0.0215$; Table 2), and they tended to decrease with increasing soil depth. These results might be related to the decrease in nutrient availability with increasing soil depth (King et al. 1999; Noguchi et al. 2005). At the study site, some nutrient content and nitrogen concentrations values were greater in topsoil than at deeper soil levels (Noh et al. 2013). The interactive effect of the observation period and soil depth was not significant for production or mortality rates (Table 2). However, fluctuations in mortality rates tended to be greater at the soil surface (0–10 cm) than in deeper soils (Fig. 3b).

Despite the fact that the seasonality of FP rates was insignificant, the FP rate exhibited a significant relationship with soil temperature ($P = 0.0126$; Fig. 4). The FP rate was positively correlated with soil temperature, which resulted from an increase in the FP rate because of rising temperatures throughout the seasons (Figs. 1, 3). Fine root growth was promoted by rising soil temperatures due to the increase in nutrient mineralization (Pregitzer et al. 2000). On the other hand, the FP rate did not show a significant relationship with soil water content, and the FM rate did not show any relationship with soil temperature and soil

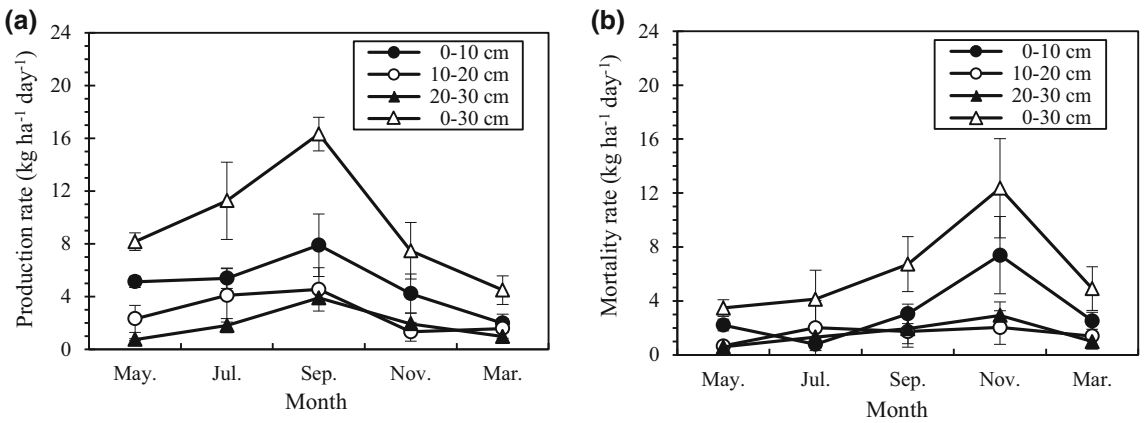

Fig. 3 Seasonal changes in the fine root production rate (**a**) and mortality rate (**b**) in a *P. densiflora* forest. Data were measured from March 27, 2013 to March 2, 2014 at soil depths of 0–10, 10–20, and 20–30 cm. *Vertical bars* indicate the standard errors of the means (n = 6)

Table 2 Split-plot design ANOVA for the effects of period and soil depth on the production and mortality of fine roots

Parameter	Source of variation	DF	MS	*F*	*P*
Production	Period	4	4.82	2.06	0.1007
	Depth	2	21.19	9.06	0.0008**
	Period × depth	8	0.89	0.38	0.9227
Mortality	Period	4	7.82	3.60	0.0164*
	Depth	2	9.50	4.37	0.0215*
	Period × depth	8	2.63	1.21	0.3264

* $P < 0.05$; ** $P < 0.001$

DF degree of freedom; *MS* mean square; *F* *F*-value from ANOVA; *P* *P* value from ANOVA

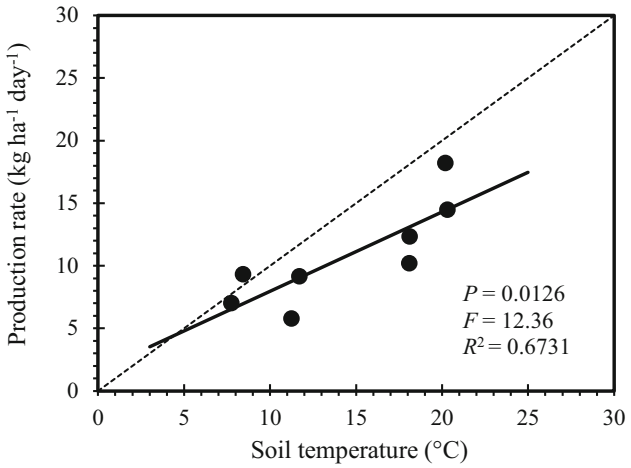

Fig. 4 The relationship between fine root production rate and soil temperature in a *P. densiflora* forest from March to November 2013

water content. In contrast to the results of this study, some previous studies have reported the close relationship of FP and FM to soil water content, since soil water content could influence the availability of water and nutrients (North and Nobel 1997; Yuan and Chen 2010).

