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# Do caliche nodules in loessial profiles affect root growth?

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## Abstract

*Background* Caliche nodules, the product of the leaching and deposition of calcium carbonate in the soil, are widely distributed in loessial profiles on the Loess Plateau in China. Their presence leads to complex interactions between plant roots and the soil.

*Aims and Methods* We studied the interactions between caliche-nodule and water content and their effects on the biomass, morphology and vertical distribution of roots of Caragana (*Caragana korshinskii* Kom.) for two years using a soil-column experiment. Four root parameters (biomass, diameter, length density and surface-area density) in various diameter classes were compared and analyzed.

*Results* Water and caliche-nodule contents significantly affected root biomass and morphology. Both coarse- and fine-root biomasses were highest at a nodule content of 30%, did not differ significantly from

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W. Yushen Colloge of Agriculture, Shanxi Agricultural University, Taigu 030801, China those in fine earth (nodule-free) but were significantly higher than at the other two nodule contents. The nodules affected fine-root biomass when water content was <60% of field capacity. Higher water content corresponded to higher biomass. Roots become thick and short in loess containing nodules. High contents of nodules (50%) negatively affected root biomass at every depth and caused larger percentage of fine roots to concentrate in the shallower layers.

*Conclusion* Our results demonstrate that plants growing in the loess containing caliche nodules show some degree of adaptability in root morphology. High nodule content is adverse to accumulation of root biomass. Caliche nodules should be given more consideration when investigating the dynamics and habits of plant growth on the Loess Plateau.

Key words Rock fragments  $\cdot$  root biomass and morphology  $\cdot$  depth distribution  $\cdot$  soil water  $\cdot$  the Loess Plateau

# Abbreviations

Four rock-	fragment	
contents:	$R_1$	fine earth (0% caliche-
		nodule content)
$R_2$		10% caliche-nodule
		content

R <sub>3</sub>	30% caliche-nodule
	content
R <sub>4</sub>	50% caliche-nodule
	content
Four water treatments:	
W <sub>1</sub>	80-100% of field capacity
W <sub>2</sub>	60-80% of field capacity
$W_3$	47-60% of field capacity
	(average water content in a
	wet year)
$W_4$	32-47% of field capacity
	(average water content in a
	dry year)
RB	root biomass
AB	aboveground biomass
R/S	root-to-shoot ratio
RD	root diameter
RLD	root length density
RSAD	root surface-area density

## Introduction

Many studies have reported that the presence of rock fragments in the soil influences plant growth and root elongation (Babalola and Lal, 1977a,b; Grewal et al., 1984; Danalatos et al., 1995; Heisner et al., 2004; Qin et al., 2015; Mi et al., 2016), because rock fragments affect the physical, chemical and biological conditions of the soil (e.g. porosity, mechanical resistance, nutrient content and composition, pH and microbial composition). Roots are organs connecting plants to soils, so they play an important role in the growth of plants. The morphology and distribution of roots in soil profiles are affected by soil properties, environmental factors and the genetic characteristics of the plants (Marshall and Waring, 1985; Gale and Grigal, 1987; Donnelly et al., 2016). Studying the effects of rock fragments on the characteristics of root systems, such as root growth and the uptake of water, is therefore important for fully understanding the habits and ecological adaptability of plant growth in stony soils, which is needed to design reasonable strategies for managing vegetation in areas where stony soils dominate.

Rock fragments affect root growth in both agricultural and forestry systems. Previous studies, however, have provided inconsistent conclusions (Table 1). Some studies have reported that the presence of rock fragments could improve the growth and development of root systems due to increases in root branching, nutrient content and populations of microbial species. For example, Jackson et al. (1972) demonstrated that blueberries (Vaccinium angustilolium Ait.) in stony soil germinated earlier and had more stems and root branches. Jongmans et al. (1997) found fungal hyphae in the pores of feldspars and hornblendes in podzol E soil horizons and granitic bedrock in European coniferous forests. The fungi exuded organic acids capable of dissolving minerals, which helped plants to use nutrients stored in the rock. Heisner et al. (2004) reported that rock fragments in the soils of the southern Black Forest in Germany had a cation exchange capacity (CEC) within the same order of magnitude as that of fine earth and that mycorrhizae and fine roots adhered to 70% of the fragments, which were conducive to the absorption of nutrients. Du et al. (2017) found that rock fragments embedded in the soil of the alpine steppe on the northern Tibetan Plateau promoted root growth, perhaps because most of the rock fragments in their study area were small (2-10 mm in diameter), providing many soil pores acting as water reservoirs for plants.

The unique thermal conditions of stony soil, with higher temperatures in high alpine ecosystems, and lower fine-earth bulk densities due to higher rockfragment contents are conductive to root penetration. The presence of rock fragments therefore optimizes the environment for root growth.

Other studies, however, have indicated that roots developed abnormally due to the higher resistance to root growth in stony soils. Roots in regions with shallow soil layers based on the bedrock (such as karst areas) are mainly concentrated in the upper layers and in the pores or cavities of the bedrock (Schwinning, 2013; Estrada-medina et al., 2013). Roots that penetrate the bedrock play an important role in plant growth. About one quarter to one third of the total root length in 12-year-old forests in southwestern Oregon was in the rocky layer, where the roots could absorb sufficient water from the rock for satisfying the needs of the plants, even though the water content of the soil above the rocky layer was below the wilting coefficient (Zwieniecki et al., 1994). The roots in this study were morphologically adapted to the cracks in the rock, and the root cortex was flattened, with "wing-like" structures on the sides of the root stelae (Zwieniecki et al., 1995). Total nutrient and water

Effects	Rock fragment	Soil	Plant	Climate	Source
No	Unrecorded type 2.5 cm thick gravel layer 3-8 mm	Clayey loam	Hybrid forage sorghum	Semiarid steppe climate	Unger (1971)
Positive	Unrecorded type 16-43.2%	Loam, sandy loam and clayey loam	Blueberries	Greenhouse	Jackson et al. (1972)
Positive	Unrecorded type Depth to gravel from 5 to 10 cm	Sandy loam	Maize	Greenhouse	Babalola and Lal (1977a)
Positive Positive Negative Negative	Unrecorded type 10% 25% 50% 75%	Sandy loam and clay	Maize	Greenhouse	Babalola and Lal (1977b)
Positive	Limestone 10-20%	Terra Rossa, clay	Apple	Mediterranean climate	Magier and Ravina (1984)
Negative	Metasedimentary rock	Clayey loam	Forests	Mediterranean climate	Zwieniecki et al. (1994;1995)
Positive Positive Positive	Feldspars Hornblendes Granitic bedrock			Temperate continental climate	Jongmans et al. (1997)
Positive Positive Positive Positive	Granite Gneiss Sandstone Glacial deposits		Forests	Montane climate	Heisner et al. (2004)
Negative	Limestone bedrock	Leptosols, loam		Tropical climate	Estrada-medina et al. (2013)
Positive	Unrecorded type 15-35%	Cryosol, Sandy soil	Grass	Cold and semi-arid plateau monsoon climate	Du et al.(2017)
No No Positive Positive Positive	Schist 25% 50% 75% 25% 50% 75%	Cinnamon, loam	Artemisia vestita Bauhinia brachycarpa	Subtropical monsoon climate	Hu et al.(2021)

 
 Table 1
 Effects of rock fragments (mainly for type and content) on root growth for different types of soil, plants and climatic conditions

contents also tend to decrease as the effective soil volume for root growth decreases (Ercoli et al., 2006; Qin et al., 2015).

