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Soil factors that contribute to the abundance and structure of the diazotrophic community and soybean growth, yield, and quality under biochar amendment

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Abstract

Background A 2-year field trial was conducted to test the effect of biochar addition (0, 15, 30, and 45 t hm^{-2}) on soil properties, nutrients, diazotrophic community diversity, abundance, and structure, and soybean growth, yield, and quality. Furthermore, we aimed to explore the responses of diazotrophs, grain yield, and quality to nine soil environmental factors. Rhizosphere soil and plant samples were collected after harvest.

Results Biochar application resulted in a lower soil bulk density (γ_d) but higher total organic carbon (TOC), effective phosphorus (AP) and total nitrogen (TN). Compared with untreated soil, the diversity index of diazotrophic bacteria in biochar-amended soil decreased, but the abundance of diazotrophic bacteria increased. The microbial community remained stable when a small amount of biochar was applied but changed as biochar amount increased. Furthermore, biochar reduced the proportion of unique nitrogen-fixing bacteria, but did not affect that of common nitrogen-fixing bacteria between biochar-amended and untreated soils, and increased the relative abundance of *Bradyrhizobium* (B9 vs. B0) and *Sinorhizobium* (B18 or B21 vs. B0) involved in symbiotic nitrogen fixation. The main components and content of fatty acids (except for stearic acid) and the content of protein and soybean oil remained stable under biochar application. The low biochar treatment (15 t hm^{-2}) promoted soybean growth and yield. Redundancy analysis suggested that TN greatly influenced the diazotrophic community structure at the phylum and genus levels, and that pH, TOC, and NO_3^--N greatly influenced grain yield and quality.

Conclusions Soil diazotroph environment can be improved by targeted farmland implementation based on changes in soil physicochemical properties, which would benefit biological N fixation in agricultural soils and further increase economic benefit.

Keywords Biochar, Diazotroph, Soya fatty acid, Soil properties, Soil nutrient

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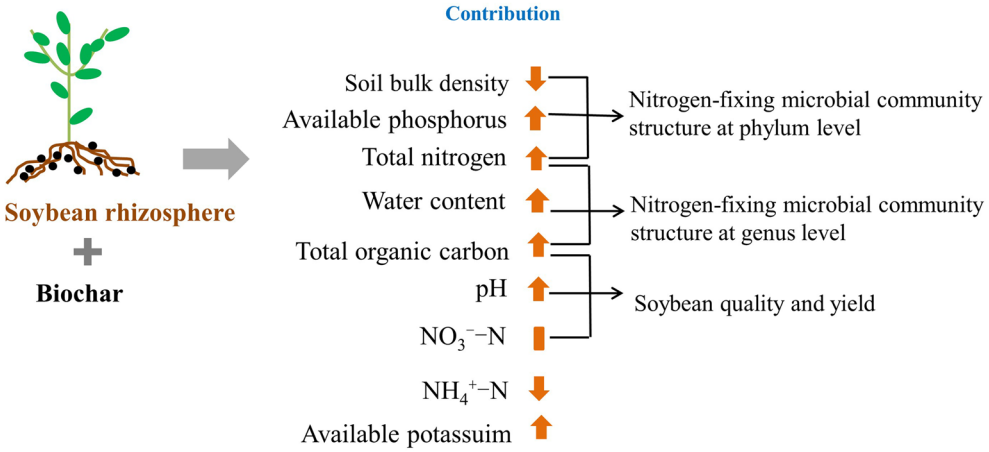
Graphical Abstract



Introduction

Soybean (*Glycine max* L. Merr) is one of the main legume and oilseed crops worldwide [14]. It is a critical component of global food security and a source of protein in the human diet and animal feed, and an increasingly important oil for biofuel production [11]. Over the past few decades, soybean production has increased because of increased cultivation area and improved management of the soil environment, including soil amendment, which has practical significance in improving soybean yield and quality, and the strategic importance of revealing the underlying mechanism.

Nitrogen (N) is generally regarded as the most critical determinant of crop productivity among mineral nutrients. In particular, soybean yield is closely related to crop N uptake [21]. The two main pathways for soil N input are organic N addition and biological N fixation by N-fixing bacteria. Specifically, although many studies have shown that N fertilizer can promote soybean growth and increase grain yield [8, 10, 27], more is not necessarily better. Thus, an excess of N fertilizer may increase greenhouse gas emissions, and long-term excessive use may even cause soil acidification [12, 22]; more importantly, the over-use of N fertilizer may increase the risk of a N imbalance in soybean production, thereby affecting carbon balance, soil health, and sustainable agricultural development [4]. Alternatively, soil amendment might be used instead of N fertilizers in soybean cropping to reduce the side effects of excess N addition. A case in point, and fully in compliance with the concept of sustainable development promoted by green agriculture, biochar, a porous and carbon-rich material, is harmless and its use entails no threat to the soil environment [25].

Biological N fixation benefits agricultural systems without reducing soil productivity or causing environmental pollution [7]. Soybean N fixation can reach 40–180 kg hm^{-2} through rhizobia [18]. Thus, research on N-fixing microorganisms is of practical significance for soybean production, such that, a clear understanding of diazotrophic activity can guide the soybean industry.

Soil physical and chemical properties may change after biochar application. Previous studies have reported that biochar benefits crop yield owing to its high carbon content, abundant micropores, large specific surface area, and strong adsorption capacity [1, 19, 26]. However, the influence of biochar on N-fixing microorganisms in the soybean–soil system has received much less attention, despite the well-known fact that soybeans form a highly effective symbiotic N-fixation system with rhizobia. Among the few studies available, Yu et al. [30] and Palansooriya et al. [17] reported that biochar addition affected microbial activity, abundance, and community composition. Further, the relationships between N-fixing microorganisms and soil physicochemical properties have not been comprehensively studied in biochar-amended rhizosphere soils. Similarly, the responses of soybean yield and quality to the rhizosphere habitat of biochar-amended soils have been ignored.

Considering the peculiarities of the soybean-soil system, we designed our study of N-fixing microorganisms in the soybean rhizosphere to gain novel insights into the soil-microbial interactions in the soybean rhizosphere. Here, we hypothesized that (1) the application of biochar can improve soil structure and fertility and provide a good living environment for diazotrophic communities; (2) the application of biochar will increase soybean yield

and quality by improving physicochemical soil properties and diazotrophic community characteristics. To test these hypotheses, a 2-year field experiment was conducted on soybeans grown on a biochar-amended soil amended. Specifically, our goals were (1) to evaluate the effects of biochar addition on soil physical and chemical properties, nutrient content, and soybean growth, yield, and quality; (2) to quantify the effects of biochar addition on the diazotrophic community; and (3) to further explore the interactions between rhizospheric soil environmental factors and the diversity, abundance, and community structure (at both phylum and genus levels) of N-fixing microorganisms, soybean yield, and quality.

