Fine root morphological traits determine variation in root respiration of *Quercus serrata*

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Summary Fine root respiration is a significant component of carbon cycling in forest ecosystems. Although fine roots differ functionally from coarse roots, these root types have been distinguished based on arbitrary diameter cut-offs (e.g., 2 or 5 mm). Fine root morphology is directly related to physiological function, but few attempts have been made to understand the relationships between morphology and respiration of fine roots. To examine relationships between respiration rates and morphological traits of fine roots (0.15–1.4 mm in diameter) of mature *Quercus serrata* Murr., we measured respiration of small fine root segments in the field with a portable closed static chamber system. We found a significant power relationship between mean root diameter and respiration rate. Respiration rates of roots < 0.4 mm in mean diameter were high and variable, ranging from 3.8 to 11.3 nmol CO$_2$ g$^{-1}$ C0$_2$/s, compared with those of larger diameter roots (0.4–1.4 mm), which ranged from 1.8 to 3.0 nmol CO$_2$ g$^{-1}$ s$^{-1}$. Fine root respiration rate was positively correlated with specific root length (SRL) as well as with root nitrogen (N) concentration. For roots < 0.4 mm in diameter, SRL had a wider range (11.3–80.4 m g$^{-1}$) and was more strongly correlated with respiration rate than diameter. Our results indicate that a more detailed classification of fine roots < 2.0 mm is needed to represent the heterogeneity of root respiration and to evaluate root biomass and root morphological traits.

Keywords: closed static chamber system, immediate field measurements, mean root diameter, root CO$_2$ efflux, specific root length.

Introduction

Root respiration is a major source of carbon dioxide (CO$_2$) efflux from forest soils and plays an important role in ecosystem-level carbon cycling. The contribution of root respiration to total soil respiration of forest ecosystems ranges from one-third to more than one-half, depending on the measurement methods, forest type and season (Bowden et al. 1993, Nakane et al. 1996, Epron et al. 2001, Dannoura et al. 2006a).

Respiration of fine roots (generally defined as < 2 mm in diameter) has been estimated to be higher than that of coarse roots (Desrochers et al. 2002, Dannoura et al. 2006b). Fine root respiration is critical for physiological functions such as maintenance, growth and nutrient uptake of trees (George et al. 2003). The physiological functions of fine roots contribute a large proportion of net primary production in carbon cycling of forest ecosystems (Vogt et al. 1986, 1996, Gower et al. 1996).

Many studies have estimated fine root respiration but only few have attempted to measure it in the field, because of the numerous difficulties associated with such measurements (Epron et al. 2001, Kominami et al. 2008, Marsden et al. 2008a, 2008b). Respiration rates of fine roots can be highly variable (e.g., Pregitzer et al. 1998, Dannoura et al. 2006b). This is probably because of the arbitrary classification of fine roots based on diameter rather than on an anatomical or physiological basis (e.g., Majdi et al. 2005).

Recent studies have shown that fine root systems are composed of individual roots with heterogeneous morphological traits and physiological functions, and that fine root morphology is directly related to physiological function (Eissenstat et al. 2000, Pregitzer et al. 2002). For example, metabolic activity of fine roots is higher for the lower order and smaller diameter fine roots (Pregitzer et al. 1998, 2002, Guo et al. 2004). Moreover, individual roots within the fine root system have variable turnover rates, which are the
result of heterogeneous production, mortality and decomposition (Wells and Eissenstat 2001, King et al. 2002, Hishi and Takeda 2005, Hishi 2007). Therefore, we must develop a new classification of fine roots, which considers their physiological functions, to understand their contribution to ecosystem carbon cycling.

