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Partitioned nitrogen fertilisation in peanut rhizosphere and geocarposphere drives specific variation soil microbiomes

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Abstract: Peanut is a plant characterised by belowground fruiting that absorbs nutrients not only through its roots but also through its pods. However, little is currently known regarding the species of bacteria that contribute to nutrient absorption and utilisation in this plant's pod and root zones. This study examined the effects of root and pod area nitrogen (N) fertiliser application on peanut rhizosphere and geocarposphere microbial communities and functions. Using two peanut cultivars [nodulated Huayu 22 (H) and non-nodulated NN-1 (B)], we applied the following four treatments: no N fertiliser (HT1, BH1); N applied to geocarposphere soil (HT2, BT2); N applied to rhizosphere soil (HT3, BT3), and N applied to both rhizosphere and geocarposphere soil (HT4, BT4). The results revealed that compared with HT1 and BT1, the HT3, HT4, BT3, and BT4 treatments promoted increases in total plant accumulated N of 11.2, 30.1, 38.5, and 9.9%, respectively. Moreover, N input contributed to an increase in the abundance of bacteria colonising the surrounding pods, which differed significantly from bacteria colonising the rhizosphere. Among the top four bacterial phyla detected, we recorded a significant increase in the relative abundances of Proteobacteria and Gemmatimonadetes in response to treatments HT2 and HT4, whereas the highest relative abundances of Acidobacteria and Actinobacteria were detected in HT3 plants. Regarding cultivar B, we detected increases in the relative abundances of Bacteroidetes and Gemmatimonadetes in response to the BT2 and BT4 treatments, and in the relative abundance of Actinobacteria in BT3 treated soil. The findings of FAPROTAX functional analysis revealed clear differences among the T2, T4, and T3 treatments of two peanut cultivars concerning the functional groups with the highest relative abundances. These findings will make a considerable contribution to enhancing our understanding of the effects of N fertilisation on soil microbial structure and function in the rhizosphere and geocarposphere of peanuts and can provide a basis for identifying beneficial bacteria for promoting N utilisation and yield enhancement.

Keywords: *Arachis hypogaea* L.; pod zone; zoot zone; bacterial composition and diversity; nitrogen application

In recent decades, there has been an increasing focus on the status of the natural environment, emphasising the sustainable development of agricultural production. Consequently, traditional fertilisation practices are gradually being replaced by quantitative fertilisation regimes based on actual crop demands. In this regard, with the continuing development of fertilisation technology, plant nitrogen (N) uptake and utilisation efficiency have been substantially improved.

As an important oil economy crop in China, peanut (*Arachis hypogaea* L.) makes an important contribution to China's edible oil supply, food processing, and farmers' income (Arya et al. 2016, Zhao et al.

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2020, Yang et al. 2022). N nutrition is recognised as one of the main factors limiting peanut growth and yield (Moreau et al. 2019, Michalczyk et al. 2020). With increases in N application within a certain range, there have been increases in crop yields in recent years (Liang et al. 2023). As a geocarpic plant, peanut can take up nutrients through its roots and pods (Hou et al. 2022, Li et al. 2024). For example, some researchers have reported that peanuts absorb nutrients such as sulfur, calcium, and zinc through the shell, which are subsequently translocated to the shoots (Beringer and Taha 1976, Zharare et al. 2010), whereas the findings of other studies have indicated that the N content of shells declines under conditions of N deficiency in the geocarposphere zone (Inanaga et al. 1990, Zhang et al. 2017).

