Rock Fragment Content in Soils Shift Root Foraging Behavior in Xerophytic Species

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Research Article

Keywords: Root hair, mycorrhizal colonization, fine-root functional traits, root branching order, gravel content, dry ecosystem

Posted Date: December 16th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1165085/v1

Abstract

Aims Root traits associated with resource foraging, including fine-root branching intensity, root hair and mycorrhiza, may change in soils with various physical structure indicated by rock fragment content (RFC), while how these traits covariate 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 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 3^{rd}- and 4^{th}-order roots at high RFCs (50% or 75%). 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. 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 subshrub and shrub species took different approaches to foraging in the rocky soil through distinctive trait syndromes of fine-root components.

Introduction

Fine-root traits are key determinations of plant and ecosystem functioning, congregating several components such as fine-root length, root hair length and density and mycorrhizal colonization. Collectively, these fine-root traits help determine the absorption 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 aspects of fine-root traits with different soil conditions should provide insight into how plants to cope with environmental gradients (Hodge, 2004; Almeda 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), these coarse partials are widespread in soil around world and play important roles in soil properties (Poesen and Lavee, 1994; Qin et al., 2015; Gargiulo et al., 2016, Zhang et al., 2016). Rock fragment content (RFC) can

affect other physiochemical parameters such as soil bulk density and porosity, water and nutrient availability (Poesen and Lavee, 1994; van Wesemael et al., 2000; Rytter, 2012; Gargiulo et al., 2016; Zhang et al., 2016; Ceacero et al., 2020) and microbial composition (Certini et al., 2004; Hong et al., 2021), which can manipulate performance of roots vertical distribution and plant growth (Qin et al., 2015; Mi et al., 2016; Hu et al., 2021). Therefore, we expect fine-root foraging behavior linked with root branching intensity, root morphology, root hair and mycorrhizal traits and their syndromes could be influence by changes in RFC.

The analysis of fine-root function by branching order has shown considerable promise for better understanding the function of the absorptive root system (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 between species (Comas and Eissenstat, 2009; Kong et al., 2014; Pagès and Kervella, 2018; Freschet et al., 2021). For example, root branching intensity negatively correlated with soil depth, but positively correlated with soil water content under heterogeneous soils (Wang et al., 2021). In addition to root branching intensity, fine-root structures and functions differentiated markedly with root orders. Typically, the most distal root tips (e.g., first- and second-order roots) are thinner with thicker cortex, higher nitrogen (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), yet 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; Jakobson 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 root exudation constitute other important pathways in foraging, particularly for immobile nutrients like P (Jungk 2001; Jakobsen et al., 2005; Lambers et al., 2017). 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), 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 typically positively related with 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 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, native to arid valleys of the Hengduan Mountain region, were chosen due to their ecological importance and divergent growth forms: *Artemisia vestita*, a fast-growing, tufted subshrub (up to 1.8 m maximum height) with shallow root system, and *Bauhinia brachycarpa*, a slow-growing, dwarf and highly-branched shrub (up to 1.5 m height) with deep rooting profile (Hu et al., 2021). 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 architecture, root hair, mycorrhizas and anatomy 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 and different species. We hypothesize that: 1) root hair density and mycorrhizal colonization decreases with increases in branching order; 2) root hairs and mycorrhizas will be promoted with increasing RFC; and 3) trade-offs exist between root length, root hairs and mycorrhiza along root orders and the RFC gradients.

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 m 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 subshrub and shrub species. As a result of the rocky soil, water stress, and infertile environment 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 ℃. Rock fragment content 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 during the two

years prior to the start of this study. Soils are classified as Cinnamon (Bao et al., 2012), with a stony loam texture 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}). Typically, *A. vestita* has shallow root development and is distributed along gentle slopes with relatively few rock fragments in soil. In contrast, *B. brachycarpa* typically has deeper roots and generally grows on steep slopes with high RFC (Hu et al., 2021). Each treatment had 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.

To obtain desired RFCs, fine soil particles and rock fragments (10-20 mm 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 soils through a 2 mm sieve, air-drying the soils for a week, 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 phyllite) which is commonly found in the regional soil 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 a sufficient amount of material. After crushing, the rock materials were first passed through a 10 mm sieve and then through a 20 mm sieve to obtain rock fragments with a diameter of 10-20 mm. The schist rock 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 S 1). 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) was immediately put on ice, transported to laboratory within 4 hours 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, total root length and root length of each branch order. 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):

Root branching intensity (No. cm^{-1}) = Total number of root tips (No.) / Total 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 ℃, 120 W, 45 kHz) for 10 minutes, 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 (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:

Root hair density (No. mm^{-2}) = Number of root hairs (No.) / Root section surface area (mm^2)

Root hair surface area (mm^2) = Root hair average surface area (mm^2, πdh) ´ 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 at 90 ℃ for 20-40 min (treatment time varied with different plants and root orders), acidified in HCl at room temperature for 1 min and stained with acid fuchsin at 90 ℃ 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).