Soil properties, the characteristics of the rock fragments (e.g. proportional mass or volume, size, degree of weathering, shape, lithology and position in the soil), plant species and climatic conditions can potentially influence root growth in stony soils (Table 1). Unger (1971) reported that the presence of a band of gravel in the subsoil did not greatly significantly affect root distribution of hybrid forage

sorghum (*Sorghum vulgare Pers.* × *Sorghum vulgare var. sudanense*). Babalola and Lal (1977a, b) found that root growth of maize depended on the content and position of rock fragments; the small number of rock fragments (<10%) in the clayey soil improved root growth, but the mechanical resistance to growth increased with gravel content, which led to extensive branching and the aberration of root-tip cells, and root length increased from 5 to 10 cm. Babalola and Lal (1977b) also demonstrated that roots were longer in

clayey soil with rock fragments than in sandy soil due to the better conditions of water and air, and that roots were shorter in soil with small rock fragments (4-8 and 8-15 mm in diameter) than in soil with larger rock fragments (15-40 mm). Magier and Ravina (1984) also found that the roots of fruit trees were more developed in stony soil under appropriate conditions of irrigation and fertilization; the number of roots was highest when the proportional volume of rock fragments was 10-20%. An optimal rock-fragment content within a moderate range can therefore benefit root growth. Optimal rock-fragment content, however, can vary with soil texture, fragment type and plant species. Grewal et al. (1984) demonstrated that yields of rainfed crops decreased as the rock-fragment content increased, but yields remained high for legumes with large fibrous roots when the proportional volume of rock fragments was 40%. Planting species with welldeveloped roots may therefore be more advantageous in stony soils, which could be one of the characteristics for selecting crops in stony areas. A recent study by Hu et al.(2021) demonstrated that rock fragments not only affected vertical root distribution and biomass accumulation, but also the relative allocation of biomass to roots. The R/S ratios for two shrubs (Artemisia vestita and Bauhinia brachycarpa) were highest at the highest rock-fragment content of 75%. Also, B. brachycarpa was likely better adapted to soil conditions associated with high contents of rock fragments compared with A. vestita.

Previous research has made important progress in determining the characteristics of root morphology and distribution in stony soils, which has helped us to understand the characteristics of plant growth and to clarify the relationships among plants, soils and rock fragments in stony areas. The factors of rock fragments influencing root growth, however, are complex. The effects of rock fragments on root growth are variable due to variations in the characteristics of rock fragments, soil properties and plant species among regions.

The thick loess widely distributed on the Loess Plateau of China differs from the soil in regions with shallow soil layers underlying bedrock. Caliche nodules are often present in loessial profiles due to the natural deposition and concretion of calcium carbonate. The spatial distribution of these nodules in surface soil and their effects on filtration, saturated water conductivity and soil erosion have been studied (Gong et al., 2018a,b; Ma et al., 2010; Zhou et al., 2009; Ma and Shao, 2008; Zhu and Shao, 2008). Gong et al. (2018a) reported a maximum coverage of caliche nodules in the surface soil near 20%, with  $\geq$ 65% of all nodules 10-50 mm in diameter. Filtration and saturated water conductivity generally decrease as caliche-nodule content increases (Ma et al., 2010; Zhou et al., 2009; Ma and Shao, 2008). These studies of caliche nodules in the soils of the Loess Plateau, however, did not consider plant growth. Mi et al. (2016) reported that plants were negatively affected when caliche-nodule content was 50% but provided no details on root biomass or morphology. Clarifying whether or how caliche nodules in the soil profile affect root development is necessary. More details should be described and analyzed for roots in soil containing caliche nodules, such as diameter, length and proportion of total biomass. The objectives of this study were therefore to determine the effect of caliche-nodule content on root growth, identify interactive effects of nodule and water contents and determine whether and how caliche nodules and soil depth affect root biomass and root morphology. Based on existing research results, we hypothesized that (i) the effects of caliche nodules on root growth would vary with caliche-nodule content and would be positive at moderate nodule contents and negative at high nodule contents, (ii) the effects of nodule content on root growth would be stronger under conditions of water shortages than under conditions of sufficient water, and (iii) the presence of caliche nodules would affect the patterns of allocation of biomass of plants and the vertical distribution of roots.

## Materials and methods

## Plant materials and the sampling site

*Caragana korshinskii* Kom., a shrub belonging to the legume family, was selected as the experimental plant. *C. korshinskii* has been widely planted on the Loess Plateau to improve the local ecological environment and provide feed for animals due to its high tolerance to harsh environments (especially drought) and high ecological and economic value. *C. korshinskii* roots consist of a main root and developed and multistage lateral roots.

Caliche nodules and fine earth used in the experiments were collected from the topsoil nearly 30 cm thick in the Liudaogou catchment of the northern Loess Plateau (38°46'-38°51'N, 110°21'-110°23'E; 1081.0-1273.9 m a.s.l.), approximately 14 km west of Shenmu County, Shaanxi Province, China. This area has a semiarid continental climate with a mean annual temperature of 8.6 °C, accumulated temperature  $\geq 10$  °C of 3232 °C and mean annual precipitation of 412 mm, with a range of 251.3-693.7 mm. The daily relative humidity (RH), air temperature (AT) and solar radiation (SR) in 2014 and 2015 are shown in Fig. 5 of the Appendix. The average RH, AT and SR from May to September in 2014 and 2015 were 55.6 and 50.3%, 19.2 and 19.7 °C, 20.1 and 20.6 MJ m<sup>-2</sup>, respectively. The ranges of RH, AT and SR in 2014 and 2015 were 14.5-97.3% and 14.4-97.9%, 6.8-26.3 and 9.0-27.7 °C, 2.5-31.7 and 1.7-31.3 MJ m<sup>-2</sup>, respectively.

The fine earth in this area is an aeolian loess rich in carbonates, and the pH ranges from 8.5 to 8.8. The carbonates in wet years leach downward during rain. The carbonates in dry years are deposited in the soil and form caliche nodules or even a petrocalcic horizon (Fig. 1A). The petrocalcic horizons are broken into calcareous rock fragments by the combination of climatic conditions and human activity. Processes such as uplifting and cultivation have subsequently redistributed the nodules within the upper soil profile, where they may influence plant growth and root development. Caliche nodules in the Liudaogou catchment mainly develop and form on paleosols such as Wucheng and Lishi loess due to the wet paleoclimate and abundant vegetation (Zhu and Shao, 2008).

The fine earth and caliche nodules (Fig.1B) were transported to the laboratory and air-dried. All of the fine earth was then passed through a stainless steel sieve with a 2-mm mesh. Plant residues in the fine earth were removed. The fine earth and caliche nodules were sampled and used to measure the main physical properties before the experiment was conducted. The initial water contents of the fine soil and rock fragments were 0.0060 and 0.0056 m<sup>3</sup>  $m^{-3}$ , respectively. The average sand, silt and clay contents of the fine earth were 80.3, 13.6 and 6.1%, respectively (International Society of Soil Science System). The fine earth was a sandy loam classified as a Glossisol in the WRB classification. The nodules were mainly composed of particles with diameters of 20-30 mm and were capable of storing water (Table 2).

**Fig. 1** Photographs of the loessial profile with petrocalcic horizons (**A**) and caliche nodules samples (**B**)



Petrocalcic horizons

Caliche nodules samples

	Table 2	Main	physical	properties	of the	caliche	nodules
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Size distribution (%)						Bulk density (g cm <sup>-3</sup> )	Saturated water content (m <sup>3</sup> m <sup>-3</sup> )	Shape
Particle size (mm)	2-10 2.15	10-20 6.69	20-30 52.92	30-40 11.38	>40 26.86	2.05	0.16	Ellipse or blocked

#### Soil composition and treatments

A soil-column experiment was conducted under natural conditions at the Shenmu Erosion and Environmental Research Station of the Institute of Soil and Water Conservation, Chinese Academy of Sciences. The station is in the Liudaogou catchment. We reconstituted soils with fine earth (particle diameter <2mm) mixed with different gravimetric proportions of calichenodules of 10 ( $R_2$ ), 30 ( $R_3$ ) and 50% ( $R_4$ ), representing low, medium and high rock-fragment contents, respectively. The fine earth (no nodules,  $R_1$ ) was used as a control. The soils were then carefully mixed by hand, with air-dried fine earth and nodules placed in PVC columns (100 cm high and 20 cm in diameter). The bottoms of the columns were covered with corrosion-resistant plates. The small gap between the column wall and the corrosion-resistant plate was sealed with silicon sealant.

