Rock fragment content in soils shift root foraging behavior in xerophytic species

Hui Hu · Weikai Bao · David M. Eissenstat · Long Huang · Jian Liu · Fanglan Li

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Abstract

Aims Root traits associated with resource foraging, including fine-root branching intensity, root hair, and mycorrhiza, may change in soils that vary in rock fragment content (RFC), while how these traits covary at the level of individual root branching order is largely unknown.

Methods We subjected two xerophytic species, Artemisia vestita (subshrub) and Bauhinia brachycarpa (shrub), to increasing RFC gradients (0%, 25%, 50%, and 75%, \( v^{-1} \)) in an arid environment and measured fine-root traits related to resource foraging.

Results Root hair density and mycorrhizal colonization of both species decreased with increasing root order, but increased in third- or fourth-order roots at high RFCs (50% or 75%) compared to low RFCs. The two species tend to produce more root hairs than mycorrhizas under the high RFCs. For both species, root hair density and mycorrhizal colonization intensity were negatively correlated with root length and root diameter across root order and RFCs. Rockiness reduced root branching intensity in both species comparing with rock-free soil. At the same level of RFC, A. vestita had thicker roots and lower branching intensity than B. brachycarpa and tended to produce more root hairs.

Conclusion Our results suggest the high RFC soil conditions stimulated greater foraging functions in higher root orders. We found evidence for a greater investment in root hairs and mycorrhizal symbioses as opposed to building an extensive root system in rocky soils. The two species studied, A. vestita and B. brachycarpa, took different approaches to foraging in the rocky soil through distinctive trait syndromes of fine-root components.

Keywords Root hair · Mycorrhizal colonization · Fine-root functional traits · Root branching order · Gravel content · Dry ecosystem
Abbreviations
RFC  Rock fragment content
AMF  Arbuscular mycorrhizal fungi
RHA  The ratio of root hair area per unit root area to AMF colonization rate

Introduction

Root traits such as fine-root length, root hair length, and density and mycorrhizal colonization are key determinations of plant and ecosystem functioning and congregating several components. Collectively, these fine-root traits help determine the absorptive function of roots. They have evolved a variety of syndromes that enable them to respond to changing soil physical structure, water and nutrient contents, and soil microbial communities (Hodge 2003, 2004; Jakobsen et al. 2005; Freschet et al. 2018, 2021). Plasticity in multiple fine-root traits in different soil conditions should provide insight into how plants cope with environmental gradients (Hodge 2004; Alameda and Villar 2012; Bardgett et al. 2014; Freschet et al. 2018). Morphological and structural traits of fine-roots have been widely discussed in plant functional ecology (Ma et al. 2018; Kong et al. 2019; Freschet et al. 2021). Patterns of root architecture can also be described by branching orders and total branching intensity. However, there persists a significant gap in understanding at the level of individual root branching order how root functional traits shift with environmental gradients.

A key aspect of soil physical structure is content of rock fragments (diameter > 2 mm). The coarse particles are widespread in soils around the world and play important roles in soil properties (Poesen and Lavee 1994; Qin et al. 2015; Gargiulo et al. 2016, Zhang et al. 2016). Contents of water and nutrients are reduced in soils with high rock fragment contents (RFCs) (van Wesemael et al. 1996; Rytter 2012; Mi et al. 2016; Ceacero et al. 2020), because such coarse-textured soils have lower water-holding capacities and deeper infiltration than finer-textured soils (Schenk and Jackson 2005; Fan et al. 2017). However, carbon (C) and nitrogen (N) may be more concentrated around rock fragments (Zheng et al. 2021; Lai et al. 2021, 2022) due to a favorable microclimate for root growth and decomposition of organic materials (Poesen and Lavee 1994; van Wesemael et al. 2000; Rytter 2012). It was also found that the C and N inputs through rock weathering are comparatively heterogeneous across different soils and lithology in different study regions (Houlton and Morford 2015). Variations in RFC can affect other physicochemical parameters such as soil bulk density, porosity, and hydrological processes (Poesen and Lavee 1994; van Wesemael et al. 2000; Gargiulo et al. 2016; Zhang et al. 2016). It is also able to alter soil microbial composition (Certini et al. 2004; Hong et al. 2021), as well as vertical distribution of roots and overall plant growth (Qin et al. 2015; Mi et al. 2016; Hu et al. 2021). Therefore, we expected that variation in RFC would alter fine-root foraging behavior linked branching intensity, root morphology, root hair, and mycorrhizal traits.

The analysis of fine-root function by branching order has shown considerable promise for better understanding the functioning of absorptive root systems (Pregitzer et al. 2002; Guo et al. 2008; McCormack et al. 2015; Lavely et al. 2020). Root branching intensity controls exploration through the soil matrix and shows substantial variation in heterogeneous soil and among species (Comas and Eissenstat 2009; Kong et al. 2014; Pagès and Kervella 2018; Freschet et al. 2021). For example, root branching intensity is negatively correlated with soil depth but positively correlated with soil water content in heterogeneous soils (Wang et al. 2021). In addition to root branching intensity, fine-root structures and functions differentiate markedly with root orders. Typically, the most distal root tips (e.g., first- and second-order roots) are thinner with thicker cortex, higher N concentration, and higher mycorrhizal colonization than higher order, more basal roots, implying they are more active in nutrient uptake (Pregitzer 2002; Pregitzer et al. 2002; Guo et al. 2008). Nonetheless, the pattern of functional differentiation along root orders in response to changes of soil physical structure remain poorly known.