This phenomenon is unique to peanuts and distinguishes this plant from soybean, pea, and other leguminous crops. It can be attributed to the peanut gynophore, which is sensitive to light and gravity and can transport fertilised ovules into the soil. Subsequent subterranean development of pods enables these structures to take up soil nutrients and mature belowground (Zhao et al. 2015). We accordingly speculated that there might be certain differences in the effects of fertilisation on the root area and geocarposphere zone concerning peanut growth. Moreover, given the different anatomical structures of pods and roots, the soil microenvironment of pods differs from that of the rhizosphere (Garren 1966). Based on fatty acid methyl ester analysis, Kloepper et al. (1992) discovered that there were clear effects of fertilisation on the geocarposphere, and it has also been demonstrated that the microbial population density in geocarposphere soil exceeds that of bulk soil (Kloepper and Bowen 1991, Xu et al. 2021). Previous studies have revealed that by isolating beneficial microbial species in bulk and geocarposphere soil, rhizosphere bacterial diversity can enhance root-soil interactions, and beneficial microorganisms can promote plant growth by increasing soil available nutrient content, enhancing their resilience. For example, arbuscular mycorrhizal fungi, which establish symbiotic associations with host plants, can promote significant increases in the host plants' absorption and utilisation of water and nutrients *via* an extensive external mycelial network (Li et al. 2019). Furthermore, it has been reported that plant growth-promoting rhizobacteria significantly contribute to sustainable agriculture, mainly concerning plant pest control and biofertiliser preparation (Abdel-Gayed et al. 2019, Sahib et al. 2020). For example, Xiao et al. (2014) isolated biocontrol bacteria with antagonistic activity against the fungus *Aspergillus flavus* in the peanut geocarposphere. Consequently, plant-associated bacteria can play particularly important roles in maintaining plant health, which may be attributed to the abundance of plant growth-promoting rhizobacteria. In the case of peanuts, however, the composition and function of bacteria associated with plant N uptake have yet to be sufficiently established. Given the anatomical differences between the roots and pods of peanuts (Garren 1966), it can be inferred that there are differences in the composition of microbial communities colonising the rhizosphere and geocarposphere.

It is well established that the rhizosphere of plants is the zone of soil characterised by the highest levels of microbial activity and that crop growth and development are closely associated with the composition of microbial communities colonising rhizosphere soil (Fuke et al. 2021). To date, although several in-depth studies on rhizosphere microorganisms have been conducted based on high-throughput sequencing technology, for belowground crops such as peanuts, there have been comparatively few studies that have examined the composition of rhizosphere and geocarposphere soil microbial communities, and the differences between the rhizosphere and geocarposphere zones concerning microbial community structure and function are generally poorly understood. Consequently, in this study, we sought to examine the effects of N application on the composition of the soil microbiota colonising microbial rhizosphere and geocarposphere zones. Our findings in this study will accordingly provide a theoretical basis for screening peanut-related beneficial microorganisms.

MATERIAL AND METHODS

Experimental site. The study was conducted from June 15 to September 30, 2021, at the Laixi Experimental Station, Shandong Peanut Research Institute, Qingdao City, Shandong Province, China (latitude 36°48'47"N, longitude 120°30'17"E). The climate in this region is one of temperate monsoon, with an average air temperature of 11.7 °C and an annual rainfall of 635.8 mm. For this study, we performed pot experiments in a greenhouse with a transparent plastic-covered roof and four open sides. The soil used for cultivating peanut plants was collected at 0–20 cm

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depths from nearby agricultural fields, the physical and chemical properties of the soil are as follows as described by Liang et al. (2022): total N, 0.9 g/kg; organic carbon, 9.7 g/kg; available P, 96.7 mg/kg; available K, 79 mg/kg; and pH = 5.9. The pots were arranged in a completely randomised block design, with three replicates for each treatment.

Experimental design and treatment details. As peanut cultivars, we used the nodulated Huayu 22 (H) and non-nodulated NN-1 (B), the growth and developmental characteristics of which are similar, although the cultivars differ concerning N utilisation patterns. We sowed four peanut seeds in each pot containing 20 kg of soil. Once the plants were at the seedling stage, we removed one seedling, leaving three seedlings in each pot for subsequent treatment. Plants of each cultivar were subjected to one of the following four treatments: a control without fertilisation (BT1 and HT1), nitrogen fertilisation in the geocarposphere without N application in the rhizosphere (BT2 and HT2), N fertilisation in the rhizosphere without N application in the geocarposphere (BT3 and HT3), and N fertilisation in both the rhizosphere and geocarposphere (BT4 and HT4). All plants were supplied with the same amounts of N (0.9 g/pot), P and K fertilisers, thereby ensuring equivalent nutrient levels in the soil. The details of these treatments are presented in Table 1.

