ORIGINAL ARTICLE

PLANT SPECIES BIOLOGY WILEY

Initial burst of root development with decreasing respiratory carbon cost in *Fagus crenata* Blume seedlings

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Funding information
Gobierno de Aragón, Grant/Award Number: H09_20R; Japan Society for the Promotion of Science, Grant/Award Numbers: 16H04871, 19H01158, 19H01161, 19H02987, L-14560

Abstract

As terrestrial plants are rooted in one place, their metabolism must be acclimatized to continuously changing environmental conditions. This process is influenced by different metabolic traits of plant organs during ontogeny. However, direct measurement of organ-specific metabolic rates is particularly scarce, and little is known about their roles in whole-plant metabolism. In this study, we investigated size scaling of respiration rate, fresh mass and surface area of leaves, stems and roots in 65 seedlings of *Fagus crenata* Blume (2 weeks to 16 months old). With the increase in plant mass, the proportion of roots in whole plants increased from 20.8 to 87.3% in fresh mass and from 12.8 to 95.0% in surface area, whereas there was only a 15.6 to 60.2% increase in respiration rate. As a result, the fresh-mass-specific and surface-area-specific respiration rates in the roots decreased by 85% and 90%, respectively, and these decreases were significantly size dependent. However, such a size-dependent decrease was not observed for the surface-area-specific respiration rate in the leaves and stems. It is likely that this rapid root development is specific to the early growth stage after germination and would help plants acquire water and nutrients efficiently (i.e., at relatively low respiratory carbon costs). Overall, it is probable that the establishment of *F. crenata* forests and survival of *F. crenata* seedlings could be promoted by substantial root growth, with a reduction in respiratory carbon cost.

KEYWORDS
biomass partitioning, root respiration, root surface area, root/shoot ratio, whole-plant metabolic scaling

1 | INTRODUCTION

Metabolism, which includes various fundamental biological processes that transform energy and materials, helps organisms to adapt to changing environmental conditions (Brown, Gillooly, Allen, Savage, & West, 2004; Sibly, Brown, & Kodric-Brown, 2012). Therefore, the metabolic rate has profound physiological, ecological and

evolutionary implications (Enquist, Tiffney, & Niklas, 2007; Glazier, 2015), and it could be key for understanding and predicting the effects of climate change on individuals and ecosystems (Brown et al., 2004; Sibly et al., 2012).

In general, the metabolic rate of an organism (i.e., the respiration rate, R) scales with body size. In order to obtain a mechanistic insight into the regulation of metabolic rate scaling, empirical evidence should be gathered from whole-organism measurements (Mori et al., 2010). The scaling of metabolic rate is usually described as a simple power function of body mass (X):

$$R = aX^b \qquad (1)$$

where a is the normalization constant and b is the scaling exponent (slope on the log–log coordinates) (DeLong, Okie, Moses, Sibly, & Brown, 2010; Kleiber, 1932; West, Brown, & Enquist, 1997). Equation (1) represents the metabolic rate as a function of body size under different environmental and phylogenetic constraints (Banavar, Cooke, Rinaldo, & Maritan, 2014; Glazier, 2018; Mori et al., 2010; Sibly et al., 2012; West et al., 1997; Yagi, Kanda, Takeda, Ishimatsu, & Oikawa, 2010). To date, most studies on the regulation of metabolic scaling were based on indirect evidence and were aimed at constructing theoretical models to explain the scaling exponent b, which has been widely assumed to be 3/4 according to the WBE model (DeLong et al., 2010; Dodds, Rothman, & Weitz, 2001; Kleiber, 1932; Kooijman, 2010; Mori et al., 2010; West et al., 1997). From a metabolic perspective, using fresh mass as the proxy for body size (X) is important because all active components (such as enzymes) are contained in the liquid phase, and they are the ultimate sources of energy for metabolic activities (Huang et al., 2019; Thakur, Rathore, & Chawla, 2018). In evolutionary biology, comparisons of metabolic scaling among various taxa, such as prokaryotes, animals, algae and vascular plants, have been performed using fresh mass as a proxy for individual body size (Banavar et al., 2014; Makarieva et al., 2008). In this context, an empirical study with direct evidence on the metabolic rate and fresh mass of individual organisms could increase our understanding of the regulation of metabolic scaling.

