Vertical distribution and production of fine roots in an old-growth forest, Japan

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Abstract: Fine roots (≤ 2 mm in diameter) account for up to 50% of total net primary production in forests, representing a major flow of both carbon and nutrients into the soil. We investigated the vertical distribution and production of fine roots in a warm temperate old-growth evergreen broadleaved forest in southwestern Japan. We used a continuous inflow method that considered different rates of diameter-dependent root mortality, decomposition, and thickening. Fine roots were classified into two classes (≤ 1 mm and 1–2 mm diameter). The experiment was conducted over a 1-year period to collect data on the mass of live fine roots and mass of dead fine roots in January, May, November and the following January. Decomposition ratios were assessed for three intervals (January to May, May to November, and November to January). More than 70% of fine roots occurred in the 0–20 cm soil layer, and less than 4% were found in the 50–80 cm soil layer. Decomposition ratios varied seasonally in both root size classes, peaking in summer and reaching a minimum in winter. The same pattern was found for production, mortality, and decomposition. The peak rate of production was 1.62 g·m⁻²·day⁻¹ in ≤ 1 mm and 0.63 g·m⁻²·day⁻¹ in 1–2 mm fine roots. The lowest production was 0.62 g·m⁻²·day⁻¹ in ≤ 1 mm and 0.38 g·m⁻²·day⁻¹ in 1–2 mm fine roots. Total fine root production over a 1-year period was 6.61 t·ha⁻¹. A mass of 2.70 t·ha⁻¹·yr⁻¹ of dead fine roots was decomposed to return nutrients to the soil. It is concluded that a warm temperate old-growth evergreen broadleaved forest in southwestern Japan plays an important role in carbon cycle and nutrient return through a high amount of production and decomposition.

Keywords: Continuous inflow method; decomposition ratio; fine roots; mortality; production

The natural forests in southern Japan are dominated by evergreen broadleaved forests. These forests have higher production than most other forests in Japan, as a result of warmer climatic conditions (Kira, Yabuki 1977; Tran, Sato 2018). The aboveground production of these Japanese forests has been investigated in a number of studies (Tadaki 1968; Kira, Yabuki 1978; Tadaki 1995; Kubota 2003; Nagakawa et al. 2006; Tada et al. 2006), but the challenges of estimating the belowground production of fine roots (≤ 2 mm in diameter) are limited by the available information (Noguchi et al. 2007). The production of fine roots may contribute up to 50% of the total net primary production (NPP) in forest ecosystems (Tran, Sato 2018; Vogt et al. 1996; Yashiro et al. 2010; Baisyya, Barik 2011; Ohtsuka et al. 2013). Since NPP is an important indicator for the evaluation of patterns, processes, and dynamics of carbon cycling in forest ecosystems (Lou et al. 2002), changes in NPP could have global significance and change the ecosystem function and structure (Mooney et al. 1996, Chapin et al. 1998). Therefore, information on belowground production would lead to a more complete understanding of production and nutrient cycle in evergreen broadleaved forests in Japan.

Fine roots capture and transport water and nutrients required by plants (McCormack, Guo 2014).
They can survive several weeks to months, for a much shorter time than a tree’s life (Tierney, Fahey 2001). More than 80% of fine roots are distributed at a soil layer of 0–20 cm in forests (Ttran, Sato 2018), indicating lower fine root distribution at deeper soil depths. Fine roots are important in reducing nutrient losses by leaching and improving tree drought tolerance by absorbing water in deeper soil during a dry period (Christina et al. 2017, Laclau et al. 2010). Several methods have been developed to estimate fine root production, including decision matrix (Fairley, Alexander 1985), rhizotron technique (Bernier, Robitaille 2004), or continuous inflow method (Osawa, Aizawa 2012). Each method has advantages and disadvantages and estimates vary in accuracy (e.g., decision matrix is a simple method but it does not include decomposition in production estimation, leading to low accuracy; rhizotron is a more complicated method as it uses a camera to take images of fine root growth but it does not include decomposition in production estimation either; continuous inflow method requires much field and lab works but includes decomposition in production estimation). Osawa and Aizawa (2012) proposed a new method for estimating fine root production: the continuous inflow method, based on the facts that fine roots grow, die, and decompose simultaneously. This method provides a more accurate estimate of fine root production that includes decomposition of dead fine roots (Osawa, Aizawa 2012). Tran et al. (2016a) classified fine roots to two classes as ≤ 1 mm and 1–2 mm in diameter and applied the continuous inflow method (Osawa, Aizawa 2012) for estimating fine root production. The resulting estimates of fine root production were more accurate as they considered different rates of fine root decomposition, and different masses of dead fine roots and live fine roots in each size class. Our study investigated the vertical distribution of fine roots and estimated their production in a warm temperate old-growth evergreen broadleaved forest in southwestern Japan.

