

ORIGINAL ARTICLE

Respiration and morphological characteristics of fine roots of two mangrove species by root branching order

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ABSTRACT To identify the functional traits of fine roots in mangrove species, we evaluated the root respiration rate and morphological traits, namely mean root diameter, specific root length (SRL), and root tissue density (RTD), using the root branching order classification in the two mangrove species. Although root respiration rates varied widely (0.63–12.76 and 0.42–6.55 nmol CO₂ g⁻¹ s⁻¹, respectively), there was a trend toward higher respiration rates for the smaller diameter roots. Only root respiration rates of first-order roots of *Bruguiera gymnorhiza* were significantly higher than those of higher order roots. There were differences in SRL, RTD, and root diameter between first two order roots and forth-order roots of both species, except for SRL of second- and forth-order roots of *B. gymnorhiza*. First-order roots of *Rhizophora stylosa* differed significantly from that of *B. gymnorhiza* in respiration rate, SRL and RTD, showing the characteristics of species. Fine root respiration and morphological traits by order classification may reflect differences in the functional traits of roots. Further research on the physiomorphological differentiation of fine roots would improve our understanding of various tolerance and adaptation strategies for environmental stress, among mangrove species.

Key words: *Bruguiera gymnorhiza*, Fine root respiration, *Rhizophora stylosa*, Root morphology, Root branching order

INTRODUCTION

Mangrove habitats are restricted to the tidally influenced coastal margins of the tropics and subtropics; therefore, their habitats are threatened by rising sea-levels. In response to the recent rapid sea-level rise, mangroves have maintained their habitat by investing enormous amount of carbon into producing fine roots, which are the source material for belowground litter and mangrove peat (e.g., Fujimoto et al. 2023, McKee et al. 2007, Ono et al. 2015, 2022). In solution culture experiments, the response and tolerance to hypoxic stress vary among mangrove species, with *Rhizophora* spp. maintaining root productivity by reducing the loss of fixed carbon through root respiration at low oxygen tensions (McKee 1996). The formation of

new root biomass consumes carbon not only for the production of root structures but also for metabolic energy, that is, growth respiration, especially for fine roots with high productivity and rapid turnover rates (Janssens et al. 2002). In changing environments, carbon investment in fine roots is an important adaptation strategy, reflecting species-specific physiological mechanisms.

Unlike coarse roots, whose respiration rate can be explained by the proportion of living and dead tissue, fine root respiration is largely due to the energy required for nutrient and water uptake, root growth, and cellular maintenance (Makita et al. 2012). In addition, fine root respiration correlates with morphological traits, such as root diameter, specific root length, and root tissue density (e.g., Eissenstat et al. 2000, Makita et al. 2009, 2012, Chen et al.



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2010), which may be indicators of fine root physiological functions (Pregitzer et al. 2002, Ostonen et al. 2007). The structural characteristics of roots could also be important indicators for assessing their contribution to maintaining soil volume in mangrove forests, although specific root length has rarely been evaluated in mangroves (Krauss et al. 2014). Knowledge of the morphological and respiratory traits of different functional roles of fine roots will be useful for understanding species-specific survival strategies to optimize carbon allocation to the roots and responding to environmental instability.

Makita et al. (2012) noted that, in several tropical tree species, the respiration rates of roots less than 1 mm in diameter were highly variable among and within species, requiring the evaluation of fine roots through a detailed classification based on physiological functions. While classification by arbitrary root diameter size (e.g., 0.5, 1 or 2 mm) has been widely used as an indicator of root functional morphology in studies of fine root carbon dynamics, the position of an individual root on branching root system may be a more accurate indicator of root function than root size (Pregitzer et al. 2002, McCormack et al. 2015). Xiong et al. (2017) assessed fine root dynamics in mangroves using the root branching order classification (the first two order roots belonging to the ephemeral absorptive group and the higher order roots (but < 2 mm in diameter) belonging to the perennial transport group). They found that the ephemeral absorptive group had a higher turnover rate and greater sensitivity to soil environmental conditions than that in the other group. The distal roots of mangroves are more responsive to salinity than the proximal roots (Burchett et al. 1984). It is possible that the respiratory and morphological traits of fine roots vary among individual roots with different physiological roles and/or among mangrove species.

