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Exogenous glucose modulated the diversity of soil nitrogen-related bacteria and promoted the nitrogen absorption and utilisation of peanut

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Abstract: Exogenous carbon (C) not only regulates plant growth but also provides energy for microbes and improves the soil environment. We hypothesised that exogenous C could improve plant growth by affecting the soil environment. Therefore, pot experiments were conducted and peanut cvs. Huayu 22(H) and NN-1(B) were used under three different treatments (the control, single nitrogen (N), and N combined with glucose (CN)). The results showed that the abundance and diversity of N-fixing bacteria are obviously influenced by the C and N, and exogenous C can promote the restoration of microbial diversity. The relative abundances of Burkholderiales were increased under HCN and BCN to 9.8% and 9.5%, respectively, compared to the control (3.9%, 2.5%). The abundance of N fixation bacteria increased mainly due to the soil nutrient change. In comparison with the single N treatment, the addition of the C significantly decreased the soil NH_4^+-N and NO_3^--N contents by 31.0% and 13.3%, respectively. And the activities of soil urease and nitrogenase were significantly increased. Compared to the control, single N significantly limited the root development, while the addition of C played a promoting role in root growth. Plant N accumulation increased compared with the control, but there was no significant difference between N treatment and CN treatment. These results indicated that exogenous C promoted soil microorganism activity and strengthened plant growth by changing the soil environment.

Keywords: *Arachis hypogaea* L.; legume; macronutrient; microbial community

Peanut (*Arachis hypogaea* L.), a typical and economically important legume, has industrial advantages in ensuring the safety of edible oil in China (Fabra et al. 2010, Shen et al. 2019). Legume is very sensitive to nitrogen (N). N is one of the essential elements for peanut growth and development. Generally, N nutrition not only directly affects plant N metabolism but also affects the formation of peanut morphological organs and biological N fixation, ultimately affects peanut yield and grain quality (Furlan et al. 2017). N fertiliser also had a significant effect on soil carbon (C), N availability and its' balance, which was crucial to soil quality and crop nutrient absorption. However, increasing N rates is unlikely to be effective in increasing yields, as N use efficiency declines at high N levels, especially inhibition of the N fixation of legume (Tilman et al. 2002). In addition, imperfect fertiliser management

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results in inconsistent and inappropriate application of fertiliser in agricultural production, with low N use efficiency and soil degradation. At present, soil degradation has become increasingly serious, with the decline of soil organic C and C/N ratio. How improve the quality of cultivated land and promote crop growth is an urgent problem for agricultural production.

Studies have shown that exogenous C sources such as sucrose and glucose play an important role in the coordination of soil nutrients and crop growth. Adding an appropriate proportion of C can not only increase the storage of soil organic C and improve soil productivity but also provide C source for microbial growth, stimulate microbial growth and metabolism, as well as improve soil enzyme activity (Ning et al. 2021), finally promote nutrient absorption in roots and plant (Lau and Lennon 2012, Sun et al. 2022). As an organic amendment, it can improve soil physiochemical properties and plant growth and is an important complementary process to soil C loss (Hansen et al. 2017, Han et al. 2018). It can also provide a large amount of C source for soil microorganisms, especially for nitrogen-fixing bacteria, which are very sensitive to the soil environment. Earlier studies have shown that organic amendment, in addition to impacting the microbial community (Cui et al. 2022), also alters the composition of soil microbial communities, such as *Firmicutes* (Tian et al. 2015), *Actinobacteria* (Cui et al. 2022), and *Acidobacteria* (Wang et al. 2021). However, research has proved that continuous years of N fertilisation resulted in an increase in soil ammonium N (NH_4^+-N) and nitrate N (NO_3^--N) residual, significantly reducing microbial biodiversity and inhibiting the growth of some N fixation bacteria. In contrast, when a small amount of N input with an increasing C/N ratio, this condition was more favourable to the growth of N fixation bacteria (Roesch et al. 2006, Coelho et al. 2008). Liao et al. (2017) and Rahav et al. (2016) confirmed that single organic fertiliser or mineral fertiliser in combination with inorganic fertiliser was beneficial to the maintenance of soil N fixing microbial function. Furthermore, many studies reported that different kind of organic materials performed differently on crop growth and soil nutrients. And differences in soil parameters can strongly influence the microbial community's activity, which may mediate plant genotype effects on microbial communities and related nutrient cycling (De Vries et al. 2017). While there is a lack of information on different genotype cultivars of peanuts under different organic materials. It is necessary to understand whether crop traits differ among genotypes in ways that influence their performance under organic addition.

Carbon plays a pivotal role in plant nutrient balance; optimum soil C/N ratio can regulate plant growth and development. In the comparison of complex structure organic materials (straw or organic manure), the C sources (glucose or sucrose) with a simple structure can rapidly deplete NH_4^+ and then enhance microbes to assimilate more NO_3^- in the soil. While there is no clear understanding of how carbon regulates peanut growth and the soil environment. Therefore, this work aims to investigate the response of peanut growth, root development, nitrogen absorption and soil bacteria under exogenous C supply with different cultivars (Figure 1). Our work can further predict the regulation of exogenous C on plant growth and development, dynamics of microbial communities and soil environment in agricultural ecosystems.