Materials and methods

Soil and biochar preparation

The field experiments reported herein were conducted at the Experimental Station of the Northwest Agriculture and Forestry University in Yangling, Shaanxi Province (34°17' N, 108°24' E, 520 m a.s.l.), China. The region has a warm temperate and monsoon climate, with average annual rainfall, temperature, evaporation, and sunshine hours of 630 mm 13 ℃, 1500 mm, and 2163.8 h, respectively. The average frost-free period extends for over 210 days. The meteorological data recorded during the tests are shown in Additional file 1: Fig. S1. Before the trial (2020), and prior to soil (0–20 cm topsoil layer) amendment the soil was determined as a loam with a bulk density (γ_d) of 1.37 g cm^{-3}. Total organic carbon (TOC), total nitrogen (TN), available phosphorus (AP), and available potassium (AK) contents were approximately 8400, 860, 15, and 150 mg kg^{-1}, respectively. Soil pH was measured at 8.16. Biochar was generated by pyrolyzing fruitwood under O_2-limited conditions via N_2 purging. The preparation protocol has been described in detail by Xu et al. [28]. The biochar prepared was sent to the test Center of Northwest A & F University to test its basic properties. The content of water, ash, volatiles, and total sulfur and hydrogen were approximately 7.30%, 5.59%, 4.98%, 0.15%, and 0.72%, respectively.

Experimental design and sampling

Soybean cultivar Zhonghuang No.13 was obtained from the Institute of Crops, Chinese Academy of Agricultural Sciences. Soybean seeds were sown and harvested on June 13 and September 30, 2020, and 2021, respectively. The area of each experimental plot was 2 m×3 m, and plots were laid in a randomized block arrangement. Plant and row spacings were 10 and 20 cm, respectively. Four biochar dosages were applied to the experimental plots, namely 0, 15, 30, and 45 t hm^{-2}, corresponding to 0, 9, 18, and 27 kg per 6 m^2 (labeled as B0, B9, B18, and B27, respectively), of the experimental plots. Biochar was applied by spraying on the soil surface and subsequently plowing to a depth of 0–20 cm. Three replicates were included per treatment. Each plot received compound fertilizer (N-P-K = 24–15-6%) as a basal application. The plots were irrigated and weeded regularly during the growth period. Crop management adopted local standard practices.

Soil samples were collected during the last harvest (September 30, 2021). At the time, 8–10 plants were randomly selected from each experimental plot, and soil samples within 0–2 mm of the root circumference were collected using the shaking method. Soil samples from each plot were mixed evenly, impurities such as roots were removed, and then samples were screened using a 2-mm screen. The samples were divided into two parts: one was air-dried for the determination of basic soil physical and chemical properties, while the other portion was stored at − 80 ℃ for soil DNA extraction. Soil samples were used to determine the physicochemical properties (water content θ, γ_d, and pH), nutrient (TOC, AP, AK, TN, NH_4^+-N, and NO_3^--N), and diazotrophic community characteristics (diversity index, *nifH* gene copies, operational taxonomic unit (OTU) numbers, and species diversity). Plant samples were collected at five growth stages and three plants were randomly selected for growth measurements (plant height, stem diameter, and leaf area). Three plants were randomly selected, split into roots, stems, leaves, and pods using scissors, and the dry weights of the different organs were measured. Soybeans were collected at maturity and air dried. The samples were used to determine fatty acid composition (palmitic, stearic, oleic, linoleic, and linolenic acid), quality (protein, oil, K, and P), and yield (100-grain weight, number of pods per plant, and grain yield).

Soil and plant physicochemical analysis

For soil samples, the θ, γ_d, and pH were determined by the oven-drying method, cutting-ring method, and potentiometric method, respectively. The AK and AP contents were determined using flame photometry. TOC content was determined using the potassium dichromate method. Meanwhile, TN content was determined using an elemental analyzer (Flash EA 1112, Thermo Finnigan), and NH_4^+-N and NO_3^--N contents were determined using a continuous colorimetric flow system (Skalar SAN + + System, Netherlands) after extraction with 1 mol L^{-1} KCl for 1 h and filtration through Whatman quantitative filter paper.

Total plant P and K were determined using vanadium molybdate yellow colorimetry and flame photometry, respectively. In turn, plant protein content was determined using near-infrared spectroscopy. Fatty acid composition and oil content were determined according to

the Chinese National Standard Methods (GB/T 5510-2011 and GB/T 14488.1-2008). Plant height, stem diameter, and leaf area were measured at key reproductive stages using tape and Vernier calipers. Biomass of the different plant organs was measured using the oven-drying method. Grain yield was determined after two to three weeks of sun-drying to the standard 13.5% water content; in addition, 100-grain weight and the number of pods per plant were also obtained.

DNA extraction and real-time PCR

Soil DNA was extracted using a PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) following manufacturer instructions. Genomic DNA purity and quality were checked using 1% agarose gels. Quantitative PCR (qPCR) was performed using an ABI7500 fluorescence quantitative PCR instrument (Applied Biosystems, California, USA). Specific *nifH* genes of nitrogen-fixing bacteria were amplified using the primers *nifH-F* (5'-AAAGGYGGWATCGGYAARTCCACC AC-3') and *nifH-R* (5'-TTGTTSGCSGCRTACATSGCC ATCAT-3') [20]. The qPCR reaction system was as follows: 2×Taq MasterMix, 10 μl (Kang Wei Century, JiangSu, China), 10 μmol·L^{-1} PCR specific primers F and R 0.5 μl each. The ultrapure water was added to 18 μl, and the corresponding 2 μl DNA was added. The qPCR methodology followed the protocol described by Ma et al. [16]: 95 ℃, 30 s; 40 PCR cycles (95 ℃, 5 s; 60 ℃, 40 s). Standard curves of qPCR were generated using tenfold dilutions of plasmid DNA-containing target genes. All PCR procedures were performed in triplicate. The amplification efficiency was 90.1%, $R^2 = 0.99$.

Illumina Miseq sequencing and bioinformatics analysis

Purified PCR products obtained from all soil samples analyzed were sent to a biotechnology company (Aoweisen, Beijing, China) for high-throughput sequencing on an Illumina MiSeq platform. The Fastq data were demultiplexed and quality filtered by Trimmomatic (v0.36) and Pear (v0.9.6), and then merged using Flash (v1.20) and standard procedures. UPARSE [5] was used to cluster Operational Taxonomic Units (OTUs) at 97% similarity level. Alpha diversity indices were analyzed using the Mothur software. The OTU representative sequences were compared and analyzed by using RDP Classifier [24] algorithm with a confidence threshold of 70%. Taxonomic information at each level was obtained to investigate the correlation between sample composition and differences in community structure.

Statistical analysis

One-way ANOVA and Duncan's multiple comparison (LSD, $\alpha = 0.05$) were used in SPSS (v19.0) software to test the significance of differences in soil physicochemical properties, diazotroph abundance, and plant growth indices among the different biochar treatments. Correlation analysis was performed using Pearson's correlation analysis. Spearman's correlation was used to analyze the relationships among *nifH* gene abundance, diversity index, soil properties, and crop efficiency index. The Vegan package of R software was used to draw a heat map and Venn map to study the community composition and differences in diazotrophs. Based on the Bray–Curtis distance, principal coordinate analysis (PCoA) and similarity analysis (ANOSIM) were performed to estimate the differences in diazotroph community structure in different biochar treatments [3]. Analysis of the differential abundance of diazotrophic bacterial genera and OTUs using the ALDEx2 method was performed using Welch's *t*-test, and the Benjamini and Hochberg *p*-value correction was applied. Redundancy analysis (RDA) was carried out in Canoco 5.0 software after standardization of numbers to explore the relationship between the diazotrophic bacterial community structure, crop benefits, and environmental variables.