Sample collection and measurement methods. At the end of the treatment period, the peanut plants were harvested with samples being divided into two portions, one of which was used to determine the dry matter content after drying at 80 °C for 48 h. In contrast, the second portion was dried to determine the total N contents of different organs using the Kieldahl method. In addition, soil samples were collected from the roots and pods of harvested plants. Soil tightly adhering to the roots and pods were collected as single rhizosphere or geocarposphere soil samples and were stored at –80 °C for subsequent DNA extraction and analysis. According to the manufacturer's instructions, total DNA was isolated from rhizosphere and geocarposphere soils using a PowerSoil® DNA isolation Kit (MO BIO Laboratories, Shanghai China). The quality and quantity of the extracted DNA were assessed electrophoretically by running samples on 1.8% agarose gels, and DNA concentration and purity were determined using a NanoDrop 2000 UV-Vis spectrophotometer (Thermo Scientific, Wilmington, USA). The bacterial 16S rRNA gene was amplified using the specific primers 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3') incorporating barcode sequences. Both the forward and reverse 16S primers were tailed with sample-specific Illumina index sequences to facilitate deep sequencing. PCR was performed in a total reaction volume of 20 µL containing 5–50 ng DNA template, 0.3 µL of forward primer (10 µmol), 0.3 µL of reverse primer

Table 1. Nitrogen (N) application to the rhizosphere and geocarposphere of peanuts

Cultivar	Treatment	Serial number	N pplication area
NN-1 (B)	R0-P0	BT1	no fertiliser
	R0-PN	BT2	geocarposphere rhizosphere no fertiliser
	RN-P0	BT3	geocarposphere no fertiliser rhizosphere
	RN-PN	BT4	geocarposphere rhizosphere
HY (H)	R0-P0	HT1	no fertiliser
	R0-PN	HT2	geocarposphere rhizosphere no fertiliser
	RN-P0	HT3	geocarposphere no fertiliser rhizosphere
	RN-PN	HT4	geocarposphere rhizosphere

B (NN-1) – a non-nodulated peanut cultivar; H (Huayu 22) – a nodulated peanut cultivar; R – rhizosphere; P – geocarposphere; BT1 and HT1 – no fertiliser applied to cultivars B and H, respectively; BT2 and HT2 – fertiliser applied to the geocarposphere; BT3 and HT3 – fertiliser applied to the rhizosphere; BT4 and HT4 – fertiliser applied to both the rhizosphere and geocarposphere

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(10 µmol), 5.0 µL of KOD FX Neo Buffer, 2.0 µL of dNTPs (2 mmol each), 0.2 µL of KOD FX Neo 0.2 µL, and ddH_2O up to 20 µL. The reaction program comprised an initial denaturation at 95 °C for 5 min, followed by 20 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 40 s, with a final extension at 72 °C for 7 min. The amplified products were purified using an Omega DNA purification kit (Omega Inc., Norcross, USA) and quantified using a Qsep-400 fragment analyser (BiOptic, Inc., New Taipei City, Taiwan). High-throughput sequencing analysis of bacterial rRNA genes was performed on the purified pooled samples using the Illumina Hiseq 2500 platform (2 × 250 paired ends) at Biomarker Technologies Corporation (Beijing, China). The 16S rRNA gene sequences obtained in this study have been uploaded to the NCBI Sequence Read Archive (SRA) database, with accession numbers BioSample SAMN36903181–SAMN36903188 and PRJNA1003662.

Statistical analysis. One-way analysis of variance (ANOVA) was conducted using SPSS 23.0 (SPSS, Inc., Chicago, USA). Alpha diversity was used to assess the diversity of the soil microbial community, with analysis being performed using Mothur version 1.30 (http://www.mothur.org/). As an index of dissimilarity between metabolic gene families, we used Bray-Curtis dissimilarity, the results of which were visualised by performing principal component analysis (PCA) using FactoMineR and the ggplot2 package in R software. Analytical data were processed using BMKCloud (http://www.biocloud.net) and functional predictions of the microbial community were performed using FAPROTAX version 1.1 (Louca et al. 2016). All bar graphs were generated using Sigmaplot 12.5 software (Systat Inc., San Jose, USA).

RESULTS

Effects of rhizosphere and pod fertilisation on plant biomass and N accumulation. Having determined the dry weights of harvested plants, we found that fertilisation in different soil zones had differing effects on the dry matter contents of the two peanut cultivars. Compared with the HT1 plants of the Huayu 22 cultivar, we detected no significant difference in the dry matter of the plants of this cultivar when receiving rhizosphere and geocarposphere fertilisation. Contrastingly, in the case of the NN-1 cultivar, compared with the BT1 plants, rhizosphere fertilisation was found to be more effective in promoting an increase in plant biomass (Figure 1A). Among all the assessed treatments, the combined rhizosphere and geocarposphere fertilisation (T4) proved to be the most effective in promoting an enhancement in total dry matter content. Furthermore, in plants subjected to this combined treatment, we found that the changes in pod weight were similar to those of total plant biomass (Figure 1B). Concerning the accumulation of total N by plants, compared with the HT1 and BT1 treatments, we recorded increases of 11.2, 30.1, 38.5, and 9.9% in response to the HT3, HT4, BT3, and BT4 treatments, respectively (Figure 2A). Compared with these treatments, we detected lower amounts of accumulated total N in those plants receiving geocarposphere fertilisation [HT2