MATERIAL AND METHODS

Study site and experimental design. The experiment was conducted from June to September 2021 in Laixi Experimental Station, Shandong Peanut Research Institute (36°48'47''N, 120°30'17''E). The climate is temperate monsoon with an average air temperature of 11.7 °C and an annual rainfall of 635.8 mm. The soil is classified as alfisol. And the soil used for the pot experiment was collected at 0–20 cm depths from agricultural fields. The collected soil was acidic (pH = 5.9), contained a total N 0.9 g/kg, organic carbon 9.7 g/kg, available P 96.7 mg/kg and available K 79 mg/kg. The soil pH was measured with a compound electrode (PE-10, Goettingen, Germany) using a soil-to-water ratio of 1:2.5. Soil organic carbon was analysed by oxidising organic C with potassium dichromate ($K_2Cr_2O_7$). The total N was measured using the Kjeldahl method. K content was analysed flame-photometrically. P content was extracted from 0.5 mol/L $NaHCO_3$ and then measured by the molybdenum antimony colourimetric method. Peanut seeds cvs. Huayu 22 (H) and NN-1(B) were surface disinfected and then placed in plastic pods (37-28-34 cm, top diameter-bottom diameter-height). Each of the four seeds was planted into a pot containing 20 kg of soil. After emergence, three seedings per pot were selected. At the seeding stage (30 days after emergence), we applied C and N with equal pure P and K levels (0.9 g P/pot, 0.9 g

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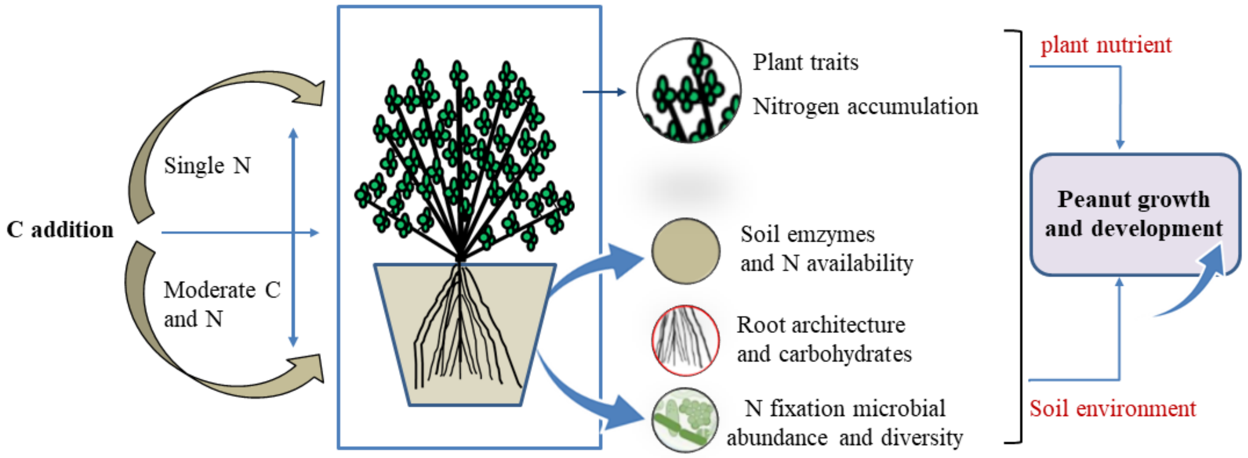


Figure 1. Concept diagram of the effect of exogenous carbon (C) on the soil-microorganism-peanut systems

K/pot). Hereafter, three treatments will be referred to by abbreviations based on different fertilisation strategies: no N and C fertilisation (H0 and B0); only mineral N fertilisation (HN and BN, 0.9 g N/pot); organic material and N addition with C/N ratio of 5 (HCN and BCN, 0.9 g N/pot, 11.25 g glucose/pot)). The organic materials C (glucose) and N (urea) were used. All the urea and glucose were added to the soil as a water solution. During the growing stage, all the pots were irrigated the equal water to keep a reasonable soil water content, according to 70% of field water capacity. Three replicate samples were prepared for each treatment. The peanuts were cultured in large waterproof sheds for three months before being harvested. At the maturity stage, plant pods, stems and leaves, and roots were harvested and were then rinsed with water to remove any attached soil. Subsequently, the samples were divided into two parts, with one part to measure plant height, branch number, number of leaves in the main stem, lateral branch length, root morphology and root enzyme activity. And the other part was dried to measure their biomass, N content, and carbohydrate contents (in roots). The soil in each pot was also collected: one fraction of fresh soil was passed through a sieve with a 2-mm mesh size and used in the measurement of soil content ammonium N (NH_4^+-N), nitrate N (NO_3^--N) and soil enzyme activity; and the other fraction was immediately frozen at –80 °C and used for DNA extraction to determine nitrogen-fixing bacteria community structure.

Sample analysis. The total N content of organs in the plant was analysed using the Kjeldahl method (Wang et al. 2017b). Root morphology was scanned by the scanner and analysed using the WinRHIZO root analysis system. The carbohydrate contents were measured by using an enzyme reagent kit. The ammonium N (NH_4^+-N) and nitrate N (NO_3^--N) levels were analysed following extractions of fresh soil with 2 mol/L KCl for 0.5 h and using a continuous flow analyser (Jones and Willett 2006). Soil urease and nitrogenase activities were assayed as described by Gong et al. (2019).

The soil microbial DNA was extracted from 1.0 g of fresh soil three times using the Power Soil TM kit (QIAGEN Laboratories, Carlsbad, Germany) according to the instructions. The universal prokaryote primers POI F(5'-TGCGAYCCSAARGCBGACTC-3') and POI R(5'-ATSGCCATCATYTCRCCGGA-3') were used to analyse the nifH gene. This primer set provides comprehensive coverage with the highest taxonomical accuracy for bacterial sequences. Paired-end sequencing of the nifH gene amplicons was carried out using Illumina NovaSeq 6000 platform at Biomarket Biotechnology Co., Ltd. (Beijing, China). The 16S rRNA gene sequences obtained in this study have been deposited in the NCBI Sequence Read Archive (SRA) database with accession number BioSample SAMN29628703–SAMN29628708, BioProject PRJNA857514.

Statistical analysis. The data were expressed as means ± standard errors of triplicate measurements. And all the data were analysed using one-way analysis of variance (ANOVA), followed by Fisher's least significant differences (*LSD*) test to determine the differences between the individual treatments (SPSS 23.0 for Windows, IBM Corp., Chicago, USA). *P*-values of < 0.05 were considered to be significant.