The amounts of nodules and fine earth necessary to fill the columns for each treatment were based on the average bulk density (2.05 g  $\text{cm}^{-3}$ ) and proportion of the nodules and on the bulk density of the fine earth (fixed at 1.33 g cm<sup>-3</sup>). The average bulk density for treatments R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> was 1.35, 1.42 and 1.45 g cm<sup>-3</sup>, respectively. Nodules and fine earth were added to the columns from the bottom in layers (10 cm each). During the filling of the columns, a tube (2 mm ID) 50 cm in length was buried in each column to provide irrigation water during the stage of controlling water. The tube was buried in the soil at a depth of about 35 cm. The surfaces were covered with 2 cm of vermiculite to minimize evaporation. We ignored surface evaporation, so the loss of water was attributed to absorption by roots. Twelve replicate columns were prepared for each rock-fragment treatment, for a total of 48 columns. All columns were exposed to the environment except during rains, when a plastic shelter was placed above the columns to prevent rainwater from entering the columns.

Four water treatments were established for each treatment of rock-fragment content: 80-100% ( $W_1$ ), 60-80% ( $W_2$ ), 47-60% ( $W_3$ , average water content in a wet year) and 32-47% ( $W_4$ , average water content in a dry year) of field capacity. Each treatment had three replicates. All columns were randomly distributed among the four treatments of nodule content. The corresponding volumetric water contents of the fine earth for  $W_1$ ,  $W_2$ ,  $W_3$  and  $W_4$  were 15-19, 11-15, 9-11 and

6-9%, respectively. The gravimetric water content of the fine earth ( $\theta_{mfe}$ , g g<sup>-1</sup>) was calculated as the volumetric water content divided by the bulk density. The gravimetric water content of the rock fragments ( $\theta_{mrf}$ , g g<sup>-1</sup>) was calculated using the empirical equation under stable conditions:

$$\theta_{mrf} = a \left( 1 - e^{-b\theta_{mfe}} \right) \tag{1}$$

where a and b are empirical parameters. The values of a and b were obtained by measuring a series of fineearth and rock-fragment water contents under equilibrium conditions. a and b in our study were 0.082 and 14.429, respectively (N=35, R=0.88, SE=0.012; N is the number of samples, R is the correlation coefficient for the fit and SE is the standard error of the estimation).

The gravimetric water content of the stony soil was determined by:

$$\theta_{mT} = R_m \theta_{mrf} + (1 - R_m) \theta_{mfe} \tag{2}$$

where  $\theta_{mT}$  (g g<sup>-1</sup>),  $\theta_{mfe}$  and  $\theta_{mrf}$  are the water contents of the stony soil, fine earth and rock fragments, respectively, and  $R_m$  is the gravimetric rock-fragment content. The gravimetric water content of each treatment obtained using equation (2) is presented in Table 3.

The filled columns were left to subside naturally for two weeks. The height of the soil core in the PVC columns was 90 cm. The depth of subsidence was in the range of 0-5 cm. The first irrigation was performed from the surface of the columns to attain a water content of the soil columns of 50% of field capacity. The columns were sealed with plastic wrap and left for a week to balance the water. C. korshinskii seeds were sown in the columns in August 2012. Seedlings were thinned to two plants per column when the plants were about 10 cm tall. Before controlling water, water was added into the columns at regular intervals from the surface to attain a water content of 50% of field capacity to keep the plants alive and provide for healthy development. Water was controlled during the growing seasons (May to September) in 2014 and 2015. Water was added to the columns through the irrigation tube at the stage of controlling water.

Water content was controlled by weighing the columns regularly (2-4 d) using an electronic balance (1 g) and adding water to the columns through

Table 3 Treatments tested in the soil-column experiment

Rock-fragment treatment		Wate	Water treatment					
	Caliche-nod- ule content (%)		Calculated range of water content (%)	Actual average water content (%)				
R <sub>1</sub>	0	W <sub>1</sub>	11.3-14.3	12.5±0.11				
		$W_2$	8.3-11.3	9.7 <u>±</u> 0.08				
		$W_3$	6.8-8.3	7.3±0.06				
		$W_4$	4.5-6.8	6.1±0.03				
$R_2$	10	$W_1$	10.8-13.6	12.0±0.12				
		$W_2$	8.0-10.8	9.5±0.10				
		$W_3$	6.6-8.0	7.2 <u>±</u> 0.06				
		$W_4$	4.4-6.6	6.0 <u>±</u> 0.03				
$R_3$	30	$W_1$	9.9-12.2	11.0 <u>±</u> 0.09				
		$W_2$	7.5-9.9	8.7 <u>±</u> 0.06				
		$W_3$	6.2-7.5	6.8 <u>±</u> 0.04				
		$W_4$	4.3-6.2	5.8 <u>±</u> 0.03				
$R_4$	50	$W_1$	8.9-10.8	9.5 <u>±</u> 0.07				
		$W_2$	7.0-8.9	8.0 <u>±</u> 0.03				
		$W_3$	5.9-7.0	$6.5 \pm 0.05$				
		$W_4$	4.2-5.9	$5.5 \pm 0.02$				

the water supply tube. The amount of water added to the columns after each weighing was determined by subtracting the residual amount of water in the columns from the upper limit of water amount in each water treatment. The changes in plant weight over the 2-4 d were ignored due to the low proportion of total water loss. The weight of the water in the column was calculated by subtracting the weight of an empty column, the fresh weight of the plant, the weights of the water supply tube and vermiculite layer and the dry weight of the reconstituted soils from the total weight. The fresh weight of the plant varied over time so was estimated using the doublefactor allometric equation (H, cm; D, mm) (Sileshi 2014)  $Ln(B) = a * Ln(D^2 * H) + b$ , where a and b are empirical coefficients and H, D and B (g) are the height, basal diameter and dry weight of the aboveground biomass (75 °C) for a single plant. a and b were obtained by measuring H, D and B for 106 plants in the study area. The ranges of H and D were 12-167 cm and 2.25-9.94 mm, respectively. The best curve fit was obtained at a=0.94 and =4.79, where  $R^2$ =0.98 and SE=0.20. The average water content during the study is presented in Table 3. The actual water content of all treatments was controlled within the calculated range, as expected.

#### Plant measurements and root analyses

Leaves and branches were collected at the end of September 2015. Each column was then carefully cut longitudinally to obtain an intact soil core with roots. The core was then cut transversely every 10 cm measured from the soil surface, and the sections were packed into numbered plastic bags. Nine samples with roots were collected from each column. We manually removed most roots from the fine earth and rock fragments from the stony soils. The residual samples of fine earth were then wet-sieved through a 0.3-mm mesh screen to obtain the residual fine roots. Fine roots adhering to the nodules were also manually removed. Roots in the nodules (if present) were also removed after destroying the nodules. All roots were washed to remove soil particles.