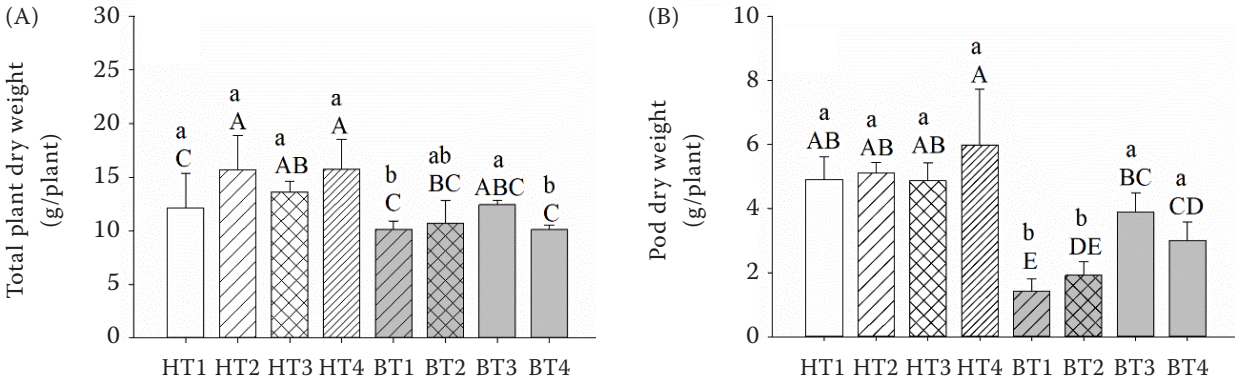

Figure 1. Effects of rhizosphere and geocarposphere fertilisation on peanut biomass. Uppercase letters indicate a significant difference at the $P < 0.05$ level among all the treatments. Lowercase letters indicate a significant difference among treatments within the same variety at the $P < 0.05$ level. B – peanut cultivar NN-1; H – peanut cultivar Huayu 22; BT1 and HT1 – no fertiliser applied to cultivars B and H, respectively; BT2 and HT2 – fertiliser applied to the geocarposphere; BT3 and HT3 – fertiliser applied to the rhizosphere; BT4 and HT4 – fertiliser applied to both the rhizosphere and geocarposphere

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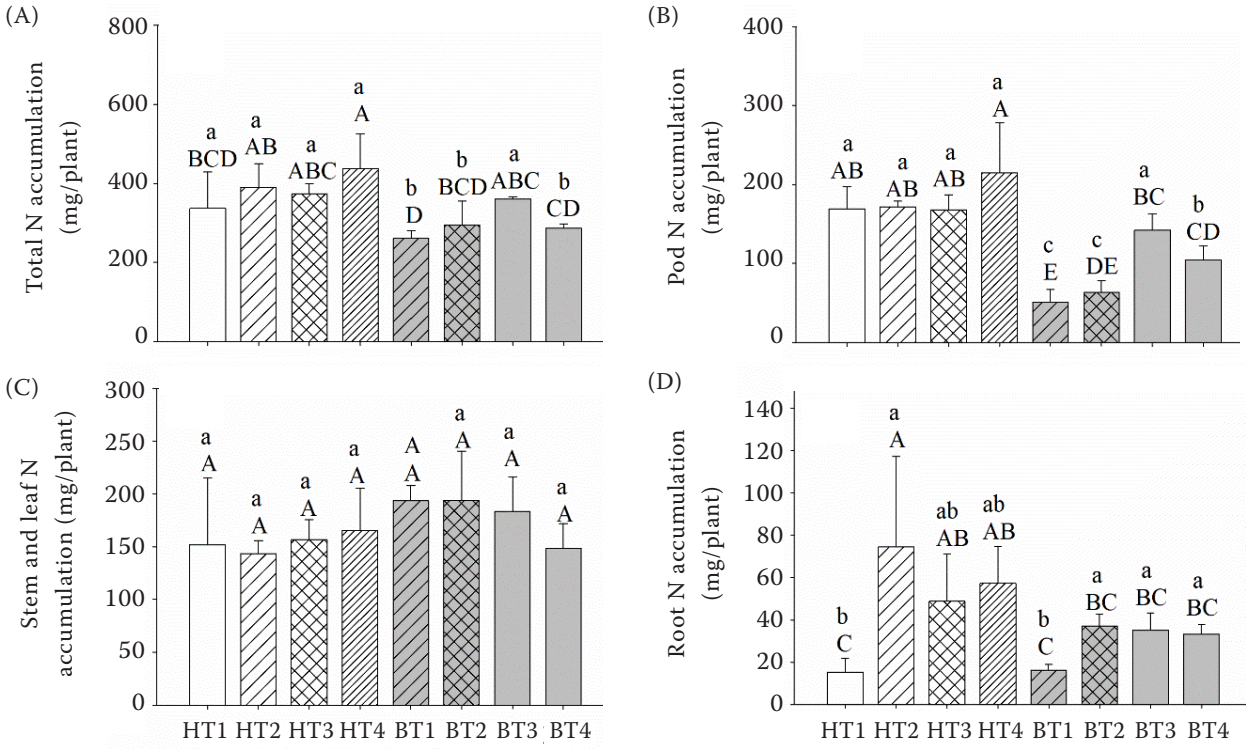