Both root hairs and mycorrhiza can dramatically improve resource uptake due to a low carbon (C) cost per unit surface area (Jungk 2001; Jakobsen et al. 2005; van der Heijden et al. 2015). Arbuscular mycorrhizal colonization generally decreases with increasing root order (Guo et al. 2008) and increases with N or phosphorus (P) limitation (Li et al. 2015; Li et al. 2019; Han et al. 2020). In addition to mycorrhizal fungi, root hairs and cluster roots constitute...
other important pathways in foraging, particularly for immobile nutrients like P (Jungk 2001; Jakobsen et al. 2005; Lambers et al. 2008). Elongation of root hairs was faster and their density was higher under low P, allowing a larger soil volume exploited by each root cylinder (Jungk 2001; Waisel and Eshel 2002; Jakobsen et al. 2005). While no study so far has determined the variation of root hairs among root orders, it would be expected that root hairs would be most proficient in the younger, lower order roots with an intact epidermis (Eissenstat and Yanai 1997). Based on the findings that mycorrhizal fungal colonization and root hairs increased with N or P limitation (Jungk 2001; Jakobsen et al. 2005; Li et al. 2015; Li et al. 2019; Han et al. 2020) and soil nutrient content decreased with increasing RFC (Rytter 2012; Ceacero et al. 2020; Hu et al. 2021; Huang 2021), we assumed mycorrhizal colonization and root hairs may increase under high RFC.

Trait syndromes of fine-root components along environmental gradients or biomes were recently discussed for the multidimensional economic spectrum and predicted plant strategies (Liese et al. 2017; Kong et al. 2019; Bergmann et al. 2020; McCormack et al. 2020; Pierick et al. 2020; Freschet et al. 2021). Both mycorrhizal colonization and root diameter are typically positively correlated to root cortical thickness across root branching orders, which may lead to synergies between root functional traits in response to resource variation (Freschet et al. 2021). Adjustment of traits like root length, root hair length, and density or mycorrhizal colonization intensity may similarly adjust in a systematic manner to resource limitation or stress conditions (Jungk 2001; Jakobsen et al. 2005; Freschet et al. 2021). It is reasonable to assume that a trade-off may exist with changing RFC, due to the functional alternative between root length, root hairs, and mycorrhizas (Jungk 2001; Jakobsen et al. 2005).

In this study, we observed fine-root traits of two xerophytic species responding to the RFC gradients (0%, 25%, 50% and 75%, v v⁻¹) in the arid valley environment of western China. The two species, Artemisia veitita and Bauhinia brachycarpa, are native to arid valleys of the Hengduan Mountain region and were chosen due to their regional ecological importance and divergent growth forms (shrub and subshrub). Whereas the difference in plant adaptations to microhabitat heterogeneity is recognized based on above-ground measurements (Li et al. 2008; Jin et al. 2018; Hu et al. 2021), the ecological significance of variations in fine-root traits is not well known. We examined root branching intensity, root hair length and density, mycorrhizal colonization intensity, and cortex thickness across five branching orders. Our objectives were to investigate (1) how these components varied with RFC gradients and (2) whether the functional differentiation among root orders was affected by varying RFC similarly for the two different species. We hypothesize that (1) root hairs and mycorrhizal colonization would be promoted with increasing RFCs, in accordance with previous findings that mycorrhizal fungal colonization and root hairs increased with N or P limitation (Jungk 2001; Jakobsen et al. 2005; Li et al. 2019) that was associated with increasing RFCs (Ceacero et al. 2020; Huang 2021); (2) root hair density and mycorrhizal colonization decreased with increases in branching order as a result of decreased nutrient absorptive function in higher root orders (Pregitzer 2002; Pregitzer et al. 2002; Guo et al. 2008); and (3) trade-offs exist between root length, root hairs, and mycorrhizal colonization among root orders and RFC gradients, attributed to the similar function between root length, root hair and mycorrhizal colonization (Jungk 2001; Jakobsen et al. 2005; Freschet et al. 2021).

Materials and methods

Study site

The study was conducted at the Jingzhou Hill of Maoxian County in Minjiang Arid Valley, Sichuan, China (31°70′ N, 103°87′ E, altitude 1637 m). RFC reaches up to 65% (g g⁻¹) in the bottom of typical arid valleys in this region (altitude 1650 m) (Bao et al. 2012). The arid valley ecosystem (altitude 1200 to 3600 m) is widely distributed throughout the Hengduan Mountain range (Xu et al. 2008a; Li and Bao 2014). These regions are often composed of moderately sparse vegetation patches dominated by xerophytic subshrub and shrub species (Bao et al. 2012; Li and Bao 2014). As a result of the rocky soil, water stress, and soil infertility limits vegetation development (Xu et al. 2008a, 2008b; Wu et al. 2008; Li et al. 2008; Qu et al. 2017). A key factor influencing soil heterogeneity in this region arises from variation in
rock fragments throughout the soil profile (Tetegan et al. 2011; Bao et al. 2012; Rytter 2012).