Root biomass (RB, g), diameter (RD, mm), length density (RLD, cm cm<sup>-3</sup>) and surface-area density (RSAD, cm<sup>2</sup> cm<sup>-3</sup>) were obtained by measuring and scanning the root samples. Individual root samples were separated into coarse roots (>2 mm) and fine roots ( $\leq 2$  mm) and further dissected to facilitate scanning. Root length (RL) of the coarse roots was measured with a ruler to a precision of 1 mm. Coarse-root RD was measured with a digital caliper to a precision of 0.01 mm. Fine roots were further divided into four diameter categories (RD<sub>1</sub>: 0-0.5, RD<sub>2</sub>: 0.5-1.0, RD<sub>3</sub>: 1.0-1.5 and RD<sub>4</sub>: 1.5-2 mm) for a more detailed analysis of root morphology. All fine-root samples were placed on transparent Perspex plates, digitized at 300 dpi and measured using WinRHIZO Pro 2013d image-analysis software (Régent Instruments Inc., Quebec City, Canada) in conjunction with an Expression XL1000 flatbed scanner (Seiko Epson Corporation, Suwa, Japan) for determining RD, RLD and RSAD. Finally, the root samples were oven-dried (80 °C, 24 h), and root biomass was determined.

### Data analysis

The root parameters (the means of the two plants in each PVC column) were compared among the rockfragment treatments and water treatments using twoway ANOVAs. Pairwise multiple comparison tests of the least significant difference (LSD) were carried out to identify differences between treatments. The data were examined to ascertain whether the variables were normally distributed, and the variances were homogeneous. The multi-way ANOVAs were carried out using IBM SPSS Statistics 25 for Windows (IBM Inc., Stanford, USA). Differences at the 0.05 level were considered significant. Graphs were plotted using Origin 2018C software (OriginLab, Northampton, USA).

The root extinction coefficient ( $\beta$ ) was used to analyze the relationship between the vertical distribution of the roots and soil depth using the vertical-distribution model of roots proposed by Gale and Grigal (1987):

$$Y = 1 - \beta^d \tag{3}$$

where Y represents the ratio of the number of roots in each transverse soil section to the total number of roots, and d represents the depth of the section. The value of  $\beta$  was obtained by simulating the vertical distribution of the root system for each treatment. A larger  $\beta$  indicates a larger percentage of the root system in the deeper sections, and a smaller  $\beta$  indicates that more of the root system is concentrated in the sections near the surface. The value of  $\beta$  is independent of root volume and density. The simulation was performed using Origin 2018C software. The fit of the vertical-distribution curves was always significant and quite good ( $R^2$ =0.892-0.998).

## Results

### Root-growth response

In the nodule treatments, the main roots bypassed the nodules and continued to grow in sub layers. The roots were twisted and bent in the stony soils, especially in the treatments with high nodule contents. Roots rarely grew into the nodules. Most roots were distributed in the fine earth and grew around the nodules. The roots were dense in the concave side of the fine earth when nodules were removed from the cross-section of the soil core. A small amount of fine roots were also attached to the surface of the nodules.

RB, RLD and RSAD in each diameter category (coarse roots and four categories of fine-root diameters, 0-0.5, 0.5-1.0, 1.0-1.5 and 1.5-2.0 mm) were

all significantly affected by nodule and water content (Tables 4 and 5). The diameter of coarse roots also differed significantly among the water contents but not the nodule contents. In contrast, the diameter of fine roots differed significantly among the nodule contents but not the water contents. These results also indicated significant interactions between the nodule and water contents for fine-root biomass. The higher *F* and lower *p* for roots with smaller diameters indicated that nodule content affected the absolute values and proportions of thin roots more than thick roots (Tables 4 and 5). The impacts on RB, RLD and RSAD were weaker for nodule content (*F*=3.714-0.021, *p*=0.000) than water content (*F*=16.417-43.192, *p*=0.000).

#### Root biomass and R/S ratios

RB was highest in  $R_3$  (36.6±3.99 g), followed by  $R_1$ (33.5±9.68 g),  $R_2$  (30.3±3.41 g) and  $R_4$  (20.9±3.26 g). RB differed significantly between  $R_1$  and  $R_4$ ,  $R_3$ and  $R_4$  and  $R_2$  and  $R_3$ . Variations in coarse-root biomass with nodule content were similar to the variations in total RB (Fig. 2A). Effects of nodule content on fine-root biomass varied with water content. In  $W_1$ , none of the four nodule treatments affected fine-root biomass. In  $W_2$ , fine-root biomass was significantly higher in  $R_3$  than  $R_2$  and  $R_4$  but did not differ significantly between  $R_3$  and  $R_1$ . In  $W_3$  and  $W_4$ , fine-root biomass was significantly lower in  $R_4$  than the other nodule treatments. High water content corresponded to high RB for both coarse and fine roots.

AB was also significantly affected by nodule and water contents (Table 4 and Fig. 2B). AB differed significantly between  $R_1$  and  $R_4$ ,  $R_3$  and  $R_4$ . High water content corresponded to high AB. The R/S ratio did not vary with nodule or water content (Table 4 and Fig. 2C), indicating that the patterns of biomass allocation did not vary with the presence of caliche nodules and water stress.

#### Parameters of root morphology

Total RLD for  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  was 0.526±0.071, 0.341±0.051, 0.432±0.033 and 0.322±0.045 cm cm<sup>-3</sup>, respectively. Total RASD for  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  was 0.110±0.015, 0.082±0.007, 0.101±0.011 and 0.070±0.010 cm<sup>2</sup> cm<sup>-3</sup>, respectively. Increasing nodule content from 0 to 10, 30 and 50%, decreased total

**Table 4** Effects of caliche-<br/>nodule and water contents<br/>on the root parameters

Root parameter		Caliche content	-nodule	Water of	ontent	Water content × caliche-nod- ule content	
		F	р	F	р	F	р
RB	Total	11.85	0.000	38.96	0.000	0.760	0.653
	Coarse roots (RD≥2 mm)	12.93	0.000	30.98	0.000	1.051	0.424
	Fine roots (RD<2 mm)	3.868	0.018	34.36	0.000	2.636	0.021
AB		13.01	0.000	69.56	0.000	1.768	0.114
R/S ratio		1.454	.0.246	1.249	0.198	1.883	0.091
RD	Mean	0.382	0.767	0.738	0.537	1.504	0.189
	Coarse roots (RD≥2 mm)	0.936	0.435	2.742	0.039	1.180	0.340
	Fine roots (RD<2 mm)	9.756	0.000	0.458	0.713	0.411	0.920
RLD	Total	7.963	0.000	22.36	0.000	1.208	0.324
KLD	Coarse roots (RD≥2 mm)	7.939	0.000	43.19	0.000	1.555	0.172
	Fine roots (RD<2 mm)	8.234	0.000	21.92	0.000	1.198	0.330
	(1.5≤RD<2 mm)	4.345	0.011	22.77	0.000	0.932	0.511
	(1≤RD<1.5 mm)	6.116	0.002	25.52	0.000	0.987	0.470
	(0.5≤RD<1 mm)	7.596	0.001	27.22	0.000	1.272	0.290
	(0≤RD<0.5 mm)	8.787	0.000	16.42	0.000	1.263	0.294
RASD	Total	7.097	0.001	26.36	0.000	1.370	0.242
	Coarse roots (RD≥2 mm)	9.531	0.000	38.68	0.000	0.849	0.578
	Fine roots (RD<2 mm)	6.620	0.001	23.75	0.000	1.361	0.246
	(1.5≤RD<2 mm)	3.714	0.021	18.18	0.000	1.208	0.324
	(1≤RD<1.5 mm)	5.952	0.002	25.00	0.000	1.239	0.307
	(0.5≤RD<1 mm)	7.555	0.001	24.88	0.000	1.446	0.210
	(0≤RD<0.5 mm)	7.216	0.001	24.66	0.000	1.431	0.216

These results were obtained using two-way ANOVAS. *F*, used for significance tests; *p*, probability associated with *F*. RB, RD, RLD and RASD represent root biomass, diameter, length density and surface-area density, respectively. AB and R/S represent aboveground biomass and the root-to-shoot ratio, respectively.