Figure 2. Effects of rhizosphere and geocarposphere fertilisation on peanut biomass and plant nitrogen (N) accumulation. Uppercase letters indicate a significant difference at the $P < 0.05$ level among all the treatments. Lowercase letters indicate a significant difference among treatments within the same variety at the $P < 0.05$ level. B – peanut cultivar NN-1; H – peanut cultivar Huayu 22; BT1 and HT1 – no fertiliser applied to cultivars B and H, respectively; BT2 and HT2 – fertiliser applied to the geocarposphere; BT3 and HT3 – fertiliser applied to the rhizosphere; BT4 and HT4 – fertiliser applied to both the rhizosphere and geocarposphere

(15.9%) and BH2 (13.0%)]. In the case of Huayu 22, we detected no significant differences among the treatments concerning total N accumulation in plants. In contrast, for the NN-1 cultivar, the BT3 treatment was found to enhance N accumulation significantly. Furthermore, pod N accumulation per plant was similar to total plant N accumulation (Figure 2B). In contrast, compared with the controls, we detected no significant differences in the N accumulation of stem + leaves and root for two peanut cultivars (Figures 2C, D). These findings indicate that pod fertilisation can enhance plant N absorption and accumulation, which, combined with the rhizosphere N, had the best effects on plant dry weight and N accumulation.

Alpha diversity-based comparison of peanut rhizosphere and geocarposphere microbial communities. A total of 1 920 689 valid sequences were obtained from the 24 assessed samples, with each sample comprising 79 744 valid sequences on average. The α diversity of bacterial communities was evaluated using the ACE, Chao1, Shannon, and Simpson indices. As shown in Table 2, the NN-1 cultivar showed significant differences in the α diversity of bacterial communities in the rhizosphere and geocarposphere zone soils, as determined based on the ACE and Chao1 indices. Among the treatments, soils receiving the BT2 and BT4 N applications were found to have the highest α diversity indices with values that differed significantly from those obtained in response to other treatments. The application of N to either the geocarposphere or rhizosphere soil was observed to have a clear influence on α diversity. In the cases of the Shannon index, the BT2 and BT3 treatments to the geocarposphere and rhizosphere soils, respectively, were found to promote significant increases in bacterial diversity. For the Huayu 22 cultivar, compared with the no N application treatment (HT1), we recorded significantly higher values for richness estimators ACE and Chao1 for bacteria in soil samples from pots receiving application N to the geocarposphere or rhizosphere and with N applied to both rhizosphere and geocarposphere

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Table 2. Alpha diversity of the rhizosphere and geocarposphere bacterial communities of peanuts in response to partitioned fertilisation

