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## Biochar distribution mode in soil affects the vegetative peanut growth, nitrogen uptake and nitrogen-fixing bacteria activity

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**Abstract:** Biochar plays an important role in agricultural production as it can improve soil fertility, promote nutrient adsorption and enhance plant growth. However, the distribution of biochar in the soil significantly impacts its application effect. In order to investigate the impact of non-uniform biochar distribution on soil nutrient uptake, root shape, peanut development, and the makeup of soil microbial communities, we carried out greenhouse peanut pot studies. This experiment followed a completely randomised design with four treatments, each with three replications. The four treatments were as follows: no biochar application (B0); concentrated biochar application near seeds (B1); relatively concentrated surface application of biochar (B2), and uniformly dispersed application of biochar (B3). The findings demonstrated that, compared to the no-biochar scenario, the aboveground and root nitrogen uptake was significantly ( $P < 0.05$ ) improved by the B2 treatment, increasing by 42.79% and 51.39%, respectively, compared to the control group. Additionally, it reduced the concentrations of  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N in the soil. The B2 treatment also significantly ( $P < 0.05$ ) increased the net photosynthetic rate and aboveground dry matter weight, increasing by 196.85% and 53.96%, respectively, compared to the B0 treatment. The B1 and B3 treatments also demonstrated a higher promoting effect. The growth of the root system and the quantity of root nodules were promoted by the addition of biochar. The number of root nodules in the B2 treatment was 72.22% higher than that in the control group. In terms of microbial and bacterial communities, the addition of biochar increased the number of nitrogen-fixing bacteria to a certain extent, while the relative abundance of soil bacterial communities showed no significant differences. In general, the non-uniform distribution of biochar in the soil significantly affected peanuts' vegetative growth and developmental effects. The relatively concentrated surface application of biochar treatments contributes to improving plant nutrient uptake and root system development. This provides a more effective application method for agricultural personnel to apply biochar fertiliser in the future.

**Keywords:** photosynthesis; root morphology; nitrogen content; organic carbon; enzyme activity

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Peanuts are a significant oilseed crop growing extensively in tropical and subtropical areas (Zheng et al. 2023). China's peanut production reached 18.3 million tons by 2022, with 4.8 million hectares under cultivation (Ren 2023). After soybean and rapeseed, this is the third-largest area used for peanut cultivation among popular oilseed crops. Since China has a large population and a pressing need to address its food shortage, raising peanut yields and expanding the area under cultivation are crucial steps in the growth of the country's peanut business. The application of biochar could enhance the soil carbon and nitrogen ratios, soil organic carbon, and other physicochemical features, which were consistent with the rise in peanut production, according to Tan et al. (2021) investigation into the impact of biochar with organic fertiliser on peanut planting. Soil cadmium pollution is a pressing issue in the peanut production process. Shao et al. (2022) discovered that the application of biochar in conjunction with foliar selenium sprays can lessen the cadmium toxicity of peanuts and increase peanut output. This implies that meeting the nutritional requirements of peanuts can be achieved primarily through the use of biochar in agricultural production (Yuan et al. 2022). Biochar is a C-rich solid that is produced by high-temperature pyrolysis of agricultural waste materials such as straw, wood, etc., under nutrient-restricted conditions (Chen et al. 2013). The physicochemical properties of biochar prepared from different raw materials vary greatly (Chen et al. 2015, Wang et al. 2017a, Tran et al. 2018). Because of its high surface area, porosity, and several functional groups, biochar is frequently employed as a soil amendment (Xu et al. 2015, Zong et al. 2023). In addition to being used as a fertiliser to increase crop growth (Sim et al. 2021), biochar applied to the soil can also raise the soil pH and soil cation exchange capacity (Baiaomonte et al. 2015), improve soil agglomeration and water retention (Liu et al. 2024), decrease environmental pollution from chemical fertilisers, and increase fertiliser utilisation rates (Sun et al. 2018).

Previous studies on biochar have concentrated on different biochar types and their application rates. For example, Wang et al. (2021) applied 0, 10, 20, and 40 t/ha of biochar to the soil and found that the application of 10 t/ha of biochar enhanced photosynthesis and increased peanut yield. Similarly, Komariah et al. (2023) found that the application of 7.5 t/ha biochar can significantly increase the physicochemical properties, such as the number of peanuts, the number of peanut pods, and the weight

of dry matter of peanuts. Using the fruits of *Cassia fistula* and *Caesalpinia* sp. as well as the bark of *Eucalyptus globulus* as raw materials, Swagathnath et al. (2019) produced biochar and studied its impact on rice development and the soil microbial ecology associated with it. The findings demonstrated that the application of biochar lengthened the roots and enhanced the bacterial community. Specifically, 1.5% fruits of *Cassia fistula* biochar increased rice shoots by 18% compared to the control group, and 0.5% barks of *Eucalyptus globulus* biochar made the new rice shoots 12% longer than the control group. However, the effects of different distribution states of biochar in the soil on the physicochemical properties of crops are often overlooked by researchers, and some studies have found that uneven application of fertilisers can lead to changes in plant growth and development, dry matter, yield and other physicochemical factors. Yuan et al. (2023) examined the effects of different fertiliser application positions (orthotropic, lateral), as well as different depths of fertiliser application (10 cm, 15 cm), on nutrient utilisation and maize yield. Using the hole application method, fertiliser application at 15 cm in the lateral position was more effective. Using the strip application method, fertiliser application at 15 cm in the orthotropic position was more effective.

In actuality, it is common to find biochar applied in non-uniform ways, such as on the surface, close to the seeds, or through uniform fertilisation. Under the same application amount of biochar, it is particularly important to study the influence of biochar distribution position on peanut growth and development, avoid unreasonable fertilisation, explore the most effective application method of biochar and clarify the mechanism. We conducted research on the effects of various biochar distribution strategies on peanut growth and soil nutrient changes based on pot planting experiments in greenhouses. We also analysed the effects of biochar on soil nutrient uptake, root morphology, and soil microbial community structure indexes. Finally, we clarified the effects of non-uniform distribution on peanut growth, which can provide significant theoretical and technological foundations for producing high-quality and effective peanuts.

## MATERIAL AND METHODS

The biochar used in this study was prepared by charring peanut shells at 450 °C for 4 h in a muffle

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Table 1. Initial physical and chemical parameters of biochar used in this study

Biochar properties	Value
pH	8.85
Carbon (g/kg)	678.45
Nitrogen (g/kg)	7.73
Phosphorus (g/kg)	1.2
Specific surface area (m <sup>2</sup> /g)	38.42
Pore volume (cm <sup>3</sup> /g)	0.024

furnace. It was in powder form, and the powder was passed through a 60-mesh sieve. The properties of the biochar are shown in Table 1.

### Experimental design

The experiment was conducted in the greenhouse (36°48'N, 120°29'E) of the Shandong Peanut Research Institute. The soil is characterised as brown soil (Haplic luvisol in FAO Soil Classification System). The physical and chemical properties of the soil are

as follows as described by Yang et al. (2024): organic matter of 16.7 g/kg, total available phosphorus (AP) of 24.35 mg/kg, available nitrogen (AN) of 81.45 mg/kg, and available potassium (AK) of 30.54 mg/kg, pH = 6.42. On July 12, 2023, three peanut seeds with uniform size were planted in pots filled with 4 kg of dry soil. The peanut cultivar was Flower Yuk 22 (the main cultivar in the area), and an equal amount of biochar was applied to the soil as a fertiliser to provide nutrients to the peanuts. After emergence, two peanut plants with the same growth were left in each pot. The experiment was a completely randomised design and divided into four treatments: no biochar application (B0); concentrated biochar application near seeds (B1); relatively concentrated surface application of biochar (B2), and uniformly dispersed application of biochar (B3), with three replicated pots in each group treatment (Figure 1). The experimental period was 55 days, which was at the flowering stage of peanuts. The B0 treatment had no additives, and biochar additions in the B1, B2 and B3 treatments were all 1% by weight of the soil (Huang et al. 2023). In the B1 treatment, biochar was added