The bold type indicates that the impacts were significant at  $p \le 0.05$ .

RLD by 35, 18 and 39% and RLD of fine roots by 35, 19 and 37%, respectively. Root elongation was therefore limited in the stony soils, especially those with high nodule contents (50% rock-fragment content in Fig. 3A). In contrast, the presence of nodules increased RD, especially for fine roots (Fig. 3B). Increasing nodule content from 0 to 10, 30 and 50% increased RD for fine roots by 10, 16 and 7%, respectively. Fine-root diameter was still higher at 50% nodule content, but the difference was smaller than for the treatments with lower nodule contents, indicating that root diameter would not increase, and could even decrease, if nodule content was sufficiently high. The parameters of root morphology excluding fine-root

diameter, were not significantly affected by nodule content in  $W_4$  (Figs. 3A, B and C).

### Root classification

Roots were firstly divided into two groups: fine roots and coarse roots. We could not determine whether the coarse or fine roots were more easily affected by nodule content, because the root parameters responded differently. Nodule content significantly affected surface area and the production of root biomass more for the coarse than the fine roots (Table 4). Nodule content, however, significantly affected root diameter and length more for the fine than the coarse Table 5Effects of waterand caliche-nodule contentson the proportions of thefine-root parameters foreach diameter class for allroots

					Water content ×caliche-nod- ule content	
	F	р	$\overline{F}$	р	F	р
Coarse roots (RD≥2 mm)	1.140	0.348	4.002	0.016	2.239	0.046
Coarse roots (RD≥2 mm)	6.055	0.002	2.679	0.064	0.849	0.578
Fine roots (1.5≤RD<2 mm)	7.122	0.001	0.139	0.936	1.674	0.138
(1≤RD<1.5 mm)	6.341	0.002	0.759	0.526	1.525	0.183
(0.5≤RD<1 mm)	9.935	0.000	5.627	0.003	1.581	0.164
(0≤RD<0.5 mm)	10.407	0.000	2.310	0.096	1.654	0.143
Coarse roots (RD≥2 mm)	1.681	0.191	2.682	0.064	1.156	0.356
Fine roots (1.5≤RD<2 mm)	9.597	0.000	0.358	0.783	1.674	0.138
(1≤RD<1.5 mm)	3.319	0.033	1.516	0.230	1.525	0.183
(0.5≤RD<1 mm)	4.417	0.011	3.697	0.022	1.581	0.164
(0≤RD<0.5 mm)	9.097	0.000	1.491	0.236	1.654	0.143
	Coarse roots (RD $\geq$ 2 mm) Coarse roots (RD $\geq$ 2 mm) Fine roots (1.5 $\leq$ RD<2 mm) (1 $\leq$ RD<1.5 mm) (0.5 $\leq$ RD<1 mm) (0 $\leq$ RD<0.5 mm) Coarse roots (RD $\geq$ 2 mm) Fine roots (1.5 $\leq$ RD<2 mm) (1 $\leq$ RD<1.5 mm) (0.5 $\leq$ RD<1 mm) (0.5 $\leq$ RD<1 mm) (0 $\leq$ RD<0.5 mm)	F           Coarse roots (RD $\geq 2$ mm)         1.140           Coarse roots (RD $\geq 2$ mm) <b>6.055</b> Fine roots (1.5 $\leq$ RD<2 mm)	F $p$ Coarse roots (RD≥2 mm)         1.140         0.348           Coarse roots (RD≥2 mm)         6.055         0.002           Fine roots (1.5≤RD<2 mm)	$F$ $p$ $F$ Coarse roots (RD>2 mm)1.1400.3484.002Coarse roots (RD>2 mm)6.0550.0022.679Fine roots (1.5 $\leq$ RD<2 mm)	F $p$ $F$ $p$ Coarse roots (RD≥2 mm)1.1400.348 <b>4.0020.016</b> Coarse roots (RD≥2 mm) <b>6.0550.002</b> 2.6790.064Fine roots (1.5≤RD<2 mm)	F $p$ $F$ $p$ $F$ Coarse roots (RD≥2 mm)1.1400.348 <b>4.0020.0162.239</b> Coarse roots (RD≥2 mm) <b>6.0550.002</b> 2.6790.0640.849Fine roots (1.5≤RD<2 mm)

These results were obtained using two-way ANOVAs. *F*, used for significance tests; *p*, probability associated with *F*. RB, RLD and RASD represent root biomass, length density and surface area density, respectively. The bold type indicates that the impacts were significant at  $p \le 0.05$ .

roots, particularly the finest roots (Table 4). Root length and surface area were further divided into four diameter categories for analysis. Mean fine-root

RLD and RASD in the  $RD_1$  diameter class (0-0.5 mm) were significantly higher in  $R_1$  than the other three nodule treatments, indicating that the presence

Coarse root (A)80 Fine root Root biomass (g) 60 40 20 0 (B)80 2 Above-ground biomass 70 Above-ground biomass (g) 60 50 40 30 20 10 0 (C) R/S ratio ab T 0.9 R/S ratio (g g<sup>-1</sup>) þ 0.6 0.3 0.0 **R**<sub>1</sub>  $R_1$  $R_1$ **R**<sub>1</sub>  $R_1$  $R_2$  $R_3$  $R_4$  $R_2$ R<sub>3</sub>  $R_4$ R<sub>2</sub> R<sub>3</sub>  $R_4$  $R_2$ R<sub>3</sub>  $R_4$ **R**<sub>2</sub> R<sub>3</sub>  $R_4$ W<sub>3</sub> W<sub>1</sub>  $W_2$  $W_4$ W" Treatment

Fig. 2 Root biomass, aboveground biomass and the root-to-shoot (R/S) ratio of coarse and fine roots for three replicates. Different letters indicate significant differences between caliche-nodule contents at  $p \le 0.05$  (one-way ANOVA). The error bars represent standard errors, and W<sub>µ</sub> represents the overall mean for the set of water contents Fig. 3 Mean root length density (RLD, cm cm<sup>-3</sup>), diameter (RD, mm) and surface-area density (RSAD, cm<sup>2</sup> cm<sup>-3</sup>) for three replicates. Different letters indicate significant differences between rock-fragment contents at  $p \leq 0.05$  (one-way ANOVA). The error bars represent standard errors, and Wµ represents the overall mean for the set of water contents



of nodules negatively affected the growth of the finest roots. Mean RLD and RASD in  $RD_2$  (0.5-1 mm),  $RD_3$  (1-1.5 mm) and  $RD_4$  (1.5-2 mm) were highest in  $R_1$  and  $R_3$  and significantly higher than in  $R_2$  and  $R_4$  (Table 7 in the Appendix), which were similar to the total values of RLD and RASD. RLD and RASD were lower in all diameter classes as water content decreased, also similar to total RLD and RASD. Mean RLD and RASD in  $RD_1$  did not differ significantly between  $W_1$  and  $W_2$ .

The percentage of RLD or RASD was equal to RLD and RASD in each diameter class divided by the

total for each treatment. Fine-root length and surface area were represented mainly by finer roots (Tables 7 and 8 in the Appendix). Approximately 80-85% of fine-root length was represented by roots with diameters <1.0 mm for all treatments, and approximately 50% were <0.5 mm. More than 60% of fine-root surface area was represented by roots with diameters <1.0 mm for all treatments. R<sub>1</sub> and R<sub>4</sub> had the highest percentages of roots in RD<sub>1</sub>, significantly more than for R<sub>2</sub> and R<sub>3</sub> (Table 8 in the Appendix). The variation in the percentages of the other diameter classes, however, was the opposite (i.e. lower in R<sub>1</sub> and R<sub>4</sub> than R<sub>2</sub> and R<sub>3</sub>).