Fine root respiration rates have been inferred from laboratory measurements of root nitrogen (N) concentration, oxygen consumption or the reduction of triphenyltetrazolium chloride method of roots sampled in the field (Ryan et al. 1996, Zogg et al. 1996, Pregitzer et al. 1998, Comas et al. 2000, Burton et al. 2002, Richter et al. 2007). Attempts have also been made to measure tree fine root respiration directly in the field by the closed chamber method (Ryan et al. 1996, Clinton and Vose 1999, Burton et al. 2002, Burton and Pregitzer 2003). However, these studies are characterized by potential problems with their method of measurements, such as the effects of root excision disturbance, wound respiration and increasing CO₂ concentration inside the chamber. Recently, Marsden et al. (2008a, 2008b) reported that immediate measurement after root excision is critical for accurate estimation of root respiration; however, they also showed that their results were associated with high uncertainty and concluded that a more extensive knowledge of the effects of morphological and physiological factors on respiration rates is required.

Here, we used a portable closed static chamber system to measure respiration of small fine root segments immediately after sampling in the field. Our objective was to elucidate the relationships between respiration rate and morphological traits of the fine roots of Quercus serrata Murr. trees in a broad-leaved secondary forest in Japan. We focused on mean root diameter and specific root length (SRL; m g⁻¹) of the fine root segments. Specific root length is the ratio of length to biomass of a root segment and is an indicator of root benefit to root cost, assuming that root length is proportional to resource acquisition and root mass is proportional to construction and maintenance (Eissenstat and Yanai 1997, Ostenen et al. 2007a, 2007b). In addition, we measured root N concentration and its relationship with root respiration rate, because several studies have shown that fine roots with high N concentrations have high respiration rates (Ryan et al. 1996, Pregitzer et al. 1998, Burton et al. 2002). From these data, we inferred how the morphological traits of the fine roots, such as mean root diameter and SRL, influence the fine root respiration rate of Q. serrata at the study site.

Materials and methods

Study site

The study was carried out at Yamashiro Experimental Forest (YMS), Kyoto, in a mountainous region of western Japan (34°47′ N and 135°50′ E; 180–250 m a.s.l., 1.7 ha). The forest consists of deciduous broad-leaved species (mainly Q. serrata) and evergreen broad-leaved species (mainly Ilex pedunculosa Miq.). Mean annual precipitation is 1449 mm, and mean temperature is 15.5 °C. The soils are Regosols of sandy loam or loamy sand and contain fine granite gravel (53% by mass) (Kaneko et al. 2007). The soil layer is generally thin and immature. Kominami et al. (2008) give further details on the study site.

The YMS is an Asia Flux site. The amount of CO₂ flux between the forest ecosystem and the atmosphere has been monitored by meteorological towers by the eddy covariance method (Kominami et al. 2008). To cross-validate the net ecosystem production, the eddy covariance method and biomass production (Goto et al. 2003), photosynthetic rate (Miyama et al. 2003), decomposition rate of coarse woody debris (Jomura et al. 2008), soil respiration (Tamai et al. 2005) and root respiration (Dannoura et al. 2006a) have been evaluated at this site (Kominami et al. 2008). Dannoura et al. (2006b) measured the respiration rates of both fine roots (0–2 mm in diameter) and coarse roots (2–250 mm) of Q. serrata, the dominant tree species in the field with a closed chamber system equipped with an infrared CO₂ gas analyzer (IRGA; LI-820, Li-Cor, Lincoln, NE). We also selected the fine roots of Q. serrata for measurement because it is one of the dominant species in the YMS, comprising 33% of the standing biomass per stand basal area (Goto et al. 2003).

Measurement system

We measured the respiration rates of small fine root segments (< 2 mm in diameter) with a closed static chamber system immediately after sampling a fine root in the field. The system comprised an infrared gas analyzer (IRGA, GMP343, Vaisala, Finland) attached to a small cylindrical chamber (0.308 l), which is suitable for measuring the respiration rates of small fine roots. The temperature in the chamber was measured with a copper constantan thermocouple. The GMP343, which is a silicon-based non-dispersive infrared sensor, is an excellent, accurate and rugged probe-type instrument for ecological measurements.