Table 1. Analysis of α diversity of soil nitrogen-fixing bacteria communities in different treatments

Treatment	ACE	Chao1	Simpson index	Shannon index
B0	266.905^{aA}	268.633^{aA}	0.976^{aA}	6.644^{abA}
BN	149.159^{cB}	146.5^{cC}	0.939^{aA}	5.585^{cA}
BCN	227.625^{bA}	227.542^{bB}	0.969^{aA}	6.481^{abA}
H0	288.446^{aA}	288.399^{aA}	0.985^{aA}	6.793^{aA}
HN	166.597^{cB}	172.944^{cB}	0.962^{aA}	5.934^{bcA}
HCN	297.841^{aA}	306.389^{aA}	0.982^{aA}	6.687^{abA}

Lowercase letters in the same column indicate a significant difference level of 0.05 among all the treatments. Uppercase letters in the same column indicate a significant difference level of 0.05 among the treatments within the same cultivar. B – peanut cv. NN-1; H – peanut cv. Huayu 22; B0 and H0 – no C and N sources applied; BN and HN – single N source applied; BCN and HCN – C and N source all applied

RESULTS AND DISCUSSION

Exogenous C and N increased microbial diversity. Analysis of the response of exogenous C on soil microbial diversity and abundance (Table 1) revealed that the average Shannon index of the soil bacterial communities under BCN (6.481) and HCN (6.687) treatment were higher than under BN (5.585) and HN (5.934). Compared to the control, a single N addition decreased the microbial diversity. While there was no significant difference under different treatments of the same cultivar for the Simpson and Shannon index. The ACE and Chao1 index bacterial communities significantly differed between the treatments. The results indicated that the abundance and diversity of N-fixing bacteria are obviously influenced by the C and N sources, and exogenous C can promote the restoration of microbial diversity. The 16S OTUs (operational taxonomic units) exhibited similar changes in the soil of treatments (Figure 2). There were unique 16S OTUs under CN treatments (BCN:70 OTUs; HCN:65 OTUs) than under single N application. Of the 16S data reads in this study, the bacterial sequences were mainly affiliated with 8 orders (Figures 3A, B). Among the identified order, Myxococcales (10.76–18.14%) and Rhizobiales (16.76–20.78%) were the most abundant taxa. And at the family level, Bradyrhizobiaccae (12.48–21.10%), Anaecromyxobacteraceae (9.89–18.44%) and Zoogloeaceae (3.99–6.47%) were the most abundant taxa. In N-fixing bacterial communities, the relative abundance of major bacterial order Rhizobiales and Burkholderiales increased under the CN treatments compared to the control,

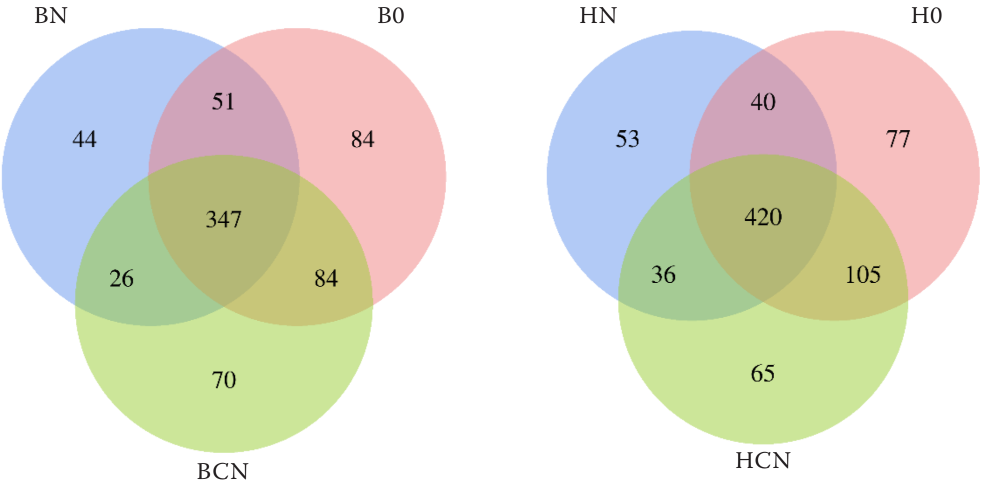


Figure 2. 16S OTUs (operational taxonomic units) Venn diagrams of peanut soil in different fertiliser treatments. B – peanut cv. NN-1; H – peanut cv. Huayu 22; B0 and H0 – no C and N sources applied; BN and HN – single N source applied; BCN and HCN – C and N source all applied