AMF colonization intensity (%) = ∑ (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) / 30 ×100%

AMF colonization rate = ∑ (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) / 30

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

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 Safranin-Fast Green (2%). Root samples were then dehydrated 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 structure 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 cortical 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 cm^3) collected from each plot. Soil samples were collected to a depth of 20 cm in each plot. Soil moisture content (SMC) was measured by oven-drying fresh subsamples at 105 ℃ until the weight became constant. Remaining soil portions 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) soil-water suspension (SevenEasyS20, Mettler Toledo, USA). TC and TN were determined by combustion

in an Elemental Analyser (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

Three-way analysis of variance (ANOVA) was used to examine the effects of RFC, plant species, root order and their interactions on root traits. Principal component analysis (PCA) was performed using the root branching intensity and average values of fine-root traits on five root orders to obtain an overview of the multidimensional fine-root functions of each species at different gravel levels. 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, $P < 0.05$); otherwise, data were analyzed by a non-parametric test (Kruskal-Wallis). 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 ($Y = X^{0.5}$). Data were analyzed using SPSS 25.0 (International Business Machines Corporation (IBM, USA). PCA was implemented through the “vegan” package of R software (version 4.0.4; R Core Team, Austria). Linear fit graph and histogram were generated using Origin 2018 (OriginLab Corporation, 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).

Results

Root branching

Root branching intensity, root length and root diameter of both species significantly changed with variation in RFC ($P < 0.01$, Table S2), but for some traits, patterns changed differently between species (Fig. 1 and 2). Root branching intensity in both two species were highest in rock-free soil, and decreased in rocky soil (Fig. 1a and b). Additionally, total root length of two species did not change significantly with an increase in RFC (Fig. 1c and d). For both species, root length and diameter of lower orders (first two or three order) were not significantly different with variation in RFC, while higher orders had the largest root length in the 25% or 50% RFC and had thicker roots in the rock-free soil (Fig. 2). Across root orders, *B. brachycarpa* had higher root branching intensity and thinner roots than *A. vestita* (Fig. 1-2 and S2). Root length and root diameter of the two species varied significantly between 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).

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). Under 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 that in *B. brachycarpa* at all levels of RFC (Fig. 3; Table S2).

AMF colonization intensity of both species differed by RFC ($P < 0.001$), and significant interspecific variation was also found ($P < 0.001$, Table 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 mycorrhizas than *A. vestita* (Fig. 3. g and h), and the two species showed opposite trends in mycorrhizal colonization of the first three root orders with increasing RFC (Fig. 3. g and h).

Root hair traits and mycorrhizal colonization 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 thickness and cortical cell density were influenced by RFC ($P < 0.001$), and plant species ($P < 0.01$, Table S2). The cortex of most root orders thickened in high RFC (50% or 75%), except for the first-order roots of *A. vestita* (Fig. 4a and b). 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. 4c and d). The cortex of the fifth order disappeared in *B. brachycarpa*; however, it still existed in *A. vestita* (Fig. 4).

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. 4 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. 4 and S5).

Relationships among root traits

In both species, the ratio of root hairs area per unit root area to AMF colonization rate (RHA) changed with RFC ($P < 0.001$), and interspecific variation was also significant ($P < 0.001$, Table S2). RHA of *A. vestita* was maximum in first- to fourth-order roots at 75% RFC, indicating that the relative increased in root hairs was more dramatic than that of mycorrhizas (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 in *A. vestita* was much higher than that of *B. brachycarpa* (Fig. 5; Table 1). In this case, root hair abundance of *A. vestita* was much larger than mycorrhiza (RHA > 1, Fig. 5a), while mycorrhizas magnitude of *B. brachycarpa* was relatively larger than that of root hairs (RHA < 1, Fig. 5b).

Table 1
The ratio of root hair area per unit root area to AMF colonization rate (RHA) of two xerophytic species variated among rock fragment content (RFC). (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 (mm^2) / Root section surface area (mm^2). AMF colonization rate = $\sum$ (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) / 30. 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.

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

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. 6). However, we did not find significant correlations of root hair traits with cortical cell density in *B. brachycarpa* (Fig. 6).