The aim of this study was to clarify the order- and species-specific respiration and morphological traits of mangrove fine roots in order to improve our understanding of the physiological function of fine roots. We examined root respiration and morphology (i.e., root diameter, specific root length, and root tissue density) for each root branching order and compared them in two mangrove species, *B. gymnorrhiza* and *R. stylosa*. Our hypotheses were as follows: (1) distal lower order roots, which require energy for nutrient and water uptake, would have higher respiration rates, similar to trends in terrestrial trees; (2) to acquire nutrients, water, and oxygen in high-salinity, frequently inundated habitats, *R. stylosa* produces thinner and longer roots than *B. gymnorrhiza*, suggesting that it employs a low-cost strategy, such as lower tissue density and/or lower

root respiration, to maintain an expansive root network; (3) the relationship between root morphological traits and respiration varies across root orders. In higher-order roots, for example, RTD decreases due to the development of aerenchyma, and their role as transport tissues with low metabolic activity becomes dominant. Consequently, respiration rates remain low, regardless of SRL or RTD.

MATERIALS AND METHODS

Study site

The study site was located in Funaura Bay on Iriomote Island in subtropical region of southwestern Japan (24° 21' N, 123° 45' E) (Fig. 1). The mean annual temperature, the maximum and minimum monthly mean temperatures and the mean annual precipitation from 1991 to 2020 were 23.9 °C, 28.9°C, 18.5°C and 2,240 mm, respectively (Japan Meteorological Agency).

Root sampling and respiration measurements were conducted in the *B. gymnorrhiza* and *R. stylosa*, which are the most common species in the mangrove forests of Iriomote Island (Nakasuga 1979). Details of stand structure and tidal environment of the study site, as shown, were provided by Fujimoto et al. (2021), who established permanent plots in each of these stands, and conducted the tree censuses and continuous tidal level monitoring. In their permanent plot established in the *B. gymnorrhiza* stand (plot code: IFB1), which was dominated by *B. gymnorrhiza* with only a few *R. stylosa* at its seaward edge, mean stem diameter, tree height, and stand density were 8.9 cm, 4.5 m

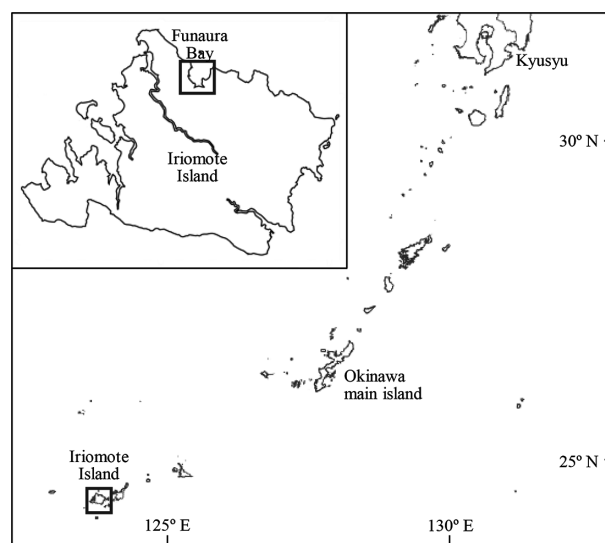


Fig. 1. Location map of Iriomote Island and the study site.

and 3,200 stems ha^{-1} , respectively, in 2018. In the plot established in the *R. stylosa* stand (IFR1), which was dominated by *R. stylosa*, and *B. gymnorrhiza* was sparsely distributed, these values were 4.9 cm, 2.4 m and 10,933 stems ha^{-1} for *R. stylosa* trees, and 3.9 cm, 2.4 m and 1,244 stems ha^{-1} for *B. gymnorrhiza* trees. The submergence rate of the total number of hours per year was calculated to be 24–46 % from the seaward edge to approximately 50 m landward in the *R. stylosa* forest and 30 % in the *B. gymnorrhiza* forest. The field survey was conducted in the vicinity of IFB1 and IFR1.

Root samplings and measurements of root respiration

Field surveys were conducted during drying-out conditions in daytime (between 1000 and 1800 h) in May 2022 and April 2023 for *B. gymnorrhiza* and *R. stylosa*, respectively. Soil blocks were excavated with a shovel from the top 20 cm of the sediment near the knee roots of *B. gymnorrhiza* and the prop roots of *R. stylosa* at randomly selected points. The identification of each root species was based on the tissue structure of the root cross sections and the color of the epidermis. The roots were gently washed out of the soil blocks with sea or river water and then cut at the base of the fourth-order roots. Root order was defined as follows: the most distal and unbranched root as the first-order root, the basal side from the junction of the first-order root as the second-order root, and closer to the proximal end of the root as the higher-order root (Pregitzer et al. 2002). Sample roots from the first- to fourth-order were separated by root order using pruning shears and tweezers and loosely wrapped in tissue paper moistened with seawater to prevent drying until measurement.