To study the scaling of metabolic rate of individual organisms, it is important to understand the size scaling of organ respiration. This is because individual organisms are complex systems that depend on the integrated performance of various organ structures and their functions (Glazier, 2015; Oikawa & Itazawa, 2003). In plants, biomass allocation to the leaves, stems (including the main stems and branches) and roots must be balanced for their growth (Bloom, Chapin III, & Mooney, 1985; Ferrio, Kurosawa, Wang, & Mori, 2018; Franklin et al., 2012; Niklas & Enquist, 2002; Shipley & Meziane, 2002), and the root/shoot biomass ratio is likely to change during ontogeny and shows size dependency as a consequence of allometric, physiological and ontogenetic conditions in plant evolution (Ackerly et al., 2000; Enquist & Niklas, 2002; Gedroc, Mcconnaughay, & Coleman, 1996; Iwasa & Roughgarden, 1984; Ledo et al., 2018; Mcconnaughay & Coleman, 1999; Modrzyński, Chmura, Tjoelker, & Thomas, 2015; Poorter et al., 2012). Therefore, metabolic scaling in plants involves size-dependent allocation of fresh mass, surface area and respiration rate among organs in individual plants. However, there are only a few empirical studies on the size scaling of allocation of energy, that is, the distribution of respiration rate among organs in individual plants (Kong & Fridley, 2019).

Fagus is one of the most widespread and dominant genera in temperate deciduous broadleaf forests in the circumpolar Northern Hemisphere (Fang & Lechowicz, 2006), and the species of this genus are relatively drought sensitive (Bolte et al., 2016; Peuke, Schraml, Hartung, & Rennenberg, 2002; Wagner et al., 2010). *Fagus crenata* Blume is one of the most typical canopy tree species of cool temperate forests that is widely distributed across Japan (Hiraoka & Tomaru, 2009; Tateishi, Kumagai, Suyama, & Hiura, 2010). Pure *F. crenata* forests are often established in snowy areas along the Japan Sea (Shimano, 2002). During the first year after germination, *F. crenata* seedlings have a high mortality rate because of various environmental factors, such as drought stress, insufficient light and fungal pathogens (Ichihara & Yamaji, 2009; Sahashi, Kubono, & Shoji, 1994; Yamaji & Ichihara, 2012). Thus, this period is considered as the bottleneck phase in the population. To date, little is known about the whole-plant physiology of *F. crenata* seedlings in this phase.

In this study, we assessed the size scaling of fresh mass, surface area and respiration rate in the leaves, stems and roots of 65 *F. crenata* seedlings (2 weeks to 16 months old). We assumed that the size-dependent shifts in fresh mass, surface area and respiration rate among plant organs in a certain growth stage represent fundamental adaptations for that growth stage. Based on this assumption, we hypothesized that there is a size-dependent shift in the allocation of fresh mass, surface area and respiration rate that is specific to the early growth stage, which is characterized by high mortality. To test this hypothesis, we also assessed the root/shoot ratio of fresh mass in various plant growth stages, from seedling to mature tree stages, using the data from our previous studies (Mori et al., 2010; Ono et al., 2013). Testing our hypothesis could help to understand how

seedlings survive the first year after germination when mortality is especially high and help describe the population dynamics of *F. crenata*.

Previous studies have revealed the geographical variation of *F. crenata*, mainly for aboveground parts, such as morphological and physiological traits of leaves (Ishii, Horikawa, Noguchi, & Azuma, 2018; Tateishi et al., 2010; Yamazaki, Yoda, Takahashi, Sonoike, & Maruta, 2007). However, despite the importance of roots for individual plant growth, these studies have rarely focused on roots. Therefore, the understanding of the ontogenetic change in shoot and root respiration at the whole-plant level in *F. crenata* seedlings would help advance our understanding of their ecophysiological responses in relation to population dynamics in the bottleneck phase in the local environment. In this study, we used *F. crenata* seedlings of one of the most typical snowy regions in Japan, where we focused on one haplotype of *F. crenata* and investigated the ecological significance of ontogenetic change in root and whole-plant respiration. Ultimately, the understanding of the geographic variation in respiration rate at the whole-plant and organ level, which is expected to be related to differences in the amount of snowfall in winter, would help to interpret the geographic differences in physiological traits as adaptations to various local environment.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

Our fieldwork included collection of *F. crenata* seeds in a Japanese national forest in the city of Tsuruoka. The fieldwork was permitted by the Shonai District Forest Office and did not involve any endangered or protected species.

