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Genotypic variations appear in fine root morphological traits of Cryptomeria japonica trees grown in a common garden

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Abstract

Aims

Fine root morphological traits regulate the belowground resource acquisition strategies of trees. However, genotypic variations isolated from environmental variations within tree species remain unclear. Thus, this study aimed to clarify the effects of genotypic variations on the fine root morphology of three types of Japanese cedar (*Cryptomeria japonica*), which were derived from different provenances but were grown in a common garden.

Methods

In the three geographically different provenances of *C. japonica* stands (Yanase, Yoshino, and Yaku), fine root morphological traits, such as root length and diameter and specific root length (SRL), were measured for each root up to the 4th branching order.

Results

Yaku cedar exhibited root traits distinct from the other provenances, such as growing longer and thinner roots in 1st root order to enhance root surface area. In addition, the SRL patterns with root orders and higher root tissue density suggested a resource conservation strategy corresponding to the low aboveground production of Yaku cedar. Yanase cedar demonstrated a significantly higher root branching ratio and specific root tips than the other provenances, indicating a strategy for investing resources in absorptive roots. Although Yoshino cedar had traits similar to that of Yanase cedar or intermediate between Yanase and Yaku cedars, high aboveground production may be achieved by nutrient acquisition with root exudates instead of absorptive root growth.

Conclusion

Genotypic variations appeared in fine root morphological traits of *C. japonica* trees, even when grown in the same environments, implying distinct resource acquisition strategies characterized by each provenance.

Introduction

In forest ecosystems, belowground environmental conditions are generally more spatially heterogeneous and complicated compared with the aboveground environments owing to multiple factors in various chemical materials, edaphic physical structures, and diverse microbiota compositions (Schoenholtz et al. 2000; Gallardo 2003; Townsend et al. 2008). Therefore, perennial plants, which are anchored at a fixed position throughout their lifespan, change their resource acquisition strategies by regulating their root functional traits to adapt to each environment. Because of this adaptation, plants have belowground functional diversity with inter- and intraspecific variations, especially in the distal root systems (Ma et al. 2018; Weemstra et al. 2021).

Generally, roots less than 2 mm in diameter are referred to as fine roots (e.g., Pregitzer et al. 2002) and play a primary role in water and nutrient uptake in the belowground parts. Furthermore, fine roots are divided into absorptive and transport roots based on their physiological functions (McCormack et al. 2015). Thus, there is functional heterogeneity in individual roots within fine root systems, which appears as phenotypic variations in anatomical and morphological traits (Hishi 2007; Hishi et al. 2017). Fine root morphological traits, namely, root length, diameter, and branching order, are closely related to physiological functions, such as absorptive and transport capacities, lifespan, and mycorrhizal colonization, at the individual root level (McCormack et al. 2012; McCormack et al. 2015; Hishi et al. 2017) and are sensitive to changing environments at the population level (Ostonen et al. 2007; Kramer-Walter et al. 2016; Doi et al. 2017; Wada et al. 2019). Specific root length (SRL), which is determined as root length per root mass, strongly reflects physiological traits such as absorptive capacity and lifespan (Eissenstat 1991; Ostonen et al. 2007; McCormack et al. 2012). In general, the absorptive capacity and turnover rate (i.e., the inverse of lifespan) of individual roots increase with increasing SRL and decreasing root tissue density (RTD). Moreover, reducing root diameter enhances the absorptive efficiency per invested carbon cost. Therefore, plant physiological characteristics and environmental adaptations can be evaluated by measuring root morphological traits (Pregitzer et al. 2002; Guo et al. 2008; McCormack et al. 2015). Furthermore, apart from pioneer roots that elongate during the initial phase of the root system development (Eissenstat and Achor 1999; Zadworny and Eissenstat 2011; Nakahata 2020), root functional types such as absorptive and transport roots are predetermined by root branching orders (Pregitzer et al. 2002; Hishi 2007; Tawa and Takeda 2015; Hishi et al. 2017). Thus, the evaluation of morphological traits for each root order in well-developed distal root systems is useful for identifying significant differences in adaptive root responses among plant individuals or populations.

In fine root morphological traits, inter- and intraspecific variations are caused by genotypic factors derived from evolutionary backgrounds (i.e., intergenerational adaptation) and environmental factors determined by the environmental circumstances where individuals grow (i.e., intragenerational acclimation). The evolutionary background of plants regulates their root morphological traits (Guo et al. 2008; Ma et al. 2018). Nevertheless, even within the same species, fine root morphological traits change considerably with environmental conditions possibly due to altitudinal or latitudinal gradients (Ostonen et al. 2007; Zadworny et al. 2016; Zadworny et al. 2017; Weemstra et al. 2021) and variations in soil chemical properties (Doi et al. 2017; Wada et al. 2019). Therefore, elucidating how the root morphological traits are predetermined by genotypic background and how these root traits have plasticity against growing environments is essential for understanding and predicting the response of trees and forest ecosystems to the environmental changes expected over the next several decades.

To date, interspecific variations in fine root morphological traits among different environments have been investigated using root order classification (Pregitzer et al. 2002; Eissenstat et al. 2015) and intraspecific variations of those have been conducted (Zadworny et al. 2016; Doi et al. 2017; Zadworny et al. 2017; Wada et al. 2019). The former often involves species variation caused by site-specific environments, and distinguishing trait variations based on genotypic or environmental factors is difficult in such cases. In contrast, the latter can reveal variations owing to environmental factors if the species is not

genotypically differentiated. However, genotypic variations due to geographical differences are expected in the belowground and aboveground morphological traits of trees. Genotypic variations of aboveground parts within a single species have been recognized in foundation species distributed across broad regions: for example, Japanese beech (*Fagus crenata*) and cedar (*Cryptomeria japonica*D. Don) in the Japanese Archipelago (e.g., Hiura 1998; Osada et al. 2015; Azuma et al. 2023), and European beech (*Fagus sylvatica*), Scots pine (*Pinus sylvestris*), and Norway spruce (*Picea abies*) in Europe (e.g., Vilà-Cabrera et al. 2015; Benavides et al. 2021).