Root branching intensity and root diameter, length, root hair, mycorrhizal colonization and cortical traits (means of five root orders) variation was assessed using RDA analysis with RFC and soil properties together as potential explanatory variables (Fig. 7). 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. 7a), while mycorrhizal colonization in *B. brachycarpa* was larger at high RFC (Fig. 7b). 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, root hair density and mycorrhizal colonization intensity decreased with increasing root order, but at high RFC (50% or 75%) the decrease was much reduced. In supporting our third hypothesis, root length was negatively correlated with root hair and mycorrhiza, 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 of water and nutrient availability (Fitter and Stickland, 1991; Kong et al., 2014; Liese et al., 2017; Freschet et al., 2018). We found that root branching intensity of both species had greatest values in rock-free soil (Fig. 1). Soil water and nutrient content decreased significantly with RFC (Fig. S6) and was closely related with the decrease of root branching intensity in rocky soil (Fig. 7). This finding is consistent with observations in temperate forest where root branching intensity was lower in the water reduction treatment (Wang et al., 2021). For both species, root length of the fourth- and fifth-order roots were greater under 25% or 50% RFC, whereas root length of the first two or three orders did not differ significantly between different RFCs (Fig. 2 a and b). Increasing root length in high root order 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. 2 c 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 demonstrated that in such heterogeneous soil the combination of branching pattern and morphological traits (elongation or thicken) along root order was a strategy for the plant to locally adapt the root density to resource variation.

Our results were consistent with our first and second hypothesis that root hair density and mycorrhizal colonization should decrease with the increasing root order, but increase at higher RFC (Fig. 3 and S4). Importantly, the functional differentiation across the five root orders changed with RFC in both species. High RFC (50%-75%) facilitated production of root hairs in high root orders (Fig. 3 a-f) and increased AMF colonization intensity in the fourth order (Fig 3 g and h), which likely improved absorptive capacity in the high root orders. The high RFC led to the decrease of soil nutrient content (Fig. S6; Rytter 2012), presumably driving the increases of root hairs (Fig. 3 a-f) and mycorrhizal colonization (Fig. 3 g and h) in higher root orders. Because infertile soil conditions often promote root hair and mycorrhizal production (Jungk 2001; Li et al., 2015; Freschet et al., 2018; Li et al., 2019; Han et al., 2020). Moreover, the high RFC typically reduces root-soil contact (Bengough, 2003), so that the root system might increase root-soil contact by increased root hair and mycorrhizal production (Jungk 2001; Jakobson 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 at the fourth-order roots of both species increased under high RFC (50% or 75%) (Fig. 4 a and b). A thicker cortex can provide greater space for mycorrhizal colonization (Brundrett, 2002; Comas et al., 2012; Kong et al., 2016), and also facilitate higher AMF colonization intensity (Fig 6), 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. 4 c and d).

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. 6 and S2), supporting our third hypothesis that a trade-off exists between root length and root hair and mycorrhiza 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 (Jakobson et al., 2005; Freschet et al., 2021).

Variation in the RHA revealed shifts between root hairs and mycorrhizas along the RFC gradients (Fig. 5; Table 1). We found that RHA was maximum in high RFC (50% or 75%), suggesting plants tend to increase root hairs more than mycorrhizas to cope with resource limitation in high RFC soil. The thinner roots might result in steep increases in root hair density with increased RFC (Fig. 6 and 7). Jakobson et al. (2005) also found mycorrhizal colonization and root hair abundance can act as alternative pathways of nutrient acquisition among different non-woody plant genotypes. Plants may encourage root hair development at the expense of mycorrhizal fungi where soil contact and resources are limited (Jungk 2001; Jakobson et al., 2005; Lambers et al., 2017; 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 RFC was associated with decreasing soil water content (Fig. 7 and S6; Hu et al., 2021). Because root hairs can reduce the sharp decline of matric potential of the root epidermis at high RFC, which may limit hydraulic failure under drought stress (Carminati et al., 2017). Another possibility is that at high RFC, there is reduced mycorrhizal fungal inoculum, resulting in lower colonization that then promotes more root hair development. Low soil organic matter and nutrient (N and P) contents in rocky soils (Fig. S6; Rytter, 2012; Huang, 2021), may be as a key factor limiting microbial growth and abundance (Certini et al., 2004; Yoshitake et al., 2018; Huang, 2021). Therefore, we surmise that plants bias towards increasing root hairs at high RFC in arid ecosystems due to a combination of water limitation and low mycorrhizal inoculum.