2.2 | Plant material and experimental design

In October 2013 and 2015, we collected seeds from trees in a mature forest of *F. crenata* in Tsuruoka, Yamagata prefecture (38°32′N, 139°56′E), and sowed them in December in both years. This forest is one of the most representative *F. crenata* forests along the Japan Sea; therefore, the collected seeds belong to the haplotype prevalent on the Japan Sea coast (Hiraoka & Tomaru, 2009; Okaura & Harada, 2002). The seeds collected in 2013 germinated in mid-April 2014 and those collected in 2015 germinated in mid-April 2016, and almost all the germinated seedlings were healthy. In late April of 2014 and 2016, we transplanted approximately 100 2-week-old healthy seedlings to 0.9-L pots and placed them under

natural light conditions outside the Yamagata University Tsuruoka Campus (38°44′N, 139°49′E). The pots were filled with a mixture of commercially available leaf mold (Hirota Shoten Co. Ltd, Tochigi, Japan) and Kanuma pumice (Tachikawa-Heiwa Nouen Co. Ltd, Tochigi, Japan), which retains moisture and allows air permeability in the soil. The monthly mean temperature in Tsuruoka ranged from 1.9°C in January to 25.1°C in August (an annual mean of 13.6°C) in 2015 and from 2.2°C in January to 26.4°C in August (an annual mean of 13.5°C) in 2016. The annual precipitation was 1,556 mm in 2015 and 1,992 mm in 2016. We watered the pots well every day for 20 days after germination; thereafter, we only watered them on days of insufficient rain. During the experiment, almost all the seedlings, including those that were transplanted to pots and those not transplanted, remained healthy, and none of them died.

We conducted 12 measurement campaigns in the period from 2015 to 2016. In each measurement, we harvested four to six seedlings that were grown in pots and measured the fresh mass (kg), surface area (m^2) and respiration rate ($\mu mol\ CO_2\ s^{-1}$) of their roots, stems and leaves. In 2015, the measurements were conducted two times in August, using 15- to 16-month-old seedlings that germinated in mid-April 2014. In 2016, the measurements were conducted two times at 2- or 3-week intervals every month from early May to early September using 2-week- to 5-month-old seedlings that germinated in mid-April 2016. These seedlings were selected to represent the widest possible range of seedling growth stages. The total number of harvested and measured seedlings was 65 and the whole-plant fresh mass ranged from 41.2×10^{-5} to 23.5×10^{-3} kg (Table 1).

2.3 | Measurement of respiration rate and surface area

Respiration rate was measured following the method developed by Mori et al. (2010). Immediately after excision, the plants were sprayed with water and the respiration rate was measured within 20 min; the plant material was wrapped in wet paper to prevent transpiration. Following the guidance of previous studies on organ respiration rate (Hasibeder, Fuchslueger, Richter, & Bahn, 2015; Mori et al., 2010; Mori & Hagihara, 1988; Mori & Hagihara, 1991), soil particles adhering to the roots were removed by washing. Subsequently, the plants were separated into roots, stems and leaves. These washing and separation processes did not have any effect on the measured values of whole-plant respiration rate, as reported by Mori et al. (2010). Different plant organs were placed in custom-made aluminum chambers (80 or 160 cm^3)

TABLE 1   Minimum and maximum values of fresh mass, surface area and respiration of whole plant, roots, leaves and stems at the individual level among all seedlings ($n = 65$)

	Fresh mass ($\times 10^{-5}$ kg)		Surface area ($\times 10^{-4}$ m^2)		Respiration rate ($\times 10^{-4}$ μmol CO$_2$ s^{-1})	
	Min.–max.	Max./min.	Min.–max.	Max./min.	Min.–max.	Max./min.
Whole plant	41.2–2,350.0	57.0	23.6–1,210.0	51.3	8.7–131.0	15.1
Roots	7.0–1,730.0	247.1	2.5–998.0	399.2	0.8–52.6	65.8
Leaves	19.3–172.0	8.9	19.2–185.0	9.6	4.6–53.3	11.6
Stems	8.5–480.0	56.5	1.4–32.9	23.5	1.1–42.3	38.5
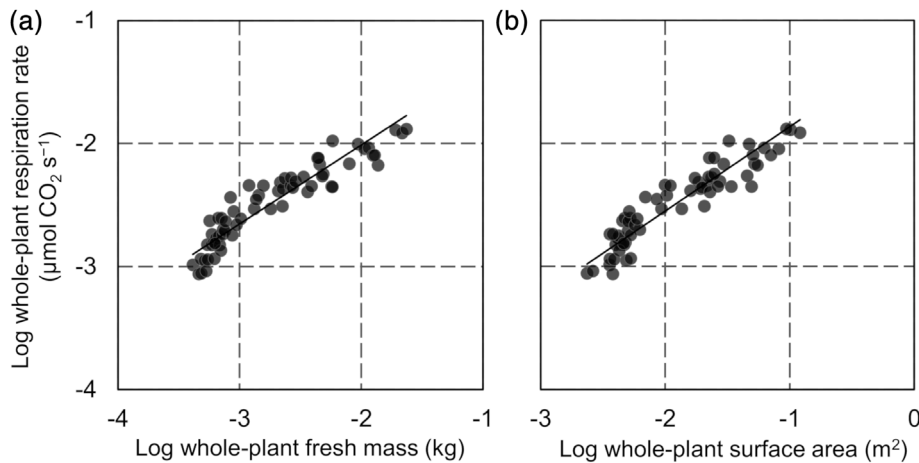
FIGURE 1   Results of the reduced major axis (RMA) analysis showing the relationships between whole-plant respiration and (a) whole-plant fresh mass and (b) whole-plant surface area. In each analysis, $n = 65$