C. japonica, an evergreen conifer, is broadly distributed across the Japanese Archipelago and comprises geographically differentiated or cultivated populations that exhibit various genotypic and phenotypic adaptations to the local environments (Nishizono et al. 2014; Hiura et al. 2021; Ohta and Hiura 2023; Saito et al. 2023; Tsumura 2023). Thus, *C. japonica* is one of the most suitable species for investigating genotypic variations in morphological traits based on broad geographic variations and cascading effects within a single species (Hiura 2023). However, genotypic variations in the fine root morphological traits of a single species remain unclear because it is difficult to separate the genotypic variations from trait variations owing to the growing environments. To quantify the effects of intraspecific genotypic variation in root morphological traits while ignoring environmental effects, it is required to verify the differences among trees with different genotypic backgrounds that have grown in a common environment. Therefore, the aim of this study was to clarify the influence of genotypic variations on the belowground resource acquisition strategy of three genotypes of Japanese cedars (*C. japonica*), which are derived from different provenances but grown in a common garden, by evaluating the distal root morphological traits for each root branching order.

Three similar-aged (ca. 50 years old) *C. japonica* stands, comprising cedars from the Yanase, Yoshino, or Yaku provenances, have already been investigated for aboveground growth and physiological characteristics in a common garden where this study was conducted (Ohta et al. 2019; Azuma et al. 2023). The growth characteristics and productivity (e.g., the annual stem height and radial growth rates and litterfall production) of Yaku cedar were significantly lower than those of Yanase and Yoshino cedars in this common garden. In addition, Yaku cedar showed higher sapwood density and lower crown depth than the other two cedar provenances. Meanwhile, root nitrogen concentration, which increases with increasing SRL (Jia et al. 2013), and root exudation rate of low-molecular-mass organic acids (LMMOA) in Yoshino cedar was possibly higher than those in the other two cedar provenances (Ohta et al. 2019), indicating distinct root morphological traits and nutrient acquisition strategies compared with the Yanase cedar which exhibits similar growth characteristics to Yoshino in the aboveground parts (Azuma et al. 2023). Based on the findings of these studies, different belowground resource acquisition strategies for Yanase, Yoshino, and Yaku cedars were hypothesized as follows: 1) Yanase cedar has root morphological traits that indicate short-lived absorptive root traits (e.g., high SRL) in lower root orders to enhance the turnover rate and maintain nutrient acquisition because of the high productivity of the aboveground parts. 2) Yoshino cedar has high SRL corresponding to the high root nitrogen concentration and a distinct foraging strategy for allocating resources to root exudation rather than to absorptive root growth as Yanase cedar does. 3) Yaku cedar is relatively restricted in carbon resources from

photosynthesis because of low leaf production and therefore increases the root surface area of distal roots per root volume by decreasing the diameter or extends the root lifespan (i.e., lower SRL) by moderating the relationship between the diameter and SRL at the whole root system level to enhance carbon use efficiency.

Materials and methods

Study site and plant materials

This study was conducted using three stands of Japanese cedars (*C. japonica*), which were derived from different provenances but were grown in a common garden in Wakayama Experimental Forest of Hokkaido University, Wakayama, Japan, at 33° 40’ N, 135° 40’ E. *C. japonica* is the principal species of temperate coniferous forests, which comprise a considerable proportion of the forest area in Japan (Forestry Agency 2021). The elevation of the study site is ca. 240 m above sea level. The mean air temperature and annual precipitation are 14.7°C and 3564 mm, respectively, which were measured at the Nishikawa meteorological station (1985–2019; Japan Meteorological Agency; 33° 38’ N, 135° 43’ E) located ca. 5 km away from the study site. In this region with the high precipitation, the study site is covered with very thin and weakly developed mineral soil without horizon differentiation (Regosol, based on the World Reference Base) (Kanda et al. 2018). The common garden is located on a south-facing slope across which the soil color (gray), texture (silt and gravel), and depth (< 10 cm) are uniform (Ohta et al. 2019).

Three stands comprised clonal cedars derived from different provenances of Yanase, Yoshino, and Yaku, which were established between 1967 and 1973, with a density of ca. 2000 trees ha^{-1}, and were ca. 49, 46, and 52 years old, respectively in 2018. These stands were located on a south-facing slope within 50 m of each other and were not managed until the experimental field survey was conducted in 2016 (Ohta et al. 2019). The native habitats of the three provenances are in the southwestern region of Japan, ranging from the Yakushima Island (30° 18’ N, 130° 30’ E) to the central part of the Kii Peninsula (33° 53’ N, 135° 41’ E). The native habitats of Yanase, Yoshino, and Yaku receive high precipitation (see Azuma et al. 2023) and are covered by very thin soils, the basement rocks of which are sandstone and shale, sedimentary rocks, and granite, respectively (Geological Survey of Japan 2015). The Yoshino and Yanase cedars had relatively high aboveground growth rates during the early growth stage (ca. <50 years old) than the Yaku cedar (Takashima et al. 2009; Itaka et al. 2013; Nishizono et al. 2014). Moreover, similar relative growth patterns were observed in each stand of the present common garden. In August 2015, mean tree height and diameter at breast height were 15.4 m and 19.2 cm, 18.3 m and 23.1 cm, and 10.9 m and 18.6 cm in Yanase, Yoshino, and Yaku plantations, respectively (Ohta et al. 2019). The chemical properties of fine roots and soil in the three stands were investigated in 2016 (Table S1). The soil properties had been differentiated among stands, presumably due to plant-soil interactions during stand growth (Ohta et al. 2019). Further details regarding the site, stand locations, and growth characteristics of each cedar provenance are mentioned in Ohta et al. (2019) and Azuma et al. (2023).