Mean annual precipitation is 495 mm, with 83% of the precipitation falling during the growing season from May to October (Maoxian County Meteorological Station, 2 km from the study site). Mean annual potential evaporation is 1332 mm and mean annual temperature is 15.6 °C. RFC ranges from 1 to 65%; the dominant rock fragment has a particle size >10 mm (Bao et al. 2012). The site was historically cultivated for agricultural crops; potatoes and celery were planted 2 years prior to the start of this study. Soils are classified as cinnamon, characterized by a clay loam with coarse texture and low fertility (Bao et al. 2012) and a typical soil depth of 50–70 cm.

Experimental design

A randomized complete block design was used to assess eight treatments as combinations of two plant species and four RFCs (0%, 25%, 50%, and 75% volumetric contents, v v⁻¹) with four replicates (plots). Making a total of 32 plots in the study. Each plot was represented by a pit, with a dimension of 1-m long, 1-m wide by 0.5-m deep and a 50-cm spacing between plots. A. vestita is a fast-growing, tufted subshrub (up to 1.8 m maximum height), with a shallow root system. It is distributed along gentle slopes with relatively few rock fragments in soil. and B. brachycarpa, a slow-growing, dwarf and highly-branched shrub (up to 1.5 m height). It has a deep rooting profile and generally grows on steep slopes with high RFC (Hu et al. 2021). They can represent typical survival strategies and provide knowledge on root trait syndromes at the root branch level of xerophyte shrubs in arid ecosystems. From a management perspective, these two xerophytes were selected to enhance understanding of survival of plants from seed germination under the natural conditions of arid valleys where soils can be quite gravel.

To obtain desired RFCs, fine soil particles and rock fragments (10–20 mm in diameter) were collected and mixed uniformly in each pit. The soils used were taken from the pits. Specifically, the soil at 0–50 cm depth was excavated from each pit. After that, the walls of each pit were lined with polyethylene film to prevent interference of external conditions, and the bottom of the plot was unlined to allow natural drainage. Fine soil fractions were obtained by passing the dried soils (air-drying for a week) through a 2-mm sieve, and mixing the soils from all pits uniformly. Soil samples (n=6) were collected to determine initial soil properties, which included total phosphorous (TP) of 0.61±0.01 g kg⁻¹, total nitrogen (TN) of 2.31±0.02 g kg⁻¹, and total carbon (TC) of 15.3±0.09 g kg⁻¹. In this study, thin-bedded limestone (dominated by phylite) which is commonly found in the region was used as the rock fragment. It was collected from the sieved soils that had been excavated from the pits and additionally from nearby land to obtain sufficient material. After crushing, the rock materials were first passed through a 20 mm sieve and then through a 10 mm sieve to obtain rock fragments with a diameter of 10–20 mm. The thin-bedded limestone had a density of 2.56±0.03 g cm⁻³ (n=12), as measured by the water displacement method (Wang et al. 2017). After a uniform mixing, the fine soil particles and rock fragments were filled back into the pits at desired RFCs in April 2018. Each plot was then irrigated with 100 L water and left to stabilize the soil.

In April 2018, plots were seeded at 0.5-1 cm depth in a regularly spaced pattern of 9 points in each pit (25 cm equidistant between two points). For both species, the seeds were collected from their natural habitats in the arid Minjiang River valley (31°42′N, 103°53′E, altitude range of 1600–1920 m) in fall 2017, air-dried for 4–8 days, and stored at room temperature (10–25 °C) until sowing. Before sowing, all the seeds were disinfected by immersion in 2.5% NaClO for 1 h. Seedlings were watered weekly after sprouting to prevent early losses. They were thinned two months after sprouting (Hu et al. 2021), leaving four average-sized seedlings per plot and about 50 cm between seedlings (Fig. S1). The plots were bi-weekly weeded to ensure normal growth of plants.

Measurements

Root sampling

A plant with average growth was selected in each plot for sampling roots (see Table S1). In September, 2019, we collected several complete root segments containing 5 levels of branching to a soil depth of 20 cm. The roots collected from each pit were divided into two subsamples. One subsample (three root segments surrounded with soil) was immediately put on ice, transported to laboratory within 4 h of sampling, and frozen for architecture analysis. The other subsample was washed in deionized water and fixed in
Formalin-Aceto-Alcohol (FAA) solution (90 ml 50% ethanol, 5 ml 100% glacial acetic acid, 5 ml 37% methanol) for assessing root hairs, mycorrhizal colonization and anatomical traits.