that were impenetrable to light. The CO$_2$ concentration within the chamber was measured every 5 s for approximately 30–300 s using an infrared CO$_2$ analyzer (GMP343; Vaisala, Helsinki, Finland) and normalized to 20°C under the assumption that Q$_{10}$ = 2. The variation in temperature within the chambers during the measurements was at most 1°C.

Leaf surface area was measured using a leaf area meter (LI-3100C; LICOR, Lincoln, NE, USA). Stem surface area was calculated as the sum of the surface area of stem and branch sections from their diameter and length, assuming that the stem and branches were cylindrical. Root surface area was determined using image analysis software WinRhizo version 2016a (Regent Instruments, Quebec, Canada). Root images for analysis were obtained using a flatbed scanner (Epson Perfection V800, Seiko Epson, Suwa, Japan) at the resolution of 800 dpi.

2.4 | Data analysis

Scaling relationships were analyzed using a simple power function on log–log coordinates based on reduced major axis (RMA) regression (Niklas & Hammond, 2014) of the log-transformed version of Equation (1) (for all analyses, $n = 65$) using PAST statistical software (Hammer, Harper, & Ryan, 2001). We assessed the scaling of respiration rate vs. fresh mass and surface area for the whole plant and leaves, stems and roots. For each plant organ, we also assessed the scaling of surface area vs. fresh mass, and fresh-mass- and surface-area-specific respiration rate vs. whole-plant fresh mass. Finally, we assessed the scaling of root/shoot ratio of fresh mass, surface area and respiration rates vs. whole-plant fresh mass. In addition, we assessed fresh-mass-based root/shoot ratio from our previous research, ranging from current year of germination to approximately 80 years of age ($n = 264$, Mori et al., 2010; Ono et al., 2013), with that of the 65 seedlings.

These regression analyses were conducted with fresh mass as a proxy for body size or organ size following the methods described by previous studies (Makarieva et al., 2008; Mori et al., 2010). As a proxy for size scaling, fresh mass would better reflect physiological properties of organs than dry mass (Huang et al., 2019; Thakur et al., 2018). Mori et al. (2010), who directly measured whole-plant respiration of trees ranging from small seedlings to large trees, also used fresh mass as a proxy for body size to analyze the size scaling of whole-plant respiration

TABLE 2 Results of reduced major axis (RMA) regression showing the relationships among respiration, mass and surface area of whole plant, roots, leaves and stems

	Exponent (b)	95% CI of b	Normalization constant (a)	95% CI of a	R^2
Respiration rate vs. fresh mass					
Whole plant	0.642	0.576–0.699	0.1870	0.1190–0.2660	0.869
Roots	0.706	0.637–0.767	0.1570	0.0928–0.2400	0.901
Leaves	1.120	0.990–1.250	8.2800	2.9300–21.6000	0.730
Stems	0.799	0.736–0.868	0.2840	0.1660–0.4950	0.833
Respiration rate vs. surface area					
Whole plant	0.685	0.620–0.743	0.0660	0.0485–0.0850	0.868
Roots	0.630	0.587–0.672	0.0295	0.0232–0.0365	0.929
Leaves	1.150	0.962–1.300	0.7190	0.2510–1.6000	0.722
Stems	0.964	0.886–1.050	0.7530	0.4080–1.4200	0.867

Note: In all regressions, $n = 65$ and $p < .001$. CI, confidence interval.