Field survey for sampling of intact fine root systems in the common garden

Field surveys for root sampling in the common garden were conducted twice in 2018 and 2019 consecutively. In August 2018, three individuals representing typical growth were selected from each of the three provenances to evaluate aboveground growth and physiological characteristics (Azuma et al. 2023) and root morphological traits. Subsequently, two distal root systems that included individual roots from 1st to 4th root orders were sampled from each selected individual tree as follows.

To sample intact living fine root systems of each individual tree, distal parts of the root systems were exposed at a 5–10 cm deep position within approximately 1 m of an individual stem by digging soil from the stem base along coarse roots toward distal parts to ensure individual identification. Exposed root systems were carefully sampled by detaching them from the soil and detangling other roots using hands and a pair of pruning scissors to ensure that intact root systems completely covered individual roots from 1st to greater than 4th root order without any loss. The sampled root systems were then carefully rinsed with pure water to remove the attached soil. Subsequently, they were wrapped with wet wipes and packed into plastic bags to prevent them from drying and maintain freshness. The samples were immediately placed in a refrigerator until the morphological analyses were performed.

Field root sampling was repeated for three different individuals for each stand in August 2019. Thus, 36 root systems were sampled and processed for the morphological analyses.

Morphological analysis of distal root systems based on root order

Samples of root systems were cut into individual roots and separated according to 1st, 2nd, 3rd, and 4th root order classifications counted from root tips (Pregitzer et al. 2002), using a pair of tweezers and a knife while observing the roots with a microscope (Doi et al. 2017). Roots greater than 4th root order were excluded from the analyses. After cutting and classifying, individual roots of each order were scanned using optical flatbed scanners (GT-X970; Seiko Epson Corp., Nagano, Japan) to measure the morphological traits.

Scanned images were analyzed to evaluate the mean root length (cm) and diameter (mm) and the number of individual roots (no.) for each root order within a root system using the WinRHIZO 2013 (Regent Instruments Inc., Quebec, Canada), a root image analysis software. Subsequently, samples were oven-dried to a constant mass at 65°C, and root mass (g) was weighed.

To further evaluate the morphological traits of the distal root systems, specific root length (SRL; m g^{-1}) and root tissue density (RTD; mg mm^{-3}) for each root order, root branching ratio (RBR; no. $no.^{-1}$) for each pair of consecutive root orders, specific root tip number (SRT; no. mg^{-1}), and specific root tip length (SRTL; cm g^{-1}) for each root system were calculated. SRL was determined as the root length per root

mass. RTD was calculated as the root dry mass divided by the fresh root volume which was estimated based on root length and diameter assuming a cylindrical shape. RBR is defined as the individual root number of the [*n*]th root order per root number of the [*n* + 1]th root order (Kong et al. 2014). SRT and SRTL were calculated by dividing the root tip number (i.e., the number of 1st order roots) and root tip length (i.e., the number or length of 1st order roots) by the total root mass from 1st to 4th order roots, respectively (Godbold et al. 2003; Hishi et al. 2017).

Reconstruction of fine root systems using morphological traits by root branch orders

The schematic diagram of distal root systems from 1st to 4th root orders was constructed by referring to mean root length, diameter, and branching ratio for Yanase, Yoshino, and Yaku cedars, respectively. In the diagram, root length and diameter were assumed to be the same within each cedar type. Individual roots were colored with different thicknesses, representing significant differences in SRL verified by statistical analyses within each root system.

Statistical analysis

Differences in the mean values of root length and diameter, SRL, and RTD among the three provenances (Yanase, Yoshino, and Yaku) were examined within each root order. In addition, those among four root orders (1st, 2nd, 3rd, and 4th root orders) were tested within each provenance, respectively, using the generalized linear mixed model (GLMM) with the restricted maximum-likelihood (REML) estimation method. Similarly, differences in the RBR among the provenances for each pair of root orders (1st to 2nd, 2nd to 3rd, and 3rd to 4th root order) and in the SRT and SRTL for whole root systems (from 1st to 4th root order) were examined using the same GLMM framework. In the GLMM, the effect of the fixed factor of provenances or root orders was tested as qualitative variables, assuming a normal error distribution of response variables, considering the identity link function, and including random variation of individual trees.

Correlations of root diameter either with root length, SRL, or RTD were evaluated using the GLMM with the REML estimation, where fixed effects of the logarithm of root diameter, tree provenance, and their interaction were analyzed, assuming a gamma distribution for response variables with the log link function and considering random variation of the individual trees.

Significant differences among provenances or root orders were determined based on if the *P* value was less than 0.05. These statistical calculations were performed using the R statistical software (version 3.5.0; R Development Core Team 2018), and the GLMM estimation was implemented using the R package “lme4” (Bates et al. 2015).

Results

For 2nd, 3rd, and 4th root orders, means of individual root length were not significantly different among the three provenances (GLMM, $P > 0.48$; Fig. 1a), whereas for 1st root order, the root length was higher in

Yaku cedar than in the others ($P = 0.002$). The mean diameter of 1st order roots in Yaku cedar was significantly lower than that of the others ($P = 0.004$; Fig. 1b), which is consistent with our 3rd hypothesis, although the differences were not significant among the provenances for 2nd and higher root order levels ($P > 0.15$). There were no significant differences in the SRL among the provenances for all root orders ($P > 0.07$; Fig. 1c). However, compared with the other provenances, mean SRL of Yanase cedar for 2nd root order was marginally higher, whereas that of Yaku cedar for 3rd root order was relatively low. Overall, the mean SRL of Yanase and Yoshino cedars varied largely between the 3rd and 4th root orders ($P < 0.001$), whereas the difference of SRL in Yaku cedar was not significant ($P = 0.056$). The SRL of Yaku cedar showed significant differences at 1st and 2nd root orders from 4th root order level ($P < 0.001$; Fig. 1c). The RTD varied significantly with different root orders, exhibiting increasing trends with increasing root orders in each provenance (Fig. 1d). Significant differences in the RTD among the three provenances were observed in 2nd and 3rd root orders, suggesting the RTD values of Yaku cedar were greater than those of the other two provenances ($P < 0.02$; Fig. 1d).