Root architecture

The three intact root segments of each pit were washed and dissected into five branching orders (1–5 orders) with the most distal root tips labeled as first order following Strahler’s stream ordering system (Pregitzer et al. 2002; Guo et al. 2008). Roots of each order were then scanned (Epson V800, Seiko Epson Corp. Japan) at 600 dpi. Images were analyzed using WinRhizo 2020 (Regent Instrument, Canada) to determine root tip numbers, sum root length (sum length of total root in the five orders) and root length (average length of individual root in each branch order). Both root length and specific root length can reflect the length of root exploring the soil (Freschet et al. 2020). Root length is considered a key trait deciding for nutrient acquisition (Crain and Dybzinski 2013; Freschet et al. 2020). Sum root lengths of the five orders can also intuitively reflect the size of target root segment. We did not include measure of specific root length (SRL), although it is often regarded as a core trait for root economics. SRL is normally highly (negatively) correlated with root diameter (Comas and Eissenstat 2009; Kong et al. 2014; Pierick et al. 2020), and it unsurprisingly decreased with increasing root order (Lavely et al. 2020; Yang et al. 2021). And root diameter was measured by cross-section with a microscope when assessing root hairs. Root branching intensity was calculated using the following equation (Comas and Eissenstat 2009):

\[
\text{Root branching intensity (No. cm}^{-1}\text{)} = \frac{\text{Total number of root tips (No.)}}{\text{Sum root length of five orders (cm)}}
\]

Root hairs

For each plant species, 15 root segments of 1 cm length were randomly selected for each root order, thoroughly cleaned with a sonicator (15 °C, 120 W, 45 kHz) for 10 min, and placed in FAA. Root hair characteristics were determined by sectioning and an image analysis system that consists of a microscope (Olympus BX53F, Japan) connected to a video camera (Toupcam, Hangzhou ToupTek Photonics, China) and an interfaced computer with an analytical software (ToupView, Hangzhou ToupTek Photonics, China). The measured characteristics included root hair diameter, length and total number in the root section (Green et al. 1991; Tomasello et al. 2018; Freschet et al. 2020). We also determined the thickness and diameter of root sections. Subsequently, density and area of root hairs, and root hairs surface area per root surface were calculated using the following equations:

\[
\text{Root hair density (No. mm}^{-2}\text{)} = \frac{\text{Number of root hairs (No.)}}{\text{Root section surface area (mm}^2\text{)}}
\]

\[
\text{Root hair surface area (mm}^2\text{)} = \text{Root hair average surface area (mm}^2\text{)} \cdot \text{Root hair number}
\]

Root hairs surface area per unit root surface = Root hair area (mm$^2$) / Root section surface area (mm$^2$)

Arbuscular mycorrhizas

The arbuscular mycorrhizal fungi (AMF) colonization intensity was determined by the acid fuchsin staining method (Kormanik and McGraw 1982, but without phenol). The main steps were clearing, dyeing, color separation, microscopic examination. The samples fixed in FAA were cleared by incubating in KOH solution (15%, m v$^{-1}$) at 90 °C for 20–40 min (treatment time varied with different plants and root orders), acidified in HCl (2%, v v$^{-1}$) at room temperature for 1 min and stained with acid fuchsin at 90 °C for 15 min (Kormanik and McGraw 1982; Hodge 2003). Then the stained roots were observed for mycorrhizal colonization in each root under a microscope (Olympus BX53F, Japan). Colonization length of AMF was measured using ToupView (Hangzhou ToupTek Photonics, China) in each root segment (averaged in 1 cm length), and a total of 30 root segments were counted using the random sampling method for each root order in each pit. AMF colonization intensity was calculated as the percentage length of roots colonized by mycorrhizal fungi (%) (Treseder 2013; Freschet et al. 2020). The ratio of root hair area per unit root area to AMF colonization rate (RHA) represents the magnitude comparison of change in root hair and mycorrhizal colonization along RFC gradients.
Root anatomy

For assessing root anatomy, 10 individual roots were randomly selected of each root order from the FAA root samples (in the high root order less than 10 root samples, the maximum number was tested). The root samples of each branch order were stained with Safra-nin-Fast Green (2%). Root samples were then dehy-
derated in 70, 85, 95 and 100% ethanol in sequence, and embedded in paraffin to cut into 8-µm thick tapes with a microtome. Pictures of the anatomical struc-
ture of the root section were taken at all orders under a microscope (Olympus BX53F, Japan). After that, the thickness of the root cortex and the density of corti-
cal cells (Guo et al. 2008; Freschet et al. 2020) at each level were analyzed using an image analysis software (ToupView, Hangzhou ToupTek Photonics, China).

Soil sampling and soil properties

Bulk density and non-capillary porosity of the 0–20 cm soil layer were determined from the mass of dry soil in cores of known volume (100 cm³) collected from each plot. Soil samples were collected to a depth of 20 cm in all treatments (Fig S6). Fresh sub-
samples were oven dried at 105 °C until the weight became constant to measure soil water content (SWC). Remaining soil subsamples were air-dried and passed through a 100-mesh (0.15 mm) sieve for determining pH, TC, TN and TP. Soil pH was determined in a 1:2.5 (w v⁻¹) soil-water suspension (Sev-
enEasyS20, Mettler Toledo, USA). TC and TN were determined by combustion in an Elemental Analyse-
ry (Elementar Vario MAX, Germany), and the TP was measured using the sulphuric acid-soluble perchlorate acid- molybdenum antimony colorimetric method (Hu et al. 2016).