Root respiration measurements were performed in the field as soon as possible after sampling. Root samples (0.007–0.95 g dry weight) were placed by root order in four different sizes of closed chamber systems consisting of plastic cylinders (0.231 and 0.217 l) coupled to an infrared gas analyzer (GMP343, Vaisala, Finland) and aluminum cylinders (0.051 and 0.035 l) coupled to an infrared gas analyzer (GMP251, Vaisala, Finland). A suitable chamber size was selected based on the thickness and volume of the sample roots. The CO_2 concentration and temperature in the chamber were measured every 30 s for 15 min in a location out of direct sunlight and recorded on a data logger (GL220, Graphtec corp., Japan) and a handheld CO_2 meter (GM70, Vaisala, Finland) connected to the chamber with GMP343 and GMP251, respectively. The temperature in the chamber was measured using a copper constantan thermocouple in

the former system and a temperature sensor built into the probe in the latter system. Makita et al. (2013) indicated that root excisions can lead to an overestimation of the root respiration rate and found that the respiration rate of roots excised 10 or more times was significantly higher than that of unexcised roots in an experiment with 17-month-old seedlings of *Cryptomeria japonica*. However, wounded respiration was not considered in this study because preliminary measurements of *B. gymnorrhiza* roots at the same site did not show significant differences between the multiple-excised and non-excised roots.

The root respiration rate (R_r , $\text{nmol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$) was calculated from the following formula using the slope of the linear increase in CO_2 concentration:

$$R_r = \Delta C_{\text{CO}_2} * \frac{V_c}{V_{\text{air}}} * \frac{273.2}{273.2 + T} * \frac{10^3}{DW}$$

where ΔC_{CO_2} is the rate of increase of the CO_2 concentration (ppm s^{-1}), V_c is volume of chamber (l), V_{air} is standard gas volume (22.41 l), T is mean temperature in the chamber ($^{\circ}\text{C}$), and DW is dry weight of root sample (g). For ΔC_{CO_2} , the slope of the regression line over a 10-min period with the highest coefficient of determination ($r^2 > 0.7$) was used; the first minute was excluded to avoid the effects of air disturbance due to chamber closure. T was calculated by averaging the temperatures within the same time period as the value of ΔC_{CO_2} . Because the respiration rate of plants is strongly dependent on temperature, all measurements were standardized to the root respiration rate at 25°C ($R_{r,25^{\circ}\text{C}}$, $\text{nmol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$) with the temperature coefficient Q_{10} which is the proportional increase in respiration per 10°C warming using following relationship:

$$R_{r,25^{\circ}\text{C}} = R_r / Q_{10}^{(T-25)/10}$$

The Q_{10} values have been reported to range from 1.1 to 2.9 for the respiration of plant roots (Atkin et al. 2000) and from 1.48 to 2.68 for mangrove species (Curran et al. 1986, McKee 1996). In this study, the Q_{10} value was assumed to be 2.0.

After respiration measurements, the samples were placed in plastic bags with little water and brought to the laboratory in a moistened state. Sixty (4 orders $\times$ 15 intact roots) and forty-four (4 orders $\times$ 11 intact roots) root samples were collected, of which two samples (both first-order roots) and six samples (2, 3 and 1 sample of first-, third-, and fourth-order roots, respectively) were not used to calculate the root respiration of *B. gymnorrhiza* and *R. stylosa*, respectively, because the coefficient of determination for the regression line was low ($r^2 < 0.7$).

Measurements of root morphology

Each root sample was placed in a Petri dish with little water and scanned on a grayscale at 600 dpi on a flat-bed scanner with bidirectional light source illumination (GT-X970, Epson, Japan) for morphological analysis. The scratches on the Petri dish and non-root organic matter captured in the scanned images were manually filled with white, and the images were binarized to have a white background and black roots to clarify the root boundaries using the GNU Image Manipulation Program (GIMP; GIMP Team, USA). The mean root diameter (mm), total root length, and volume of the root samples were determined using WinRHIZO Pro 2013 (Regent Instruments, Canada), a software specifically designed for root image analysis. After scanning, the root samples were oven-dried at 65°C for 48 h and weighed. Specific root length (SRL; m g^{-1}) and root tissue density (RTD; g cm^{-3}) were calculated from total root length, volume, and dry weight of the samples. Because it is difficult to measure thin and voluminous fine roots directly, morphological indices were obtained through the aforementioned image analysis.

To confirm the morphological characteristics of roots visually, root cross-sections were also observed. Root samples were collected at the study site and fixed in 3 % glutaraldehyde solution. Then, the samples were dehydrated through a graded ethanol series and embedded in epoxy resin. They were sectioned at 30–40 μm by a microtome and stained in safranin. Enlarged images of root cross section were taken by using a digital camera (EOS KISS X3, Canon, Japan) attached to a light microscope (BX50–32, OLYMPUS, Japan).