The RBR between the 2nd and 3rd order roots was significantly higher in Yanase cedar than the other two provenances (GLMM, $P = 0.02$; Fig. 2), though no significant difference among the provenances was found in the other pairs of root orders ($P > 0.75$).

The SRT was the highest in Yanase cedar compared with the other provenances (GLMM, $P < 0.001$; Fig. 3a), as the mean value of Yanase cedar was approximately twice that of Yaku cedar. There was no significant difference in the SRTL among the three provenances ($P = 0.16$; Fig. 3b), although the mean SRTL decreased in the following order: Yaku > Yoshino > Yanase cedars.

For all root orders, the relationships between individual root diameter and length indicated concave patterns in which the root length increased with increasing root diameter (Fig. 4a). These relationships were consistent among the three provenances (GLMM, $P > 0.33$). In contrast, the SRL decreased exponentially with increasing root diameter (Fig. 4b). Although the regression curves seem to differ among the provenances, the differences in slopes in the current GLMM were marginal and not significant between the Yanase and Yaku cedars ($P = 0.06$). This result does not support our 3rd hypothesis, which considers that Yaku cedar moderates this relationship by extending its root lifespan (i.e., decreasing the SRL) at the whole root system level. Furthermore, the RTD did not demonstrate a significant relationship with the root diameter for any cedar provenance ($P > 0.27$; Fig. 4c).

Discussion

As *C. japonica* is one of the most widely distributed foundation species across the Japanese Archipelago, quantifying its intraspecific variation in functional traits will help us understand how tree species moderate their physiological functions under changing environments in terms of functional ecology and evolutionary biology (Hiura 2023). This study is a few that assessed intraspecific variations in fine root morphological traits caused only by different genotypic backgrounds using three provenance types of *C. japonica* trees planted in a common garden and suggested that intragenerational tree

acclimation to the growing environment, that includes soil chemical alteration by plant-soil interactions (Ohta et al. 2019), is possibly affected by genotypic backgrounds. Several previous studies evaluating fine root morphological and anatomical traits of *C. japonica* focused on trait variations among root orders and their plasticity with environmental conditions and stand age (Tawa and Takeda 2015; Hishi et al. 2017; Wada et al. 2019). Although fine root morphological traits have plasticity against growing environments (Doi et al. 2017; Zadworny et al. 2017; Wada et al. 2019), our common garden experiment suggests that intraspecific variations in fine root morphology can be determined not only by environmental conditions but also by genotypic differences in trees.

The SRL of Yanase and Yoshino cedars changed largely at 3rd root order from that at 4th root order in which transport roots were presumed, while the significant change from 4th root order appeared at 2nd root order in Yaku cedar. Root orders up to 2nd or 3rd levels are regarded as absorptive roots because they possess intact cortical cells, whereas root orders greater than them are considered transport roots (McCormack et al. 2015; Freschet and Roumet 2017). Therefore, these results indicate that the root order in which root physiological functions shift largely between short-lived absorptive and long-lived transport roots differs among the provenances (Fig. 5). The absorptive roots of Yaku cedar might be confined to more distal parts (1st and 2nd root orders) of the root system compared with Yanase and Yoshino cedars. In the previous studies, anatomical analyses of each root order for *C. japonica* demonstrated that high-absorptive primary roots without a cork cambium were found in 1st and 2nd order roots while higher-order roots comprised almost of less-absorptive secondary roots with a cork cambium (Tawa and Takeda 2015; Hishi et al. 2017), which corresponds with the changes in root functional types with root orders of Yaku cedar. Alternatively, the analyses for each diameter class suggested 0.6 mm as a rough boundary for distinguishing primary and secondary (i.e., absorptive and transport) roots in *C. japonica* (Tawa and Takeda 2015), which is satisfied between 3rd and 4th root orders in Yanase and Yoshino cedars. Therefore, these previous reports do not prevent our interpretations that the proportions of absorptive roots can gradually change with root orders and those in 2nd and 3rd order roots might be affected by genotypic backgrounds.

On the other hand, tree height, canopy depth, and litterfall in the Yaku stand were lower than those in the other stands (Ohta et al. 2019; Azuma et al. 2023), thus restricting the growth and productivity of Yaku cedars. Given that SRL is strongly correlated with the turnover (i.e., lifespan) and respiration of individual roots (McCormack et al. 2012; McCormack et al. 2015; Miyatani et al. 2018), Yaku cedar might conserve resources for root growth and maintenance costs by limiting the absorptive function only to lower-order roots in response to aboveground growth limitation. Moreover, the relatively high RTD (Fig. 1d) in our study corresponds with the high sapwood density in the aboveground parts (Azuma et al. 2023), suggesting that wood anatomy can be strongly affected by genotypic backgrounds. In contrast to root length and SRL, which varied with root diameter, the RTD was not correlated (Fig. 4). The positive link between root and stem tissue densities and the fact that higher values correspond with lower relative growth rates have also been reported in interspecific comparisons (Kramer-Walter et al. 2016).