Statistical analyses

Linear mixed models ANCOVAs were used to exam-
ine sources of variation in fine-root traits of the two species. In these models, the RFC and root order were treated as fixed factors, whereas the plant species was treated as random factors. We facilitated type III sums of squares to examine F-values and signifi-
cance of the fixed factors. The variance component estimates and significance of the random factor was evaluated by Variance Components Analysis. One-
way ANOVA was used to examine the significance of differences in root traits of each order among the RFCs. Data meeting the assumption of homogeneity of variance was tested by least significant difference (LSD); otherwise, data were analyzed by a non-para-
metric test (Kruskal-Wallis). The above analyses were conducted using SPSS 25.0 (International Business Machines Corporation (IBM), USA). We performed a redundancy analysis (RDA) using fine-root traits of both species for the means of five orders together with measured soil properties along the RFCs. This analysis was carried out with Canoco 5.02 (Biometris, Wageningen University and Research Centre, The Netherlands). Principal component analysis (PCA) was performed using the root branching intensity and average values of fine-root traits of five root orders to obtain an overview of the multidimensional fine-root functions of each species at different RFC levels. PCA was implemented through the “vegan” package of R software (version 4.0.4; R Core Team, Austria). Line fitting analyses were used to test the relationships between root traits across branching and across the RFCs. We also evaluated variation in patterns of fine-root traits along 1–5 orders within RFC through linear fitting analysis based on standardized data \(Y = X^{0.5}\). Linear fit graph and histogram were
generated using Origin 2018 (OriginLab Corporation, USA).

Results

Root branching, length, and diameter

For the total five root branching orders of both species, root branching intensity was higher in rock-free soil than in soils with rock fragment (Fig. 1a and b). Additionally, the sum root length of the five branching orders in the two species tended to decrease with an increase in RFC (Fig. 1c and d). In both species, root length and diameter of higher order roots (but not lower order roots, i.e. first two or three order) significantly differed among RFC treatments (Fig. 2a-d). Differences were also found between the two species, where *B. brachycarpa* had higher root branching intensity and thinner roots than *A. vestita* across root orders (Figs. 1 and 2 and S2).

Root length and root diameter in both species varied significantly across root orders (*P* < 0.001, Table S2). Root length increased from first- to fourth-order roots and declining thereafter, while root diameter increasing with root orders among different RFC (Fig. 2 and S3).

AMF colonization intensity of both species differed by RFC (*P* < 0.05, Table S2), and interspecific variation was also found (Fig. S2). For the two species, AMF colonization intensity was increased in fourth-order roots under 75% RFC (Fig. 3g and h). In general, *B. brachycarpa* had more mycorrhizal colonization intensity than *A. vestita* (Fig. 3g and h), and the two species showed opposite trends in mycorrhizal colonization of the first three root orders with increasing RFC (Fig. 3g and h).

Fig. 1 Root branching intensity and sum length of five root orders in *A. vestita* and *B. brachycarpa* varied with soil rock fragment contents. Bars represent means ± SE, *n* = 4. Differences in means were assessed with one-way ANOVA, except difference in root branching intensity means of *A. vestita* which were assessed with the non-parametric test (Kruskal-Wallis). Different lowercase letters for *A. vestita* and different capital letters for *B. brachycarpa* are significantly different. *, ** designate differences at *P* < 0.1 and *P* < 0.05, ns indicate no significant differences between treatments (*P* > 0.1)

Root hair traits and mycorrhizal colonization

In both species, root hairs occurred and root hair length and density generally increased on the third- or fourth-order roots at 50% or 75% RFC (Fig. 3). In the rock-free soil, root hairs were produced on the first three root orders in *A. vestita*, while root hairs were mainly formed on the first two orders in *B. brachycarpa* (Fig. 3). Root hair length and number in *A. vestita* were greater than those in *B. brachycarpa* at all levels of RFC (Fig. 3 and S2).

AMF colonization intensity of both species differed by RFC (*P* < 0.05, Table S2), and interspecific variation was also found (Fig. S2). For the two species, AMF colonization intensity was increased in fourth-order roots under 75% RFC (Fig. 3g and h). In general, *B. brachycarpa* had more mycorrhizal colonization intensity than *A. vestita* (Fig. 3g and h), and the two species showed opposite trends in mycorrhizal colonization of the first three root orders with increasing RFC (Fig. 3g and h).

Root hair traits and mycorrhizal colonization intensity of the two species differed significantly among root orders (*P* < 0.001, Table S2) and they disappeared in fifth-order roots (Fig. 3). Across all levels of RFC, root hair diameter increased with root order, while root hair length, root hair density and AMF...
Colonization intensity decreased with increasing root order (Fig. 3 and S4).

**Root anatomical traits**

Cortical cell density was influenced by RFC ($P < 0.01$, Table S2), and plant species (Fig. S2). The cortex of most root orders thickened in high RFC (50% or 75%), except for the first-order roots of *A. vestita* (Fig. 2e and f). Cortical cells in first-order roots of *A. vestita* and in second- and third-order roots of *B. brachycarpa* were denser under 75% RFC (Fig. 2g and h). The cortex of the fifth order disappeared in *B. brachycarpa*; however, it still existed in *A. vestita* (Fig. 2).