rate. Our statistical inference to compare the scaling exponents was based on the 95% confidence interval (CI). In the case of no overlap among 95% CIs between groups, the differences among them were significant.
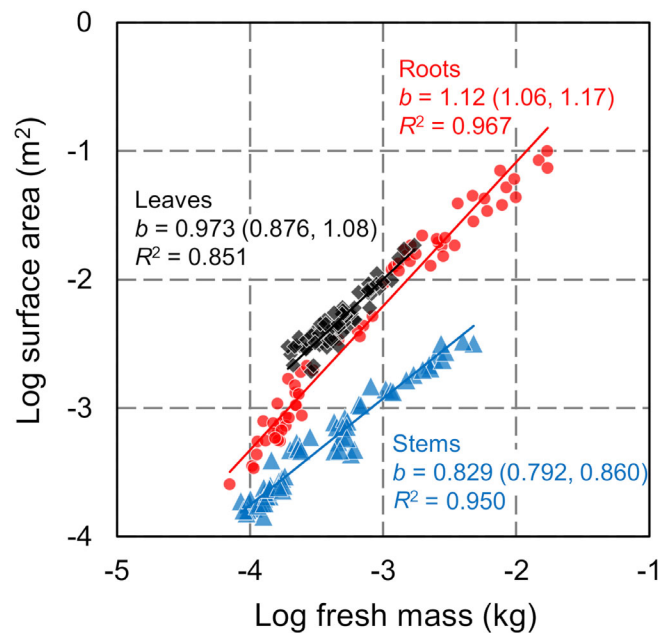
3 | RESULTS

3.1 | Size scaling of respiration and surface area

Size scaling of whole-plant respiration rate vs. whole-plant fresh mass and whole-plant respiration vs. whole-plant surface area showed significantly negative allometry (i.e., $b < 1$). There was no significant difference in the exponents between these relationships at the 95% CI level (Figure 1a,b, Table 2).

The roots showed a wider range of variation in surface area (399.2-fold, Table 1) than that in fresh mass (247.1-fold). As shown in Figure 2, the scaling of root surface area vs. root fresh mass was significantly positive (i.e., $b > 1$) at the 95% CI level. Compared with the variation in root surface area and root fresh mass, the variation in root respiration rate (65.8-fold) presented a narrow range. As a result, the scaling of root respiration rate vs. root surface area and root respiration rate vs. root fresh mass was allometrically negative (i.e., $b < 1$). The scaling exponent of root respiration vs. root surface area was lower than that of root respiration vs. root fresh mass, although the difference was not significant at the 95% CI level (Figure 3a,b, Table 2).

The leaves showed a similar range of variation in surface area (9.6-fold, Table 1) and fresh mass (8.9-fold), resulting in nearly isometric scaling (i.e., $b = 1$) for leaf
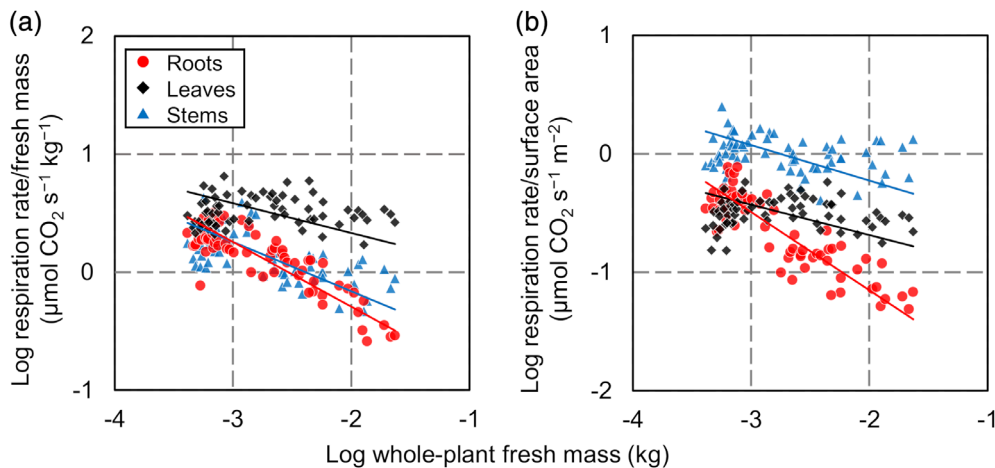

FIGURE 2 Results of the reduced major axis (RMA) analysis showing the relationships between surface areas and fresh mass of different plant organs. Black diamonds represent total leaves, blue triangles represent total stems and red circles represent total roots of individual seedlings. In each analysis, $n = 65$. The value in parentheses is 95% confidence interval (CI) of exponent b

area vs. leaf fresh mass (Figure 2). Furthermore, the variations in surface area and fresh mass were close to those in respiration rate (11.6-fold). We found isometric exponents for the scaling of leaf respiration rate vs. leaf area, and leaf respiration rate vs. leaf fresh mass (Figure 3a,b, Table 2).