We enclosed one fine root segment (about 0.2 g fresh mass) in the chamber and measured the CO₂ concentration for 16 min. The CO₂ concentration and temperature in the chamber were recorded with a data logger (NR-1000, Keyence, Japan). To avoid the effects of air disturbance caused by opening the chamber, the data for the first 5 min and last 1 min were not used. The respiration data for the middle 10-min interval were used to calculate fine root respiration (*F* _subroot_, nmol CO₂ g⁻¹ s⁻¹):

\[
F_{\text{root}} = \frac{1}{n} \left( \sum_{i=1}^{n} \frac{C_{\text{CO}_2}(t_i + \Delta t) - C_{\text{CO}_2}(t_i)}{\Delta t} \right) \frac{V_s}{V_{\text{air}}} \times \frac{273.2}{273.2 + T} \frac{10^3}{\text{DW}},
\]

*V* _s_, chamber volume (l); *V* _air_, air volume (l); *T*, temperature (°C); *DW*, dry mass (g); *n*, number of measurements; *C* _CO₂_, CO₂ concentration (ppm).
where $C_{CO_2}$ is CO2 concentration (ppm) at time $t$, $n$ is number of time intervals in the measurement period (600 s), $t_1$ is start time, $\Delta t$ is time interval, $V_c$ is volume of chamber (0.308 l), $V_{air}$ is standard gas volume (22.41 l), $T$ is chamber temperature ($^\circ$C) and DW is dry mass of the root sample (g).

We obtained at least 600 data points during the 10-min interval. During the 16-min measurement interval, the increase in CO2 concentration inside the chamber was $< 50$ ppm and the response was linear ($r^2 > 0.792$, $P < 0.001$), indicating that there were minimal effects of increasing CO2 concentration and other possible sources of error, such as system leaks and insufficient air flow during the measurement period, on the fine root respiration rate. The linear response also indicated that the chamber volume was not large relative to the size of the fine root samples. Although we did not specifically test for wound respiration, wound respiration of our samples could be minimal because we excised the root samples by cutting off only one point of attachment, whereas previous studies obtained samples by cutting off several points of attachment.

**Field measurement**

Fine root respiration was measured during the nights (2200-0500 h) of August 1 and September 12, 2007. We chose to make measurements at nighttime to minimize the change in temperature and to maintain high relative humidity during the measurements. Although we did not measure root water potential, roots sampled at night were probably subjected to less water stress than roots sampled in the daytime. Mean temperature in the chamber for August 1 and September 12, 2007 was 23.7 and 23.1 $^\circ$C, respectively. Changes in temperature during the measurement were $< 1$ $^\circ$C.

We established a 15 x 15 m plot on a slope just below a ridge where *Q. serrata* trees dominated. We excavated root samples with pruning shears and small knives from the upper 10 cm of soil at random locations under the canopy of *Q. serrata* trees. We chose a rectangular area (15 x 10 cm) on the ground and excavated two to four root segments in this area. Each root segment, which included small lateral roots, was carefully isolated from the soil and organic matter to ensure that it was not damaged and remained attached to larger roots. Then, the segment was gently washed with deionized water to remove the soil without disrupting any of the small lateral roots and was blotted with tissue paper to remove excess water. We first excised the thickest point of the root segment and immediately (within 1 min) measured root respiration. The respiration rate was measured within 10 min after the excavation with the small closed static chamber system described above. We repeated this procedure for each root sample. The total number of samples was 13 and 19 for August 1 and September 12, 2007, respectively, and mean fresh mass of the samples was 0.2 g. After the respiration measurements, the segments were wrapped in moistened tissue paper, placed on ice and transported within a few hours to the laboratory for further analysis.

**Fine root morphology and root N concentration**

Each fine root segment was scanned in gray scale at 400 dpi with a filter of 1.0 mm in an automatic threshold method (Expression 10000XL, EPSON). Total root length and mean root diameter of each segment were determined using WinRHIZO Pro 2007a software (Regent Instruments, Quebec, Canada), which is an image analysis system specifically designed for root measurement. After scanning, the segments were dried at 70 $^\circ$C for 48 h and weighed. We calculated SRL from total root length and dry mass of the segment.

Each fine root segment was then cut into smaller pieces with scissors and finely crushed with a mortar and pestle in preparation for N analysis. Total N concentration was measured with an NC analyzer (SUMIGRAPH NC-900, Shimadzu, Japan).