## Vertical root distribution

RB was used to analyze the vertical distribution of roots to give sufficient consideration to the interactive effects between nodule and water contents. RB for the coarse and fine roots varied with depth (Fig. 4).

The coarse-root profiles for  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$ indicated that 93.9, 94.6, 94.5 and 89.0% of the coarse roots were concentrated in the 0-50 cm sections, respectively. The fine-root profiles for  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  indicated that 65.3, 68.6, 67.4 and 75.1% of fine roots were concentrated in the 0-50 cm sections, respectively.  $\beta$  for the coarse-root distribution did not differ significantly between rock-fragment

Fig. 4 Variations in root biomass with soil depth and its response to calichenodule content and water content (\*). The error bars represent standard errors for the set of water contents  $(W_{\mu})$  and caliche-nodule contents  $(R_{\mu})$ . R, W and R×W represents calichenodule contents, water contents and the interaction between nodule and water contents, respectively. \*, p < 0.05; \*\*, p < 0.01.



**Fig. 5** Daily relative humidity (RH), air temperature (AT) and solar radiation (SR) from May to September in (A) 2014 and (B) 2015

$\beta$ for the calic	he-nodule contents			$\beta$ for the water contents				
Treatment $W_{\mu}$	Coarse rootsFine roots $(RD>2 mm)$ $(RD\leq 2 mm)$			Treatment $R_{\mu}$	Coarse roots (RD>2 mm)	Fine roots (RD≤2 mm)		
	R <sub>1</sub>	0.946 <u>+</u> 0.013a	0.978 <u>+</u> 0.002a	W <sub>1</sub>	0.939 <u>+</u> 0.011a	0.977±0.006a		
	$R_2$	0.936 <u>+</u> 0.020a	0.975 <u>±</u> 0.003a	$W_2$	0.942 <u>+</u> 0.015a	0.975 <u>±</u> 0.005a		
	R <sub>3</sub>	0.943 <u>+</u> 0.012a	0.976 <u>±</u> 0.004a	<b>W</b> <sub>3</sub>	0.935 <u>+</u> 0.023a	0.975 <u>±</u> 0.004a		
	$R_4$	0.961±0.020a	$0.972 \pm 0.005 b$	$W_4$	0.946 <u>+</u> 0.017a	0.975 <u>±</u> 0.003a		

**Table 6** Extinction coefficients ( $\beta$ ) of the distribution of root biomass for the rock-fragment and water contents

contents (F=1.297, p=0.292, Table 6) or the water contents (F=1.083, p=0.370).  $\beta$  for the fine-root distribution differ significantly between the nodule contents (F=6.322, p=0.002) but not the water contents (F=1.341, p=0.297). No interactive effects were identified. Mean  $\beta$  for the fine-root distribution was lower in R<sub>4</sub> than R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub>, indicating that the profile of fine roots was shallower in R<sub>4</sub>.

RB for the coarse and fine roots in each soil layer was analyzed using a two-way ANOVA, with nodule and water contents as fixed factors (Fig. 4). The results indicated that nodule content mainly affected coarse-root biomass above 50 cm and fine-root biomass below 50 cm.

## Discussion

Effects of caliche nodules on root biomass and morphology

The mechanical strength of soil restricts root growth more in stony soil than fine earth (Unger and Kaspar, 1994; Merotto and Mundstock, 1999; Schneider and Don, 2019), and the total amount of available water decreases as rock-fragment content increases (Cousin et al., 2003; Tetegan et al., 2011). The presence of rock fragments should therefore adversely affect plant growth and root development. Our results for the effects of caliche nodules, however, did not support these expectations. Root biomass was lower only at the highest nodule content. Our result was consistent with the study by Hu et al. (2021) for A. vestita, a fast-growing subshrub where root biomass was significantly lower in a treatment with the highest rockfragment content than in fine earth. The highest rockfragment content in the study by Hu et al. (2021) was 75% by volume, which was higher than that in our experiment (50% by mass  $\approx 40\%$  by volume). Our result differed from the study by Hu et al. (2021) for another plant, *B. brachycarpa*, a slow-growing shrub where all treatments with rock fragments had higher root biomass than fine earth. Plants of different species therefore responded differently to rock-fragment content, indicating the need for individualized management for different plants species in stony areas.

According to the analysis by Mi et al. (2016), water-use efficiency (WUE) was highest in soil containing 30% caliche nodule, similar to the finding by Ceacero et al. (2020) that WUE in Mediterranean ecosystems was highest at a rock-fragment content of 17% (by volume). We speculated that WUE, not the total amount of water available to plants, may have been the more important factor affecting root production. As reported by Babalola and Lal (1977a,b), Magier and Ravina(1984) and Poesen and Lavee (1994), root growth was optimal at the moderate content of rock fragments (the common range of 10-20%). Rock-fragment content beyond the optimal may thus begin to negatively affect root growth because of poor growing conditions, such as restricted availabilities of water and nutrients, excessive temperature and resistance to growth. We therefore proposed a similar hypothesis. However, optimal nodule content did not appear in this study, further demonstrating that the effects of rock fragments on plant growth were complex. A more elaborate experimental design for nodule content is therefore needed to verify whether the optimal nodule content exists.

Caliche nodules affect aboveground biomass and root biomass similarly. The nodules in our study did not significantly affect the ratio of aboveground to root biomass, indicating that the pattern of biomass distribution was not influenced by the nodules. This finding differed from the results reported by Du et al. (2017), where the ratio of aboveground to root biomass in grassland on the northern Tibetan Plateau decreased as the rock-fragment content increased, because aboveground biomass decreased, and root biomass increased. R/S between the fine earth and the soil containing rock fragments did not differ significantly for A. vestita but differed significantly only when the rock-fragment content reached 75% for *B. brachycarpa* in the study by Hu et al.(2021) in Minjiang Arid Valley in southwestern China. These conflicting results were probably due to the different soils, rock fragments, plants and natural conditions between the study areas. The fine earth, rock fragments and plant samples were taken from the natural grassland by Du et al.(2017) and the establishing experimental plots under naturally climatic conditions by Hu et al.(2021). Hu et al.(2021) irrigated water into the plots at the seedling stage of the plant. Frequent irrigation and vermiculite mulch carried out in our soil-column experiment, which could weaken effects of nodules on root growth. The frequent watering led to different soil moisture fluctuations from the natural precipitation. Tetegan et al. (2015) considered that rock fragments could serve as water reservoirs during periods when the fine earth dried and could reduce plant-water stress during a moderate drought. The frequent watering weakened this effect of the nodules. Vermiculite mulch (a common way to minimize soil evaporation for obtaining data for plant evaporation based on water loss) could effectively inhibit soil evaporation in order to make ensure water loss by roots uptake in our original consideration, however, did not consider the effects of the nodules on soil evaporation.

Roots become thick and short in loess containing caliche nodules. Our results for the effects of rock fragments on root morphology were partly consistent with the effects of soil compaction. The mechanical resistance to root penetration is higher in compacted soils (Colombi et al., 2017; Ramos et al., 2010), similar to stony soils where roots have difficulty penetrating when they encounter rock fragments. Root thickening is a common response of roots to mechanical impedance, which is the adjustment of the roots to soil strength to reduce the risk of buckling and to the decrease in mechanical stress acting on the roots during penetration. This adjustment is associated with cell turnover and rate of cell detachment at the root cap and in root mucilage (Colombi et al., 2017). Unlike the trend of root shortening and thickening with the degree of soil compaction (Colombi et al., 2017; Ramos et al., 2010), root diameter and length in our study increased at nodule contents of 10 and 30% and then decreased at a content of 50%. The presence of rock fragments creates macropores between rock fragments and fine earth (Babalola and Lal, 1977a, b; Meng et al., 2018), which is conducive to root penetration and the conservation of water and may have contributed to the longer roots in the soil containing 30% nodules relative to the soil containing 10% nodules.