The stems showed a wider range of variation in fresh mass (56.5-fold, Table 1) than that in surface area (23.5-fold), revealing a negative allometry of stem surface
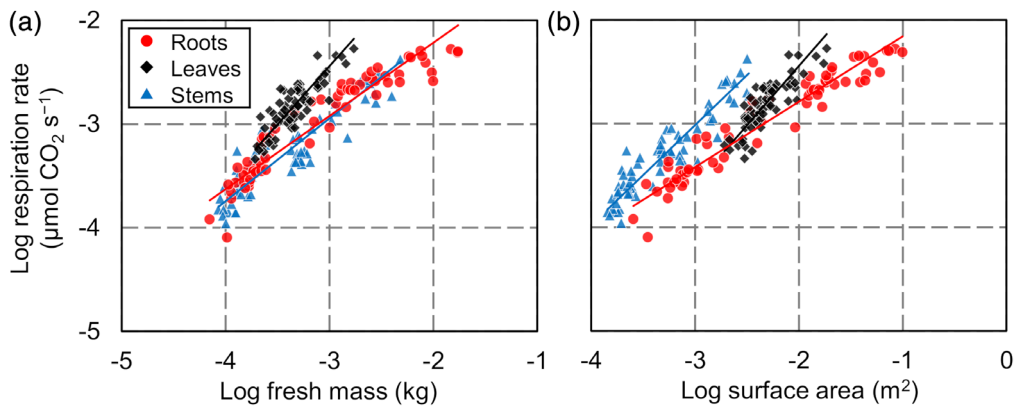
FIGURE 3 Results of the reduced major axis (RMA) analysis showing the relationships between respiration and (a) fresh mass and (b) surface area in different plant organs. Black diamonds represent total leaves, blue triangles represent total stems and red circles represent total roots of individual seedlings. In each analysis, $n = 65$. Details are compiled in Table 2

FIGURE 4 Results of the reduced major axis (RMA) analysis showing the relationships between (a) fresh-mass-specific respiration rate and (b) surface-area-specific respiration rate of plant organs in relation to whole-plant fresh mass. Black diamonds represent total leaves, blue triangles represent total stems and red circles represent total roots of individual seedlings. In each analysis, $n = 65$. Details are compiled in Table 3

area vs. stem fresh mass (Figure 2). The range of variation in stem respiration rate (38.5-fold) was narrower than that in stem fresh mass, but wider than that in stem surface area. Consequently, the scaling of stem respiration rate vs. stem surface area was nearly isometric, whereas the scaling of stem respiration vs. stem fresh mass was allometrically negative (Figure 3a,b, Table 2).

3.2 | Fresh-mass- and surface-area-specific respiration rates of plant organs

The results of the analysis of scaling of fresh-mass- and surface-area-specific respiration rate in different plant organs vs. whole-plant fresh mass are shown in Figure 4a,

b and Table 3. Among the three organs, the roots showed the steepest decrease in both fresh-mass-specific respiration rate and surface-area-specific respiration rate, and this decrease was significantly size dependent. Conversely, for leaves, both fresh-mass-specific respiration rate and surface-area-specific respiration rate did not significantly change with the increase in plant size. Finally, for stems, both fresh-mass-specific respiration rate and surface-area-specific respiration rate showed a decrease with an increase in plant size; however, compared with the decrease in the fresh-mass-specific respiration rate, the decrease in surface-area-specific respiration rate was not significantly size dependent.

The exponent for the surface-area-specific respiration rate in the roots was significantly lower than that in the

TABLE 3 Results of reduced major axis (RMA) regression: scaling of fresh-mass-specific respiration rate (μmol CO_2 s^{-1} kg^{-1}) and surface-area-specific respiration rate (μmol CO_2 s^{-1} m^{-2}) in the roots, leaves and stems with whole-plant fresh mass (kg)

|	Exponent (b)	95% CI of b	Normalization constant (a)	95% CI of a	R^2	p
Fresh-mass-specific respiration rate vs. whole-plant fresh mass						
Roots	−0.548	−0.608 to −0.489	0.0408	0.0275–0.0585	0.739	<.001
Leaves	−0.252	−0.807 to −0.207	0.6700	0.0189–0.8910	0.001	.811
Stems	−0.417	−0.497 to −0.335	0.1010	0.0624–0.1730	0.414	<.001
Surface-area-specific respiration rate vs. whole-plant fresh mass						
Roots	−0.659	−0.733 to −0.575	0.0034	0.0021–0.0057	0.763	<.001
Leaves	−0.255	−0.823 to −0.215	0.0638	0.0017–0.0833	0.003	.647
Stems	−0.299	−0.373 to −0.230	0.1500	0.0911–0.2430	0.067	.037

Note: In all regressions, $n = 65$. CI, confidence interval.

leaves and stems at the 95% CI level. On the regression line, with the increase in whole-plant fresh mass, the surface-area-specific root respiration rate declined by approximately 90% (from 0.507 to 0.0496 μmol CO_2 s^{-1} m^{-2}), and the fresh-mass-specific root respiration rate declined by approximately 85% (from 2.6 to 0.387 μmol CO_2 s^{-1} kg^{-1}). In addition, among the three organs, the size-dependent decrease in surface-area-specific respiration rate was observed only in the roots. These results indicate that only the roots exhibited a significant decrease in respiratory carbon cost on a surface-area basis and fresh-mass basis with the increase in plant mass.