**Data analysis**

Relationships between fine root respiration rates and mean root diameter, SRL or root N concentration were examined by regression analysis. Exponential and power equations have been used to describe the relationship between diameter or SRL and respiration rate of fine roots. We chose to fit the power relationship, because it had the highest $r^2$ value. The relationships between fine root respiration and root N concentration were examined by linear regression analysis. All statistical analyses were conducted using the scientific data analysis software IGOR Pro 5.0 (Wave Metrics, Inc., Lake Oswego, OR).

**Results**

The range of mean diameter, total length and dry mass of the 32 fine root segments analyzed were 0.15–1.40 mm, 0.14–2.96 m and 0.02–0.13 g, respectively. Root respiration rates measured with the closed static chamber system varied widely from 1.8 to 11.5 nmol CO2 g$^{-1}$ s$^{-1}$.

We found a significant power relationship between mean root diameter and fine root respiration rate (Figure 1, $r^2 = 0.719$, $P < 0.001$). Fine root respiration rates increased markedly with decreasing mean diameter. The respiration rates of root segments $< 0.4$ mm were very high compared with those of larger diameter segments (0.4–1.4 mm in diameter). Respiration rates of root segments $< 0.4$ mm varied widely, ranging from 3.8 to 11.3 nmol CO2 g$^{-1}$ s$^{-1}$, whereas those of larger diameter root segments varied from 1.8 to 3.0 nmol CO2 g$^{-1}$ s$^{-1}$.

The SRL of the fine root segments ranged from 1.05 to 80.39 m g$^{-1}$ (Figure 2) and fine root respiration rate
increased with increasing SRL ($r^2 = 0.786$, $P < 0.001$). Root N concentration of the fine root segments ranged from 7.5 to 17.5 mg g$^{-1}$ (Figure 3), and fine root respiration rate increased linearly with increasing fine root N concentration ($r^2 = 0.627$, $P < 0.001$).

**Discussion**

The respiration rates of our fine root segments measured immediately after sampling in the field with a closed static chamber system were within the range of published values (Zogg et al. 1996, Desrochers et al. 2002, Marsden et al. 2008a). Furthermore, our estimates of fine root respiration rates as a function of root diameter were continuous with estimates for coarse roots made by Dannoura et al. (2006b) for the same study site (Figure 4). Dannoura et al. (2006b) pooled all fine roots < 2 mm in diameter and obtained an estimate of fine root respiration ranging from 1.3 to 2.5 nmol CO$_2$ g$^{-1}$ s$^{-1}$ (transformed to rates at 24 °C based on $Q_{10}$ values), about 4.5 times lower than the maximum respiration rates in our study. The amount of fine root sample measured may explain the difference in fine root respiration rates between the two studies. Up to now, it was technically difficult to measure the respiration rates of fine roots because of their extremely small size. In several studies, a large root sample (mean fresh mass 2.0–4.0 g) was measured (e.g., Burton and Pregitzer 2003, Dannoura et al. 2006b). As indicated by our results, there is considerable variation in fine root respiration rate, especially among small diameter roots. Therefore, the respiration rate per unit dry mass of a large sample of roots may be underestimated, because, on a mass basis, large diameter roots, which have lower respiration rates, mask the higher respiration rates of roots with diameters < 2 mm. We used a small root sample (mean fresh mass 0.2 g) for each measurement to minimize heterogeneity among samples that include the roots of different diameters.
Figure 4. Relationship between mean root diameter and root respiration rate of fine and coarse roots of Q. serrata at the YMS site, western Japan. Respiration rates of fine roots (●, < 2 mm; n = 32) were measured in this study and respiration rates of coarse roots (○, 2–60 mm; n = 30) were measured by Dannoura et al. (2006b).

Consequently, the high respiration rates of the smaller diameter roots were reflected in our measurements. The effects of the amount and diameter distribution of root samples on the estimates of respiration rate are important factors to be considered when examining belowground carbon balances (Kominami et al. 2008).