Cortical thickness and cell density of both species differed significantly among root orders ($P < 0.001$, Table S2). Cortical thickness decreased with increasing root order at most levels of RFC (except in *A. vestita* at 75% RFC) (Fig. 2 and S5). Cortical cell density decreased with increasing root order in *A. vestita*, but it increased from first- to second-order roots and declined thereafter in *B. brachycarpa* (Fig. 2 and S5).
Relationships among root traits

In both species, the ratio of root hairs area per unit root area to AMF colonization rate (RHA) of each root order changed with RFC ($P<0.001$, Table S2), and interspecific variation was also found (Table 1 and Fig. S2). RHA of *A. vestita* was maximum in first- to fourth-order roots at 75% RFC, indicating that the relative increase in root hairs was more dramatic than that of mycorhizas (Table 1). For *B. brachycarpa*, RHA was stable in the first three root orders with RFC, but the fourth order were promoted to form more root hair in 50% RFC (Table 1). Overall, RHA across the five root orders in *A. vestita* was means of fourth-order in *A. vestita* and of the third- and fourth-order in *B. brachycarpa* were assessed with the non-parametric test (Kruskal-Wallis). Different lowercase letters for *A. vestita* and different capital letters for *B. brachycarpa* represent significant differences between rock fragment contents, *, ** and *** designate differences at $P<0.1$, $P<0.05$ and $P<0.01$, respectively; ns indicate no significant differences between treatments ($P>0.1$). AMF: Arbuscular Mycorrhiza fungi

Fig. 3 Root hair diameter, root hair length, root hair density and AMF colonization intensity of each root order in *A. vestita* and *B. brachycarpa* at a range of soil rock fragment contents. (Root hair and mycorrhizal colonization of the two species disappeared in fifth-order roots.) Bars represent means ± SE, $n=4$. Differences in means were assessed with one-way ANOVA, while differences in root hair length means of the second-order in *A. vestita*, and all root hair and AMF traits
much higher than that in *B. brachycarpa* (Fig. 4; Table 1). In this case, root hair abundance of *A. vestita* was much greater than mycorrhizal colonization (RHA > 1, Fig. 4a), while mycorrhizal colonization magnitude of *B. brachycarpa* was relatively larger than that of root hairs (RHA < 1, Fig. 4b).

Root hair length, root hair density and AMF colonization intensity were both negatively correlated with root diameter and root length in both species (*P < 0.001*), but were positively and strongly correlated with cortical thickness and cortical cell density in *A. vestita* (Fig. 5). However, we did not find significant correlations of root hair traits with cortical cell density in *B. brachycarpa* (Fig. 5).

Root diameter and length, root hair diameter, length and density, mycorrhizal colonization intensity, cortical thickness and cell density (means of five root orders) and root branching intensity variation was assessed using RDA analysis with RFC and soil properties together as potential explanatory variables (Fig. 6). For both species, root hair length and density, cortical thickness, cortical cell density and RHA were maximum at high RFC and low water and nutrient content, while root diameter was thickest at low RFC that correlated with low non-capillary porosity. AMF colonization intensity of *A. vestita* was greatest at low RFC (Fig. 6a), while mycorrhizal colonization in *B. brachycarpa* was greatest at high RFC (Fig. 6b). Fine-root variation in *A. vestita* was mainly explained by non-capillary porosity and TN, but in *B. brachycarpa* variation in root traits was mainly explained by TC, RFC and TN (Table S3).

### Discussion

In this study, rock fragments in the soil induced shifts in a wide array of root traits in both *A. vestita* and *B. brachycarpa*. Notably, increasing RFC stimulated production of root hairs and mycorrhizal colonization in higher order roots (third- and fourth-order) in both species. As expected of our first two hypotheses, root hair density and mycorrhizal colonization intensity decreased with increasing root order, but at high RFC (50% or 75%) the decrease was reduced. In supporting our third hypothesis, root length was negatively correlated with root hair density and mycorrhizal colonization intensity, demonstrating a trade-off between root components along root order and the RFC gradient.

Rock fragment content affected fine-root foraging behavior

Root branching is highly plastic and sensitive to variation in water and nutrient availability (Fitter and Stickland 1991; Kong et al. 2014; Liese et al. 2017; Freschet et al. 2018). In the present study, root branching intensities of both species decreased in rocky soils (Fig. 1), which was closely related to

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**Table 1** The ratio of root hair area per unit root area to AMF colonization rate (RHA) of two xerophytic species varied among rock fragment content (RFC)

| Species            | RFC | First order | Second order | Third order | Fourth order |
|--------------------|-----|-------------|--------------|-------------|--------------|
| *Artemisia vestita*| 0%  | 10.57±2.35 ab | 8.30±1.87 b  | 5.44±1.81 ab | 0 a          |
|                    | 25% | 7.74±1.27 b  | 11.48±4.09 ab| 1.82±0.51 b | 0 a          |
|                    | 50% | 10.51±0.60 ab| 12.68±3.06 ab| 1.88±0.82 ab| 0 a          |
|                    | 75% | 20.62±3.22 a | 36.74±8.02 a | 16.15±6.01 a| 3.14±2.68 a |
| *Bauhinia brachycarpa* | 0%  | 1.45±0.29 A   | 0.12±0.11 A  | 0 A         | 0 B          |
|                    | 25% | 3.38±0.50 A   | 0.91±0.45 A  | 0 A         | 0 B          |
|                    | 50% | 4.18±1.23 A   | 4.56±2.33 A  | 2.75±1.55 A | 10.79±7.12 A|
|                    | 75% | 2.08±0.38 A   | 2.59±0.99 A  | 1.50±1.49 A | 0.05±0.05 AB |