Effects of water content on root growth and the interaction of water content with caliche-nodule content

The behavior was similar between the fine earth and the stony soils, where the higher water content led to longer roots and higher surface-area density and biomass (Figs. 2A, 3A and 3C). High water content stimulates root growth, and low water content may induce withering. Our results were consistent with the conclusion by Cai et al. (2018), who compared plant growth in stony soil with rock-fragment contents up to 60% with homogeneous silty soil under different water conditions. Cui et al. (2021) also reported a positive relationship between the vertical distribution of root density and water content for *C. korshinskii*.

The coarse-root biomass in our study was affected by the nodules at all water contents, and nodule content and water content did not interact. Fine roots behaved differently from coarse roots. The effects of nodule content on fine-root biomass varied with water content. The results of the fine-root responses to the nodules supported our second hypothesis, that the effects of the nodules on root growth were more significant under low-than high-water conditions. In the two high-water treatments, fine-root biomass in each nodule treatment did not differ from that in the fine earth. In the two low-water treatments, fineroot biomass in the treatment of 50% nodule content significantly reduced (reduced by 52 and 60% in  $W_3$ and W<sub>4</sub> treatment, respectively). Water deficiency exacerbated the negative effects of nodules on fineroot growth in the soil with high content of nodules. Available water content was lowest in the lowest water and highest nodule content treatment. Available water content is possibly too low to meet the need for plant growth. Interactive effects of nodule and water content on the responses to the response of plants to soil water availability were also considered to be one of the evidences to explain interactive effects on fineroot growth and need to be further studied.

Variation of impacts of caliche nodules with root diameter and soil depth

The division of roots into fine and coarse categories are considered to be too arbitrary for identifying the difference of physiological function between different root sizes (Donnelly et al., 2016; Yu et al., 2017; McCormack et al., 2015). Studies of fine roots have confirmed that thin fine roots are more responsive to soil environmental changes than are thicker fine roots. For example, King et al. (2002), Baddeley and Watson (2005) and Montagnoli et al. (2014) indicated that the lifespans of fine roots were shorter for smaller roots with higher turnover rates compared to larger roots. Materechera et al. (1992) reported that roots with large diameters also tended to have higher percentages of penetration, Montagnoli et al. (2014) found that finer roots (RD <0.5 mm) were more responsive than larger roots (0.5 < RD < 2 mm) to soil temperature and water content. The length and surface area of fine roots easily obtained using a root scanner in our study were divided into four diameter categories to provide an opportunity for a detailed analysis of root morphology. The resulted demonstrated that the presence of nodules resulted in morphological adaptations and altered fine root distribution. The thinner the fine roots, the more they were affected by the nodules, indicating that finer roots were more sensitively responsive to the presence of nodules than were the thicker roots. The fine-root systems of C. korshinskii are mostly comprised of very fine roots ( $\leq 1$  mm). These were the longest and largest surface-area root type and play important role in root water and nutrient uptake. These results emphasize the need to divide fine roots into categories. Evaluating the structural and morphological attributes of roots of each diameter category and how they respond to caliche nodules and water content would provide more detailed information about how plants regulate themselves to adapt to the presence of caliche nodules and changes in water content.

Zhang et al. (2009) reported that the roots of mature *C. korshinskii* (twenty years old) were distributed vertically in the uppermost portion of the 3-m soil profile

in the Tengger Desert in China, with coarse roots concentrated in the upper 0.4 m and >60% of fine roots distributed in the upper 1.0 m. Bi et al. (2006) also reported that >80% of root biomass of young C. korshinskii (three years old) was distributed in the 0-60 cm layer. The roots in our study were obtained after three years of plant growth, and the control of water content began in the second year. By the end of the study, >90% of the coarse roots were concentrated above 50 cm and >90% of the fine roots were concentrated above 80 cm, indicating that the depth of the soil column was satisfactory. Vertical distribution of roots for C. korshinskii in this study was consistent with the previous studies, i.e. roots (especially for coarse roots) mainly distributed in the upper soil layers. In the treatment of 50% nodule content, nodules negatively affected coarse-root biomass at the shallower depths (-10 to -50 cm) and fine-root biomass at the deeper sections (-60 to -90 cm), respectively. In the entire profile, more fine roots were at shallower depths in the stony soils (lower  $\beta$  in Table 6), especially at the highest nodule content. The presence of caliche nodules therefore affected the pattern of the vertical distribution of the roots. High levels of caliche nodules prevented roots from extending downward due to negative effects such as high penetration resistance, relative few soils and water resources.

#### Implications and limitations of this study

Caliche nodules in the soil profile are common and natural on the Loess Plateau of China. We designed a soil-column experiment under semi natural conditions to simulate the loessial environments that had different nodule and water contents. The nodules are heterogeneously distributed in the soil or on the soil surface due to the influence of a combination of terrain, climate, pedogenesis and human activity. Zhu and Shao (2008) reported that the mass percentage of caliche in topsoil ranged from 3 to 50% in the study area. We designed different caliche nodule contents by referring the range found by Zhu and Shao (2008) to represent low, medium and high levels of nodules. The nodules in the columns were uniformly distributed throughout the entire depth of nearly 1 m where plant roots are concentrated. The scenario of the topsoil containing nodules is also common in the northern Loess Plateau.

Caliche nodules are characterized of lower water holding capacity and harder material than the fine earth. According to the study by Ma et al.(2010) and Zhou et al.(2009), nodule content was 30% and 50%, saturated hydraulic conductivity of the stony soils was significantly lower than the fine earth. Nodule content was about 10% (8% by volume), the stony soil displayed a greater saturated hydraulic conductivity compared to the fine earth (Ma et al., 2010). Water movement and porosity structure are different in the soil containing nodules from the fine earth. The presence of nodules therefore results in complicated soil circumstance for plant growth. In this soil column experiment, slow water transport and formation of large porosity would be helpful for efficient water absorption for plants and roots penetration. However, low ability of water retention and hard material would limit root growth. In the regions with high caliche nodule coverage (the top of mountain, mountain ridge, roadsides, edges of ravines, and other high elevation regions in the study by Gong et al. (2018a) and where the soil containing high nodule content, caliche nodules deserve serious consideration to develop appropriate plant management measures.

Soil water, a significant water source for plant on the Loess Plateau of China, was as another factor to explore whether effect of nodules on root growth varied with soil water condition. The results indicated that both water and nodule contents influenced root growth and the distribution of root parameters into fine and coarse roots, but interactions were rare, except in fineroot biomass. The dominant driver of root biomass and morphology was the watering level, and only the highest nodule content (50%) consistently reduced root production. Our study preliminarily and explicitly focused on the effects of caliche nodules on root development on the Loess Plateau and can provide a practical reference for developing measures of vegetation cultivation and management by predicting plant growth based on the abundance of caliche nodules.

Soil and plant undergoes a natural cycle of wetting and drying in the field. Our soil columns experiment, however, could not meet the completely natural conditions. Frequent irrigation mentioned above was different from the natural precipitation. Effects of nodules on soil evaporation were not considered. Finally, caliche nodules may affect the lateral growth of roots in the soil, which cannot be understood using soil-column experiments. Extending the study to field plots in situ or simulated under a completely natural environment will be necessary to obtain more precise and detailed information on root growth in loess containing caliche nodules.