**MATERIAL AND METHODS**

**Study site.** A permanent 200 × 200 m plot was established in 1989 in a warm temperate old-growth evergreen broadleaved forest in southwestern Japan (32°03’N, 131°12’E) for long-term ecological research (Sato et al. 1999). The study was undertaken in a subplot of 20 × 20 m inside the 200 × 200 m plot. The subplot is located on the upper slope facing west with sloping of 9–12°. The climate of the site was characterized by precipitation of about 3 000 mm yr⁻¹ with no pronounced dry season, and an average temperature of 14.2 °C (Sato et al. 1999). The lowest monthly precipitation was recorded during December with 72 mm, while the maximum was recorded during June with 467 mm. There are six months of winter (Nov-Apr) with minimum temperature dropping to 3 °C in January and six months of summer (May-Oct) with maximum temperatures up to 32 °C in July and August. The main soil type is a moderately moist brown forest soil (Sato et al. 1999).

The vegetation in the site is dominated by numerous trees of evergreen species including major species: *Distylium racemosum* Siebold & Zucc, *Persea thunbergii* (Sieb & Zucc) Kostermans, *Quercus acuta* Thunb, *Q. salicina* Thunb, and *Q. gilva* Blume. Minor species with only a few trees per hectare are deciduous broadleaved trees such as *Cornus controversa* HemsI and *Carpinus tschonoskii* Maxim. Totally, 36 evergreen broadleaved and 13 deciduous species were found in the 200 × 200 m plot with a total 4 668 individuals and basal area of 217 m² (Sato et al. 1999).

**Fine root production and vertical distribution.** The continuous inflow method (Osawa, Aizawa 2012) was applied to estimate fine root production (*P*, Eq. 1), mortality (*M*, Eq. 2), and decomposition (*D*, Eq. 3):

\[ P = (B_r - B_d) + (N_r - N_d) + \left[ -\left( N_r - N_d \right) / \gamma_y + N_r \right] \times \ln \left( 1 - \gamma_y \right) \tag{1} \]

\[ M = \left( N_r - N_d \right) + D \tag{2} \]

\[ D = -\left( N_r - N_d \right) - \left( N_r - N_d \right) / \gamma_y + N_r \right] \times \ln \left( 1 - \gamma_y \right) \tag{3} \]

where:

- \( B_r \), \( B_d \) = biomass (mass of live fine roots; g·m⁻²) at times \( t_i \) and \( t_f \), respectively (\( t_f \geq t_i \));
- \( N_r \), \( N_d \) = necromass (mass of dead fine roots; g·m⁻²);
- \( \gamma_y \) = fine root decomposition ratios between \( t_i \) and \( t_f \);
- \( B_r \), \( B_d \), \( N_r \), and \( N_d \) are obtained by sequential soil core sampling, while \( \gamma_y \) is obtained by litter bag technique.