Data analysis

Differences in root respiration rate, mean root diameter, SRL, and RTD among root orders were evaluated using two-way ANOVA and Tukey-Kramer HSD tests. Linear regression analysis was used to examine the relationships between morphological traits (SRL and RTD) and root respiration rate. The relationship between mean root diameter and fine root respiration rate has been well fitted with power functions in terrestrial studies (Makita et al. 2009, 2012, Chen et al. 2010). In this study, the variables were log-transformed and linearly approximated to apply a power function to the relationship between the mean root diameter and respiration rate. Probability plots of variables were examined to analyze for heteroscedasticity. All statistical analyses were performed using R statistical software.

RESULTS

The root respiration rates varied widely from 0.63–12.76 and 0.42–6.55 $\text{nmol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$ for *B. gymnorrhiza* and *R. stylosa*, respectively (Fig. 2a), especially for first-order root (7.95 ± 3.14 and 3.28 ± 1.74 [mean $\pm$ SD] $\text{nmol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$). The mean respiration rate by root order was higher in lower orders. Significant differences in root respiration rates were observed for both root order and tree species, as well as for their interaction (two-way ANOVA, $p < 0.001$). First-order roots of *B. gymnorrhiza* had a significantly higher respiration rate than any other order of *B. gymnorrhiza* or first-order roots of *R. stylosa* (Tukey-Kramer HSD test, $p < 0.001$). In contrast, no significant differences in respiration rate were detected among root orders in *R. stylosa* (Tukey-Kramer HSD test, all $p > 0.05$).

The SRL of *R. stylosa* was exceptionally long, especially in first-order root, where it exceeded 220 m g^{-1} (Fig. 2b). The maximum SRL for *B. gymnorrhiza* was 112.6 m g^{-1} . The SRL of the first-order roots in both species and second-order roots in *R. stylosa* differed significantly from the other orders (Tukey-Kramer HSD test, $p < 0.001$). Root respiration rate increased with longer SRL for *B. gymnorrhiza* ($r^2 = 0.19$, $p < 0.001$) and *R. stylosa* ($r^2 = 0.19$, $p < 0.01$), whereas a negative relationship between respiration rate and SRL was observed in first-order roots of *B. gymnorrhiza* ($r^2 = 0.72$, $p < 0.001$) (Fig. 3a).

The RTD ranged from 0.09–0.34 g cm^{-3} in the two mangrove species (Fig. 2c). The mean RTD for each root order was highest for second-order roots in both species, followed by first-order and third-order roots. The RTD of fourth-order roots in both species was significantly lower than that of other order roots except for third-order roots in *R. stylosa* (Tukey-Kramer HSD test, $p < 0.05$). Among the tree species, the RTD of first- and second-order roots in *B. gymnorrhiza* were lower than those in *R. stylosa* (Tukey-Kramer HSD test, $p < 0.05$). No correlation was found between RTD and respiration rate across all root orders (Fig. 3b).

As with both species, the first- and second-order roots were significantly finer than third- and fourth-order roots (Tukey-Kramer HSD test, $p < 0.05$) (Fig. 2a). All samples of the first- and second-order roots and half of the third-order roots of *B. gymnorrhiza* were less than 2 mm in diameter, which is commonly defined as fine roots. In *R. stylosa*, most third-order roots (10 of 11 samples) had a diameter of 2 mm or less, in addition to the first- and second-order roots. All first-order roots of both species, and the second-order roots of *R. stylosa* were very fine roots ($< 0.5 \text{ mm}$). There was a power-law relationship between the

root respiration rate and root diameter of *B. gymnorrhiza* ($r^2 = 0.58, p < 0.001$) and *R. stylosa* ($r^2 = 0.29, p < 0.001$) (Fig. 3c). A particularly large variation in the respiration rate was observed in the roots of *B. gymnorrhiza* and *R. stylosa* with diameters of less than 1 and 0.5 mm, respectively. The respiration rate of *R. stylosa* was lower than that of *B. gymnorrhiza* when comparing roots of similar diameters.

Developed aerenchyma air spaces were more clearly observed in higher-order roots, with *B. gymnorrhiza* exhibiting them in second- to fourth-order roots and *R. stylosa* in third- and fourth-order roots (Fig. 4).