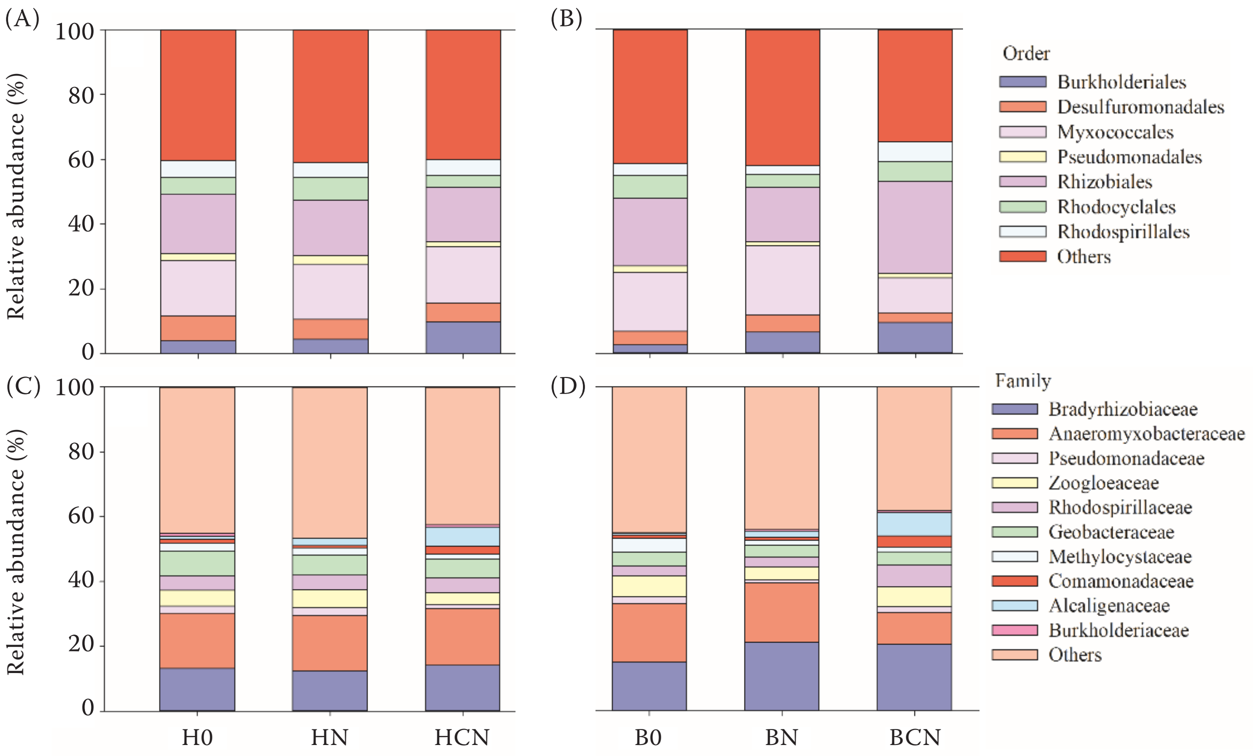
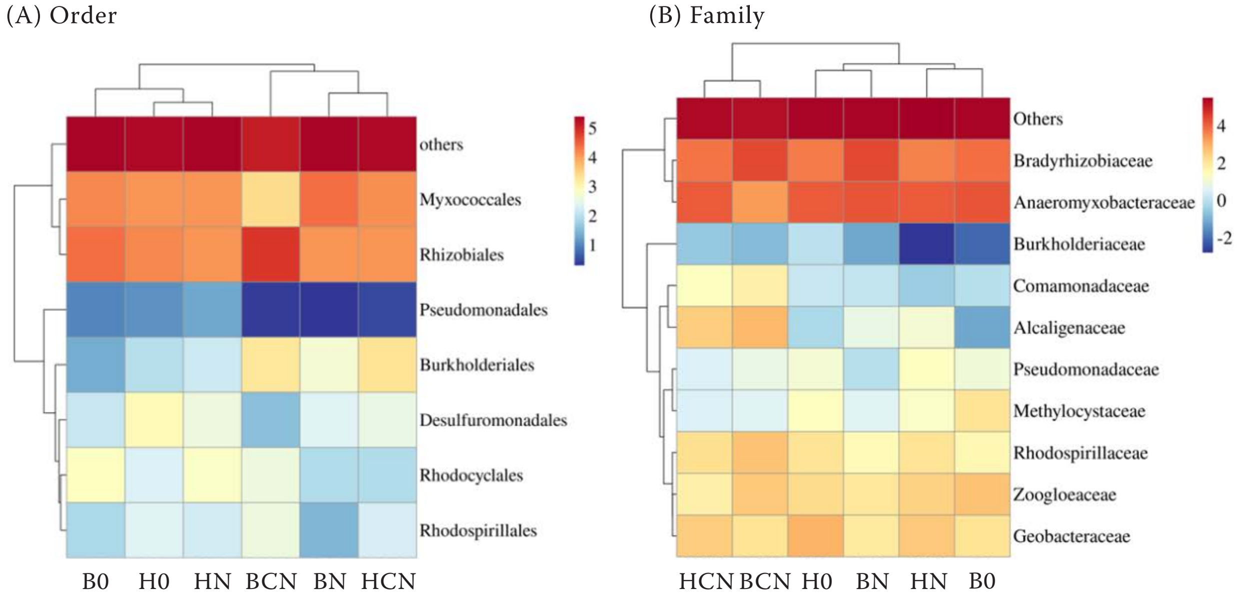


Figure 3. Relative abundance of the major order and family in each treatment. The relative abundance is the percentage of the total number of sequences in each treatment. Order and family with relative abundance < 1% are summed as "others". B – peanut cv. NN-1; H – peanut cv. Huayu 22; B0 and H0 – no C and N sources applied; BN and HN – single N source applied; BCN and HCN – C and N source all applied

whereas the order Myxococcales showed a decreasing trend (Figures 3A, B). The major bacteria of the family Bradyrhizobiaceae and Alcaligenaceae had an increased relative abundance trend. However,


(A) Order

(B) Family

Figure 4. Heatmap of the dominant nitrogen fixation bacterial order and family in different treatments. B – peanut cv. NN-1; H – peanut cv. Huayu 22; B0 and H0 – no C and N sources applied; BN and HN – single N source applied; BCN and HCN – C and N source all applied