# Conclusions

Studying roots in different diameter categories and soil depths under different water conditions in response to caliche-nodule content is necessary for understanding and predicting plant dynamics in soil containing caliche nodules on the Loess Plateau. The soil containing a moderate mass content of nodules (30%), similar to fine earth, was generally more favorable to root growth than soil with low (10%) or high (50%) mass contents of nodules. High contents of nodules negatively affected root growth. Nodules also affected root morphology. Root length density was 35, 18 and 39% lower, and fine-root diameter was 10, 16 and 7% higher, in the soils with nodule contents of 10, 30 and 50%, respectively, relative to fine earth. Higher water content led to higher root production, longer roots and larger surface areas in both the fine earth and the stony soils. Fine-root biomass varied significantly and interactively with nodule and water contents and was not affected by the presence of the nodules until the water content was <60% of field capacity. Classifying the roots based on the length and surface area of fine roots indicated that roots with smaller diameters were more significantly affected by the nodules. Coarse roots were significantly affected by nodules in the 10-50 cm soil layer, but fine roots were significantly affected by nodules in the 60-90 cm layer. A larger percentage of the fine roots were concentrated in the shallower layers in the stony soils, especially in the treatment with the highest nodule content.

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## Appendix

Table 7Fine-root length density (cm cm-3) and root surface-<br/>area density (cm2 cm3) in diameter classes from 0 to 2 mm<br/>in increments of 0.5 mm. Values are means  $\pm$  standard errors

for the set of water contents  $(W_{\mu})$  and caliche-nodule contents  $(R_{\mu})$ . Different letters indicate significant differences at  $p \le 0.05$  (one-way ANOVA).

Treatment		Root length de Root diameter	$\begin{array}{l} \text{ensity} \times 10^2 \text{ (cm} \\ \text{(mm)} \end{array}$	cm <sup>-3</sup> )		Root surface-area density $\times 10^2$ (cm <sup>2</sup> cm <sup>-3</sup> ) Root diameter (mm)				
		0-0.5	0.5-1.0	1.0-1.5	1.5-2.0	0-0.5	0.5-1.0	1.0-1.5	1.5-2.0	
$\overline{W_{\mu}}$	R <sub>1</sub>	27.19±3.60a	14.48 <u>+</u> 2.12a	4.58 <u>+</u> 0.71a	1.73 <u>+</u> 0.29a	3.47 <u>±</u> 0.47a	3.66 <u>+</u> 0.54a	2.00 <u>+</u> 0.31a	1.07 <u>+</u> 0.17a	
r	$R_2$	16.18 <u>±</u> 1.47b	10.04±1.15b	3.40 <u>±</u> 0.37b	1.34 <u>±</u> 0.14b	2.33±0.19bc	2.65±0.24b	1.56 <u>+</u> 0.14b	0.89 <u>+</u> 0.09b	
	$R_3$	20.04 <u>±</u> 2.48b	12.85±1.55a	4.56 <u>+</u> 0.49a	1.79 <u>+</u> 0.19a	2.82 <u>+</u> 0.34b	3.34 <u>+</u> 0.40a	2.03 <u>+</u> 0.22a	1.14 <u>+</u> 0.12a	
	$R_4$	16.42 <u>±</u> 2.00b	8.66 <u>+</u> 1.48b	3.01±0.51b	1.26 <u>+</u> 0.18b	2.13±0.27c	2.19 <u>±</u> 0.37b	1.34 <u>+</u> 0.22b	0.82±0.11b	
Rμ	$W_1$	27.60 <u>±</u> 1.98a	17.57±1.21a	5.84 <u>+</u> 0.40a	2.24 <u>+</u> 0.16a	3.70 <u>+</u> 0.26a	4.38 <u>+</u> 0.32a	2.52 <u>+</u> 0.18a	1.40 <u>+</u> 0.09a	
μ	$W_2$	23.61 <u>+</u> 3.11a	13.29 <u>+</u> 1.63b	4.48 <u>±</u> 0.49b	1.80 <u>+</u> 0.18b	3.18 <u>+</u> 0.38a	3.42 <u>+</u> 0.40b	2.01±0.21b	1.16 <u>+</u> 0.11b	
	$W_3$	16.80 <u>±</u> 1.90b	9.05±1.01c	3.19 <u>+</u> 0.34c	1.24 <u>+</u> 0.12c	2.33±0.25b	2.45 <u>+</u> 0.26c	1.48 <u>+</u> 0.15c	0.82±0.08c	
	$W_4$	11.82±1.35b	6.12±0.63c	2.03±0.23d	0.83±0.08c	1.54±0.16c	1.58±0.16d	0.90±0.10d	0.52 <u>+</u> 0.05d	

**Table 8** Proportions of fine-root length density and surfacearea density of each diameter class to total densities. Values are means  $\pm$  standard errors for the set of water contents (W<sub>µ</sub>)

and caliche-nodule contents (R<sub> $\mu$ </sub>). Different letters indicate significant differences at *p*≤0.05 (one-way ANOVA).

Treatment		Proportion of (mm)	root length den	sity (%)@@Ro	ot diameter	Proportion of root surface-area density (%)@@Root diameter (mm)			
		0-0.5	0.5-1.0	1.0-1.5	1.5-2.0	0-0.5	0.5-1.0	1.0-1.5	1.5-2.0
$W_{\mu}$	R <sub>1</sub>	55.9 <u>±</u> 1.5a	29.7±0.9b	9.5±0.5b	3.5±0.2c	31.6 <u>+</u> 0.9a	33.1 <u>±</u> 0.6a	18.2 <u>+</u> 0.6b	9.6 <u>±</u> 0.3c
	$R_2$	51.9±1.3b	31.3 <u>+</u> 0.6ab	10.7 <u>±</u> 0.5b	4.2 <u>±</u> 0.2b	28.8 <u>+</u> 0.9b	32.2±0.3a	18.8 <u>+</u> 0.5b	10.8 <u>+</u> 0.4b
	R <sub>3</sub>	49.7 <u>±</u> 0.7b	32.1±0.4a	11.7 <u>+</u> 0.4a	4.7 <u>±</u> 0.2a	27.2 <u>±</u> 0.5b	32.3 <u>±</u> 0.5a	20.1±0.4a	11.4 <u>+</u> 0.2a
	$R_4$	56.6±1.3a	28.0±0.9c	9.7 <u>±</u> 0.4b	4.2 <u>+</u> 0.2b	31.2 <u>+</u> 0.8a	30.5±0.68b	18.6 <u>+</u> 0.4b	11.4 <u>+</u> 0.4a
R <sub>u</sub>	$W_1$	50.9±0.8b	32.5 <u>±</u> 0.4a	10.8 <u>+</u> 0.3a	4.2 <u>+</u> 0.2a	28.5±0.5b	33.5±0.5a	19.4 <u>+</u> 0.3a	10.9 <u>+</u> 0.3a
	$W_2$	53.5±1.3ab	30.1±0.9b	10.4 <u>+</u> 0.4a	4.2 <u>+</u> 0.2a	29.4 <u>+</u> 0.8ab	31.4±0.7b	18.8 <u>+</u> 0.4a	11.0 <u>+</u> 0.5a
	$W_3$	54.6±1.5ab	29.3 <u>+</u> 0.7b	10.5 <u>+</u> 0.6a	4.1 <u>+</u> 0.2a	32.5±1.0a	31.5±0.4b	19.4 <u>+</u> 0.6a	11.0 <u>+</u> 0.5a
	$W_4$	55.1±1.8a	29.3±1.0b	9.9 <u>+</u> 0.6a	4.1±0.2a	30.6±1.2ab	31.5±0.67b	18.1 <u>±</u> 0.6a	10.6 <u>+</u> 0.3a

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