Results

Soil physicochemical properties and nutrient contents

Soil physicochemical properties and nutrient contents varied with the amount of biochar applied. As shown in Table 1, γ_d for the biochar-amended soils decreased by 2.19–6.57% compared with that for untreated soil. The higher the amount of biochar added, the lower the γ_d value. Conversely, θ, pH, TOC, AP, AK, and TN generally increased (0.58–3.46%, 0.25–0.86%, 17.79–58.72% ($p < 0.05$), 8.00–20.13% ($p < 0.05$), 0.10–0.88%, and 4.65–18.60% ($p < 0.05$), respectively), with increasing biochar amount, compared with untreated soil. However, NH_4^+-N and NO_3^--N contents did not increase linearly with biochar amount, and NH_4^+-N and NO_3^--N contents peaked in the B0 and B27 treatments, respectively.

Diversity and abundance of N-fixing microorganisms

Effects of biochar application on soil diazotrophic diversity index

Chao1 refers to the species richness index, which estimates the number of OTUs in the community. The Shannon and Simpson indices were used to estimate the diversity of the microorganisms in the samples. The good coverage of each sample was approximately 99%, which indicated that the amount of sequencing data was reasonable and large enough to reflect most microbial information in the samples. Compared with untreated soil, Chao1, Shannon, and Simpson index for biochar-amended soil decreased by 39.41–62.50%, 14.69–31.03%, and 1.04–15.63%, respectively. No significant decrease in

Table 1 Soil physicochemical properties and nutrient contents in biochar-amended soil

Treatment	Soil physicochemical properties			Soil nutrient contents					
	θ/%	γ_d/(g/cm^3)	pH	TOC/(g/kg)	AP/(mg/kg)	AK/(mg/kg)	TN/(g/kg)	NH_4^+-N/(mg/L)	NO_3^--N/(mg/L)
B27	17.90 ± 0.27a	1.28 ± 0.02c	8.23 ± 0.04a	13.38 ± 0.55a	18.02 ± 0.47a	152.17 ± 2.25a	1.02 ± 0.01a	1.23 ± 0.03b	0.38 ± 0.01a
B18	17.50 ± 0.02a	1.31 ± 0.02bc	8.18 ± 0.02b	11.43 ± 0.37b	17.99 ± 0.55a	151.25 ± 1.50a	0.95 ± 0.02b	1.21 ± 0.01b	0.24 ± 0.02c
B9	17.40 ± 0.28a	1.34 ± 0.02ab	8.15 ± 0.01b	9.93 ± 0.36c	16.20 ± 0.34b	151.00 ± 3.25a	0.90 ± 0.01c	1.13 ± 0.02c	0.32 ± 0.02b
B0	17.30 ± 0.06a	1.37 ± 0.03a	8.16 ± 0.01b	8.43 ± 0.32d	15.00 ± 0.53c	150.85 ± 1.87a	0.86 ± 0.02d	1.52 ± 0.02a	0.31 ± 0.01b

B27, 45 t hm^{-2} biochar addition; B18, 30 t hm^{-2} biochar addition; B9, 15 t hm^{-2} biochar addition, B0, no biochar added. θ, water content; γ_d, soil bulk density; TOC, total organic carbon; AP, available phosphorus; AK, available potassium; TN, total nitrogen. Values are means ± standard deviation. Different lowercase letters within columns indicate significant ($p < .05$) differences among treatments

Table 2 Soil diazotrophic diversity indexes for biochar-amended soil

Treatment	Chao1	Shannon	Simpson	Coverage/%
B27	637.36 ± 148.45b	4.70 ± 0.92c	0.81 ± 0.21b	99.58b
B18	394.50 ± 91.92c	4.60 ± 0.71c	0.82 ± 0.19b	99.82a
B9	407.30 ± 151.33c	5.69 ± 0.68b	0.95 ± 0.01a	99.81a
B0	1051.92 ± 191.03a	6.67 ± 0.99a	0.96 ± 0.02a	99.42c

B27, 45 t hm^{-2} biochar addition; B18, 30 t hm^{-2} biochar addition; B9, 15 t hm^{-2} biochar addition, B0, no biochar. The values represent average ± standard deviation. Different lowercase letters within columns indicate significant ($p < .05$) differences among treatments

Simpson index value was observed when a small amount of biochar was added. However, Chao1 and Shannon indices decreased significantly under these circumstances (Table 2).

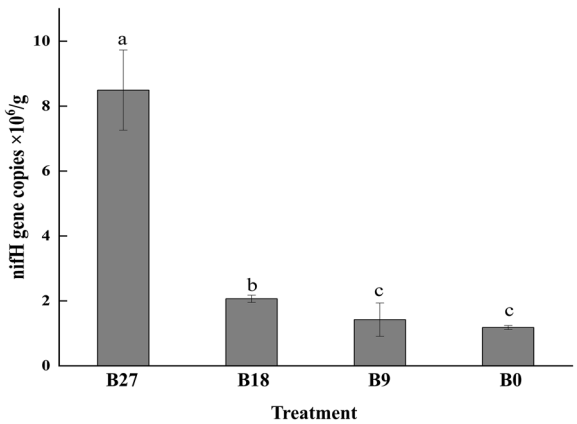


Fig. 1 Number of nifH gene copy for biochar-amended soils. B27, 45 t hm-2 biochar addition; B18, 30 t hm-2 biochar addition; B9, 15 t hm-2 biochar addition, B0, no biochar addition. Different lowercase letters indicate significant (p < .05) differences among treatments

Effects of biochar application on the copy number of soil N-fixing functional genes

Figure 1 shows that the number of *nifH* gene copies increased with increasing biochar application amount, and was approximately 4–6 times higher for the B27 treatment than for the other treatments. Significant ($p < 0.05$) differences in the number of *nifH* gene copies were observed among biochar-addition treatments. Furthermore, the effect of large amounts of biochar on *nifH* gene copy number was greater than that of small amounts of biochar. Overall, compared to biochar-unamended soil, biochar application promoted the abundance of soil N-fixing-related genes.