Interspecific differences in root foraging behaviors

We also observed interspecific differences between *A. vestita* and *B. brachycarpa* (Fig. S2; Table S2). We found that branching intensity of *B. brachycarpa* was generally higher than that of *A. vestita* (Fig. 1). *B. brachycarpa* also had finer root system than *A. vestita* (Fig. 2 and S2). Thinner and more branched root

system should favor *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).

A. vestita tended to form abundant root hair to absorb nutrients, while *B. brachycarpa* preferred symbiosis with mycorrhiza in resource uptake (Fig 5; Table 1). The greater root hair length and density of *A. vestita* should facilitate contact of the root with soil and its reach to less mobile or spatially isolated soil resources (Jungk 2001; Jakobson et al., 2005; Freschet et al., 2020), which might be a complementary function of shallow and low branching roots. Compared with mycorrhiza, root hair can rapidly obtain nutrients from the surrounding soil and effectively convert into dry matter of plants (Jakobson et al., 2005), but it might cause nutrient deficiency in the root hair zone. In *B. brachycarpa*, more mycorrhizas in undepleted soil than root hairs (Joner et al. 1995), may contribute to nearly an order of magnitude greater resource acquisition than root hairs (Jakobson et al., 2005).

Conclusion

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 general, 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. Our results verified the positive correlation of cortical thickness with mycorrhizal colonization, and found that denser cortex cell was also beneficial to mycorrhiza infection. A trade-off existed between root traits and mycorrhizas with changes in RFC. Species different in the way they coped with increasing RFC. Abundant root hairs favored *A. vestita* the quick nutrient and water uptake and convert into dry matter for maintain its fast growth. By contrast, the *B. brachycarpa* developed a larger branching and deeper root system and tended to form mycorrhizas, contributed to its success in cope with the environmental pressures caused by increased in RFC. The similarities and key differences in resource foraging strategies and functional differentiation identified a more mechanistic understand of how arid-land plant respond to rocky soils.

Abbreviations

RFC, Rock fragment content; AMF, arbuscular mycorrhizal fungi; RHA, The ratio of root hair area per unit root area to AMF colonization rate.

Declarations

Acknowledgments This study was funded by the National Key R & D Program of China (No. 2017YFC0505105) and the Second Qinghai-Xizang Plateau Scientific Expedition and Research Program (STEP)(2019QZKK0301). DME was partially supported by USDA NIFA Federal Appropriation 635 under

Project #PEN0 4744 (Accession #1023222). We thank Dr. Jian Liu for his review of the first draft of this paper.

Authors' contributions

HH, FL and WB conceived the ideas and designed methodology; HH and LH collected and analyzed data; HH and FL wrote the manuscript; DME edited the manuscript. All authors contributed critically to the draft and gave final approval for publication.