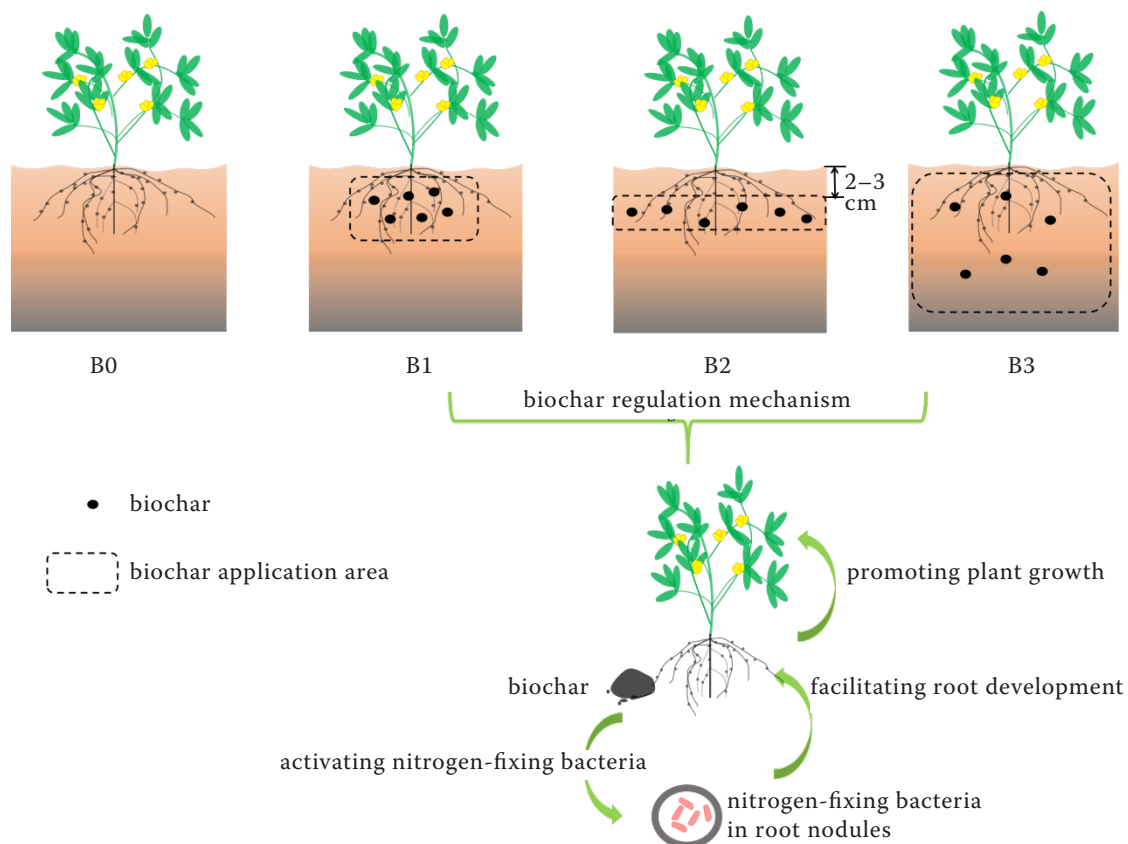


Figure 1. Distribution status of biochar in different treatments and the mechanism of biochar promoting peanuts growth and development. B0 – no biochar application; B1 – concentrated biochar application near seeds; B2 – relatively concentrated surface application of biochar; B3 – uniformly dispersed application of biochar

centrally near the inter-root of peanut seeds. In the B2 treatment, biochar was applied uniformly to the upper surface of the soil. The application position was about 2–3 cm away from the soil surface. In the B3 treatment, biochar was mixed well with the soil, and peanut seeds were planted in the soil for potting experiments to observe the growth characteristics of the crop. After sowing, water was managed according to plant growth and soil moisture content.

### Determination methods

**Peanut agronomic traits.** The effects of different treatments on the agronomic traits of peanuts were determined at 55 days after application. Growth parameters included plant height, side branch length, branch number, photosynthetic characteristics and plant dry weight. Functional leaves (inverted trifoliate leaves) of plants were selected, and photosynthetic characteristics such as net photosynthetic rate, transpiration rate, intercellular CO<sub>2</sub> concentration, and stomatal conductance were measured at 9–11 a.m. using the CIRAS-3 Portable Photosynthesis System (Amesbury, USA). Aboveground plant parts were dried to a constant weight in an oven at 80 °C and weighed to obtain dry weight (Meng et al. 2024).

**Root morphological indices.** The morphology of the root sample is good, complete and untruncated. The roots were first cleaned, and the number of root nodules was counted. Then, the morphology of the roots was scanned with a scanner and analysed using the WinRHIZO root analysis system for total root length, root surface area, root volume, and the number of tips (Liang et al. 2024). Finally, the roots were dried in an oven at 80 °C to a constant weight and weighed to obtain the dry weight.

**Total nitrogen content in plants and soils.** Plant samples were dried in an oven at 70 °C to determine total nitrogen using the Kjeldahl method (Wang et al. 2017b). Soil samples were taken immediately after 55 days of peanut harvest. The soil samples were mixed well and stored at 4 °C after sieving. Soil inorganic nitrogen (NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N) was extracted with 2 mol/L KCl solution and determined by a flow injection analyser (Jones and Willett 2006).

**Organic carbon content and soil enzyme activity.** The soil organic carbon was determined *via* wet digestion using the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> oxidation method (Yang et al. 2019). The double antibody sandwich method determined the enzyme activity with the urease ELISA (enzyme-linked immunosorbent as-

say) kit and nitrate reductase in Qingdao Sangon Biotechnology Co., Ltd (Meng et al. 2023). Urease activity was measured as follows: the purified urease antibody was coated onto the microporous plate to create the solid-phase antibody, and then urease was added successively into the micropore of the coated monoclonal antibody. It was then combined with horseradish peroxidase-labelled urease antibody to form an antibody-antibody-enzyme-conjugate antibody complex. After thorough washing, the substrate 3,3',5,5'-tetramethylbenzidine was added for colour development. The absorbance of the sample was measured using the enzyme marker at a wavelength of 450 nm, and the concentration of urease activity was calculated using the standard curve. Nitrate reductase activity was measured as follows: the purified plant nitrate reductase antibody was coated onto the microporous plate to create the solid-phase antibody. The nitrate reductase antibody was then added to the coated micropores, followed by the addition of horseradish peroxidase-labelled nitrate reductase antibody to form an antibody-antibody-enzyme-conjugate antibody complex. After thorough washing, the substrate 3,3',5,5'-tetramethylbenzidine was added for colour development. The absorbance was measured using the enzyme marker at a wavelength of 450 nm, and the concentration of nitrate reductase activity was calculated using the standard curve.

**Soil bacterial community determination.** Total soil DNA was extracted using a DNA extraction kit, and PCR amplification was performed using bacterial *nifH*/*nifH*R region primers F: 5'-AAAGGYGGWATCGGYAARTCCACCAC-3' and R: 5'-TTGTTSGCSGCRTACATSGCCATCAT-3' for target gene amplification. The PCR-amplified products were purified, and the purified samples were sent to the Illumina NovaSeq sequencing platform of Shanghai Majorbio Bio-pharm Technology Co. By splicing and filtering the reads, clustering or denoising, and performing species annotation and abundance analysis, the species composition of the samples can be revealed. Furthermore, alpha diversity, significant species differences, correlation analysis and functional prediction analysis can be performed for statistical analysis of the bacterial community structure.

### Data analysis

SPSS 27.0 data statistical software (IBM Co., Armonk, USA) was used for analysis. The Waller-



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Duncan method was used to discriminate statistically significant differences between treatments ( $P < 0.05$ ). Plotting was performed using Origin 2018. In microbiology, the community composition of peanut inter-rhizosphere soils was analysed for species abundance at the genus level, and diversity analysis was obtained using the Sobs index.

### Data availability statement

The original transcriptome data used in this study was submitted to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database with accession numbers BioSample SAMN40909140-SAMN40909151 and BioProject PRJNA1098480.