Community structure of N-fixing microorganisms

Effects of biochar application on microbial community species-composition

The species classification information corresponding to each OTU reflects the community structure of the soil samples at different levels. Figure 2 indicates that unamended and biochar-amended soils contained the same dominant species types (relative abundance > 1%), but with significant differences in species abundance distribution. As shown in Fig. 2, all N-fixing bacterial groups were clearly divided into five dominant phyla or 19 dominant genera. At the phylum level (Fig. 2a), the overall relative abundance of the *Proteobacteria* phylum was the largest, followed by the *Actinobacteria* phylum, ranging from 55.48–77.75% and 11.14–30.45%, respectively. More extensive biochar application led to an obvious increase in the relative abundance of *Proteobacteria* and *Verrucomicrobia*. Specifically, compared to the control, the relative abundance of *Proteobacteria* phylum in the B27 and B18 treatments increased by 20.93% and 29.07%, respectively, while that of *Verrucomicrobia* phylum increased by 329.70% and 92.53%, respectively. No significant differences in the abundance of any phylum were observed between the B9 and B0 treatments. In turn, at the genus level (Fig. 2b), the genera with an average

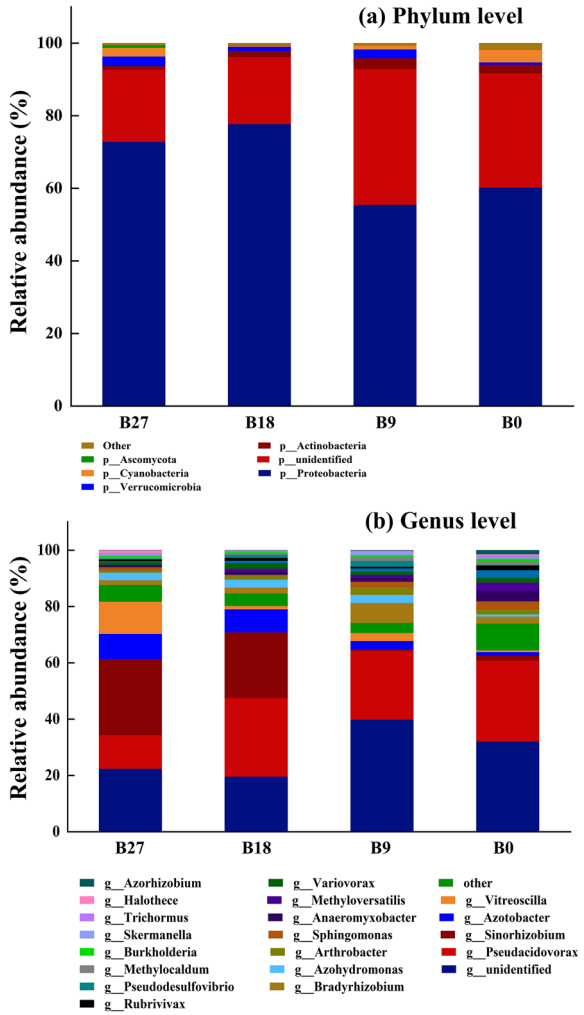


Fig. 2 Relative abundances (%) of the dominant diazotrophic across different biochar treatments. **a** Phylum level, **b** genus level. B27, 45 t hm^{-2} biochar addition; B18, 30 t hm^{-2} biochar addition; B9, 15 t hm^{-2} biochar addition, B0, no biochar addition

relative abundance greater than 5% included *Pseudacidovorax*, *Sinorhizobium*, and *Azotobacter*. Compared with unamended soil, the relative abundances of *Sinorhizobium*, *Azotobacter*, *Vitreoscilla*, *Azohydromonas*, and *Pseudodesulfovibrio* genera for biochar-amended soil treatments increased significantly, whereas those of *Skermanella* and *Halothece* increased slightly. Among the other genera with decreasing abundances, *Pseudacidovorax*, *Methyloversatilis*, and *Sphingomonas* were considerably less abundant. Cluster analysis (Additional file 1: Fig. S2) of the 20 dominant N-fixing microorganism communities further confirmed the high similarity in species composition among treatments as the amount of biochar applied increased.

Effects of biochar application on diazotrophs abundance differences

Differences in diazotroph abundance at the genus level were analyzed six times for the four treatment groups. As can be seen from Additional file 1: Fig. S3, the volcano plot comparing the B0 group with B9, B18 and B27, respectively, shows a larger absolute value of the horizontal coordinate of the scatter distribution, indicating a large difference in N-fixing bacteria between the biochar treatment and the control group. The higher the point on the graph, the smaller the value of the vertical coordinate p. Diazotrophs with significant differences ($p < 0.05$) between groups are highlighted by black dots. B9 compared to B0, with significant differences in abundance for *Skermanella*; Diazotrophic with significant differences in abundance at B18 compared to B0 included *Azorhizobium, Desulfovibrio, Pelomonas, Cylindrospermum, Azohydromonas, Trichormus*; Diazotrophs with significant differences in abundance between B27 and B0 were *Paraburkholderia, Oligotropha*, and *Skermanella*. There were fewer diazotrophic genera with significant differences between B9, B8 and B27 treated with biochar application, and no diazotrophic genera were detected with significant differences in abundance between the B18 and B27 groups.

Effects of biochar application on OTUs

Principal coordinate analysis was applied to evaluate variations in community composition among the different treatments. The first two principal components accounted for 46.68% of the total variance in the original datasets. Specifically, PC1 explained 29.87%, and PC2 explained 16.81% of the total variance in soils (Fig. 3a). The community composition for the treatment with a small amount of applied biochar (i.e., B9 treatment) was similar to that for the unamended soil. Furthermore, although the community composition for the B27 treatment was similar to that for the B18 treatment, it was significantly different from that for the unamended soil. ANOSIM also reflected significant differences in diazotrophic bacterial communities among the different biochar treatment groups (ANOSIM statistic, $R = 0.3248$, $P = 0.003$).

The clustering distances in the B27 and B18 treatments were similar, with the most significant proportion of OTU-1173. In contrast, those in the B9 and B0 treatments were similar and showed the largest proportion of OTU-1304 (Fig. 3b). A Venn diagram can be used to count the number of OTUs that are common and unique to multiple samples. This represents the overlap between groups of OTUs and their samples or subgroups in an environmental sample. There