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Figures

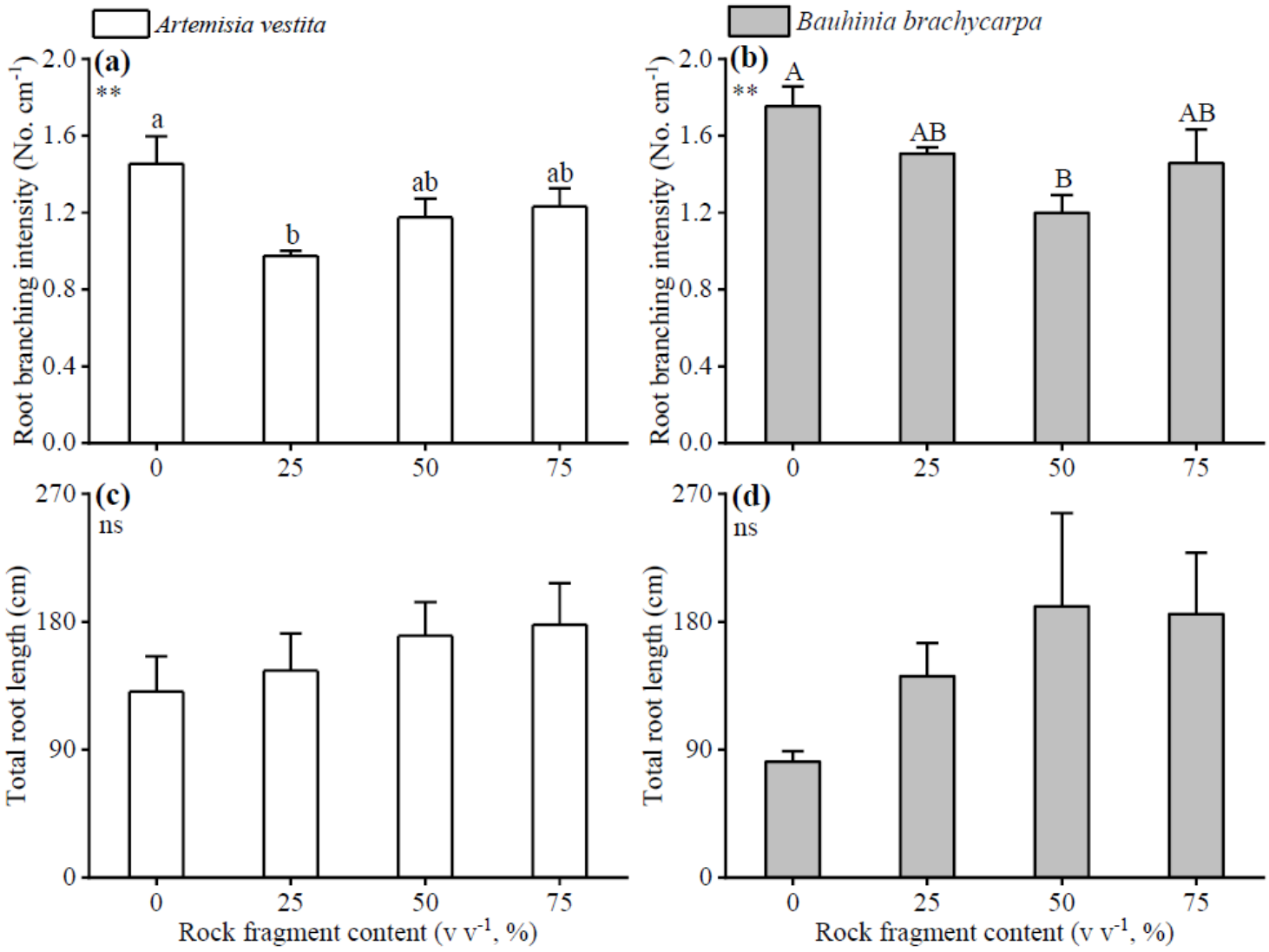

Figure 1

Root branching intensity and total 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 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, *, ** and *** designate differences at P < 0.1, P < 0.05 and P < 0.01, respectively.

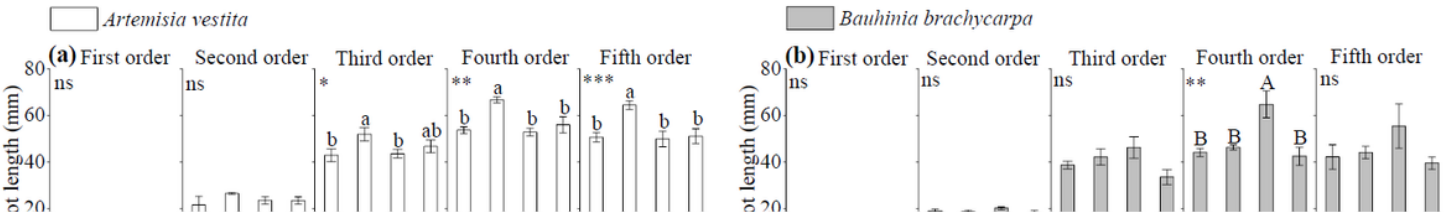

Figure 2

Root length and root diameter of each root order in A. vestita and B. brachycarpa at a range of soil rock fragment contents. Bars represent means ± SE, n = 4. Differences in means were assessed with one-way ANOVA, except differences in root length means of fourth-order in A. vestita and root length means of fourth-order and root diameter means of the third-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.

Figure 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. 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 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. AMF: Arbuscular Mycorrhiza fungi.