The annual FP and FM at the 0–30 cm soil depth were 3200.2 and 2271.5 kg ha^{-1} yr^{-1} respectively, and they were highest at the 0–10 cm depth with values of 1632.1 and 1154.6 kg ha^{-1} yr^{-1}, respectively (Table 3). The annual FM at the 0–30 cm depth corresponded to approximately 70 % of the annual FP. Moreover, the FP between July 31 and September 26 accounted for 29.6 % of the annual FP, and the FM (kg ha^{-1}) between September 27 and November 29 occupied 34.8 % of the annual fine root mortality (Table 3). According to the study by Noh et al. (2013), conducted at the same study site, the total NPP in a *P. densiflora* forest was approximately 19.0 Mg ha^{-1} yr^{-1}. Based on this result, our estimates of annual FP (3200.2 kg ha^{-1} yr^{-1}; Table 3) accounted for about 17 % of total NPP. The annual FP in our study was 1.3 to 1.9 times higher than values estimated in previous studies of Korean coniferous forests, including *Larix leptolepis*, *P. densiflora*, and *P. rigida* (1702–2394 kg ha^{-1} yr^{-1}) (Hwang and Son 2003; Son and Hwang 2003; Hwang et al. 2007; Park et al. 2010), except for the study of a *P. koraiensis* forest by Park et al. (2010) (4532 kg ha^{-1} yr^{-1}). Our estimate of annual FP was also higher than another estimate based on sequential coring in the same year and at the same site (1720 kg ha^{-1} yr^{-1}; Han, unpublished data). The differences among the studies might be attributed to the difference in measurement methods. All of the estimates of previous studies were measured by sequential coring. However, the sequential coring method underestimates fine root production when fine root growth and mortality occur concurrently (Kurz and Kimmins 1987). In *P. palustris* forests, the fine root (<0.5 mm in diameter) production estimates from minirhizotrons were 2.5–6.0 times higher than production estimates from soil coring data (Hendricks et al. 2006). Fine root production and mortality occurred simultaneously during the study period (Fig. 3). The minirhizotron technique yields more accurate estimates of fine root production than sequential coring because it is available for simultaneous measurements of

Table 3 Fine root production and mortality of *P. densiflora* from March 2013 to March 2014

Fine root	Soil depth (cm)	26 Mar. –29 May, 2013	30 May –30 Jul., 2013	31 Jul.–26 Sep., 2013	27 Sep.–29 Nov., 2013	30 Nov. –2 Mar., 2014	Total (kg ha^{-1} yr^{-1})
Production (kg ha^{-1})	0–10	327.1 (27.6)	333.6 (48.3)	457.5 (137.2)	269.6 (95.6)	244.3 (83.4)	1632.1 (104.5)
	10–20	147.6 (65.5)	253.3 (125.9)	263.2 (94.9)	84.4 (45.4)	193.8 (90.9)	942.3 (391.2)
	20–30	47.0 (4.71)	111.2 (33.3)	225.7 (26.4)	123.3 (52.3)	118.6 (44.0)	625.8 (76.2)
	Total	521.7 (42.6)	698.1 (187.0)	946.4 (81.0)	477.3 (137.2)	556.7 (69.3)	3200.2 (376.3)
Mortality (kg ha^{-1})	0–10	141.9 (27.1)	49.2 (28.7)	176.9 (42.3)	473.3 (183.4)	313.3 (78.1)	1154.6 (278.3)
	10–20	43.0 (1.63)	125.1 (125.1)	100.9 (67.8)	130.3 (79.9)	173.9 (111.1)	573.2 (372.7)
	20–30	38.0 (13.8)	82.3 (47.6)	112.8 (64.6)	187.8 (63.1)	122.8 (67.2)	543.7 (101.3)
	Total	222.9 (39.3)	256.6 (137.5)	390.6 (130.5)	791.4 (235.4)	610.0 (103.4)	2271.5 (623.9)

Data are presented as means (standard error)

fine root production and mortality in this and other studies (Steele et al. 1997; Hendricks et al. 2006). However, the results of this study might be insufficient to fully elucidate the fine root dynamics of *P. densiflora* forests, as there were only six minirhizotron tubes. Long-term observations with more replicates are required to determine the relationship between fine root dynamics and aboveground photosynthetic activity and to compare the minirhizotron and sequential coring methods.

Conclusion

We quantified FP and FM using minirhizotrons and CFs. The FP and FM changes with season and soil depth were affected by soil environmental conditions such as soil temperature and nutrient content. Moreover, the seasonality of FP and FM was likely related to plant phenology, including leaf expansion and the leaf-fall period. In future studies, it will be necessary to determine the relationship between fine root dynamics and aboveground photosynthetic activity in *P. densiflora* forests. The minirhizotron technique is a reliable method for the direct measurement of fine roots with minimal site disturbance. Minirhizotrons and conversion factors developed in this study will enable fast and accurate estimates of the fine root dynamics in *P. densiflora* forest ecosystems.

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