## RESULTS

### Changes in peanuts photosynthesis and growth

The level of photosynthesis can be determined by the net photosynthetic rate, transpiration rate, intercellular  $\text{CO}_2$  concentration and stomatal conductance. The effects on the photosynthetic parameters of peanuts are shown in Figure 2, and in general, it seems that peanuts in the B2 treatment had higher values of the indices at the flowering stage,

and the photosynthetic effect is more prominent than in other treatments. Specifically, in terms of the net photosynthesis rate (Figure 2A), there was a significant difference between the B2 and B0 treatments and the B1 treatments ( $P < 0.05$ ), and there was no significant difference between the B2 and B3 treatments. The net photosynthetic rate of B2 was the highest, which was 30.52, 69.23 and 196.85% higher than that of B3, B1 and B0, respectively. In terms of stomatal conductance (Figure 2C), the B2 treatment outperformed the other treatments by a substantial margin ( $P < 0.05$ ), and its values were higher than those of the B0 treatment by 152.45%. There was minimal variation in the intercellular  $\text{CO}_2$  concentration (Figure 2B) across the treatments. The low value of biochar-added treatments may be due to the fact that they have not shown advantages at the flowering stage. In terms of transpiration rate (Figure 2D), there was a significant difference between the B2 treatment and the other treatments ( $P < 0.05$ ), which was 91.16, 51.82, and 75.63% higher than the B0, B1, and B3 treatments, respectively. There was no difference among the other treatments, which had a small overall effect.

As shown in Figure 3, different treatments had different effects on peanut plant height and dry matter weight. Firstly, the plant height of the B1 and B2 treatments was significantly ( $P < 0.05$ ) different

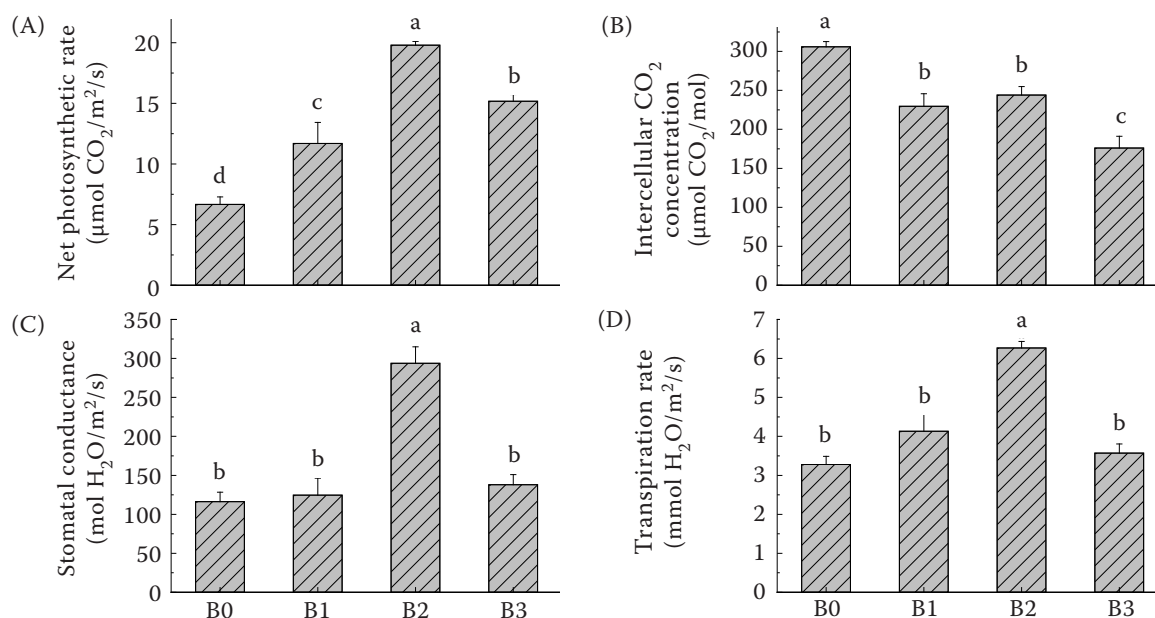


Figure 2. Effects of different distribution of biochar on photosynthesis of peanuts. (A) Net photosynthetic rate; (B) intercellular  $\text{CO}_2$  concentration; (C) stomatal conductance and (D) transpiration rate. B0 – no biochar application; B1 – concentrated biochar application near seeds; B2 – relatively concentrated surface application of biochar; B3 – uniformly dispersed application of biochar

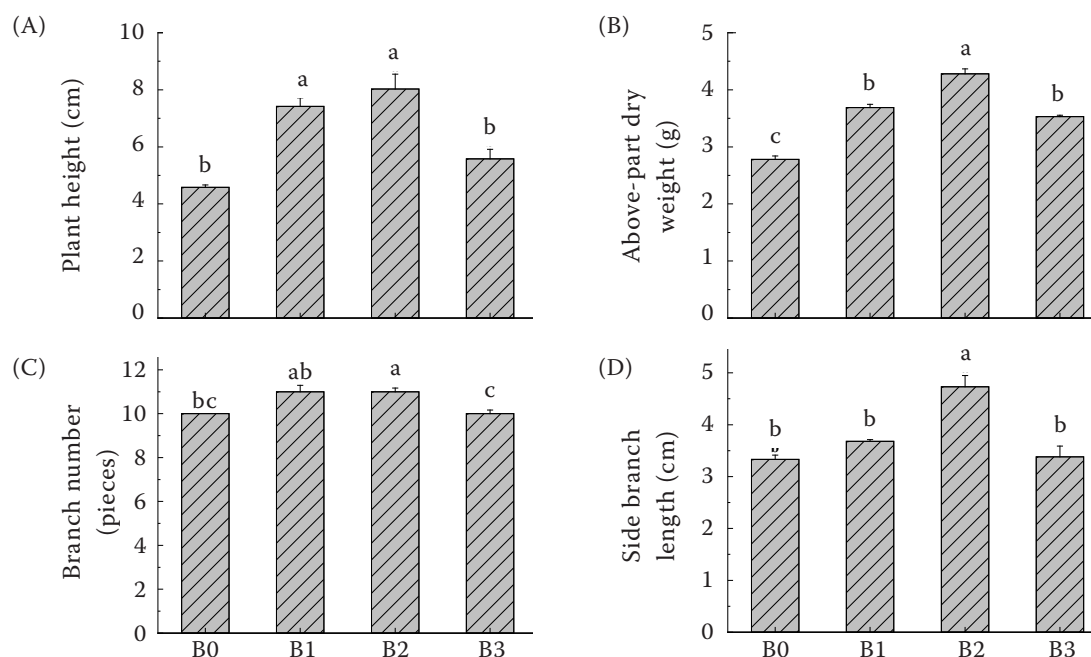


Figure 3. Effects of a different distribution of biochar on growth and developmental characteristics and dry matter weight of peanuts. (A) Plant height; (B) above-part dry weight; (C) branch number, and (D) side branch length. B0 – no biochar application; B1 – concentrated biochar application near seeds; B2 – relatively concentrated surface application of biochar; B3 – uniformly dispersed application of biochar

from that of the B0 treatment (Figure 3A). The plant height of the B1, B2, and B3 treatments increased by 62.01, 75.33, and 21.83%, respectively, compared with that of the B0 treatment. In terms of dry matter weight (Figure 3B), the values of the biochar-added treatments were significantly different from the B0 treatment ( $P < 0.05$ ). The B2 treatment was the most effective, with a dry matter weight of 4.28 g, an increase of 53.96% compared to the B0 treatment. In terms of branch numbers (Figure 3C), there was little difference between them, and the differences were weak. No statistically significant difference was observed between the side branch lengths of the B0, B1, and B3 treatments (Figure 3D). Nonetheless, there was a significant difference ( $P < 0.05$ ) in the side branch length between the B2 treatment and the other three treatments. The side branch length of the B2 treatment was 4.73 cm, which was 42.04% higher compared to the B0 treatment.

Overall, the B2 treatment had better peanut agronomic indices compared to the other treatments. Photosynthesis also reflected the changes in the growth of peanut fertility under different treatments. The application of biochar generally increased the plant height of peanuts (Figure 3A), which implies that biochar can increase plant growth. The plant

above-part dry weight trend among different treatments was consistent with plant height (Figure 3B). The treatment with added biochar promoted the accumulation of substances in peanut stems and leaves, which might be due to the larger photosynthesis rate promoting peanut growth.