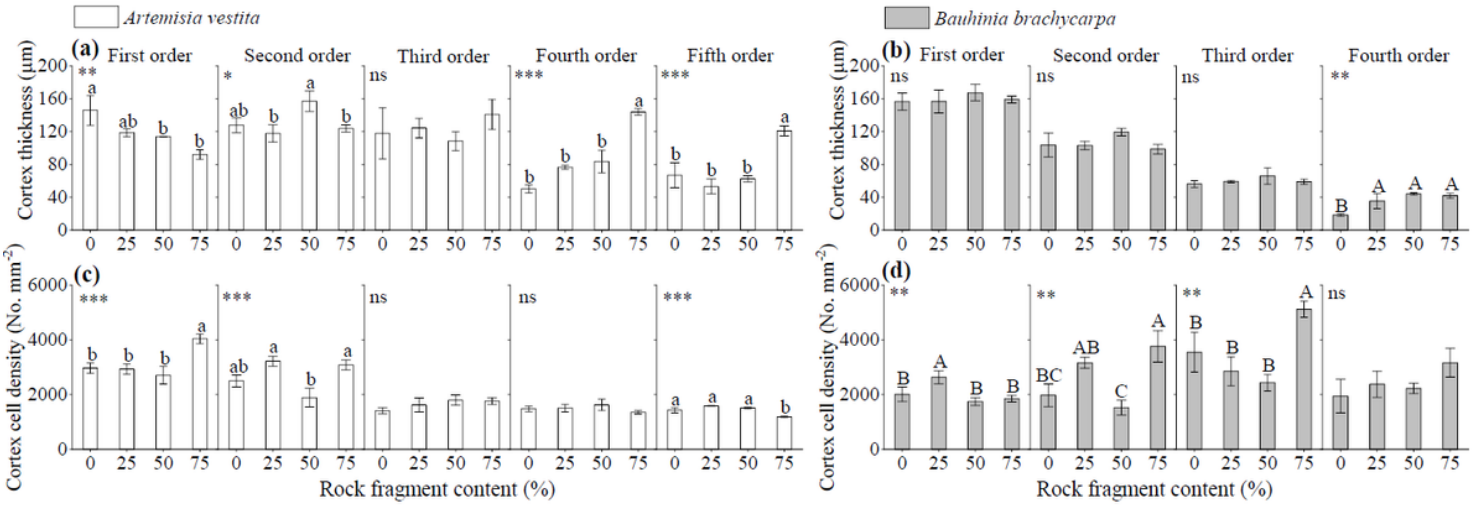

Figure 4

Root cortex thickness and root cortex cell density of each root order in A. vestita and B. brachycarpa at a range of soil rock fragment contents. Bars represent means ± SE, n = 4. Differences in means were assessed with one-way ANOVA, except differences in root cortex thickness mean of the fourth-order in A. vestita was 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.