For sequential soil core sampling, a 36-mm diameter steel tube was used to take soil cores to a depth of 21 cm on four occasions (January, May and November 2013, and January 2014). Forty soil
cores were collected at each time with a spacing of 2 × 4 m inside the subplot; if the positions for coring were located on the stump of trees, they were moved 30 cm farther from the stumps. The coring positions on four occasions were at least 50 cm apart. Fine roots were separated from the collected soil by washing in water and sieving. Dead and live fine roots were classified to diameter classes of ≤ 1 mm and between 1–2 mm (Tran et al. 2016a). Live and dead fine roots were differentiated by their colour, resilience, and structural integrity (Hishi, Takeda 2005). Fine roots were then air-dried for a week and forced air-dried in an oven at 70 °C until their mass remained constant, and live roots and dead roots were weighed separately for the two size classes.

To measure a decomposition ratio, we used the litter bag technique (Osawa, Aizawa 2012). Litter bags of an envelope type were made from special cloth. The cloth had a pore size of 0.6 μm, preventing the ingrowth of roots. Dead fine roots were collected from the field, washed free of soil, and then oven-dried at 70 °C for a constant mass. Around 1 g of oven-dried fine roots and some fine soil were placed into a litter bag of 10 × 10 cm in size. Litter bags were buried in a 20 × 20 m subplot at a soil depth of 10–15 cm. In each period of the year (Jan-May, corresponding to late winter and early summer; May-Nov, corresponding to summer; and Nov-Jan, corresponding to winter), 20 litter bags were collected. After collection the litter bags were immediately washed and sieved to collect the remaining fine roots, then oven-dried at 70 °C for remained mass. The decomposition ratio $\gamma_{ij}$ was then estimated as $\gamma_{ij} = (\text{initial mass} - \text{remained mass})/\text{initial mass}$.

To investigate the vertical distribution of fine roots, a 36-mm diameter steel tube of 1 m in length was used to core the ground by 10 cm intervals in May 2013. Fine roots were then separated from collected soil, classified to ≤ 1 mm and 1–2 mm fine roots, oven-dried at 70 °C, and weighed for dry mass.

**Data analysis.** The estimation was conducted for two size classes separately (those with diameter ≤ 1 mm and those with diameter 1–2 mm). Then decomposition, mortality, and production of all fine roots (those with diameter ≤ 2 mm) were a total from two classes. Univariate analysis of variance (ANOVA) and Duncan’s multiple-range post hoc test were employed to understand the differences in means among decomposition ratios, mortality, and production. Prior to the application of ANOVA, skewness and kurtosis were applied to test the assumption of normality and Levene’s test was used for the assumption of homoscedasticity. All analyses were conducted using SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

**RESULTS**

**Fine root vertical distribution**

Fine roots were concentrated in the upper mineral soil (Figure 1), with more than 70% above 20 cm in soil. Less than 4% occurred below 50 cm. A comparison between the two size classes at each soil depth
indicated the higher amount of ≤ 1 mm fine roots at 0–40 cm soil layers. While at deeper layers (> 40 cm) the amount of 1–2 mm fine roots was higher than that of ≤ 1 mm fine roots. The amount of ≤ 1 mm fine roots in the 0–10 cm soil layer was nearly three times that of 1–2 mm fine roots. While in the 10 to 20 cm soil layer, the amount of ≤ 1 mm fine roots was double that of 1–2 mm fine roots. Conversely, it was found at deeper layers. For example, at 50 to 60 cm soil depth the amount of 1–2 mm fine roots was double that of ≤ 1 mm fine roots (Figure 1).

**Fine root decomposition ratio**

The decomposition ratio of dead fine roots varied across seasons (Figure 2), when it was significantly higher in summer than in winter or spring. The decomposition ratio of ≤ 1 mm fine roots was higher than that of 1–2 mm fine roots. The ≤ 1 mm fine root class had a peak decomposition ratio of 0.00097 ± 0.00012 day⁻¹ during May-Nov and the lowest ratio of 0.00074 ± 0.00006 day⁻¹ during Nov-Jan. While in 1–2 mm fine root class, the highest decomposition ratio was 0.00059 ± 0.00009 day⁻¹ also during May-Nov and the lowest ratio was 0.00036 ± 0.00003 day⁻¹ also during Nov-Jan. Generally, the decomposition ratio of ≤ 1 mm fine roots was nearly double that of 1–2 mm fine roots (Figure 2).