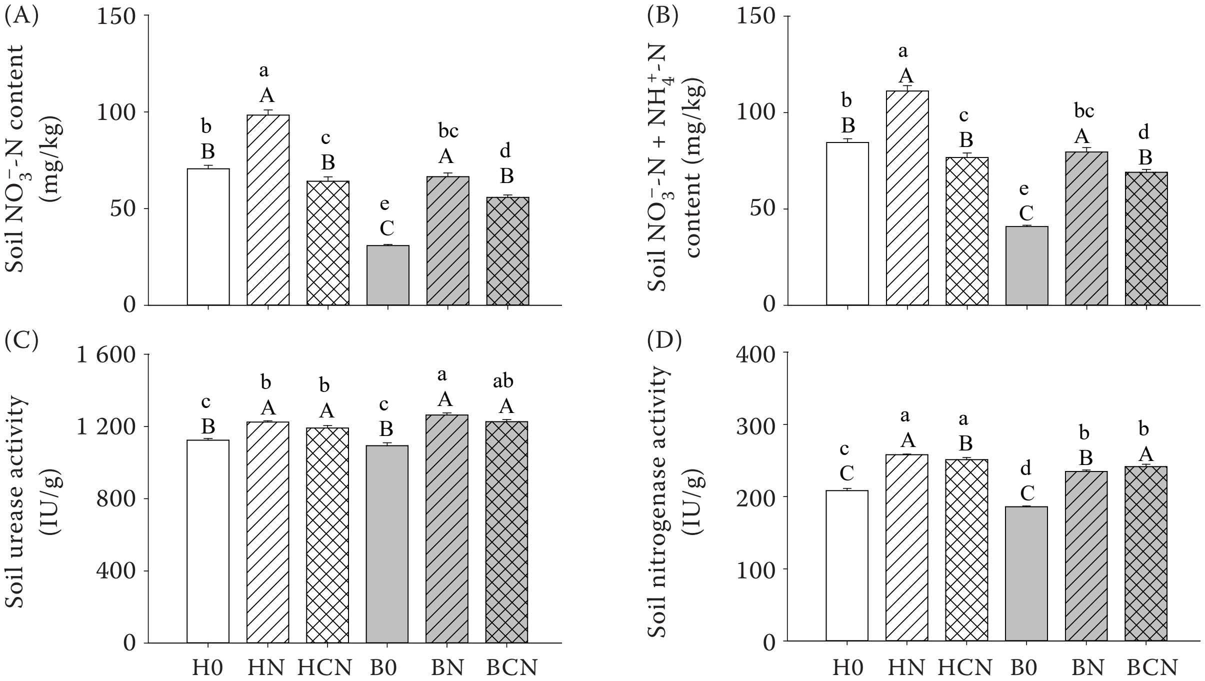


Figure 5. Effects of glucose addition on soil content of NH_4^+-N and NO_3^--N (A, B) and soil enzyme activities (C, D). B – peanut cv. NN-1; H – peanut cv. Huayu 22. B0 and H0 – no C and N sources applied; BN and HN – single N source applied; BCN and HCN – C and N source all applied. Lowercase letters indicate a significant difference level of 0.05 among all the treatments. Uppercase letters indicate a significant difference level of 0.05 among the treatments within the same cultivar

the family Anaeromyxobacteraceae was compared with the control (Figure 3C, D). This indicated that the abundance of dominant N fixation bacteria was affected significantly by the C and N sources.

The N-fixing bacteria with a high relative abundance are shown in Figure 4. The relative abundances of order Rhizobiales and Burkholderiales were increased under HCN and BCN. The relative abundances of Rhizobiales and Myxococcales were higher under H0 and B0 treatments (Figure 4A). Regarding the relative abundance of bacterial family communities, the Bradyrhizobiaceae and Alcaligenaceae were increased under HCN and BCN (Figure 4D). Under B0, H0 and BN, HN treatments, the abundance of Zoogloeaceae and Anaeromyxobacteraceae was increased and higher than in other treatments.

Effects of C and N addition on available N and soil enzyme activities. Compared to the control, two cultivars of H and B showed a similar trend in different treatments. The contents of NH_4^+-N and NO_3^--N were higher under single N treatment compared to the control and the CN treatments. The total content of NH_4^+-N + NO_3^--N increased by 31.7% and 94.6% in HN and BN treatment, respectively, compared to the control. While addition, the C significantly decreased the soil NH_4^+-N + NO_3^--N contents by 31% and 13.3%, compared to single N treatment (Figure 5A,B). Compared to the control treatment, soil urease and nitrogenase activities were significantly increased in N and CN treatments. It significantly increased by 8.9, 6.0, 15.6 and 12.2%, respectively. These results may indicate that an additional C source could activate soil N effectively to improve plant uptake and decrease the residual N.

Effects of C and N addition on root morphology, root enzyme activities and carbohydrate. According to Figure 6, the root length, root surface area, root volume, and root tip number were the highest in the control treatment. The addition of a single N source significantly reduced the root length, root surface area, root volume and root tip number of the peanut, while the addition of C played an important role in promoting the root growth of the peanut (Figure 6A–D). For peanut B, adding C and N had no obvious effects on root morphology. While for peanut H, exogenous C significantly improved root surface area and root volume. We also investigated whether C addition influences the activities of root nitrogenase and catalase (Figure 6E–H).