### Changes in peanut root characteristics

The results of peanut root morphology measurements are shown in Figure 4. There were differences in the effects of different treatments on the four indexes of root length, root surface area, root volume and root tips. Generally, the relatively concentrated surface application of biochar treatment (B2) had a better effect on all indexes. The differences in root length among the different treatments were more significant (Figure 4A). The peanut root length of the B2 treatment was 2.161 cm, which was 101.83, 29.65, and 6.56% longer than that of the B0, B1, and B3 treatments, respectively. Regarding root surface area (Figure 4B), the B2 treatment was the best, and the root area amounted to 375.07 cm<sup>2</sup>. The B2 treatment was not significantly different from the B3 treatment, but the difference between the B0 and B1 treatments was significant ( $P < 0.05$ ). Regarding root

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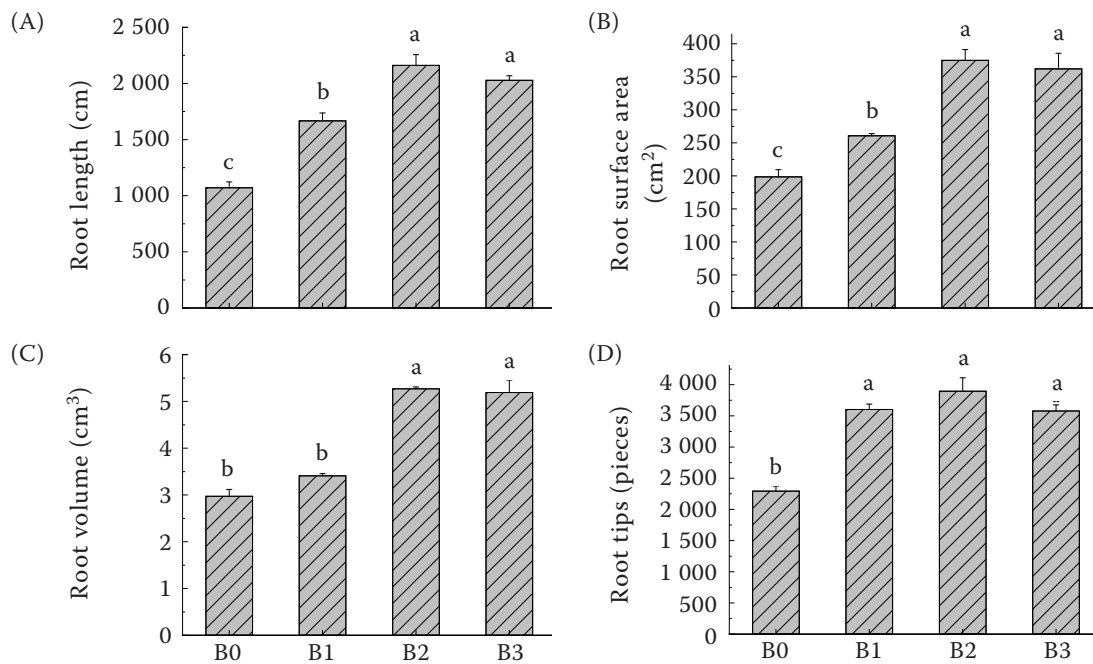


Figure 4. Effects of different distribution of biochar on root characteristics of peanuts. (A) Root length; (B) root surface area; (C) root volume, and (D) root tips. B0 – no biochar application; B1 – concentrated biochar application near seeds; B2 – relatively concentrated surface application of biochar; B3 – uniformly dispersed application of biochar

volume (Figure 4C), the B2 treatment was slightly higher than the B3 treatment, and the root volume was 5.27 cm<sup>3</sup>. The B2 and B3 treatments were significantly different compared to the B0 and B1 treatments ( $P < 0.05$ ); there was no significant difference between the B0 and B1 treatments. In terms of the root tips (Figure 4D), the numbers of root tips with biochar-added treatments were significantly different ( $P < 0.05$ ) compared to the B0 treatment. The B2 treatment had the greatest number of root tips, 3 895, which was 69.79% higher than that of the B0 treatment.

As shown in Figure 5, the root dry mass and the number of root nodules were different among treatments. Firstly, in terms of the number of root nodules (Figure 5A), the B2 treatment was significantly different ( $P < 0.05$ ). The number of root nodules in the B2 treatment was higher than in the B0, B1, and B3 treatments by 72.22, 32.86, and 60.34%, respectively. The B2 treatment differed considerably ( $P < 0.05$ ) from the B0 and B1 treatments in terms of root dry mass (Figure 5B). The B2 treatment had the heaviest root dry mass of 1.00 g, and the root dry mass of the B1, B2, and B3 treatments was 14.86, 35.14, and 31.08%

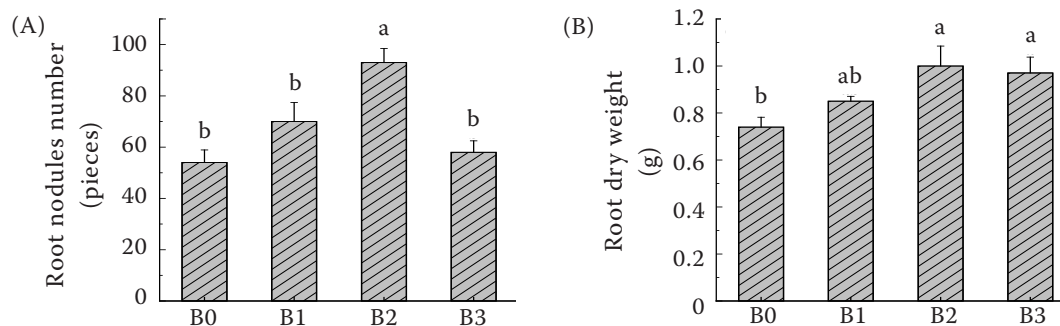


Figure 5. Effects of different distribution of biochar on root nodules development and root dry weight of peanuts. (A) Root nodules number (one root), and (B) root dry weight. B0 – no biochar application; B1 – concentrated biochar application near seeds; B2 – relatively concentrated surface application of biochar; B3 – uniformly dispersed application of biochar

higher than that of the B0 treatment, respectively. Overall, the root dry mass and the number of root nodules in the B2 treatment were superior compared to the other treatments.

### Changes in soil physicochemical factors and nitrogen absorption in peanut

The soil inorganic nitrogen content of the biochar-added treatment was relatively low (Table 2). Firstly, the biochar-added treatments led to a significant decrease in soil  $\text{NO}_3^-$ -N content compared with the B0 treatment ( $P < 0.05$ ). The B1 treatment had the lowest soil  $\text{NO}_3^-$ -N, followed by the B2 and B3 treatments, which were reduced by 46.61, 38.91, and 24.61%, respectively, compared with the B0 treatment. In other words, the biochar-added treatments were able to reduce the accumulation of  $\text{NO}_3^-$ -N in the soil. For  $\text{NH}_4^+$ -N, soil  $\text{NH}_4^+$ -N did not differ much among the four treatments, containing about 2.8–3.4 mg N in 1 kg of soil. As shown in Table 2, urease activity was higher in the soils of biochar-added treatments than in the treatment without the addition of biochar, and there were significant differences among the treatments ( $P < 0.05$ ). The highest urease activity of 417.52  $\mu\text{mol/day/g}$  soil was found in the biochar (B1) treatment, while the B2 treatment showed a slight decrease in urease activity relative to the B1 treatment. Nitrate reductase activity remained very low compared to soil urease activity, indicating extremely weak nitrate reductase activity in the soil. B1 and B2 treatments had higher nitrate reductase activity compared to the other treatments. The nitrate reductase activity of the biochar-added treatments was higher than that of the B0 treatment, with B1, B2, and B3 being 128.57, 142.86, and 85.71% higher than that

of the B0 treatment. The trends of organic carbon content in the soil among the different treatments were consistent with urease and nitrate reductase activities (Table 2). The organic carbon content in the soil was higher in the B1 and B2 treatments, with significant differences among the four treatments ( $P < 0.05$ ). The organic carbon content in B1, B2, and B3 treatments with the addition of biochar was increased by 382.69, 334.62, and 184.62%, respectively, compared to that in the B0 treatment. Compared to the B1 and B2 treatments, the B3 therapy had a less favourable impact on urease, nitrate reductase, and organic carbon content. This may be because of the application mode of the B3 treatment, the concentration of biochar near the peanut root system was small, and the fertiliser efficiency was diluted, which makes it difficult to play a good role.