**Fine root decomposition, mortality and production**

In ≤ 1 mm fine roots, decomposition (Figure 3A), mortality (Figure 3B), and production (Figure 3C) were seasonally dependent. The highest decomposition, mortality, and production occurred in summer (May-Nov) as 0.65 ± 0.12 g·m⁻²·day⁻¹, 1.11 ± 0.24 g·m⁻²·day⁻¹ and 1.64 ± 0.54 g·m⁻²·day⁻¹, respectively. The lowest decomposition, mortality, and production were found during winter (Nov-Jan), with 0.32 ± 0.05 g·m⁻²·day⁻¹, 0.45 ± 0.10 g·m⁻²·day⁻¹ and 0.62 ± 0.09 g·m⁻²·day⁻¹, respectively (Figure 3A, B, C).

In 1–2 mm fine roots, mortality (Figure 3E) and production (Figure 3F) were seasonally dependent, but decomposition was not (Figure 3D). The highest mortality and production occurred in summer with 0.41 ± 0.08 g·m⁻²·day⁻¹ and 0.63 ± 0.11 g·m⁻²·day⁻¹, respectively. The lowest mortality and production occurred in winter with 0.26 ± 0.05 g·m⁻²·day⁻¹ and 0.38 ± 0.04 g·m⁻²·day⁻¹, respectively (Figure 3E, F). The decomposition was 0.17 ± 0.03 g·m⁻²·day⁻¹ during Jan-May, 0.19 ± 0.03 g·m⁻²·day⁻¹ during Nov-Jan, and 0.25 ± 0.04 g·m⁻²·day⁻¹ during May-Nov (Figure 3D).

Decomposition, mortality, and production of all fine roots as a combination of both classes (≤ 1 mm and 1–2 mm fine roots) were seasonally dependent (Figure 4). The patterns were similar to those in ≤ 1 mm fine roots (Figure 3A–C). The highest decomposition reached 0.90 ± 0.16 g·m⁻²·day⁻¹ during summer (May-Nov) and the lowest decomposition of 0.50 ± 0.08 was g·m⁻²·day⁻¹ during winter (Nov-Jan; Figure 4).

The highest mortality of 1.52 ± 0.33 g·m⁻²·day⁻¹ was found in summer, and the lowest mortality was 0.72 ± 0.15 g·m⁻²·day⁻¹ in winter. The highest production reached 2.25 ± 0.62 g·m⁻²·day⁻¹ during summer and the lowest production was 1.00 ± 0.13 g·m⁻²·day⁻¹ during winter (Figure 4).

Over a 1-year period (Figure 5), fine root decomposition, mortality, and production were 2.70 ±
1.07 t·ha⁻¹, 4.47 ± 1.80 t·ha⁻¹, and 6.61 ± 2.75 t·ha⁻¹, respectively, for a warm temperate old-growth evergreen broadleaved forest in southwestern Japan.

**DISCUSSION**

Using one size class (a combination of all roots ≤ 2 mm in diameter) and applying the same continuous inflow method (Osawa, Aizawa 2012) indicated the fine root production of 5.66 t·ha⁻¹·yr⁻¹ in the present study site (Tran et al. 2015). The repeated method of estimation gave a slightly higher estimate of 6.61 t·ha⁻¹·yr⁻¹ (Figure 5). Such fine root production is lower than that in *Chamaecyparis obtusa* (Siebold & Zucc.) plantation in central Japan (7.55 t·ha⁻¹·yr⁻¹; Osawa, Aizawa 2012), but higher than in Bornean tropical rainforest in Malaysia (4.47 t·ha⁻¹·yr⁻¹; Katayama et al. 2019) and temperate broadleaved forest in Germany (1.38 t·ha⁻¹·yr⁻¹; Meinen et al. 2009). This indicated the importance of the method used, as there exist several methods for estimating fine root production (Fairley, Alexander 1985; Bernier, Ribitaille 2004; Osawa, Aizawa 2012; Tran et al. 2016b). Therefore, the method developed by Tran et al. (2016a) is recommended for practical application. However, this method requires much laboratory works for classifying fine roots. The difference in production results from different decomposition ratio of ≤ 1 mm and 1–2 mm
fine roots (Figure 2), which is higher in smaller fine roots. Therefore, the decomposition ratio must be treated carefully as the behaviour of fine roots in nature. If both size classes of fine roots had the same decomposition ratios, then there would be no need to separate the classes.