We found a strong relationship between the mean diameter of fine root segments and root respiration rate (Figure 1). Fine root respiration increased with decreasing root diameter even < 2 mm, which is the conventional cut-off for fine roots. In our study, respiration rates of root segments < 0.4 mm in diameter were high compared with those of larger diameter roots. This finding is in good agreement with the results of Pregitzer et al. (1998) who showed that root respiration rates were much higher for roots < 0.5 mm in diameter than for larger diameter roots and that the differences in respiration rates among the roots with different diameters reflected variation in physiological functions or metabolic activity. These findings suggest that fine roots need to be classified on the basis of physiological function rather than diameter.

There is evidence that fine root respiration differs widely with root morphology and age (Pregitzer et al. 1998, Bouma et al. 2001, Comas et al. 2002). Morphological traits of fine roots, such as SRL, are correlated with nutrient uptake and assimilation, which should be reflected in root respiration and carbon maintenance costs (Eissenstat and Yanai 1997, Eissenstat et al. 2000). The SRL of fine roots varies significantly among tree species and is higher for deciduous trees than for coniferous trees (Ostonen et al. 2007b). The difference in SRL among tree species is greatly influenced by biotic factors, including associated fungi (King et al. 2002, Pregitzer et al. 2002) and rhizosphere microbial communities (Löhmus et al. 2006). The high SRL values that we measured in the roots of Q. serrata are characteristic of a deciduous root system, and similar to those reported for Fagus sylvatica L. (Ostonen et al. 2007b). The roots of Q. serrata have symbiotic ectomycorrhizal fungi (Smith and Read 1997) and the high SRL values of Q. serrata roots are characteristic of ectomycorrhizal roots.

Specific root length is a commonly used parameter of fine root morphology and has been studied as an indicator of environmental changes (Ostonen et al. 2007b). However, the relationships between SRL of fine roots and physiological functions such as root respiration are not well known. We found a significant correlation between SRL and fine root respiration and showed that SRL explains over 60% of the variation in fine root respiration rate in the field (Figure 2). The respiration rates of fine roots < 0.4 mm in diameter were high and variable. The range of fine root diameters (0.15–0.40 mm) was narrow to explain the variability in fine root respiration rates. However, compared with fine root diameter, the SRL of roots < 0.4 mm in diameter had a wider range (11.25–80.39 m g⁻¹) and was more strongly correlated with fine root respiration rate indicating that SRL could be used to estimate the respiration rate of fine roots. Our results also suggested that there is a functional relationship between SRL and fine root respiration rate. The higher variation in root respiration rate of small roots may result from higher measurement error than for coarse roots. However, the strong relationship between SRL and fine root respiration indicates that these variations resulted from differences in root morphological traits and that SRL may be a key morphological parameter for evaluating fine root respiration.

We found a strong positive correlation between root N concentration and fine root respiration, corroborating the results of many published reports (Ryan et al. 1996, Pregitzer et al. 1998, Burton et al. 2002). It seems that the N concentration is closely correlated with growth and physiological factors such as protein concentration, nutrient assimilation and maintenance of ionic gradients (Ryan et al. 1996, Pregitzer et al. 1998). The finding that both SRL and N concentration are strongly correlated with fine root respiration rate gives support to the notion that root morphology reflects physiological function (Eissenstat et al. 2000, Hishi 2007).

Although our results indicated that SRL explained the variation in root respiration rate better than root diameter, in practice, root diameter is the most easily and quickly measured variable in the field. Recent studies, in which fine roots were classified as < 2.0 mm in diameter, revealed that these roots differed morphologically and functionally from both roots < 0.5 mm in diameter and larger roots (Joslin et al. 2006, Majdi et al. 2008). Thus, a more detailed classification of fine roots < 2.0 mm is needed to represent the heterogeneity of root functions and to evaluate root biomass and root morphological traits.

Because our data are limited to fine roots of Q. serrata in a specific plot and period, we need to clarify the
relationships between morphological traits and fine root respiration rates with data from different plots, seasons and other tree species to confirm the robustness of these relationships. If we consider both detailed morphological traits and respiration rates of fine root segments, it should be possible to classify fine roots into several physiological groups. Such a classification would enable us to gain a more precise understanding of the belowground carbon cycle in forest ecosystems, which reflects the physiological functions of tree root systems.

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