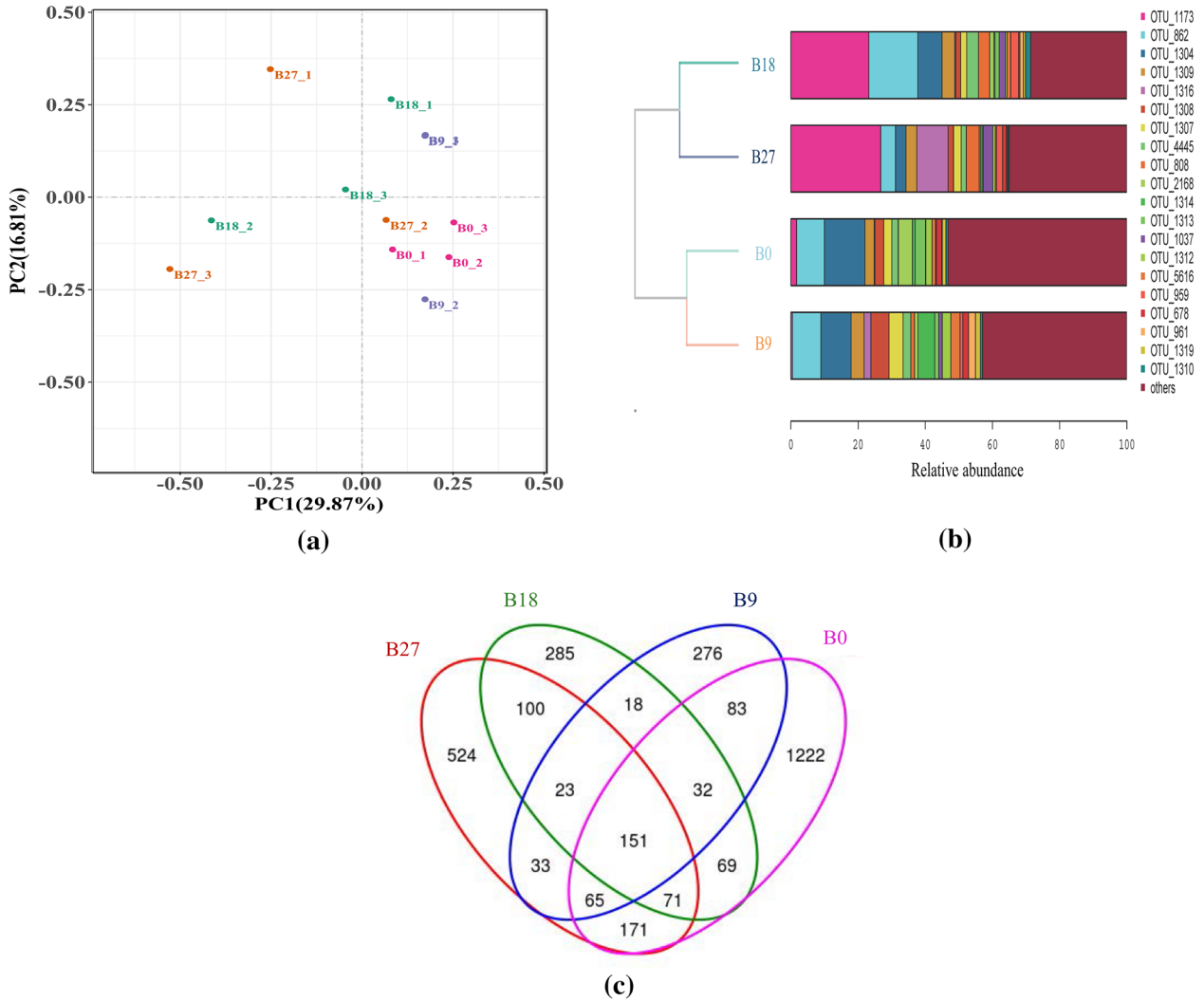


Fig. 3 Effect of biochar application on OTUs. **a** Principal coordinate analysis, **b** stacked bar chart with clustering tree, **c** Venn diagram. B0_1, B0_2, and B0_3 represent the three replicates of the B0 treatment, and other treatments are depicted in the same way. B27, 45 t hm^{-2} biochar addition; B18, 30 t hm^{-2} biochar addition; B9, 15 t hm^{-2} biochar addition, B0, no biochar addition

were 3123 OTUs from four treatments, and the number of OTUs shared by the four treatments was 151, accounting for 4.84% of the total. The number of OTUs accounted for 36.43% (1138), 23.98% (749), 21.81% (681), and 59.69% (1964) of the total for the B27, B18, B9, and B0 treatments, respectively. The proportion of the OTUs number of unique N-fixing bacteria reached 46.05% (524), 38.05% (285), 36.85% (276), and 62.22% (1222) for the B27, B18, B9, and B0 treatments, respectively; in addition, the number of OTUs that overlap reached 458, 331, and 323 in B27 and B0, B18 and B0, and B9 and B0 treatments, respectively (Fig. 3c). This finding demonstrated that the number of N-fixing bacteria first decreased and then increased with increasing biochar addition. No significant differences were observed in the proportions of common N-fixing bacteria between the biochar-amended and unamended soils.

Grain yield and quality

Biochar addition affected fatty acid composition, grain yield, and soybean quality (Table 3). Biochar application had no significant effect on the fatty acid composition of soybeans, which contained palmitic, stearic, oleic, linoleic, and linolenic acids, but neither palmitoleic nor myristic acid in 2020 or 2021. The addition of biochar had no significant effect ($p > 0.05$) on palmitic acid, linoleic acid, protein, or oil content, but significantly increased K and P content ($p < 0.05$) in the 2-year trial. The other quality indicators (stearic, oleic, and linolenic acids) did

Table 3 Effects of biochar on fatty acid composition, and grain yield and quality of soybean

years	Treatment	Fatty acid composition/%					Qualities/(g/100 g)				Yield		
		Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Protein	Oil	K	P	Pods per plant	100-grain weight/g	Grain yield/(kg/hm^2)
2020	B27	11.6 ± 0.26a	3.65 ± 0.06a	28.3 ± 0.23a	48.9 ± 1.05a	7.55 ± 0.15b	36.7 ± 0.5a	18.5 ± 0.50a	1.69 ± 0.03a	0.695 ± 0.01a	50.23 ± 2.26b	25.58 ± 0.69ab	2716.52 ± 18.21c
	B18	11.6 ± 0.25a	3.62 ± 0.12a	26.0 ± 0.14c	50.7 ± 1.13a	8.01 ± 0.21a	35.7 ± 0.4a	18.6 ± 0.60a	1.72 ± 0.07a	0.680 ± 0.01a	55.8 ± 2.12a	25.12 ± 0.41c	3006.78 ± 29.24b
	B9	11.7 ± 0.18a	3.59 ± 0.08a	26.4 ± 0.08b	50.4 ± 0.55a	7.93 ± 0.15ab	36.3 ± 0.7a	19.7 ± 0.50a	1.47 ± 0.06b	0.631 ± 0.02b	56.2 ± 3.33a	26.33 ± 0.42a	3885.46 ± 28.47a
	B0	11.5 ± 0.09a	3.60 ± 0.08a	26.6 ± 0.01b	50.4 ± 1.2a	7.91 ± 0.23ab	36.9 ± 0.5a	18.7 ± 0.60a	1.46 ± 0.04b	0.647 ± 0.02b	47.8 ± 3.29c	24.67 ± 0.31c	2695.36 ± 13.45c
2021	B27	10.3 ± 0.22a	3.73 ± 0.03ab	27.7 ± 0.04b	49.6 ± 0.4a	8.67 ± 0.20a	35.6 ± 0.6a	15.1 ± 0.20a	1.70 ± 0.05a	0.611 ± 0.01a	48.0 ± 3.06bc	24.56 ± 0.29c	2727.65 ± 17.75c
	B18	10.6 ± 0.13a	3.76 ± 0.06a	26.8 ± 0.02c	50.0 ± 1.0a	8.84 ± 0.04a	35.0 ± 0.3a	15.3 ± 0.80a	1.63 ± 0.04bc	0.582 ± 0.02bc	51.0 ± 2.65ab	24.27 ± 0.56c	3009.21 ± 36.42b
	B9	10.3 ± 0.16a	3.49 ± 0.08cd	28.0 ± 0.03a	49.4 ± 0.6a	8.81 ± 0.13a	35.0 ± 0.4a	14.5 ± 0.80a	1.67 ± 0.04ab	0.602 ± 0.01ab	51.8 ± 4.32a	27.64 ± 0.52a	4137.87. ± 34.34a
	B0	10.4 ± 0.17a	3.58 ± 0.09c	27.9 ± 0.02 ab	49.3 ± 0.7a	8.82 ± 0.17a	35.3 ± 0.3a	15.5 ± 0.64a	1.58 ± 0.09d	0.574 ± 0.02c	44.2 ± 3.51c	26.12 ± 0.29b	2728.6 ± 27.61c