Moreover, the conservative strategy of Yaku cedar in terms of morphological traits is implicated in 1st order roots that were significantly longer and thinner than those of Yanase and Yoshino cedars (Fig. 1a, b). The longer and thinner roots have more efficient root absorption owing to the enhanced length (i.e., SRTL; Fig. 3b) and surface area of absorptive roots per unit of root volume. These results support our 3rd hypothesis regarding the Yaku cedar strategy. However, the SRL change with root diameter did not differ from the other provenances contrary to our expectation (Fig. 4b). In addition, differences in the 1st order root morphology among the tree provenances might be related to mycorrhizal associations. Some *C. japonica* fine roots can form arbuscular mycorrhizas (AM) primarily in the 1st root order (Hishi et al. 2017; Matsuda et al. 2021). Because distal roots associated with mycorrhizal fungi become larger in diameter than those of non-infected roots (Kong et al. 2014; Eissenstat et al. 2015; Valverde-Barrantes et al. 2016), the 1st order roots of Yanase and Yoshino cedars, which were larger in diameter than those of Yaku cedar and other *C. japonica* stands (Wada et al. 2019), might have specific strategies for nutrient acquisition enhanced by AM colorizations.

As Yaku cedar has distinctly low aboveground productivity during the early growth stage in its native habitat (Takashima et al. 2009; Itaka et al. 2013) and in the common garden (Ohta et al. 2019; Azuma et al. 2023), fine root morphological traits of Yaku cedar were also differentiated from trees of other provenances, despite large differences in soil chemical properties between the present common garden and its unique native habitat on Yakushima Island where higher soil phosphorus contents are observed owing to volcanic ash (Mukai et al. 2016). Furthermore, Yaku cedar has a relatively long genetic distance from Yanase and Yoshino cedars (Tsumura 2023), implying the limits of functional plasticity against growing environments and the consistency of inherent characteristics determined by the genetic background.

In contrast, Yanase and Yoshino cedars elongated short-lived absorptive (i.e., high SRL; Fig. 1c) roots more than did Yaku cedar probably because of the relatively high aboveground production indicated by leaf litterfall (Ohta et al. 2019). High aboveground productivity enables sufficient carbon resource supply to the belowground parts. Positive feedback mechanisms might exist in which high carbon resource allocation to roots promotes water and nutrient acquisition, further enhancing aboveground productivity. In Yanase cedar, the SRL in 2nd root order exhibited high values similar to those in 1st root order (Fig. 1c), and the RBR between the 2nd and 3rd root orders was higher than that in the other provenances (Fig. 2), resulting in high SRT also (Fig. 3a). These results support our 1st hypothesis, indicating the possibility that Yanase cedar has a high absorption capacity, even in the 2nd root order, and invests the most resources in fine root growth and respiratory activities among the three provenances. *C. japonica* stands with high fine root biomass tends to have thicker roots (Hirano et al. 2017), which indicates Yanase cedar might invest more resource to fine root biomass growth than did Yaku cedar (Fig. 1b). Inorganic nitrogen concentrations in the soil promote root elongation and branching (Giehl and von Wirén 2014). Even though the same environments were assumed in the stand establishment, the edaphic conditions of each cedar stand have gradually changed over the years due to the positive feedback of plant-soil interactions that fertilize soil nutrient conditions by litter supply and root exudation. Therefore, the high RBR of Yanase cedar might have been induced by the edaphic conditions

derived from the positive feedback in its stand, which has the higher soil nitrate concentration compared with the Yoshino stand (Ohta et al. 2019).

Yoshino cedar demonstrated root morphological traits that were almost similar to Yanase cedar at the individual root level (e.g., root length and diameter) (Fig. 1) and intermediate values at the whole root system level (e.g., RBR and SRT) (Fig. 3 and Fig. 4) in the other two provenances. There were no remarkable characteristics of root morphology in Yoshino cedar among the three provenances. Nevertheless, the aboveground production such as litterfall production and relative growth rate of Yoshino cedar was equivalent to or better than that of Yanase cedar (Ohta et al. 2019; Azuma et al. 2022). Considering the contribution of the root exudation (Grayston et al. 1997), the high productivity of Yoshino cedar can be interpreted as a distinct resource acquisition strategy, which is consistent with the 2nd hypothesis of this study. Ohta et al. (2019) investigated soil and root chemical properties and root exudation of LMMOA in the same stands of Yanase, Yoshino, and Yaku cedars and revealed that the root exudation rates of LMMOA from distal root systems were higher in Yoshino cedar than in Yanase and Yaku cedars. Although there is a concern that the exudation of LMMOA is a part of total root exudation, high root exudation can enhance soil microbial activity and further accelerate the mineralization of organic nitrogen and other nutrient availability (Dakora and Phillips 2002). Hence, Yoshino cedar might compensate for nutrient acquisition that succeeds in high aboveground productivity by investing carbon resources in root exudation presumably instead of producing roots with absorptive morphological traits compared with Yanase cedar. Although total carbon invested in root exudates remains unknown, Yoshino cedar improves nutrient conditions in the rhizosphere by using root exudates of LMMOA, thereby resulting in higher nitrogen concentrations in the fine roots than the other cedar types (Ohta et al. 2019). Although this study focused on differences in fine root morphological traits due to genotypic variations, local adaptation owing to geographical characteristics can be integrated into resource acquisition strategies by altering root morphological and soil chemical characteristics.