As can be seen from Figure 6, the nitrogen content of the above-ground part, as well as the root system, increased after biochar application. Regarding nitrogen content in the aboveground part (Figure 6A), the nitrogen content per peanut seedling of B1 and B2 treatments was 107.09 and 110.63 mg, respectively, which were not significantly different from each other. Among them, the B2 treatment increased nitrogen by 42.79% compared with the B0 treatment. Regarding root nitrogen content, the values were generally lower due to peanuts' flowering stage, but the biochar treatments were better than the B0 treatment (Figure 6B). Except for the B1 and B3 treatments, which did not show a significant difference, there was a significant difference between the other treatments ( $P < 0.05$ ). The nitrogen content of the root system of a single peanut plant in the B1, B2 and B3 treatments increased by 30.82, 51.39, and 40.50% compared with that of the B0 treatment, respectively.

Table 2. Effects of a different distribution of biochar on changes in effective nitrogen content, enzyme activities and organic carbon content in the soil

Treatment	$\text{NH}_4^+$ -N	$\text{NO}_3^-$ -N	Urease activity in soil	Nitrate reductase activity in soil	Organic carbon content in soil
	(mg N/kg soil)	(mg N/kg soil)	( $\mu\text{mol/day/g}$ soil)	( $\mu\text{mol/day/g}$ soil)	(%)
B0	$3.13 \pm 0.052^b$	$33.08 \pm 0.645^a$	$247.44 \pm 7.530^d$	$0.14 \pm 0.006^c$	$0.52 \pm 0.008^d$
B1	$3.07 \pm 0.045^{bc}$	$17.66 \pm 0.759^d$	$417.52 \pm 13.392^a$	$0.32 \pm 0.003^{ba}$	$2.51 \pm 0.083^a$
B2	$3.44 \pm 0.064^a$	$20.21 \pm 0.694^c$	$375.32 \pm 11.421^b$	$0.34 \pm 0.004^a$	$2.26 \pm 0.051^b$
B3	$2.88 \pm 0.083^c$	$24.94 \pm 1.115^b$	$332.07 \pm 1.712^c$	$0.26 \pm 0.010^b$	$1.48 \pm 0.053^c$

Waller-Duncan method was used to distinguish the differences between treatments ( $P < 0.05$ ), and the confidence interval was 95%. B0 – no biochar application; B1 – concentrated biochar application near seeds; B2 – relatively concentrated surface application of biochar; B3 – uniformly dispersed application of biochar



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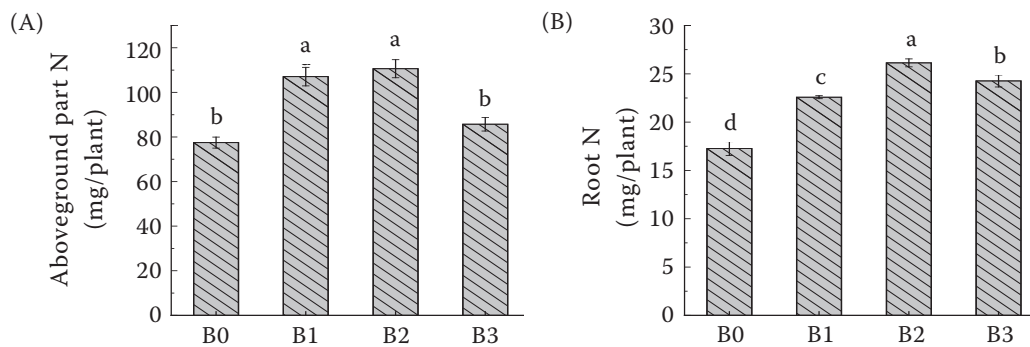


Figure 6. Effects of a different distribution of biochar on the nitrogen content of (A) aboveground part and (B) root. B0 – no biochar application; B1 – concentrated biochar application near seeds; B2 – relatively concentrated surface application of biochar; B3 – uniformly dispersed application of biochar

As seen in Figure 7, the inorganic nitrogen content in the soil was negatively correlated with nitrogen accumulation in the plant. The nitrogen content absorbed by peanut plants in the B1 treatment was 1.89% higher than in the B2 treatment, which was not statistically different. The nitrogen content contained in peanut plants in the B0 treatment was 39.55%, which was 21.45% less than in the B2 treatment. This result showed that biochar promoted the absorption of nitrogen by peanut plants to some extent.

### Changes in soil microbial diversity

**Community diversity analysis.** By sequencing the soil microbial diversity of each treatment, the different treatments (B0, B1, B2, B3) yielded 15 678, 19 223, 16 154, and 17 925 valid sequences at the flowering stage, respectively. Based on the number of bacterial OTUs (Figure 8A), the dilution curves of the different treatments gradually levelled off with the increase in sequencing depth, indicating that the amount of sequencing was sufficient to cover all microorganisms in the samples. This can directly reflect the reasonableness of the sequencing data volume.

Principal coordinates analysis (PCoA) showed (Figure 8B) that the first and second coordinate axes explained 34.34% and 23.83% of the diversity, respectively, which together explained 58.17% of the diversity. B0 and B3, B1 and B2 treatments intersected more on the PCoA plot, indicating that the difference in soil microbial community structure between B0 and B3, B1 and B2 treatments was not obvious. The larger distance within the group of B0 treatments may be due to the more complex soil environment and uneven distribution of genera. However, B1 and B0, B3 treatments and B2 and B0, B3 treatments were

obviously separated on the PCoA plot. The results showed that the soil microbial community structure of B1 and B2 treatments significantly differed from that of B0 and B3 treatments.

**Community relative abundance analysis.** The effect of the different treatments on the relative abundance of soil bacterial communities is shown in Figure 9. Microbial species obtained from the four different treatments were categorised into 2 domains, 2 kingdoms, 7 phyla, 10 classes, 17 orders, 26 families, 34 genera and 66 species. Sequences that could not be classified were defined as unclassifiable. At the genus level (Figure 9A), the four different treatments had the same dominant bacterial genera with insignificant differences. In descending order of relative abundance, the top eight genera with higher relative abundance

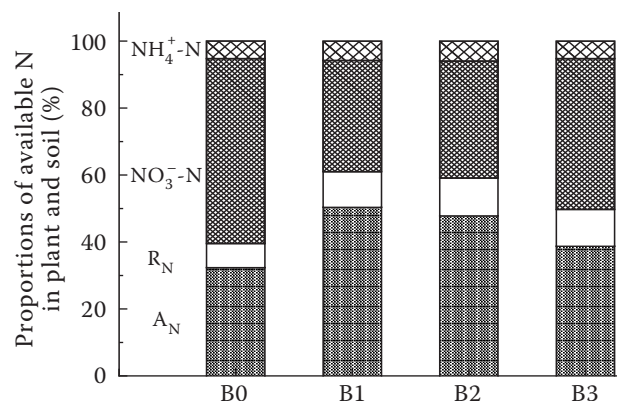


Figure 7. Changes in the proportion of plant and soil available nitrogen in different distributions of biochar. B0 – no biochar application; B1 – concentrated biochar application near seeds; B2 – relatively concentrated surface application of biochar; B3 – uniformly dispersed application of biochar; A<sub>N</sub> – aboveground part nitrogen (N); R<sub>N</sub> – root N