3.3 | Ontogenetic shift in the root/shoot ratio

As shown in Figure 5, slope b for the scaling of root/shoot ratio of surface area was significantly larger than that of fresh mass and respiration rate at the 95% CI level. In addition, there was no significant difference between slope b for the scaling of root/shoot ratio of fresh mass and that of respiration rate. Slope b of root/shoot ratio was 1.19 for surface area (95% CI, 1.01–1.34, $R^2 = 0.748$), 0.807 for fresh mass (95% CI, 0.689–0.910, $R^2 = 0.756$) and 0.637 for respiration rate (95% CI, 0.492–0.754, $R^2 = 0.447$). From the smallest to the largest studied plants, the root/shoot ratio on these regression lines increased from 0.146 to 18.9 for the surface area (12.8 to 95.0% in whole-plant surface area), from 0.263 to 6.88 for fresh mass (20.8 to 87.3% in whole-plant fresh mass) and from 0.184 to 1.51 for respiration rate (15.6 to 60.2% in whole-plant respiration rate).

Therefore, the roots showed not only the largest decrease in fresh-mass- and surface-area-specific respiration rates (Figure 4a,b), but also the largest increase in fresh mass and surface area among the three organs.
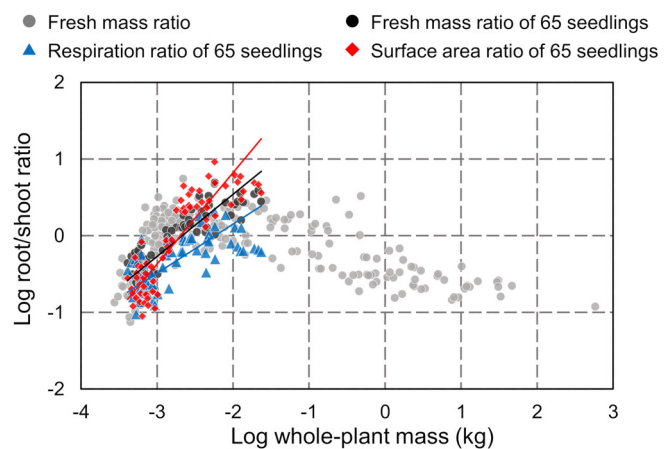
FIGURE 5 Plot diagram of the root/shoot ratios and whole-plant fresh mass of *Fagus crenata* from germination to mature tree stages. Black circles, red diamonds and blue triangles represent the root/shoot ratio of fresh mass, surface area and respiration rate, respectively, in the 65 seedlings shown in Figures 1–4. For these values, regression lines were obtained using the reduced major axis (RMA) regression analysis (each analysis, $n = 65$ and $p < .001$). Gray circles represent the root/shoot ratio of fresh mass obtained from our previous studies ($n = 264$)

These results revealed that the negative allometry of whole-plant respiration rate (Figure 1a,b) was caused by the increase in the proportion of roots in whole-plant fresh mass and surface area, as well as by the decrease in fresh-mass- and surface-area-specific root respiration with the increase in plant size.

In a plant mass greater than that of the largest one of the 65 measured seedlings, the root/shoot ratio showed a gradual decrease with the increase in whole-plant fresh mass to approximately 0.1–0.3 (10–20% of the whole-plant fresh mass around 10–1,000 kg). This indicates that the observed size-related increase in the root/shoot ratio is specific to the seedling stages.

4 | DISCUSSION

In this study, to the best of our knowledge, we showed for the first time that *F. crenata* seedlings rapidly increase the root surface area available for water and nutrient absorption, and decrease respiratory carbon costs in roots, which are apparent in the decrease in surface-area-specific root respiration rate (Figure 4b). Considering the decrease in fresh-mass root/shoot ratio in plants with a higher whole-plant fresh mass (Figure 5), the rapid root development with a reduction in respiratory carbon costs is probably specific to the early growth stage of *F. crenata*. Root growth has been suggested to be important for seedlings to avoid nutrient and water deficiency (Lloret, Casanovas, & Peñuelas, 1999; Padilla, Miranda, & Pugnaire, 2007). The increase in mass allocation to the shoots after the early growth stage must be preceded by an increase in water and nutrient absorption via the root surface (Padilla et al., 2007). Therefore, the initial burst of root growth may be an important step to increase photosynthetic performance and consequently accelerate shoot growth. Conversely, failure to achieve a high root/shoot ratio may be one of the physiological factors in seedling death.