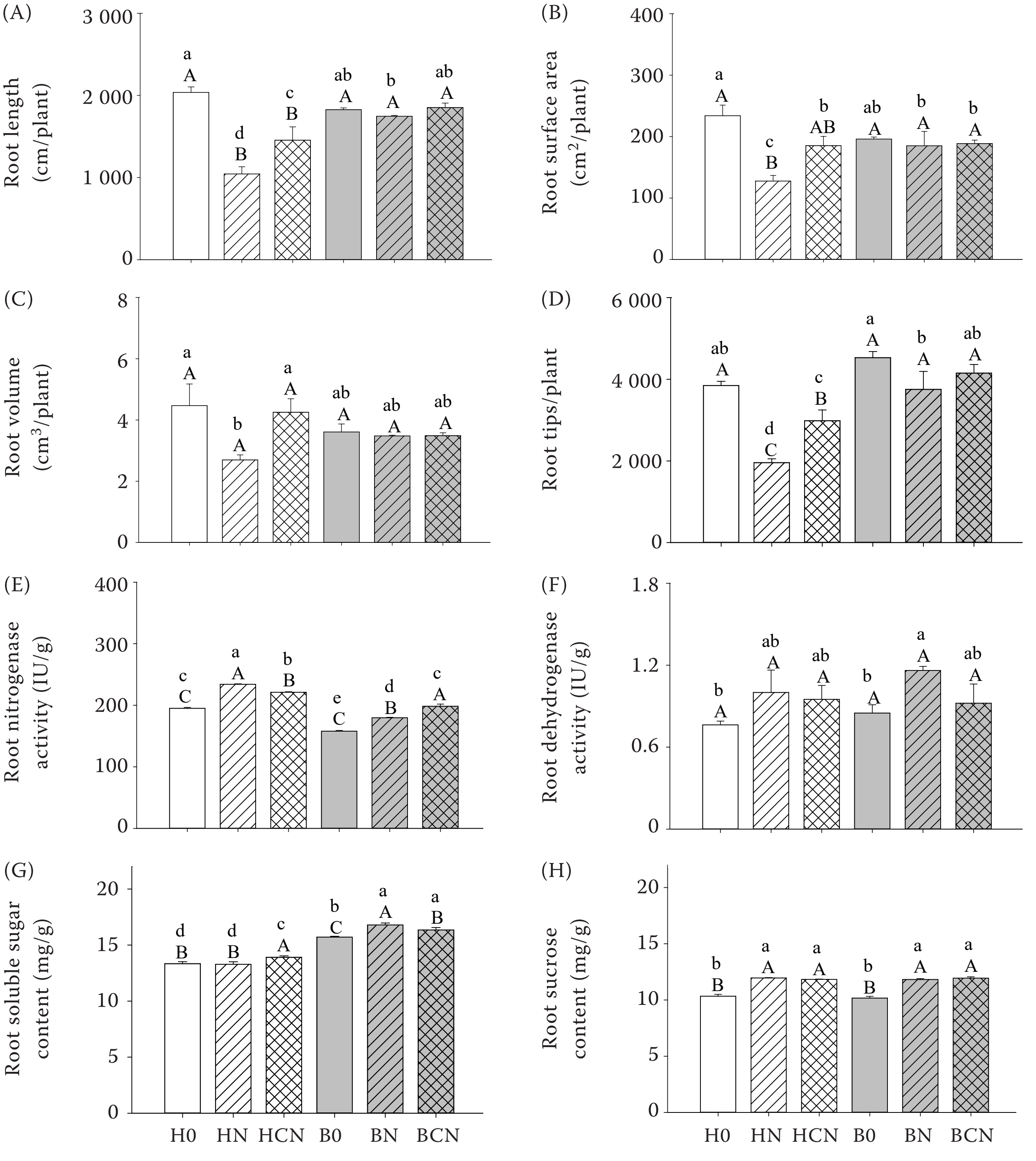


Figure 6. Effects of glucose addition on root morphology, root enzyme activities and nonstructural carbohydrate content. B – peanut cv. NN-1; H – peanut cv. Huayu 22. B0 and H0 – no C and N sources applied; BN and HN – single N source applied; BCN and HCN – C and N source all applied. Lowercase letters indicate a significant difference level of 0.05 among all the treatments. Uppercase letters indicate a significant difference level of 0.05 among the treatments within the same cultivar

It showed that the root nitrogenase and catalase had an increasing trend under different treatments, and the single N treatment was the highest among all the treatments. The root nitrogenase increased by 20, 13.3, 13.9 and 25.6% compared to control, respectively, and the catalase increased by 31.1, 24.5, 36.7 and 8.4%. In comparison to the control, the contents of sucrose and soluble sugar in roots were higher in other treatments, and there was no significant difference between single N and CN treatment (Figure 6G,H).

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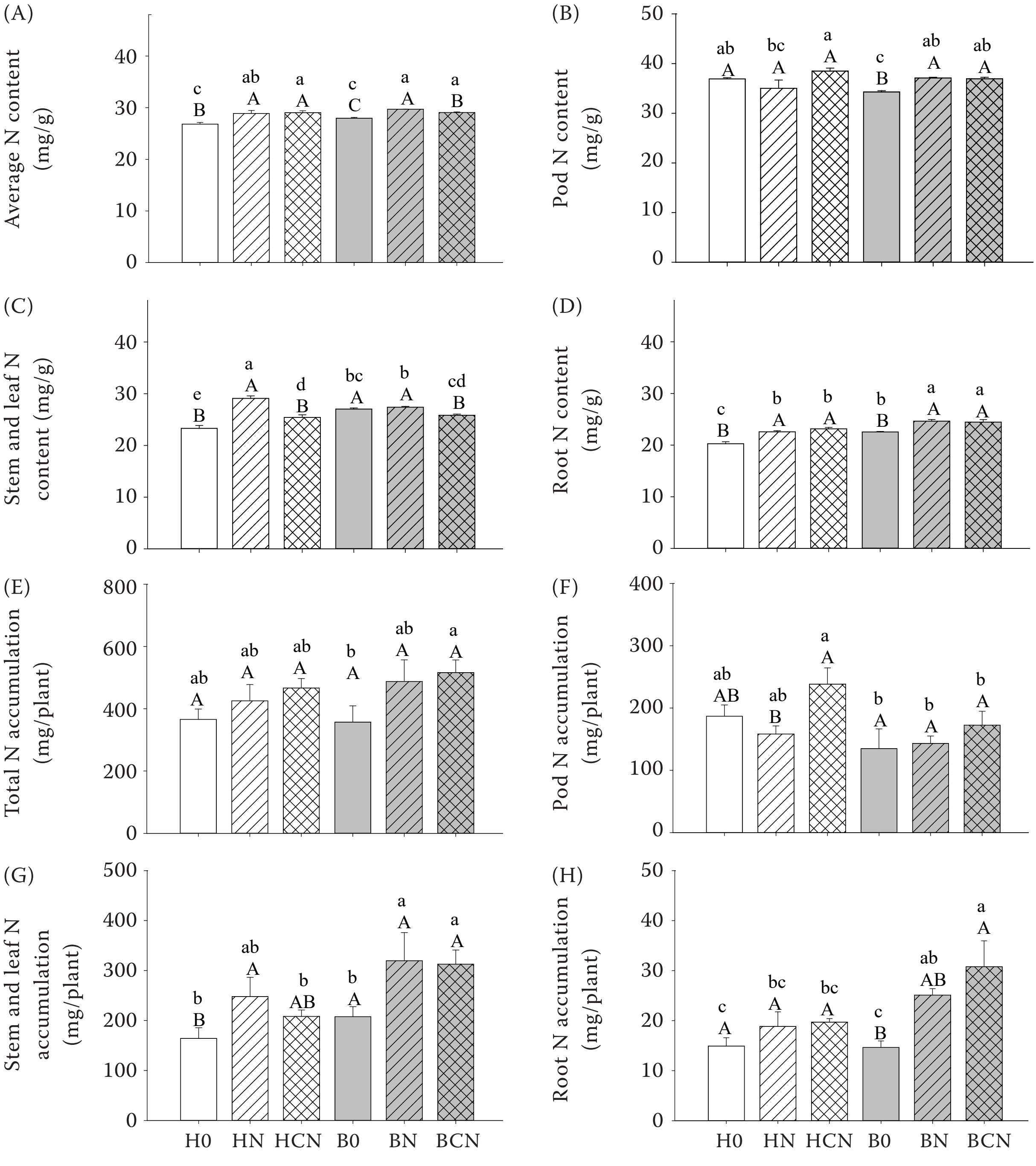