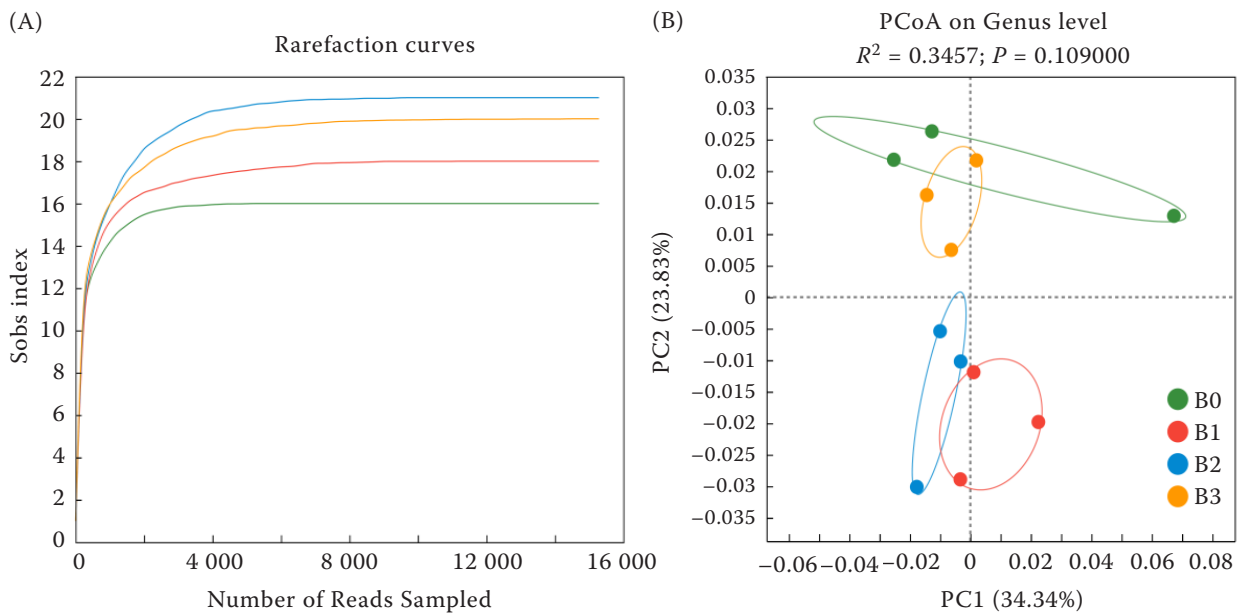


Figure 8. Analysis of soil microbial community diversity in different biochar distribution. (A) rarefaction curves on genus level, and (B) PCoA on genus level. B0 – no biochar application; B1 – concentrated biochar application near seeds; B2 – relatively concentrated surface application of biochar; B3 – uniformly dispersed application of biochar

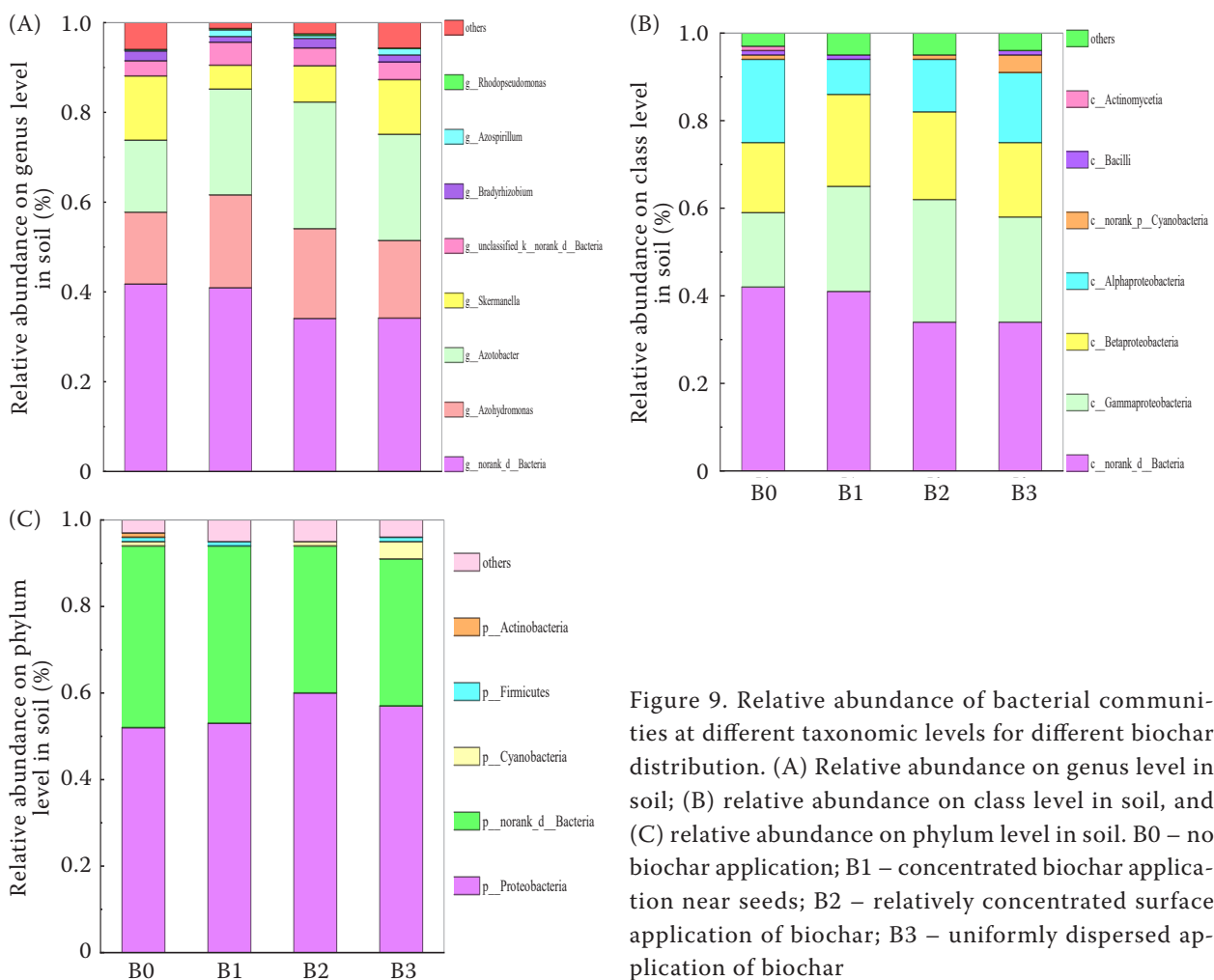


Figure 9. Relative abundance of bacterial communities at different taxonomic levels for different biochar distribution. (A) Relative abundance on genus level in soil; (B) relative abundance on class level in soil, and (C) relative abundance on phylum level in soil. B0 – no biochar application; B1 – concentrated biochar application near seeds; B2 – relatively concentrated surface application of biochar; B3 – uniformly dispersed application of biochar

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were: *g\_norank\_d\_Bacteria*, *g\_Azohydromonas*, *g\_Azotobacter*, *g\_Skemanella*, *g\_unclassified\_k\_norank\_d\_Bacteria*, *g\_Bradyrhizobium*, *g\_Anabaena*, *g\_Azospirillum*. Among them, *g\_Azohydromonas*, *g\_Azotobacter* and *g\_Azospirillum*, as nitrogen-fixing bacteria, can not only change nitrogen molecules into nitrogen atoms that can be digested and absorbed by plants but also play an active role in the natural nitrogen cycle, which is of great significance to the sustainable development of agriculture. As can be seen in Figure 9A, the numbers of *g\_Azohydromonas*, *g\_Azotobacter*, and *g\_Azospirillum* in the treatments with biochar (B1, B2, and B3) increased compared with the B0 treatment. Especially the number of *g\_Azotobacter* in the B2 treatment was significantly higher than that in the other three treatments, with a certain difference. In addition, *g\_Rhodopseudomonas*, as a non-dominant bacterial genus, is a kind of photosynthesising bacterium; the number increased after the addition of biochar, which indicated that the addition of biochar was beneficial to the photosynthesis of peanuts.

At the class level (Figure 9B), *c\_Gammaproteobacteria*, *c\_Betaproteobacteria*, *c\_Alphaproteobacteria*, *c\_Cyanobacteria*, *c\_Bacilli*, and *c\_Actinomycetia* were the dominant classes. Among them, the dominant classes in the B0 treatment accounted for approximately 54% of the total. After adding biochar, the number of dominant classes in the B1 treatment was slightly lower than that in the B0 treatment, at around 53%. This may be the result of a more complex soil environment. The percentage of dominant classes in both B2 and B3 reached about 61%, which represented a significant increase in the number of dominant classes after the addition of biochar compared to the B0 treatment.