B27, 45 t hm^{-2} biochar addition; B18, 30 t hm^{-2} biochar addition; B9, 15 t hm^{-2} biochar addition, B0, no biochar addition. θ, water content; γ_d, soil bulk density; TOC, total organic carbon; AP, available phosphorus; AK, available potassium; TN, total nitrogen. Values are means ± standard deviation. Different lowercase letters within columns indicate significant ($p < .05$) differences among treatments

not respond similarly to the biochar. Stearic acid reached its maximum level in the B18 treatment in 2021, whereas the effect was insignificant in 2020. In turn, linolenic acid content reached its maximum in the B18 treatment in 2020, whereas the effect was insignificant in 2021. Meanwhile, oleic acid content reached a maximum in the B27 treatment in 2020 and a maximum in the B9 treatment in 2021. In this study, the differences in grain yield among biochar treatments were significant ($p < 0.05$), which showed a "significant increase-significant decrease" tendency with increasing amount of biochar applied. The treatment with the highest yield was B9. The pattern of soybean yield under biochar treatment was similar in both years but was generally higher in the latter year.

Plant growth and biomass

Biochar addition affected plant height, stem diameter, leaf area index, and dry weight of different organs (Fig. 4). Plant height and stem diameter of the control group (i.e., without biochar application) were greater than those of the biochar treatment groups. This effect was reflected at different growth stages. Plant height and stem diameter followed the order B0 > B9 > B18 > B27. Considering the measured data in 2021 as an example, compared with the biochar treatment groups, plant height of the control group increased by 4.33–10.41 cm, and the stem diameter increased by 0.17–0.82 mm. In turn, leaf area index first increased and then decreased during the growth period, with that of the B9 treatment group being the largest. In the critical flowering and pod stages of soybean growth, the leaf area index followed the order B9 > B18 > B0 > B27. Lastly, at the early growth stage, the total dry weight was highest in soybean plants grown under treatment B0; however, at maturity stage, the dry weight of plants in group B0 was the lowest and that of plants in group B18 was the largest. The late increase rate of pod dry weight in the biochar treatment groups was 10.17%-30.5% higher than that in the control group, and the dry weight of other organs under different biochar treatments showed no obvious pattern.

Relationship between diazotrophic groups, grain yield and quality, and soil environmental factors

Redundancy analysis (RDA) was used to determine the relationship and influence of environmental factors such as physical and chemical indexes (θ, γ_d, pH) and nutritional indexes (TOC, AP, AK, TN, NH4+-N, NO_3^--N) with microbial community, grain yield, and quality. As can be seen in Fig. 5a, RDA analysis at the phylum level shows that axis 1 explains 21.82% and axis 2 explains 44.96%. The first three environmental factors influencing the community structure of nitrogen-fixing microorganisms are γ_d (F = 2.4, $p < 0.05$), TN (F = 2.9, $p < 0.05$), and AP (F = 3.2, $p < 0.05$). Meanwhile, RDA analysis at the genus level can be seen from Fig. 5b; which shows that axis 1 explains 25.1% and axis 2 explains 37.74%. The first three environmental factors influencing the community structure of N-fixing microorganisms are TN (F = 2.1, $p < 0.05$), TOC (F = 2.0, $p < 0.05$), and θ (F = 2.0, $p < 0.05$). Further, Fig. 5c shows that the environmental factors affecting grain yield and quality (2021) were TOC (F = 6.6, $p < 0.05$), pH (F = 3.0, $p < 0.05$), and NO_3^--N (F = 2.8, $p < 0.05$), with 23.74% explained on axis 1 and 42.34% explained on axis 2.

Table 4 lists the number of nifH gene copies correlated positively with θ, pH, TOC, TN, and NO3–N (r = 0.879, 0.890, 0.870, 0.881, and 0.710, respectively, $p < 0.01$) and AP, protein, and P (r = 0.633, 0.639, and 0.613, respectively, $p < 0.05$), but negatively with γ_d (r = − 0.663, $p < 0.05$). Diazotrophic diversity indexes all correlated positively with γ_d (r = 0.6, $p < 0.05$), in which case, Chao1 index correlated with NH4+-N (r = 0.911, $p < 0.01$) and protein (r = 0.606, $p < 0.05$), and Shannon index correlated positively with NH4+-N and linolenic acid (r = 0.618 and 0.610, respectively, $p < 0.05$) but negatively with TOC (r = − 0.581, $p < 0.05$).

Discussion

Effect of biochar on soil physicochemical properties

Biochar is widely utilized in agricultural production to improve soil habitats because of its high efficiency and environmental protection effects. Similar to the findings of Mao et al. [15], Tan et al. [23], and Zhang et al. [31], here, we found that biochar can loosen cultivated soils, further increasing porosity and water-holding capacity, which in turn enhances microbial activity. Furthermore, biochar addition maintains or promotes an alkaline soil environment, avoiding soil acidification and impoverishment resulting from the excessive application of fertilizer, which is, to some certain extent, beneficial to microbial growth [9]. In addition, biochar is rich in mineral nutrients, which may improve the absorption and availability of soil nutrients [2], showing an increasing trend towards greater TOC, AP, AK, and TN as the amount of biochar applied increases. However, as soil microorganisms are sensitive to changes caused by biochar in the soil environment, different types of bacteria involved in the N cycle can exhibit altered activities, leading to changes in the characteristics of the NH_4^+-N and NO_3^--N contents. The impact of biochar on soil N cycling is a complex issue that is affected by various factors including, soil type, biochar type, and number of years of biochar application. However, the underlying mechanisms require further investigation. Biochar can potentially increase crop yield and quality; however, excessive application may lead to reduced yield. Field studies should focus the