Means ± SE, n=4. Root hair and mycorrhizal colonization of the two species disappeared in fifth-order roots. (Differences in means were assessed with the non-parametric test (Kruskal-Wallis). Different lowercase letters for *A. vestita* and different capital letters for *B. brachycarpa* are significantly different at *P < 0.05*). AMF: arbuscular mycorrhizal fungi. Root hairs surface area per unit root surface = Root hair area (mm²) / Root section surface area (mm²). AMF colonization rate = Σ (colonization length of root segment 1 / length of root segment 1 + colonization length of root segment 2 / length of root segment 2 + colonization length of root segment 3 / length of root segment 3 + …… + colonization length of root segment 30 / length of root segment 30) / sum length of 30 roots segment. The ratio of root hair area per unit root area to AMF colonization rate (RHA) = Root hairs surface area per unit root surface / AMF colonization rate.
the significant decreases in soil water and nutrient contents with increasing RFCs (Fig. 6 and S6; Wang et al. 2021). While a higher branching intensity increases local soil exploitation, lower branching may enable larger soil exploration (Eissenstat et al. 2015; Freschet et al. 2021) and thus be the most effective strategy for the uptake of mobile soil resources such as nitrate and water (e.g. Pedersen et al. 2010). Root length of the fourth- and fifth-order roots in both species were greater under 25% or 50% RFC (Fig. 2a and b). Such increasing root length could promote plants to explore larger soil volume and acquire more nutrients (Freschet et al. 2020, 2021). Conversely, the thickest diameter of high root order (forth- to fifth-order) in both species were found in rock-free soil (Fig. 2c and d). This may be due to the decrease of soil porosity (Fig. S5; Xu et al. 2012; Gargiulo et al. 2016) and thus increased soil mechanical resistance in compact soils with low RFC, which resulting in plants with thicker roots (Clark et al. 2003). The results demonstrate that in such heterogeneous soil the combination of branching pattern and morphological traits (length or thickness) along root order was a strategy for the plant to locally adjust the root density to resource variation in space.

Our results were consistent with our first and second hypothesis that root hair density and mycorrhizal colonization intensity should decrease with the increasing root order, but increase at higher RFCs (Fig. 3 and S4). Importantly, there was strong functional differentiation across the five root orders changed with RFC in both species. High RFCs (50-75%) facilitated production of root hairs in high root orders (Fig. 3a-f) and increased AMF colonization intensity in the fourth order (Fig. 3g and h), which likely improved absorptive capacity in the high root orders. The high RFCs led to a decrease of soil nutrient content (Fig. S6; Rytter 2012), presumably driving the increases of root hairs (Fig. 3a-f) and mycorrhizal colonization (Fig. 3g and h) in higher root orders, because infertile soil conditions often promote the production of root hairs and mycorrhizas (Jungk 2001; Li et al. 2015; Freschet et al. 2018; Li et al. 2019; Han et al. 2020). Moreover, the high RFCs typically reduces root-soil contact (Bengough 2003), so that the root system might increase root-soil contact by increased root hair and mycorrhizal colonization (Jungk 2001; Jakobsen et al. 2005; van der Heijden et al. 2015). Cortical thickness in fine roots is an important aspect of nutrient uptake (Guo et al. 2008; Freschet et al. 2020). Cortical thickness in fourth-order roots of both species increased under high RFCs (50% or 75%) (Fig. 2e and f). A thicker cortex can provide greater space for mycorrhizal colonization Brundrett 2002; Comas et al. 2012; Kong et al. 2016), and also

\[
\text{AMF colonization rate} = \frac{\sum (\text{colonization length of root segment 1} / \text{length of root segment 1} + \text{colonization length of root segment 2} / \text{length of root segment 2} + \text{colonization length of root segment 3} / \text{length of root segment 3} + \ldots \ldots + \text{colonization length of root segment 30} / \text{length of root segment 30})}{\text{sum length of 30 roots segment}}
\]
facilitate higher AMF colonization intensity (Fig. 5), which is consistent with the higher AMF colonization intensity in fourth-order roots at high FRC (Fig. 3). Freschet et al. (2020) suggested that plants increase number of cortical cells to compensate for environmental stress. However, we found that cortical cell density at 75% RFC was greater at lower root orders than higher root orders (Fig. 2g and h).
Trade-offs among root components along rock fragment gradients

Another important result of this study is that root length of each root order was negatively related to root hair density and AMF colonization intensity (Fig. 5 and S2), supporting our third hypothesis that a trade-off exists between root length and root hair and mycorrhizal colonization across root orders and the RFC gradients. This finding demonstrated a coordinated response among root traits, similar to previous studies which found a negatively relationship between specific root length and mycorrhizal colonization intensity (Jakobsen et al. 2005; Freschet et al. 2021).