At the phylum level (Figure 9C), *p\_Proteobacteria*, *p\_Cyanobacteria*, *p\_Firmicutes*, and *p\_Actinobacteria* were the dominant phylum. At the phylum level, *c\_Gammaproteobacteria*, *c\_Betaproteobacteria*, and *c\_Alphaproteobacteria* were collectively called *p\_Proteobacteria*. Therefore, it can be seen in the figure that the phylum level *p\_Proteobacteria* in the B2 treatment was significantly different compared to the other three treatments.

**Analysis of factors influencing soil microbial diversity.** Through the db-RDA analysis of soil microorganisms and environmental factors under different treatments of biochar sites (Figure 10), the scatter plot shows the distribution of the samples. The points of different colours in the plot indicate the sample groups under different treatments. Red

arrows indicate quantitative environmental factors, and whether the dots are aligned with the direction of the arrows represents positive and negative correlations. The length of the environmental factor arrow can represent the influence of the environmental factor on the data of the sample group. The size of the angle between the arrow line and the sorting axis indicates the size of the correlation between the environmental factor and the sorting axis; a small angle indicates a large correlation. The quadrant where the arrow is located indicates the positive or negative correlation between the environmental factor and the sorting axis in the azimuthal direction.

At the genus level (Figure 10A), the sample points of the B2 treatment were more concentrated compared to the other three treatments, with positive correlations with soil organic carbon content and root nitrogen content and negative correlations with soil nitrate reductase. In addition, the first sorting axis mainly correlated well with soil organic carbon content ( $r = 0.9998$ ) and root nitrogen content ( $r = 0.909$ ), and the second sorting axis correlated well with soil nitrate reductase content ( $r = 0.9474$ ). The envfit function was used to test the significance of each environmental factor. The results showed that the effects of soil organic carbon content ( $r^2 = 0.801$ ), root nitrogen content ( $r^2 = 0.8133$ ) and soil nitrate reductase content ( $r^2 = 0.5849$ ) on soil microorganisms reached a significant level ( $P < 0.05$ ). At the class level (Figure 10B), the trend of the arrows is consistent with the genus level, and the sample points are more dispersed compared to the genus level.

However, at the phylum level (Figure 10C), the envfit function was used to test the significance of each environmental factor. The results showed that the soil organic carbon content ( $r^2 = 0.739$ ) and root nitrogen content ( $r^2 = 0.5744$ ) significantly affected soil microorganisms ( $P < 0.05$ ), while the effect of soil nitrate reductase content ( $r^2 = 0.2446$ ) on soil microorganisms did not reach a significant level ( $P = 0.292$ ). These results indicate that soil organic carbon content, root nitrogen content, and soil nitrate reductase content were the main environmental factors affecting soil microorganisms at the genus and class levels, whereas soil nitrate reductase had little effect on soil microorganisms at the phylum level.

## DISCUSSION

Biochar is a C-rich material with a porous structure that facilitates nutrient cycling and improves

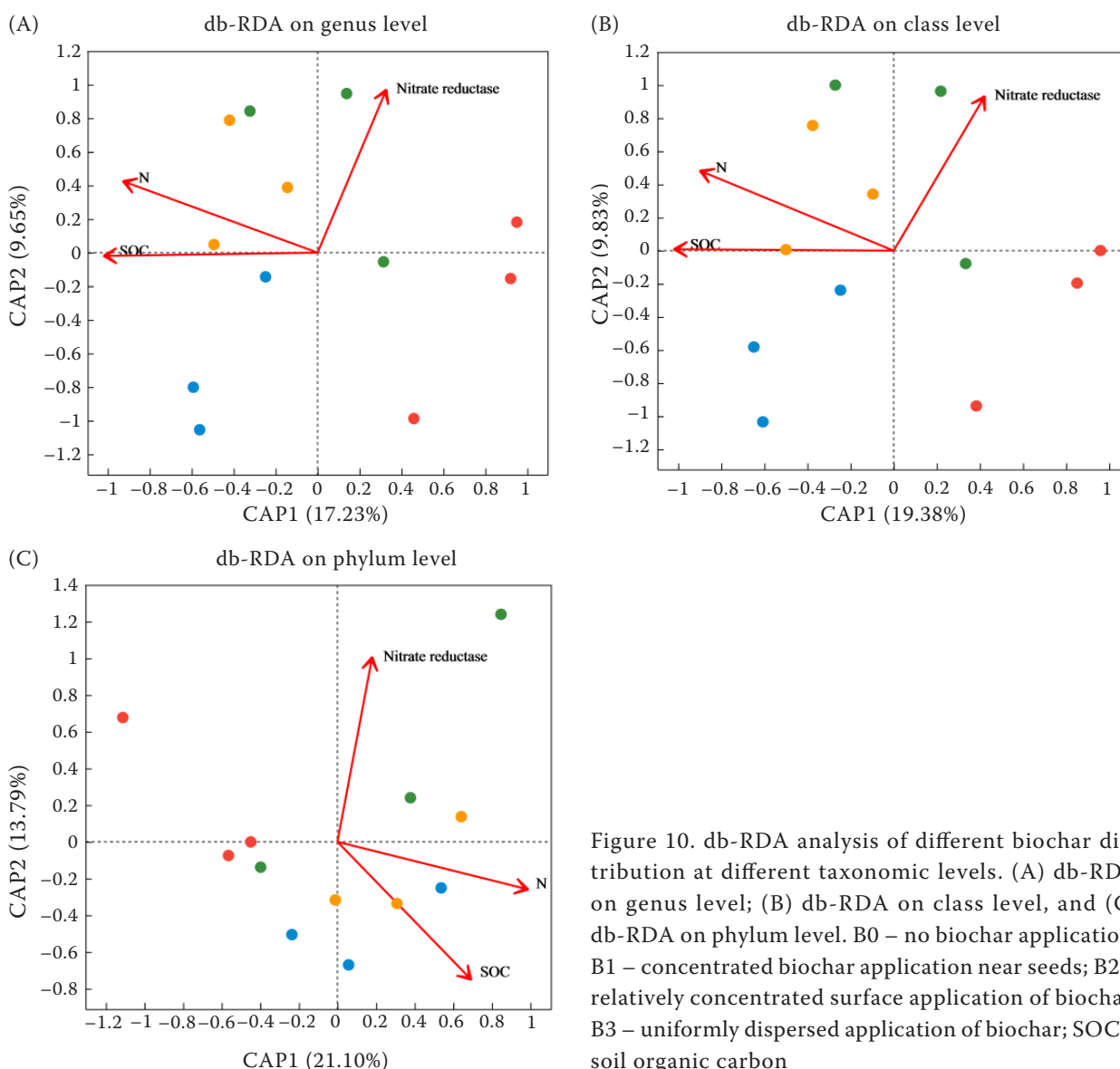


Figure 10. db-RDA analysis of different biochar distribution at different taxonomic levels. (A) db-RDA on genus level; (B) db-RDA on class level, and (C) db-RDA on phylum level. B0 – no biochar application; B1 – concentrated biochar application near seeds; B2 – relatively concentrated surface application of biochar; B3 – uniformly dispersed application of biochar; SOC – soil organic carbon

soil fertility (Khan et al. 2024), and soil application of biochar is a strategy to promote soil nitrogen cycling and increase microbial activity (Gao et al. 2014, Zhang et al. 2018, Lopes et al. 2021). In this study, we carried out experiments with peanut pots in greenhouses and investigated the changes in peanut growth, root morphology, soil nutrient uptake, and soil microbial community structure as a result of the non-uniform distribution of biochar. Compared to no biochar application, the results showed that applying biochar might decrease soil  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N concentrations and promote the utilisation of nitrogen aboveground and in the roots of peanuts. Compared to the B0 treatment, the B2 treatment's aboveground dry matter weights and net photosynthetic rate increased by 53.96% and

196.85%, respectively. This study also discovered that the application of biochar could encourage the growth of peanut root nodules and the root system; this result is consistent with Liu et al. (2024). The addition of biochar somewhat enhanced the quantity of nitrogen-fixing bacteria in the microbial communities, while the relative abundance of soil bacterial communities did not differ significantly.