DISCUSSION

Functional and morphological characteristics for each root order

Our results of an exponential increase in root respiration rate with decreasing root diameter (Fig. 3c) are consistent with those of previous studies in terrestrial forests (Makita et al. 2009, 2012, Chen et al. 2010). In particular, the respiration rate of first order roots for *B. gymnorrhiza* was significantly higher than that of higher order roots, although there was a large variation in the respiration rate (Fig. 2a, 3 a-c). The first two or three order roots, often referred to as absorptive roots, have a considerably higher uptake capacity for nutrients and water than transport roots (Guo et al. 2008, McCormack et al. 2015). The contribution ratios of nutrient uptake, growth, and maintenance to root respiration vary widely among species, depending on the nutrient demand, nutrient use efficiency, and relative growth rate of the plants (e.g., Van der Werf et al. 1988, Poorter et al. 1991, Scheurwater et al. 1998, George et al. 2003). Wetland plants adapted to hypoxia are unique in that they increase the respiratory costs for nutrient uptake to grow faster, whereas terrestrial plants increase the costs of growth respiration (Nakamura and Nakamura 2016). In the two mangrove species examined in this study, the first- or first two order roots may mainly be absorptive roots, and the high energy required for nutrient and water uptake could be reflected in the high root respiration rate of these roots.

Fine root respiration has been explained by the SRL and RTD, which increase with increasing SRL and decreasing

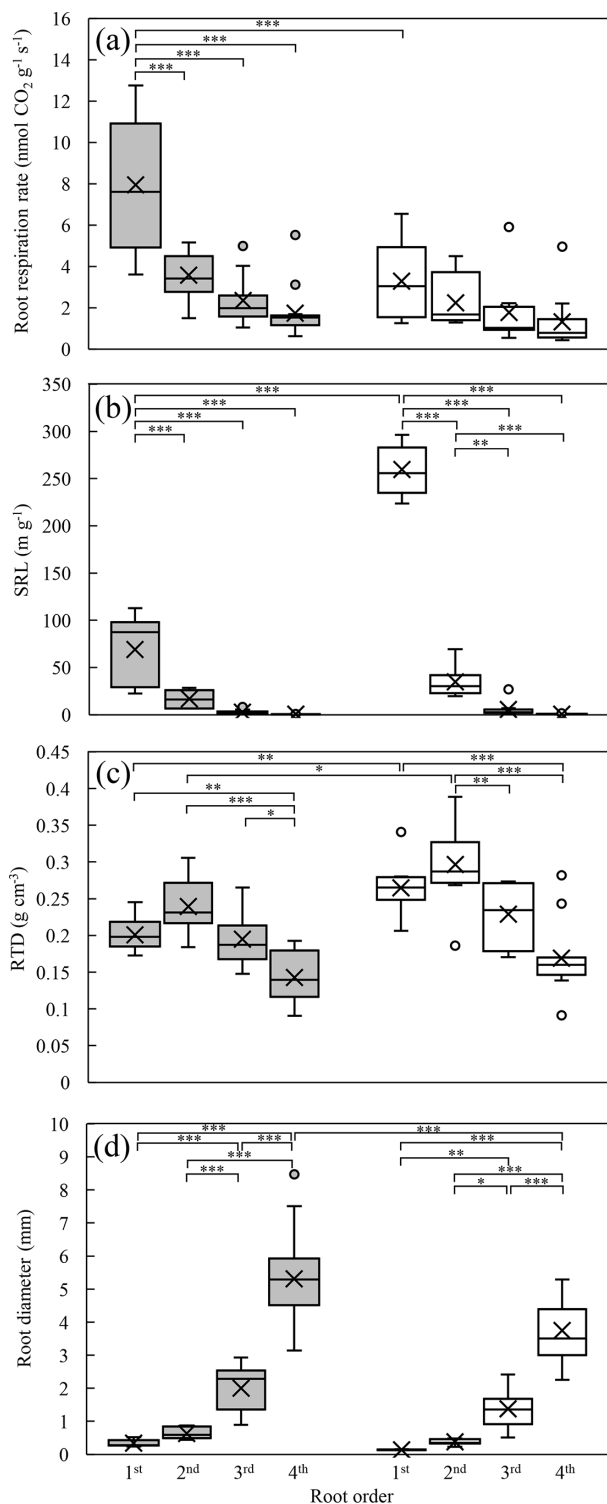


Fig. 2. Box plots of root respiration rates (a), specific root length (SRL; b), root tissue density (RTD; c), mean root diameter (d) for each root branching order in *Bruguiera gymnorrhiza* (gray) and *Rhizophora stylosa* (white). The horizontal lines and the boxes represent the median and the interquartile ranges (IQR, 25th–75th percentile ranges), respectively. The lower (higher) whiskers indicate the minimum (maximum) value within 1.5 times the IQR, with outliers shown as circles. Asterisks indicate levels of significance: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Significant levels between species were noted when there were significant differences between the same order.