In the present study, the mass root/shoot ratio of 4–5-month-old *F. crenata* (between 23.4×10^{-4} and 57.7×10^{-4} kg in whole-plant fresh mass) was 1.07–2.22 (51.7–68.9% in whole-plant fresh mass) on the regression line in our analysis of the size scaling of mass root/shoot ratio. Similar trends were observed by Peuke et al. (2002) in *Fagus sylvatica* L. In their study, the mass root/shoot ratio of 4–5-month-old *F. sylvatica* seedlings from 10 provenances which differed in annual precipitation was approximately 0.71–1.11 (41.5–52.6% for whole-plant mass). Therefore, rapid root growth in the early growth stages probably helps successful seedling establishment of not only *F. crenata*, but also other *Fagus* species. The high allocation of biomass to the roots is an important trait of shade-tolerant species, including *F. crenata*. Shade-tolerant species typically exhibit a higher mass root/shoot ratio than shade-intolerant species (Paz, 2003). The high biomass allocation to the roots is important to defend the seedlings of shade-tolerant species against multiple hazardous factors, such as herbivores and pathogens, in the understory with low light availability (Kitajima, 1994). Therefore, our findings on root development in *F. crenata* are in accordance with the expected survival strategies of shade-tolerant species.

In the early growth stages of the plants assessed in the present study (between 41.2×10^{-5} and 23.5×10^{-3} kg in whole-plant fresh mass), a large proportion of energy (respiration rate) and biomass (fresh mass and surface area) was allocated to the roots as whole-plant fresh mass

increased (Figure 5). However, after the early growth stages, as biomass allocation to the shoots increases with the increase in plant mass, energy allocation to the shoots is expected to increase. This change in preferential energy allocation to the shoots after the early growth stage may cause a gradual increase in photosynthetic performance and growth, as demonstrated by previous studies (Cavender-Bares & Bazzaz, 2000; Ishida, Yazaki, & Hoe, 2005; Thomas, 2010).

Several studies in various organisms have suggested that the negative allometry of the metabolic rate is partially due to the increase in the relative masses of organs with low metabolic rates (Atkin, 2010; Mori et al., 2010; Oikawa & Itazawa, 2003). One of the reasons for this size-dependent shift is physicochemical constraints (Atkin, 2010; Ballesteros & Luque, 2018; Mori et al., 2010), mainly gravity, which becomes increasingly important as plants grow (Enquist & Bentley, 2012). However, in a wide range of plant sizes, from seedlings to mature trees, size scaling of root respiration rate remains unclear, even after considering the importance of root system acquisition in the evolution of terrestrial plants (Kenrick & Crane, 1997; Raven & Edwards, 2017). In the present study, the negative allometry of whole-plant respiration was due to the increase in the proportion of roots in whole-plant fresh mass and surface area, and the decrease in fresh-mass- and surface-area-specific root respiration with the increase in plant size. These results emphasized that the roots impose major constraints on the scaling of whole-plant respiration rate.

The mechanism by which fresh-mass- and surface-area-specific root respiration rates decreased with the increase in root fraction remains unclear. Considering that the entire root system consists of roots of different ages, structures and functions (Eissenstat, Wells, Yanai, & Whitbeck, 2000; Weemstra et al., 2016), more extensive measurements of respiration within different parts of the root system are needed to reveal the mechanism. To date, the respiration rate of the entire root system in mature trees has been rarely measured, and scaling relationships of function between individual roots and the entire root systems are not as well established as those between leaf and whole aboveground parts (McCormack et al., 2017). In our future studies, we need to investigate whether the scaling of shoots and root respiration rates of *F. crenata* at the whole-plant level differs among different regions. As to whole-plant respiration rates that include shoots and roots, Mori et al. (2010) and Reich, Tjoelker, Machado, and Oleksyn (2006) have empirically observed that the whole-plant respiration rates are likely to be unaffected by growth conditions and would be similar within and among species. Therefore, understanding the scaling of whole-plant respiration, including shoots and

roots from seedlings to mature trees within species, would provide insight into the adaptive significance of attaining a larger body size irrespective of differences in local environmental conditions. Ultimately, studying the scaling of physiological traits of roots and shoots at the whole-plant level in various regions within species would help advance our understanding of the geographic differences at the organ level as an adaptation of the species to various local environments.

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