Figure 7. Effects of glucose addition on nitrogen (N) accumulation of peanut. B – peanut cv. NN-1; H – peanut cv. Huayu 22. B0 and H0 – no C and N sources applied; BN and HN – single N source applied; BCN and HCN – C and N source all applied. Lowercase letters indicate a significant difference level of 0.05 among all the treatments. Uppercase letters indicate a significant difference level of 0.05 among the treatments within the same cultivar

Effects of C and N addition on N absorption and accumulation in plants. Compared with the control, all the treatments with N or CN showed their potential to promote N absorption and accumulation in peanut organs (Figure 7). The N content of pod and root had an increasing trend under N and CN treatment, compared to the control (Figure 7B,D). While the N content of the stem and leaf showed an opposite trend, with C addition significantly decreasing the stem and leaf N content

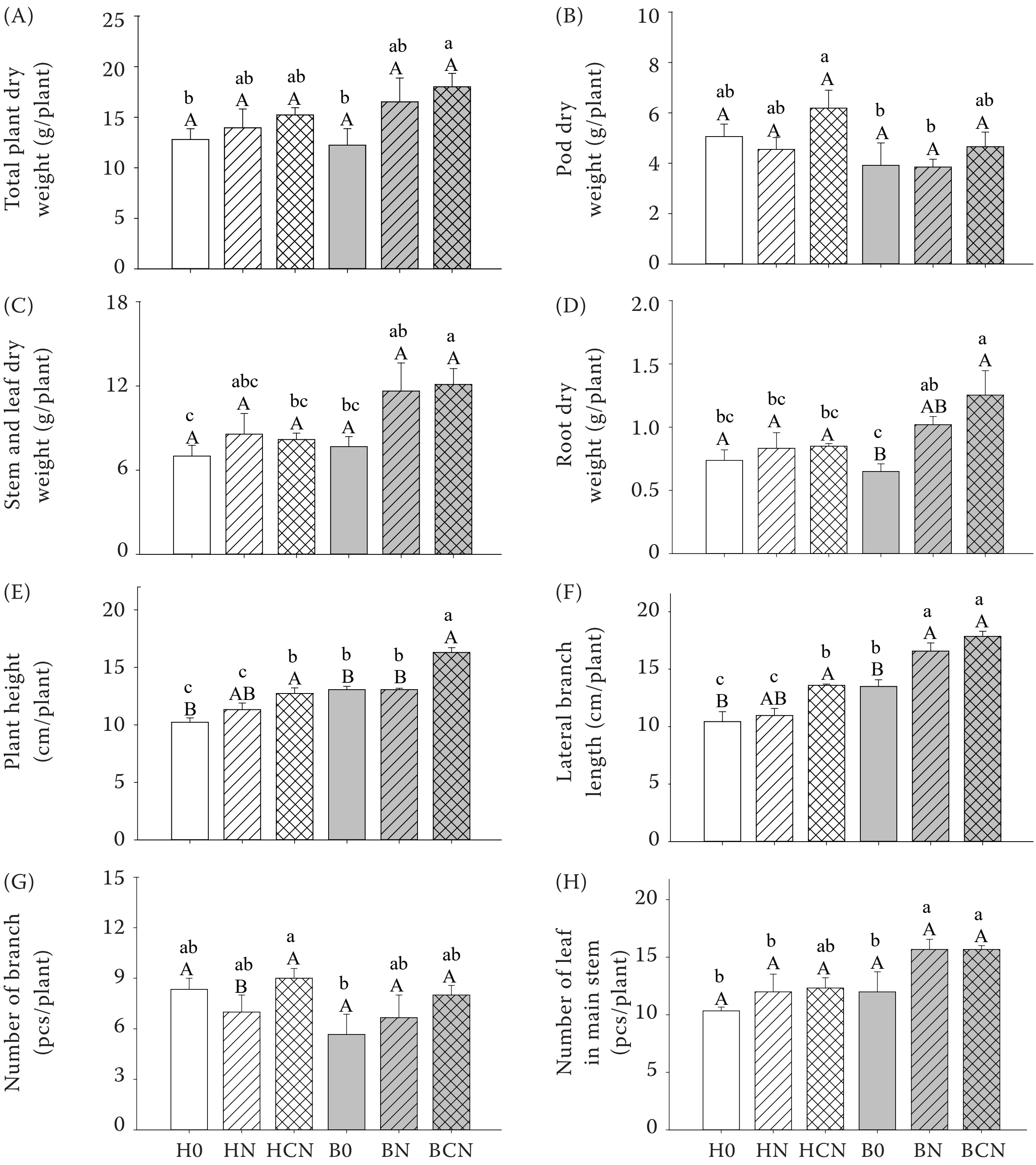

Figure 8. Effects of glucose addition on plant biomass and trait of peanut. B – peanut cv. NN-1; H – peanut cv. Huayu 22. B0 and H0 – no C and N sources applied; BN and HN – single N source applied; BCN and HCN – C and N source all applied. Lowercase letters indicate a significant difference level of 0.05 among all the treatments. Uppercase letters indicate a significant difference level of 0.05 among the treatments within the same cultivar