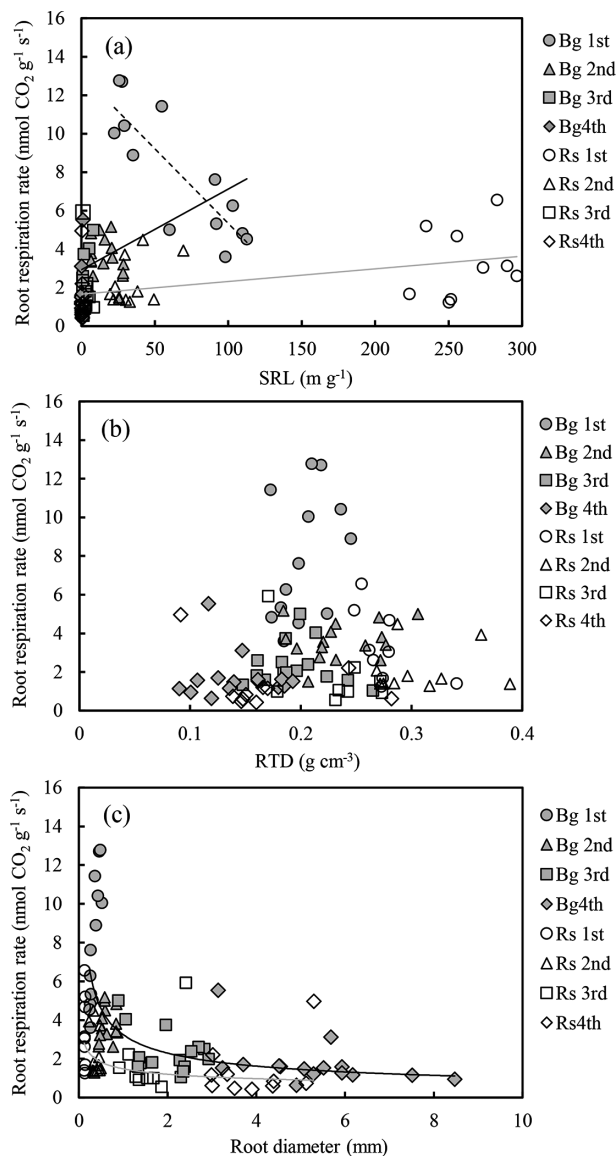


Fig. 3. Relationship between root respiration rate and specific root length (SRL; **a**), root tissue density (RTD; **b**), and mean root diameter (c) in *Bruguiera gymnorhiza* (Bg) and *Rhizophora stylosa* (Rs). The regression lines in (**a**) show positive relationships for all root samples of *B. gymnorhiza* (black solid line; $r^2 = 0.19$, $p < 0.001$, $n = 58$) and *R. stylosa* (gray line; $r^2 = 0.19$, $p < 0.01$, $n = 38$), and negative relationship for first-order roots of *B. gymnorhiza* (black dashed line; $r^2 = 0.72$, $p < 0.001$, $n = 13$). The regression lines in (**c**) show power relationships for *B. gymnorhiza* (black line; $R_r = 3.24D^{-0.50}$, $r^2 = 0.58$, $p < 0.001$, $n = 58$) and *R. stylosa* (gray line; $R_r = 1.48D^{-0.30}$, $r^2 = 0.29$, $p < 0.001$, $n = 38$).

RTD (Eissenstat et al. 2000, Makita et al. 2009, 2012, Lai et al. 2015). Although our results on the relationship between root respiration rate and SRL were also in general agreement with those of previous studies, the first-order roots of *B. gymnorhiza* had a wide range of SRL and

respiration values, with longer SRL tending to have lower respiration (Fig. 3a). Additionally, there was a negative correlation between SRL and root diameter for first order root in *B. gymnorhiza* ($r^2 = 0.90$, $p < 0.001$). The first (or first two) order roots consist of absorptive fibrous roots which do not undergo secondary growth, and exploratory pioneer roots which become transport roots with secondary growth (Zadworny and Eissenstat 2011, Shao et al. 2019). Because there is a mixture of functional types with large variations in respiration rates and morphologies within the same order roots, analysis focusing not only on root order, but also on developmental stage, may help clarify functional traits, especially in low order roots. The two mangrove species in this study did not exhibit the tendency observed in previous studies (Makita et al. 2012; Lai et al. 2015), in which higher RTD correlated with lower respiration rate, and no clear correlation was observed between RTD and respiration rate (Fig. 3b). Mangroves develop aerenchyma, a spongy tissue with large air spaces, in the inner cortex of the respiratory roots to transport and store oxygen within tissues to adapt to hypoxic waterlogged conditions (Curran 1986, Jintana et al. 1992, Pi et al. 2009). Aerenchyma formation is caused by cell separation, and the volume of intercellular spaces increases from the root meristem to the mature root tissue (Purnobasuki and Suzuki 2004, 2005). Microscopic observation of root cross-sections of different orders showed the development of aerenchyma and secondary tissue with increasing root branching order (Fig. 4). Higher-order roots with secondary growth have less metabolic activity (e.g., nutrient uptake and respiration) than lower-order roots (Jia et al. 2011). The relationship between RTD and respiration rate in higher-order roots, especially third- and fourth-order roots, can be attributed to decreased metabolic activity with the development of aerenchyma. Specifically, an increase in RTD may physically obstruct internal gas pathways, potentially limiting oxygen diffusion. However, in higher-order roots, the development of extensive aerenchyma leads to a decrease in RTD. Despite this lower density, these roots primarily function as transport conduits rather than metabolically active absorptive tissues, which likely contributes to their significantly lower respiration rates. These observations suggest that the relationship between RTD and respiration is not uniform but rather depends on the interplay between internal tissue structure and the specific functional role of the root.