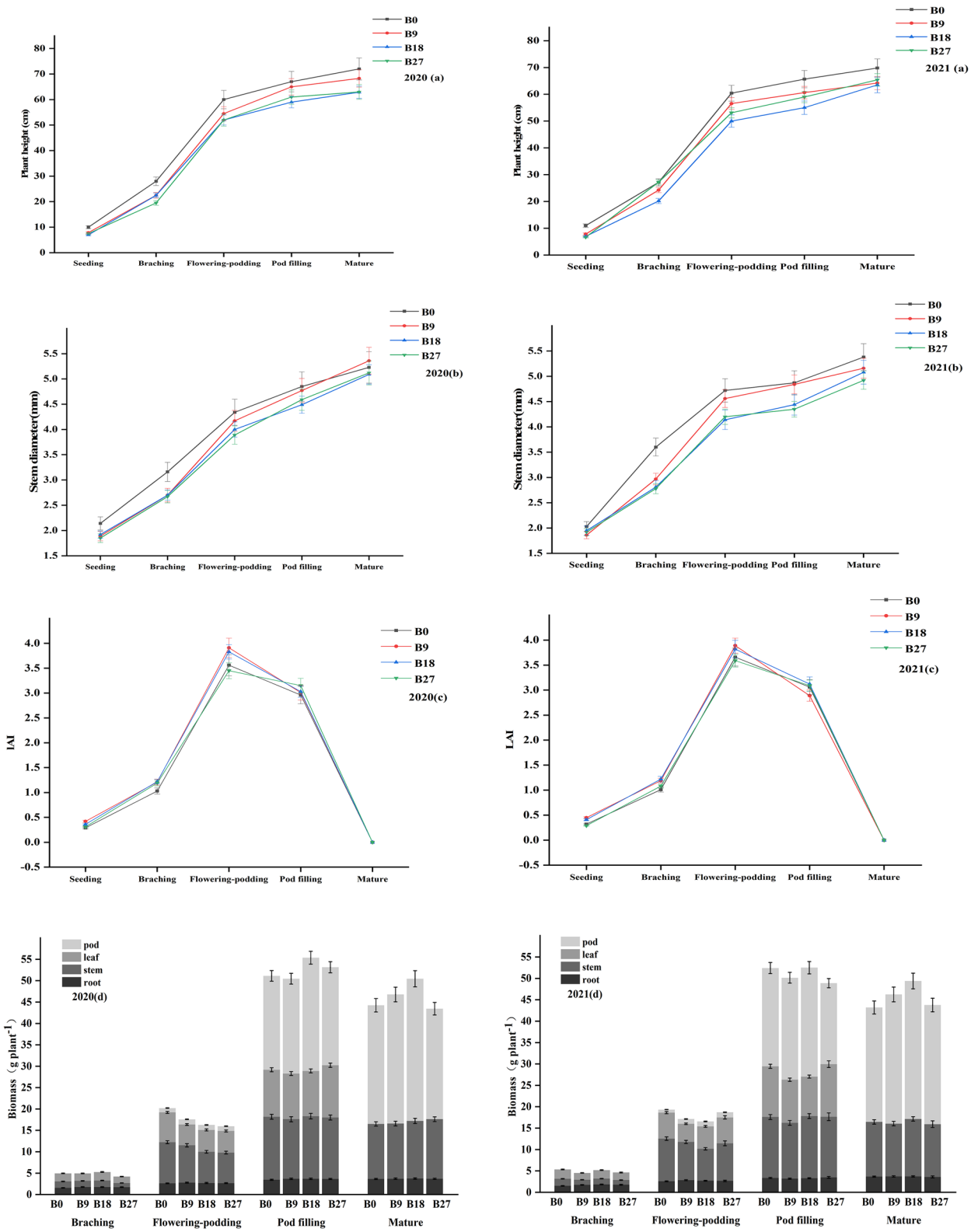


Fig. 4 Changes in plant height, stem thickness, leaf area index, and biomass of different organs under different biochar treatments in 2020 and 2021 at key soybean fertility stages. B27, 45 t hm^{-2} biochar addition; B18, 30 t hm^{-2} biochar addition; B9, 15 t hm^{-2} biochar addition, B0, no biochar addition

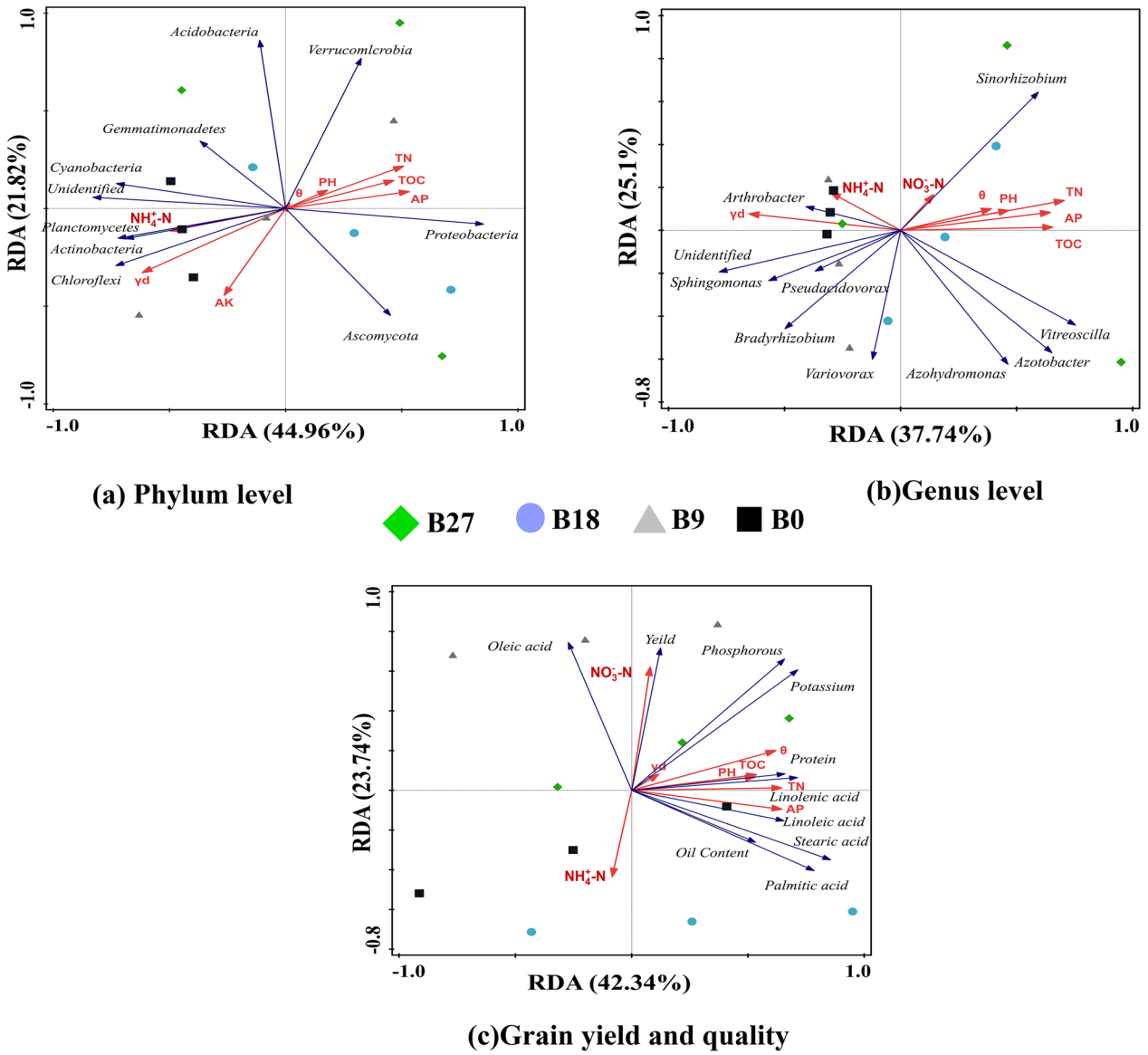


Fig. 5 RDA analysis of the relationships between diazotrophic groups, grain yield and quality, and soil environmental factors: **a** phylum level, **b** genus level, **c** grain yield and quality in 2021. B27, 45 t hm^{-2} biochar addition; B18, 30 t hm^{-2} biochar addition; B9, 15 t hm^{-2} biochar addition, B0, no biochar addition

soil–biochar–water interaction aiming to elucidate the mechanism underlying the influence of biochar in this respect.