Even under similar stand initial conditions in the same common garden, Yanase, Yoshino, and Yaku cedars possibly have different resource acquisition strategies based on their genotypic backgrounds, which was reflected in their root morphological traits (Fig. 5), exudate dynamics, and soil chemical properties altered by plant-soil interactions (Ohta et al. 2019). Although this study demonstrated the influence of genotypic factors on intraspecific variation in root morphological traits of *C. japonica* trees grown in a common garden, further investigation is required in this site to prove resource use strategies in belowground parts by estimating fine root production and biomass, and in their native habitats to understand the extent of regulation of root morphological traits by genotypic and environmental factors. The quantitative evaluation of intraspecific variations in functional traits, either due to genotypic background or plasticity against growing environments, will provide new insight to understand the limits of tree responses to changing environments that are predicted over the next several decades.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Field works for material preparation were conducted by all authors. Data collection and analyses were performed by RN. The first draft of the manuscript was written by RN and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

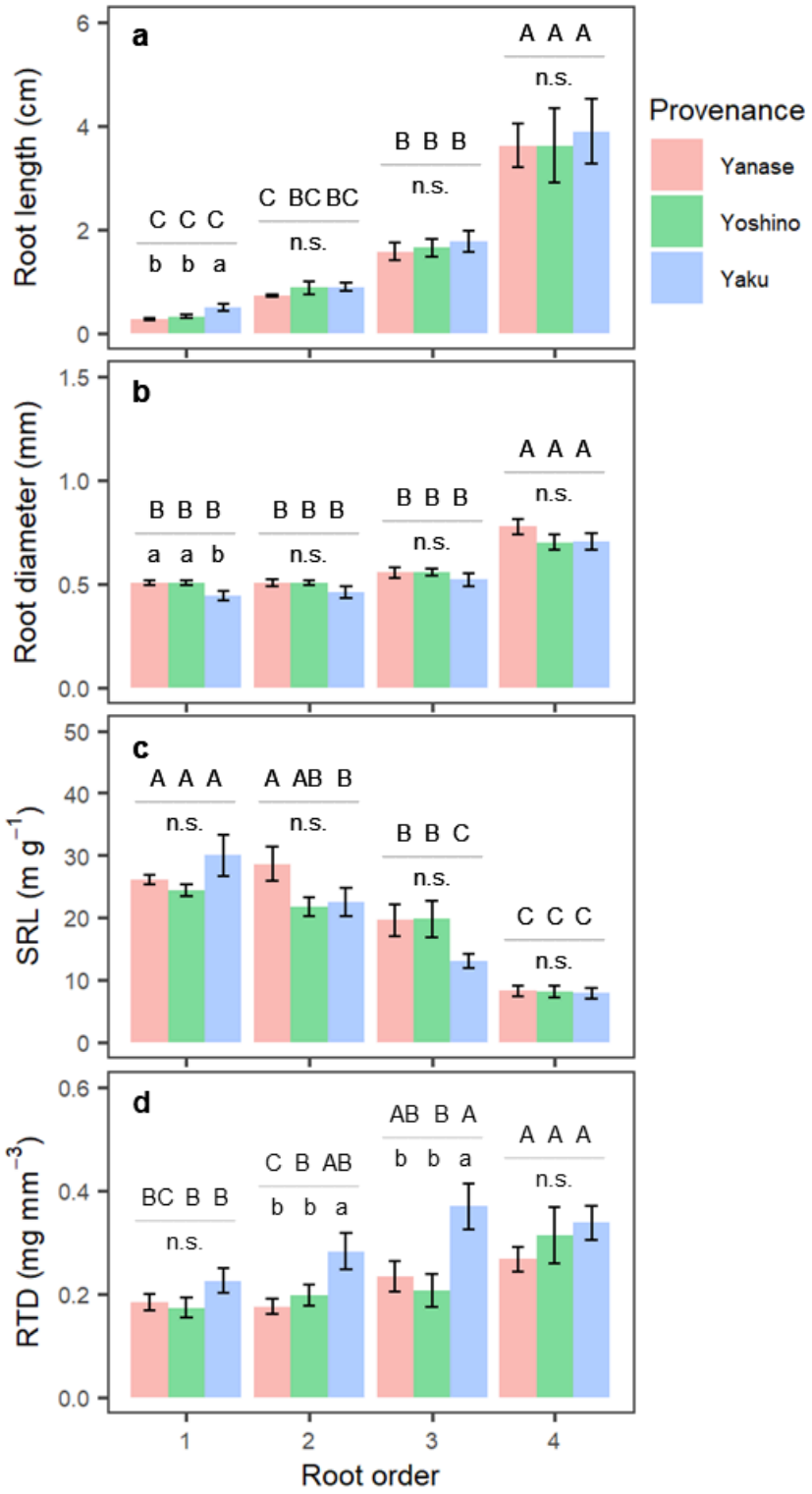


Figure 1

Mean values (± standard error, *n*= 12) in (a) root length, (b) root diameter, (c) specific root length (SRL), and root tissue density (RTD) among root orders or tree provenances. Different capital or small letters indicate significant differences among the root orders or tree provenances, respectively (GLMM, $P < 0.05$), and n.s. denotes no significant difference.