Fine root respiration rates had a stronger relationship with root order and diameter than with SRL and RTD. Because the root diameter range of each order was clearly differentiated by the root order in this study (Fig. 2d and 3c), both diameter and order classifications can be used to

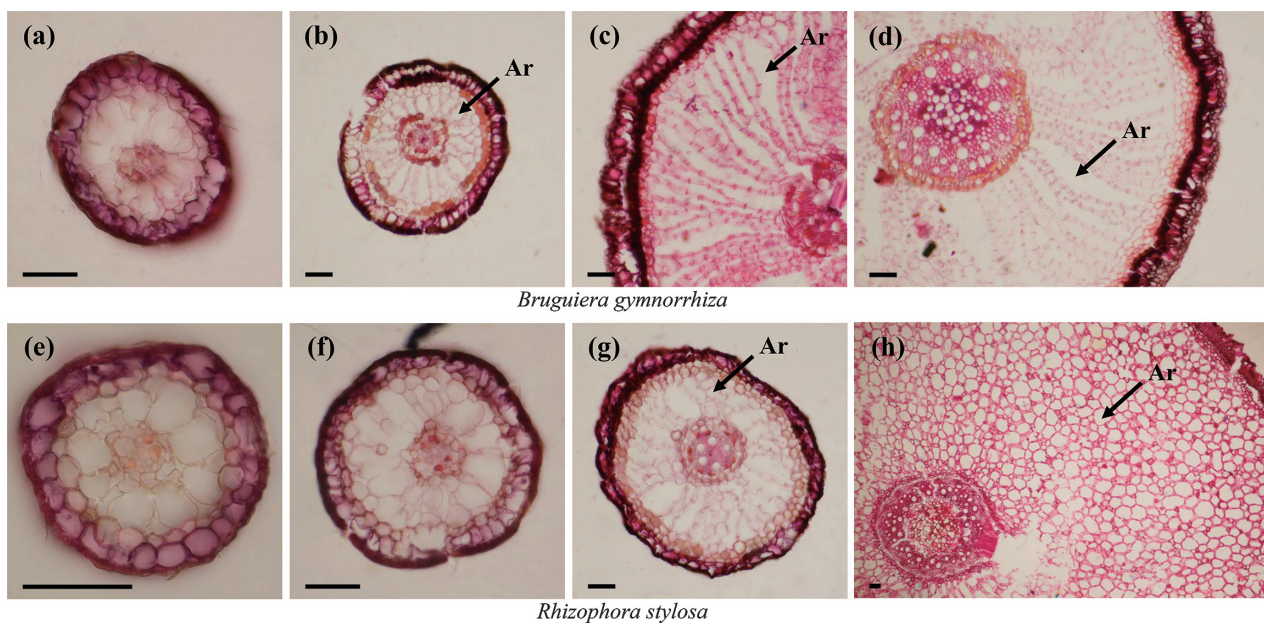


Fig. 4. Cross sections of first (a, e), second (b, f), third (c, g), and fourth (d, h) branching order roots of *Bruguiera gymnorhiza* and *Rhizophora stylosa* (bars = 200 μm). Developed aerenchyma air spaces (Ar) were more clearly observed in the higher branching orders of the roots.

evaluate the functional traits of fine roots. However, the excised root method makes measurements under conditions different from the original soil environment, whereas it is widely used as a root respiration measurement method and has the advantage of being measured separately in any arbitrary classification (Jia et al. 2011). It is also possible that root respiratory activity is reduced if root separation takes too long after sampling. It is necessary to develop a method to measure fine root respiration in an environment similar to actual soil conditions and to evaluate the change in respiratory activity caused by the exposure and removal of roots from the rhizosphere.