Variation in the RHA revealed shifts between root hairs and mycorrhizal colonization along the RFC gradients (Fig. 4; Table 1). We found that RHA was maximum in high RFCs (50% or 75%), suggesting the two species tend to increase root hairs more than mycorrhizas to cope with resource limitation under high RFCs soil in the arid valley. The thinner roots might result in steep increases in number of root hair with increased RFC (Figs. 5 and 6). Jakobsen et al. (2005) also found that mycorrhizal colonization intensity and root hair abundance can act as alternative pathways of nutrient acquisition among different non-woody plant genotypes. Plants may encourage root hair development instead of mycorrhizal fungi where soil contact and resources are limited (Jungk 2001; Jakobsen et al. 2005; Lambers et al. 2008; Freschet et al. 2021). The arid conditions may play an important role in soil properties and fine-root traits in our study. Increased abundance of root hairs in the high RFCs was associated with decreasing soil water content (Fig. 6 and S6; Hu et al. 2021). Root hairs can reduce the sharp drop in soil matrix potential adjacent to the root epidermis and root shrinkage, thus mitigating steep reductions in hydraulic conductivity caused by drought stress at high RFC (Carminati et al. 2017). In addition, root hairs are more advantageous than mycorrhizas in P-impoverished soils of this study (Fig. 4, S6 and Table 1) and others (Lambers...
et al. 2008, 2010, 2015; Teste et al. 2016). A possibility is high RFC soils are not conducive to mycorrhizal colonization for the following reasons. One is limited soil organic matter and nutrient (N and P) contents (Fig. S6; Rytter 2012; Huang 2021) limiting microbial growth and abundance (Certini et al. 2004; Yoshitake et al. 2018; Huang 2021). Secondly, higher costs to maintain the association with mycorrhizal fungi in less fertile soils may not compensate with sufficient nutrient return (Teste et al. 2016). Thus, insufficient P in impoverished soil for mycorrhizal hyphae may not be an effective plant strategy (Lambers et al. 2015).

We also observed interspecific differences between A. vestita and B. brachycarpa (Fig. S2). We found that branching intensity of B. brachycarpa was generally higher than that of A. vestita (Fig. 1). As root branching intensity strongly affects the overall number of roots and their distribution in the soil profile (Freschet et al. 2020), B. brachycarpa might have more roots and deeper root distribution than A. vestita (Hu et al. 2021; Yang et al. 2021). Root trait synergies of B. brachycarpa support the point of Stavros and Matthias (2014) that plants with a highly branched root system might respond strongly to their AM associates (Fig. 4; Table 1). B. brachycarpa also had finer root system than A. vestita (Fig. 2 and S2). Thinner, more branched and a deeper root system should provide B. brachycarpa to access to nutrients in resource-limited environment (50-75% RFC), because finer root was a prime strategy to increase the soil–root exchange surface at a lower cost (Pagès and Kervella 2018; Lu et al. 2022). Thus, B. brachycarpa could explore a larger soil volume in infertile soils via mycorrhizal fungi (Joner et al. 1995; Jakobsen et al. 2005) and more efficiently exploit local nutrient patches by high branching intensity and thin root (Freschet et al. 2021; Lu et al. 2022).

A. vestita tended to form abundant root hairs to absorb nutrients (Fig. 4; Table 1). The greater root hair length and density of A. vestita should facilitate root-soil contact and acquire less mobile or spatially isolated soil resources (Jungk 2001; Jakobsen et al. 2005; Freschet et al. 2020), which might be a complementary function of shallow and low branching roots. Compared with mycorrhizal hyphae, root hairs can rapidly obtain nutrients from the surrounding soil and effectively convert to satisfy the fast-growth of A. vestita (Jakobsen et al. 2005; Hu et al. 2021), but it might cause nutrient deficiency in the root hair zone. Root hair density and cortical thickness of low order roots in A. vestita increased more significantly with the increase of RFC (Figs. 2 and 3), which indicated that this species could adjust its absorptive capacity more quickly to cope with the change in soil resources availability. A. vestita with thick roots and low degrees of branching was considered as a resource-conserving species (Pierick et al. 2020). However, dense root hairs on the first- to fourth-order roots greatly increase its resource acquisition capacity, providing sufficient nutrients to meet the need of rapid plant growth. Therefore, root hairs may be an indispensable strategy associated with root economic spectrum, but this needs to be verified by further research.

**Conclusions**

Root architecture, morphology, root hair and mycorrhizal development and root anatomy all varied significantly with increasing RFC and associated with reduced nutrient content in soil. In support of our first hypothesis, both species increased the absorptive potential of third- or fourth-order roots by producing root hairs and increasing mycorrhiza to alleviate the environmental pressure caused by high RFC (50% and 75%). The length and number of root hair decreased with increasing root order, which was consistent with the second hypothesis. Our results supported the positive correlation of cortical thickness with mycorrhizal colonization, and found that denser cortical cells were also beneficial to mycorrhiza infection. A trade-off existed between root traits and mycorrhizas with changes in RFC, leading support our third hypothesis. Species differed in the way they coped with increasing RFC. Abundant root hairs favored rapid nutrient and water uptake by A. vestita to support fast growth. By contrast, B. brachycarpa developed a larger branching and deeper root system and tended to form mycorrhizas, contributing to its success in coping with the environmental pressures caused by increased RFC. The similarities and key
differences in resource foraging strategies and functional differentiation identified provide a more mechanistic understanding of how arid-land plant respond to rocky soils.

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