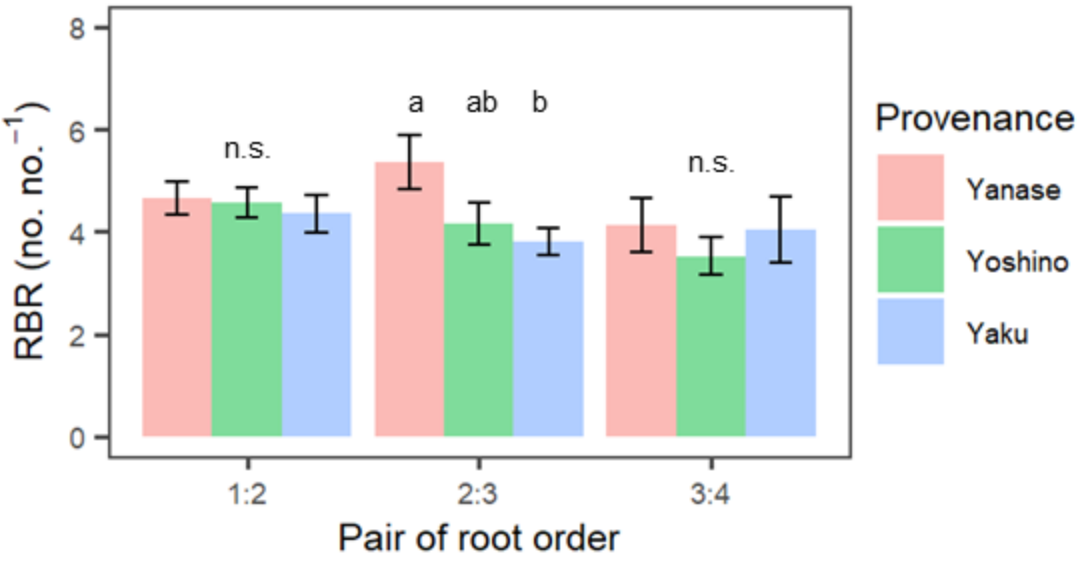


Figure 2

Mean values (± standard error, n = 12) in root branching ratio (RBR) among tree provenances for each pair of root orders. Different letters indicate significant differences among the tree provenances (GLMM, $P < 0.05$) , and n.s. denotes no significant difference.

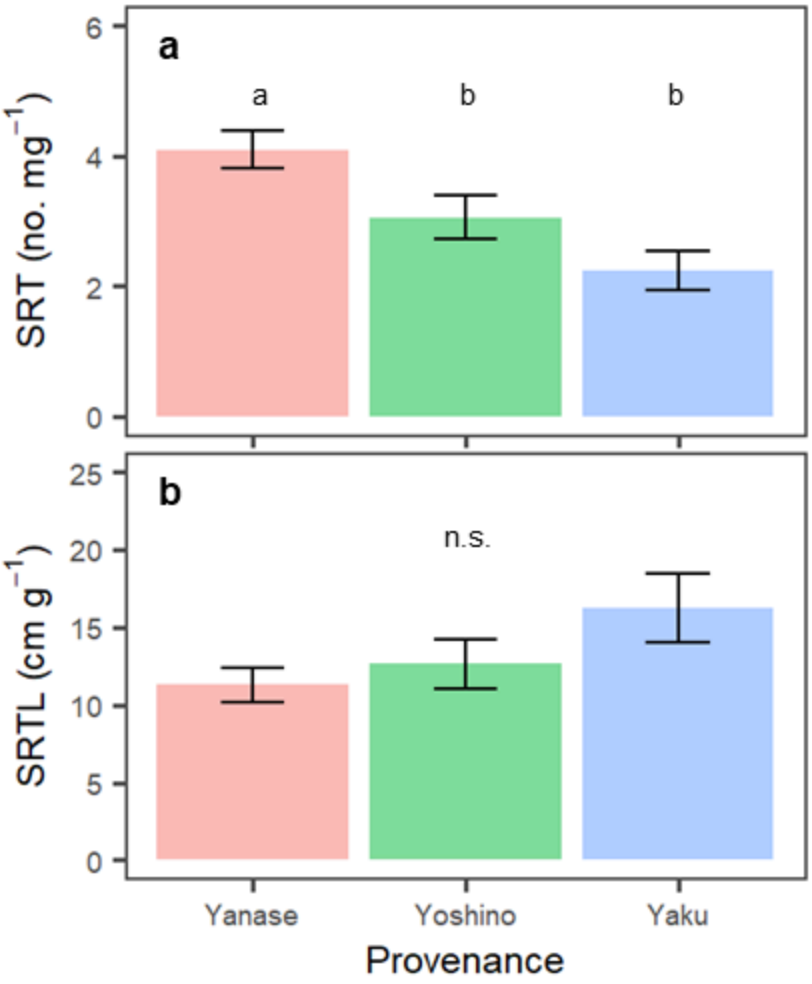


Figure 3

Mean values (± standard error, *n*= 12) in (a) specific root tips (SRT) and (b) specific root tip length (SRTL) among tree provenances. Different letters indicate significant differences among the tree provenances (GLMM, $P < 0.05$), and n.s. denotes no significant difference.