Comparison of fine root respiration and functional traits among species

The range of root respiration rates in this study was in consistent with previous studies in *R. mangle* (0.5–6 $\text{nmol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$ at 25–28°C; McKee 1996, Lovelock et al. 2006) and in *Avicennia germinans* and *Laguncularia racemosa* seedlings (4–12 $\text{nmol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$ at 25°C; McKee 1996). These rates were within the range of the root respiration rates of terrestrial trees (0.5–51 $\text{nmol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$; Burton et al. 2002, Chen et al. 2010, Comas and Eissenstat 2004, Makita et al. 2009, 2012), but the respiration rates of roots with a diameter of less than 0.5 mm in this study (1.2–12.8 $\text{nmol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$) were

lower than those of tropical terrestrial tree species (5–24 $\text{nmol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$; Makita et al. 2012).

SRL of first-order roots of *B. gymnorhiza* (≤ 0.52 mm in diameter) and second-order roots of *R. stylosa* (0.2–0.5 mm in diameter) were within the range of those less than 0.5 mm in diameter and/or first two order roots in terrestrial trees (5–140 m g^{-1} ; Comas and Eissenstat 2004, Makita et al. 2009, 2012), while first-order roots of *R. stylosa* (< 0.2 mm in diameter) were considerably longer (Fig. 2b). Longer SRL and thinner absorptive roots are thought to increase the exploration and uptake capacity of soil resources, with less carbon investment in root construction (Comas et al. 2002, Comas and Eissenstat 2004). Mangroves can grow in hypoxic and saline soil conditions not only because of their fine root morphology, which facilitates the exploration and uptake of nutrients, water, and oxygen, but also because of their unique mechanism, such as aerenchyma, that enable efficient acquisition of soil resources with low respiration per unit dry weight. Wetland species can efficiently take up and transport ions and utilize oxygen at low respiration rates through the development of aerenchyma (e.g., Armstrong and Drew 2002, Colmer 2003, Nakamura et al. 2013, Nakamura and Nakamura 2016). In addition, the amount of internal aeration and roots with aerenchyma formation varies with soil oxygen concentration within the same species (Laan et al. 1990, Jintana et al. 1992). Understanding the development of aerenchyma and the associated changes in

the respiration rate and morphology of fine roots under different environmental conditions could provide insights into the ecophysiological adaptation strategies of mangroves.

A positive linear relationship between root respiration and fine root production has been observed in Japanese coniferous forests (Sun et al. 2020). The production of *R. stylosa* roots, especially very fine roots (< 0.5 mm in diameter), which correspond to the first two order roots, was found to be significantly higher than that of thicker roots and of *B. gymnorhiza* (Fujimoto et al. 2021). Nevertheless, the lower-order roots of *R. stylosa* did not increase the respiration rate as rapidly as *B. gymnorhiza* in response to root order or diameter. Specifically, the first-order roots of *R. stylosa* exhibited a distinctive strategy characterized by a significantly higher RTD, a specifically elongated morphology, and reduced respiration rates compared to *B. gymnorhiza*. This conservative metabolic adaptation likely enables the production of large amounts of very thin absorptive roots, facilitating the efficient acquisition of oxygen, nutrients, and water under prolonged waterlogged soil conditions.

CONCLUSION

This study revealed distinct trends in root respiration and morphology across root orders in the two mangrove species, *B. gymnorhiza* and *R. stylosa*. Root respiration rates of the mangrove species increased exponentially with decreasing root order and diameter. These results support our first hypothesis, confirming that distal lower-order roots have the high metabolic activity necessary for nutrient and water uptake, which is consistent with trends observed in terrestrial trees. Our results agree with the second hypothesis, which revealed an adaptation strategy unique to *R. stylosa* in high-salinity, frequently inundated habitats. *Rhizophora stylosa* produces thinner, longer roots (higher SRL), which allows it to extend its root system more extensively than *B. gymnorhiza*. *R. stylosa* had significantly lower respiration rates and higher RTD in its first-order roots than *B. gymnorhiza*. This suggests a metabolic balancing strategy in which low construction and maintenance costs of individual fine roots sustain an expansive root system, indicating the traits optimized for efficient resource acquisition. In support of the third hypothesis, we confirmed that the relationship between root morphological traits and respiration differs among root orders. Microscopic observations showed that the development of extensive aerenchyma in third- and fourth-order roots leads to a decrease in RTD. Since these higher-order roots primarily function as transport tissues

with low metabolic activity, their respiration rates remained consistently low, regardless of variations in SRL or RTD. These findings emphasize that internal anatomical shifts, rather than simple morphological traits, determine the respiratory costs of roots in mangroves.

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