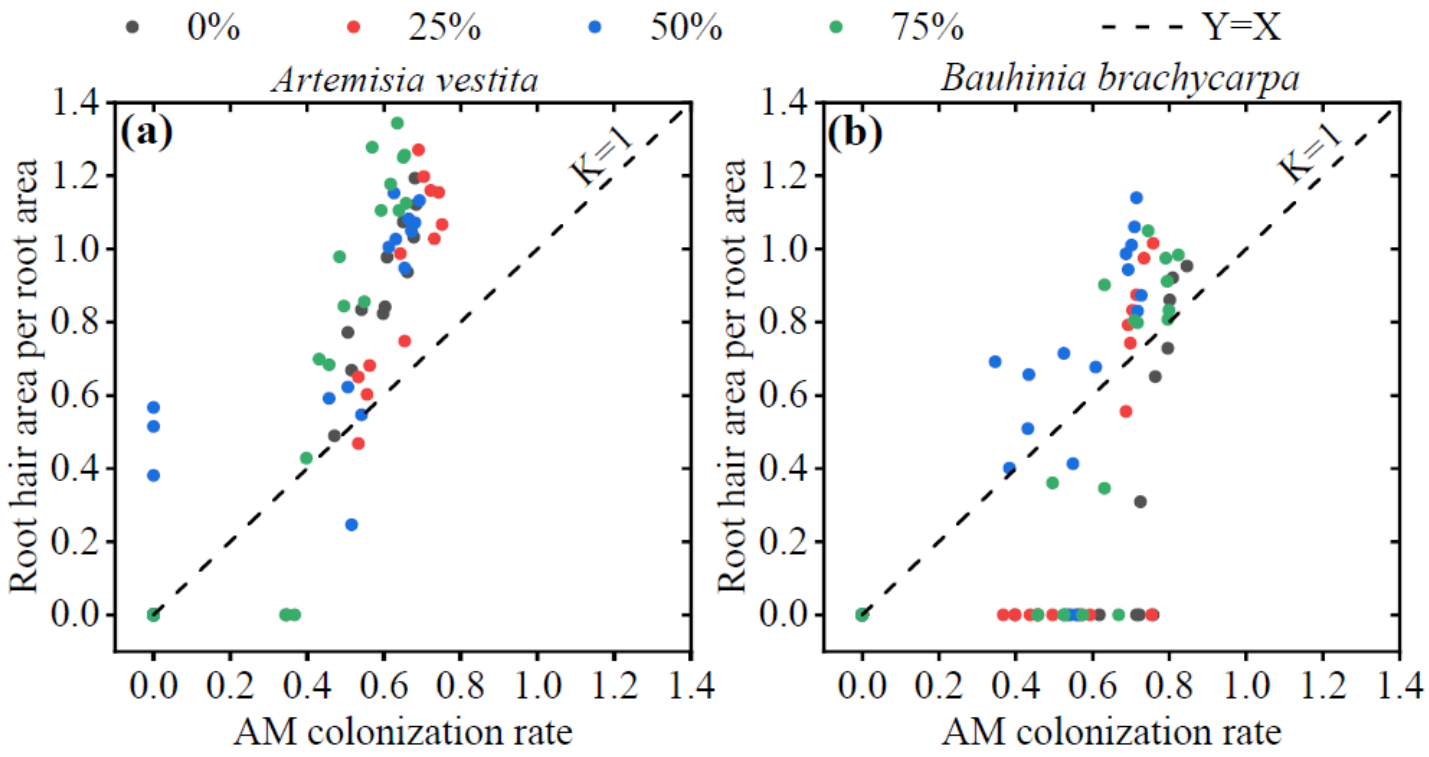

Figure 5

Root hair area per unit root area along AMF colonization rate in A. vestita (a) and B. brachycarpa (b) across rock fragment content gradient. Based on standardized data (Y=X^{0.2}). The dashed line represents the equation Y=X with a slope of 1(K = 1). AMF: Arbuscular Mycorrhiza fungi. Root hairs surface area per unit root surface = Root hair area (mm²) / Root section surface area (mm²). AMF colonization rate = ∑ (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) / 30.

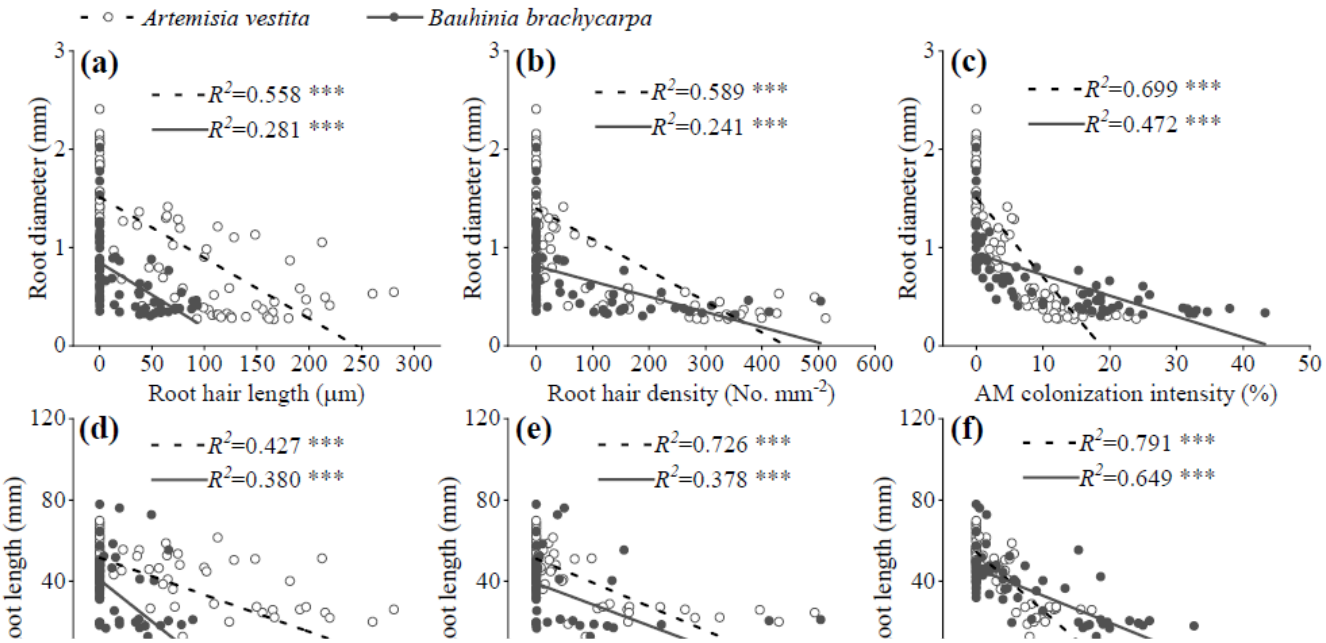

Figure 6

Relationships between root traits of A. vestita and B. brachycarpa across root orders and along rock fragment content gradient (*, ** and *** designate significant level of correlations at P < 0.05, P < 0.01 and P < 0.001, respectively)