In addition, the mechanism of biochar promoting crop growth can be obtained from the research results, as shown in Figure 1. Firstly, the results demonstrated that biochar could activate the microbial community near the root system, thereby enhancing the number of nitrogen-fixing bacteria in root nodules, which in turn increased root length, the number of root nodules, and other root characteristics. The increase

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in the number of root nodules led to an increase in the nitrogen fixation capacity of the root system, which enhanced the nutrient uptake by peanuts and further promoted the growth of peanuts (Figure 1).

**Effects of different distribution of biochar on photosynthesis, growth and developmental characteristics and dry matter weight of peanuts.** The majority of the growth indices of the biochar treatments differed considerably from the B0 treatment, according to the experimental data. Regarding photosynthesis, the net photosynthetic rate, stomatal conductance, and transpiration rate of the B2 treatment significantly differed from those of the other treatments, producing the largest  $A$ ,  $g_s$ , and  $E$  values. Meanwhile, regarding physiology, the B2 treatment was superior to the other treatments regarding plant height and dry matter weight. It can be seen that among the three treatments with biochar addition, the B2 treatment showed significant ( $P < 0.05$ ) superiority. Lu et al. (2020) investigated the effects of two methods of biochar application, namely, the mixing of biochar with topsoil (about 3–5 cm) and uniformly mixing of biochar with soil, on the growth and yield of rice. These findings indicated that the yield increased by approximately 25–36% when the biochar was mixed with topsoil but only by 11–14% when the biochar was evenly mixed with soil. This conclusion is consistent with the results of this experimental study, which can prove that applying biochar on the surface can promote the growth and development of plants. This may be because the relatively concentrated surface application of biochar can lock the evaporation of water from the soil, reduce the emission of nitrogen, and maintain the effectiveness of soil nitrogen without affecting the further development of the peanut root system (Lu et al. 2020). The B1 treatment inhibited seed germination, nutrient uptake by the root system, and the further emergence of the crop due to the excess biochar near the seeds (Yang et al. 2021). The B3 treatment is a uniform application of biochar mixed with soil, which results in low levels of biochar near the root system and higher levels of biochar in the soil below the root system, reducing nitrogen uptake by the inter-root system (Lu et al. 2020).

**Effects of different distribution of biochar on changes in plant/soil nutrient uptake.** At the root level, we observed that the B2 and B3 treatments positively affected root length, surface area, and volume compared with the B1 treatment. In addition to showing advantages in root length, surface area,

and volume, the B2 treatment showed significant ( $P < 0.05$ ) differences in root dry weight and number of root nodules compared with the other treatments. This is likely due to biochar's porous and macroporous structure, which can lock a certain amount of water under the relative surface application of biochar treatment, thus promoting root development. In addition, the porous nature of biochar provides a habitat for soil microorganisms to survive and reproduce, which significantly improves soil microbial population and activity (Jin et al. 2024), promoting activities of rhizobacteria and nitrogen-fixing bacteria, thus promoting root growth and nutrient accumulation. Similarly, the research results of Han et al. (2023) show that biochar-added treatments can improve the yield and growth characteristics of maize by increasing root length, delaying root senescence and changing the structure of the endophytic fungi community in roots.

As seen in Figure 7, the inorganic nitrogen content in the soil was negatively correlated with nitrogen accumulation in the plant. Meng et al. (2023) showed that the use of inhibitors could increase the accumulation of nitrogen in peanuts and thus reduce the inorganic nitrogen content in the soil, which is consistent with our findings. In this study, biochar, regardless of how it was applied to the soil, promoted nitrogen uptake by peanut plants to some extent and also enhanced root development. Among them, the nitrogen absorption effect was more pronounced in peanut plants treated with B1 and B2. Similarly, a previous study by Zhang et al. (2023) investigated the application of biochar and the combination of two irrigation modes (drip and subfilm drip) to regulate root development to improve peanut yield in an arid region. The experimental results showed that the application of biochar promoted peanut root development and net photosynthesis rate regardless of the irrigation mode. This may be due to the fact that the biochar powder came into direct contact with the peanut roots, which promoted nutrient cycling around the roots (Brennan et al. 2014) and facilitated nitrogen uptake by the root system, thereby improving the peanut root system as well as plant growth.

Nitrate in the plant is converted to nitrite and then to ammonium through metabolic pathways under the action of nitrate reductase, which is finally used for protein synthesis. When the nitrate content in the soil is too high, nitrate reductase in the plant does not play a role, leading to nitrate accumulation. In this study, the nitrate reductase activity was low in



the B0 treatment (Table 2), so the nitrate nitrogen content in the soil was too high, leading to a decrease in the amount of nitrogen absorbed by the crop, thus affecting crop growth. Soil urease can reflect soil nitrogen availability (Chen et al. 2014). Soil urease can decompose urea into ammonia and carbon dioxide, releasing a source of nitrogen that plants can absorb and utilise. As can be seen in Table 2, the activity of urease in the soil was increased by the addition of biochar. El-Bassi et al. (2021) reported that the use of olive mill waste to make biochar and its application to tomato potted plants found that adding biochar promoted tomato growth and increased urease activity. This is consistent with our findings. The present study showed that all three biochar treatments increased the soil's organic carbon content compared to the B0 treatment. Among them, the B1 and B2 treatments had a more prominent organic carbon content. Pang et al. (2023) found that the direct application of biochar into the soil can significantly increase the content of organic carbon in the soil and promote the retention of organic carbon, which is consistent with our research results. In addition, the addition of biochar can also improve the soil environment, enrich the structure of microbial communities, and enhance the stability of organic carbon storage (Azeem et al. 2019).

**Effects of different application methods of biochar on changes in soil microbial community structure.** Soil microorganisms are sensitive and the structure of soil microbial communities is susceptible to the influence of physicochemical properties such as soil type, water content, organic carbon and total nitrogen content (Shen et al. 2013). At the genus and phylum levels, the effects of soil organic carbon content, root nitrogen content and soil nitrate reductase content on soil microorganisms reached significant levels ( $P < 0.01$ ), indicating that these indicators are the main environmental factors affecting soil microorganisms. However, the effect of soil nitrate reductase on soil microorganisms was not significant at the phylum level, indicating that soil nitrate reductase was not a major environmental factor affecting soil microorganisms. In this study, when comparing the changes in soil microbial community structure under different biochar distribution treatments, the number of nitrogen-fixing bacteria in soil microorganisms increased after the addition of biochar treatments at the genus level. The increase in the number of *g\_Azotobacter* in the relatively concentrated surface application of biochar treatments

was particularly obvious. This may be because the moderate concentration of biochar in the vicinity of the roots in the B2 treatment was able to provide more nutrients to the root system, resulting in an increase in the number of soil microorganisms in the B2 treatment. Since *g\_Azotobacter* is a type of nitrogen-fixing bacteria that can convert nitrogen molecules into nitrogen atoms that can be absorbed by plants, the increase in *g\_Azotobacter* will enhance the performance of photosynthesis and nitrogen fixation in peanut roots. Because the number of nitrogen-fixing bacteria in the B2 treatment was relatively large, the photosynthesis, plant height, root length, root surface area, and the number of root nodules in the B2 treatment were more advantageous compared to the other three treatments. Li et al. (2024) found that, at the phylum level, the addition of biochar decreased the number of *p\_Proteobacteria* and increased the number of *p\_Firmicutes* bacteria. This result contradicts the findings of our study. However, Li et al. (2024) found that the addition of biochar increased the number of *p\_Actinobacteria* bacteria, which is consistent with our findings in this regard. The main reason for this discrepancy is that the structure and diversity of soil bacterial communities are susceptible to multiple factors.

In summary, biochar had a growth-promoting effect on the crop, while its non-uniform distribution significantly affected the growth and development of peanuts. In particular, the most noticeable improvement in peanut root development and nutrient uptake was observed with the relatively concentrated surface application of biochar treatment. Additionally, it offers the practical and theoretical foundation for enhancing crop agronomic management and judicious use of biochar fertiliser.

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