(Figure 7C). Plant N accumulation increased compared with the control. The total N accumulation under CN treatments was the highest among all the treatments (Figure 7E,F). The N accumulation of the whole plant increased by 16.2 and 27.4% for a cultivar of H and by 36.6 and 44.5% for a cultivar of B, respectively. But there was no significant difference between N and CN treatments (Figure 7E,F). And the N accumulation in the stem and leaf had a similar trend with the whole plant (Figure 7G,H).

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These results indicated that addition C promoted N transport from stem and leaf to pod.

Effects of C and N addition on plant biomass and growth. The effects of C and N addition on plant growth were analysed after harvesting (Figure 7). The results showed that other treatments significantly promoted the accumulation of total biomass, plant height, lateral branch length, branch number and number of leaves in the main stem, compared with the control, and the variation trend of these plant characteristics was basically the same trend for two cultivars. The total dry matter weight increased by 9, 19, 35 and 47% in HN, HCN, BN and BCN treatment, respectively (Figure 8A,B). In N, CN treatment of two peanut cultivars, lateral branch length and number of leaves in the main stem were significantly different from the control, with increasing by 5.2, 30.4, 20 and 30%, respectively, compared to the H0 and B0 (Figure 8B2, 8B4). Furthermore, peanut of B is more sensitive to N and C sources with a better promotion effect on growth. These results indicated that the C source significantly promoted the growth of peanuts in comparison to single N addition.

With long-term N fertilisation, the surplus of N in the farmland ecosystem increased obviously (Feng et al. 2018). Once the surplus N exceeds the absorption capacity of crops and the retention capacity of the soil, it can lead to low N use efficiency and high N losses. Studies have proved that organic fertiliser applied alone or in combination with chemical fertiliser can contribute to the restoration of fixation bacterial diversity. And these effects of fertilisation on soil may be direct, through increased nutrient availability such as N availability and C/N ratio (Wang et al. 2017a). In this study, the contents of NH_4^+-N and NO_3^--N under exogenous glucose treatments decreased significantly, but a single N treatment increased the NH_4^+-N and NO_3^--N contents (Figure 5A,B). This is mainly because adding an appropriate C source can increase soil enzyme activity, improve soil N, and promote crop N absorption. We also noticed that exogenous C and N significantly affected soil microbial diversity and abundance (Table 1). The average Shannon index of the soil fixation bacterial communities under BCN and HCN treatment was higher than under BN and HN. While, a single N addition decreased the microbial diversity. This was closely related to the exogenous C, which can provide energy when the soil microbial is limited by Tian et al. (2015), and Esperschütz et al. (2007) demonstrated that organic fertiliser, in addition to impacting the microbial community diversity, also alter the composition of soil microbial. In the present study, some dominant fixation bacteria, such as Rhizobiales and Burkholderiales at the order level and Bradyrhizobiaceae and Alcaligenaceae at the family level, increased significantly (Figure 3). The result was consistent with previous studies, which reported a strong association between the C/N ratio and the diversity of soil microbial communities (Zhao et al. 2020). Therefore, a more detailed study of resource limitation and its influence on microbial structure and function is needed, which may assist in predicting the dynamics of microbial communities and C cycling in intensive agriculture ecosystems.

Carbon source is an essential environmental factor for plant growth and development (Ljung et al. 2015). Many studies have found that exogenous sucrose or glucose can improve the dry matter accumulation of root and plant growth (Praveen et al. 2011, Wang et al. 2015). Our study demonstrated that glucose addition significantly promoted the peanut plant height, the lateral branch length and above-ground dry matter accumulation. This may be due to the exogenous C source enhancing the soil's strong positive priming effect, significantly increasing the soil C, N availability, which optimises the environment of the plant root area. Further, exogenous C improved plant root activity by strengthening the root's function of absorption and utilisation of nutrients. As we all know, the heavy supply of N often causes the inhibition of nodulation and N fixation for peanuts. When an exogenous carbon source is added to the soil with a reasonable N rate, it can provide the energy for rhizobium and finally promote N fixation. This may be another reason to improve plant growth. We also found that the peanut cultivar of B was more sensitive to the exogenous carbon, which was related to the genotypes and the utilisation characteristics of nutrient elements. In the present study, the response trend of different cultivars to C and N addition was consistent. Exogenous C significantly increased the plant N accumulation compared to the control. Therefore, exogenous C may regulate plant growth and development through improving N absorption of root and plant.

Carbon plays an important role in soil fertility and development regulation in plants. We found that N and CN addition affects peanut growth and the soil environment (Figure 1). However, under the single N treatment, the N addition had a limited effect on plant growth, while the C addition significantly promoted the accumulation of total biomass, plant height, lateral branch length and the total N accumulation of plant (Figure 8). These changes may be attributed to the improvement of the soil environment. C addition decreased the soil N residual and increased the soil urease and nitrogenase enzyme activities. It is widely accepted that the response of microbes to their environment results in the production of enzymes (Nannipieri et al. 2012). So, there may be a significant relationship between enzyme activity and the composition of bacterial communities, with relative abundance of some dominant N fixation bacteria increased. Soil enzyme activity has been acknowledged to provide a unique integrative biochemical assessment of soil function and condition and may be useful as an indicator of soil functional diversity (Epelde et al. 2008, Ling et al. 2014). And further information is necessary about the relationship between functional microbial activity and enzyme activities, as well as soil properties after C addition.

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