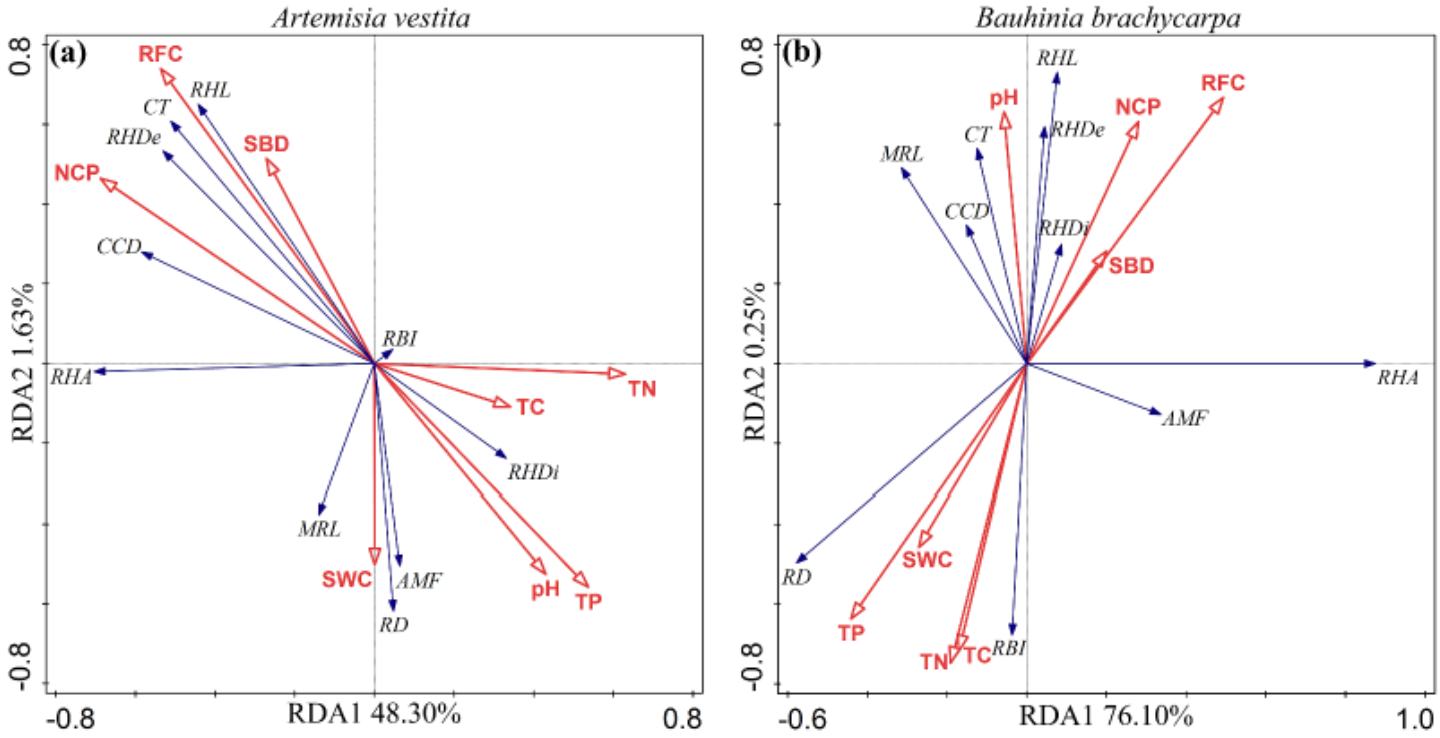

Figure 7

Redundancy analysis of fine-root traits (mean values of five root orders except RBI) and soil properties in A. vestita (a) and B. brachycarpa (b) along rock fragment content gradient. The proportion explained of Axis 1 and Axis 2 in (a) are 48.30% and 1.63%, and in (b) are 76.10% and 0.25%, respectively. Solid blue lines indicate ten fine-root functional traits. Solid red lines indicate soil properties. Abbreviations for root traits are as follow: RBI, root branching intensity; MRL, root length; RD, root diameter; RHDi, root hair diameter; RHL, root hair length; RHDe, root hair density; AMF, AMF colonization intensity; CT, cortex thickness; CCD, cortex cell density; RHA, the ratio of root hair area per root area to AMF colonization rate; SWC, soil water content; SBD, soil bulk density; NCP, non-capillary porosity; TC, total soil carbon; TN, total soil nitrogen; TP, total soil phosphorus.

Supplementary Files

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- SurpportinginformationFigS1S6andTableS1S2.docx