Effect of biochar on diazotrophic community

This study indicated that biochar addition caused a reduction in the diversity of N-fixing microorganisms concomitant with an increase in *nifH* gene abundance. Applying biochar may change the C:N ratio. In this case, plants compete with microorganisms for nitrogen, which may severely limit the growth of some microorganisms. Consequently, the diversity of N-fixing microorganisms may decrease when the C:N ratio falls below a threshold, while some dominant N-fixing microorganisms are more likely to survive and reproduce, thus increasing *nifH* gene copies. Although biochar can improve soil physicochemical properties, it is necessary to comprehensively consider the source material and the biochar application amount and the combined application of organic/inorganic fertilizers, to avoid reducing microbial diversity with excess biochar application.

Table 4 Pearson correlation coefficients (*r*) between soil properties and crop efficiency indexes, and *nifH* gene copies and microbial diversity indexes

Correlation	nifH gene copies	Chao1	Shannon	Simpson
γ_d	− 0.663 *	0.620 *	0.936 **	0.700 *
θ	0.879 **	0.017	− 0.185	− 0.056
pH	0.890 **	0.143	− 0.153	0.058
TOC	0.870 **	− 0.358	− 0.581 *	− 0.335
AP	0.633 *	− 0.458	− 0.556	0.274
AK	0.379	0.131	0.130	0.364
TN	0.881 **	− 0.294	− 0.542	− 0.331
NH_4^+-N	− 0.201	0.911 **	0.618 *	0.278
NO_3^--N	0.710 **	0.296	0.125	0.140
Palmitic acid	− 0.142	0.192	0.324	0.381
Stearic acid	0.458	0.079	− 0.102	− 0.041
Oleic acid	0.039	0.434	0.563	0.435
Linoleic acid	0.076	− 0.062	0.045	0.398
Linolenic acid	− 0.204	0.308	0.610 *	0.460
Protein	0.639 *	0.606 *	0.390	0.424
Oil	0.025	0.513	0.418	0.218
K (plant)	0.549	− 0.190	0.012	0.166
P (plant)	0.613 *	− 0.100	0.055	0.232
Grain yield	− 0.352	− 0.379	0.257	0.400

B27, 45 t hm^{-2} biochar addition; B18, 30 t hm^{-2} biochar addition; B9, 15 t hm^{-2} biochar addition, B0, no biochar addition. θ, water content; γ_d, soil bulk density; TOC, total organic carbon; AP, available phosphorus; AK, available potassium; TN, total nitrogen. * and ** indicate significant ($p < .05$) and highly significant ($p < .01$) difference, respectively

Biochar influences the composition and structure of diazotrophs by altering the cycling and supply of nutrients in biochar-amended soils [13]. Here, we found that biochar led to an obvious increase in the abundance of two phyla and three genera, of which the *Proteobacteria* and *Sinorhizobium* genera belong to the rhizobial system, and their increasing abundance was beneficial to symbiotic N fixation by the soybean–rhizobia association. Soybean plants require large amounts of N, which is biologically fixed by free and symbiotically associated N-fixing microorganisms living in the soil. Biochar increases the biological N-fixation activity by improving soil fertility and the activity of N-fixing microorganisms, thereby creating a propitious inter-root environment for crop growth.

Linking rhizospheric soil properties to diazotrophic community and soybean growth in biochar-amended soil

Xu et al. [29] pointed that soil environmental factors drive the composition and distribution of the microbial community and that the structure of the microbial community changes with the soil physical and chemical properties. Our RDA analysis indicated that biochar addition changed the physicochemical factors in the soybean rhizosphere, which in turn affected the community of N-fixing microorganisms, with significant contributions of γ_d, θ, AP, TN, and TOC; additionally, pH, TOC, and NO_3^--N were the main contributors to soybean yield and quality. Thus, soil environmental factors have various impacts on the microbial community and crop benefits derived thereof. Furthermore, among multiple indices, soil C and N contents, and soil structure reportedly have the greatest effects [6, 32]. Application of biochar to the soybean rhizosphere can improve the soil physicochemical properties and microbial communities, as well as the relationships between them. Further research should focus on their quantitative contributions to soybean yield and quality, based on reported findings.

Conclusions

As an effective soil amendment, biochar can improve the soil environment in the soybean rhizosphere. Biochar significantly increased TOC, TN, and AP. Biochar addition altered the community structure of N-fixing microorganisms. Specifically, compared with unamended soil, biochar soil-amendment reduced the diversity of N-fixing microorganisms but increased *nifH* gene abundance. The microbial community remained stable under a small amount of biochar, but changed with increasing amounts of biochar application.

Furthermore, although biochar application reduced the proportion of unique N-fixing bacteria, it did not affect that of common N-fixing bacteria. The main components and contents of fatty acids and protein and soybean oil generally remained stable with biochar addition within a single growing season. The grain yield for the B9 treatment increased by 51.65% compared with the control treatment, indicating that 15 t hm^{-2} is a suitable amount of biochar to add to the soil. RDA and correlation analysis suggested that TN had a significant influence on diazotrophic community structure at both phylum and genus levels; furthermore, pH, TOC, and NO_3^--N had significant influences on grain quality and yield. The findings reported herein were recorded for alkaline soils. Specific microbial communities contributing to grain yield and quality and the corresponding relevant mechanisms need to be experimentally elucidated for acidic soils. Finally, future field studies should also consider different forms and dosages of biochar.

Abbreviations

γ_d	Soil bulk density
TOC	Total organic carbon
TN	Total nitrogen
AP	Available phosphorus
AK	Available potassium
θ	Water content

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-023-00429-0.

Additional file 1: Figure S1. Daily maximum and minimum temperatures and precipitation for the summer soybean-growing season in 2020 and 2021. **Figure S2.** Clustering variability of N-fixing microorganisms for biochar-amended soil. B0_1, B0_2, and B0_3 represent the three replicates of the B0 treatment. Similarly for the rest of the treatments. **Figure S3.** Genus-level ALDE × 2 differential abundance test volcano plots. Features that are significantly different ($p<0.05$) are highlighted in black and marked. B27, 45 t hm^{-2} biochar addition; B9, 15 t hm^{-2} biochar addition, B0, no biochar addition.

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Author contributions
WW contributed to the design of experiments and manuscript writing. FS contributed to the analysis and interpretation of data. JD and LL finished the experiments and contributed to the data acquisition. TB contributed to the investigation, and XX was involved in drafting the manuscript and revising it critically for important intellectual content. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
The Northwest A&F University and the Kunming University of Science and Technology are aware of the cooperation and every co-author approved this submission.

Competing interests
The authors declare no competing interests.

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