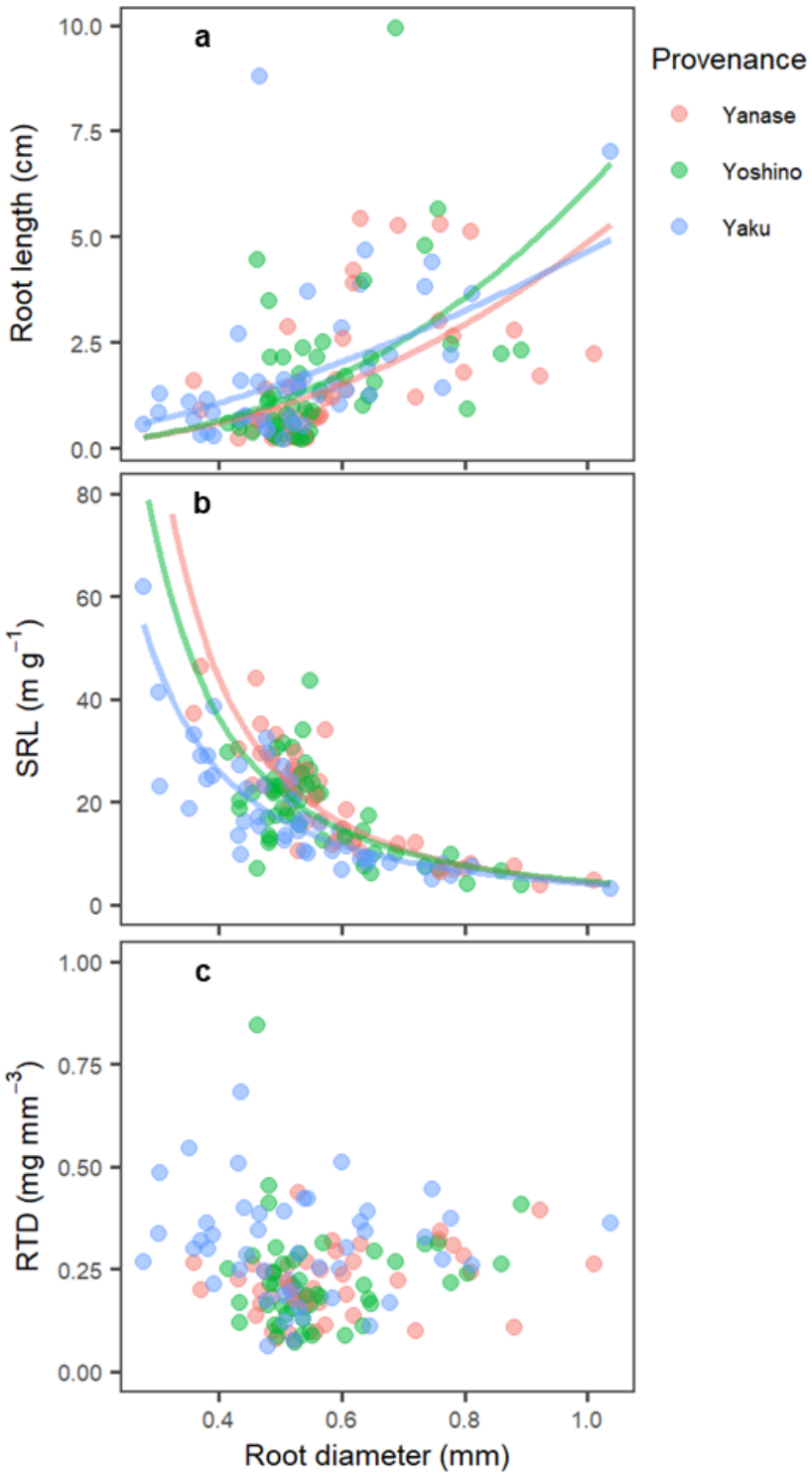
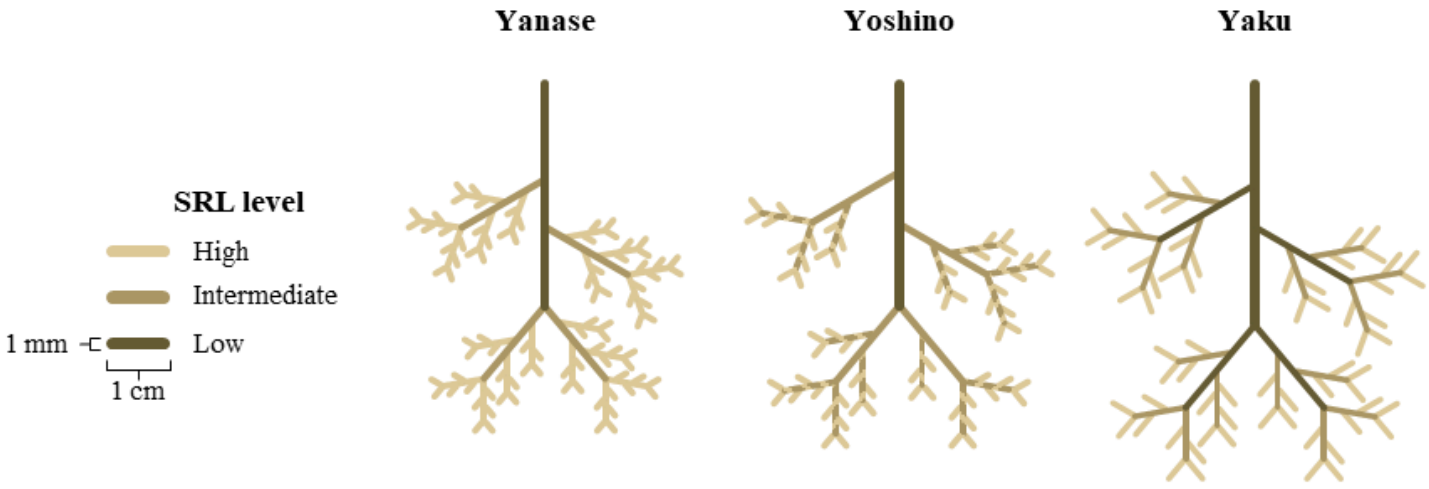


Figure 4

Relationships between root diameter and (a) root length, (b) specific root length (SRL), or (c) root tissue density (RTD) for each tree provenance. Dots indicate the mean values within each root order, and lines represent significant regression curves ($P < 0.05$) for each provenance by the GLMM analyses.



Aboveground productivity (Azuma et al. 2023)	High	High	Low
Root exudation rate (Ohta et al. 2019)	Low	High	Low
Foraging strategy	Resource consumptive for root growth	Resource consumptive for root exudate	Resource conservative

Figure 5

Schematic diagram of distal root systems from 1st to 4th root orders and summary of presumed foraging strategy for each cedar type. The root diagram was constructed by referring to mean root length, diameter, and branching ratio from our results (Fig. 1a, b and Fig. 2). Different root colors represent significant differences in specific root length (SRL) within each root system (Fig. 1c). Striped colors indicate no significant difference from just upper and lower root orders (e.g., 2nd order roots in Yoshino cedar). Aboveground productivity and root exudation rate in three cedar types were summarized from Ohta et al. (2019) and Azuma et al. (2023). Foraging strategy for each provenance was discussed in the Discussion section.

Supplementary Files

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- ESM1.pdf