The density of fine roots declined with depth, as found in other studies (Maeght et al. 2015; Tran, Sato 2018), which is logical when water and nutrient supplies are higher in upper soil layers (Pierret et al. 2016; Germon at al. 2018). Fine root production is dependent on soil water content and soil fertility (Canham et al. 2015), as higher moisture and more fertile soil lead to higher fine root production (Nguyen et al. 2019). Meanwhile, more 1–2 mm fine roots distributed at deeper soil layers (50 to 80 cm; Tran, Sato 2018) could support trees better by maximizing water absorption from deeper soil especially in the dry season (Laclau et al. 2010; Christina et al. 2017).

Decomposition ratios were seasonally dependent (Figure 2). This could be explained by the differences in temperature and soil humidity between summer and winter. Litter decomposition is influenced by surrounding environments with low temperature and soil humidity inhibiting microbial activity (Krishna, Mohan 2017; Bueis et al. 2018). High decomposition ratio (Figure 2) with high mortality (Figure 3) led to the high decomposition of fine roots in summer (May-Nov). Meanwhile, high production in summer (Figure 3 and 4) could be explained by the growing season in the present study site. Vegetation is classified as an evergreen broadleaved forest. However, at a low temperature in winter trees may much reduce growth compared to summer. Since summer is the growing season, trees require more nutrients and water for their growth. Therefore, fine root growth has to peak to physiologically balance demand and supply. Such a pattern was seen in a temperate deciduous forest (Tran et al. 2016b).

Fine root production is affected by several factors such as ages and types of forest, climate, and edaphic conditions. The fine root production in this study was 6.61 t·ha⁻¹·year⁻¹. It was 1.36 t·ha⁻¹·year⁻¹ in Quercus serrata Murray plantation in Tsukuba, Japan (Tran et al. 2016b), 5.78 t·ha⁻¹·year⁻¹ in a secondary Q. serrata forest in Ohtsu, Japan (Tran et al. 2016a). While it was 3.65 t·ha⁻¹·year⁻¹ in old-growth (Tran 2017) and 1.13 t·ha⁻¹·year⁻¹ in secondary (Tran, Sato 2018) evergreen tropical forests in Vietnam; these figures indicate that fine root production varies among sites internationally and nationally. Therefore, to deeply understand the function of fine roots in the forest carbon cycle and in nutrient return, estimating the fine root production locally is becoming important.
CONCLUSION

Fine roots were studied in a warm temperate old-growth evergreen broadleaved forest, southwestern Japan. More than 70% of fine roots were distributed at 0–20 cm soil layers, while less than 4% of fine roots were distributed at 50–80 cm soil layers. In shallow soil layers, the mass of ≤ 1 mm fine roots was higher than that of 1–2 mm fine roots. Conversely, in deeper soil layers the mass of 1 to 2 mm fine roots was higher than that of ≤ 1 mm fine roots. The fine root decomposition ratio was seasonally dependent and was higher during summer compared to winter. A similar pattern was found in decomposition, mortality, and production in both size classes, indicating higher values during summer. Total fine root decomposition, mortality, and production over a 1-year period in this study were 2.70 t·ha⁻¹·yr⁻¹, 4.47 t·ha⁻¹·yr⁻¹, and 6.61 t·ha⁻¹·yr⁻¹, respectively. The study forest plays an important role in carbon cycle and soil nutrient cycling as 4.47 t·ha⁻¹·yr⁻¹ of dead fine roots contributed to soil organic matter and 2.70 t·ha⁻¹·yr⁻¹ of dead fine roots was decomposed to return nutrients to soil and emit carbon to the atmosphere through heterotrophic respiration.

REFERENCES


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