Cultivar	Treatment	Serial number	Application area	ACE	Chao1	Shannon	Simpson
NN-1 (B)	R0-P0	BT1	–	1 520.19 ± 7.4^{b}	1 530.37 ± 3.02^{bcd}	8.46 ± 0.07^{abc}	0.99 ± 0^{a}
	R0-PN	BT2	geocarposphere	1 548.15 ± 27.93^{a}	1 557.66 ± 30.13^{ab}	8.59 ± 0.05^{ab}	0.98 ± 0^{ab}
			rhizosphere	1 501.75 ± 9.21^{bc}	1 508.64 ± 9.39^{cd}	8.42 ± 0.07^{abc}	0.99 ± 0^{a}
	RN-P0	BT3	geocarposphere	1 491.96 ± 13.65^{c}	1 503.94 ± 23.58^{d}	8.34 ± 0.09^{bc}	0.99 ± 0^{a}
			rhizosphere	1 521.62 ± 3.98^{b}	1 537.28 ± 11.36^{bc}	8.44 ± 0.07^{abc}	0.98 ± 0^{b}
	RN-PN	BT4	geocarposphere	1 559.92 ± 23.62^{a}	1 575.9 ± 21.9^{a}	8.65 ± 0.11^{a}	0.98 ± 0^{ab}
			rhizosphere	1 509.49 ± 4.47^{bc}	1 524.35 ± 11.13^{cd}	8.29 ± 0.37^{c}	0.98 ± 0.01^{ab}
HY (H)	R0-P0	HT1	–	1 502.03 ± 19.09^{c}	1 515.18 ± 23.6^{b}	8.35 ± 0.14^{bc}	0.99 ± 0^{a}
	R0-PN	HT2	geocarposphere	1 531.48 ± 6.02^{ab}	1 539.35 ± 9.66^{ab}	8.57 ± 0.09^{a}	0.98 ± 0^{bc}
			rhizosphere	1 496.18 ± 18.14^{c}	1 510.48 ± 16.26^{b}	8.25 ± 0.11^{c}	0.99 ± 0^{ab}
	RN-P0	HT3	geocarposphere	1 513.21 ± 14.19^{bc}	1 530.44 ± 23.08^{b}	8.39 ± 0.09^{abc}	0.99 ± 0^{ab}
			rhizosphere	1 550.88 ± 9.91^{a}	1 565.11 ± 17.92^{a}	8.54 ± 0.02^{ab}	0.98 ± 0^{bc}
	RN-PN	HT4	geocarposphere	1 529.73 ± 8.58^{ab}	1 537.23 ± 9.89^{ab}	8.46 ± 0.02^{abc}	0.98 ± 0^{c}
			rhizosphere	1 529.64 ± 16.02^{ab}	1 538.48 ± 21.85^{ab}	8.31 ± 0.28^{abc}	0.98 ± 0.01^{abc}

Different lowercase letters after values denote a significant difference at the 5% level. B (NN-1) – a non-nodulated peanut cultivar; H (Huayu 22) – a nodulated peanut cultivar; R – rhizosphere; P – geocarposphere; N – nitrogen; BT1 and HT1 – no fertiliser applied to cultivars B and H, respectively; BT2 and HT2 – fertiliser applied to the geocarposphere; BT3 and HT3 – fertiliser applied to the rhizosphere; BT4 and HT4 – fertiliser applied to both the rhizosphere and geocarposphere

area. Higher Shannon index values were obtained for geocarposphere soil samples compared with soil collected from the rhizosphere. In contrast, the converse was true for Simpson index values, thereby providing evidence to indicate that the geocarposphere soil of peanuts is characterised by a higher bacterial species diversity than that of rhizosphere soil. We also detected varietal differences in bacterial communities associated with plants subjected to the T4 treatment. In response to the BT4 treatment, we observed a significant difference between the geocarposphere and rhizosphere soil concerning bacterial richness and community diversity determined using the ACE, Chao1, and Shannon indices. In contrast, contrastingly, for the Huayu22 cultivar, there was no significant difference between geocarposphere and rhizosphere soils in response to the HT4 treatment.

As shown in Figure 3, the rarefaction curves of read numbers for the eight treatments were characterised by increases that eventually plateaued (Figure 3A). Furthermore, compared with the BT1, BT3, HT1, and HT3 treatments in which soil received no N application or only rhizosphere N application, we detected a significantly higher number of operational taxonomic units (OTUs) in soil that had received the BT2, BT4, HT2, and HT4 treatments. Figure 3B reveals that whereas the species evenness of bacterial communities was similar in soils receiving the eight treatments, the bacterial communities in the BT1, BT3, HT1, and HT3 soils were characterised by a lower species richness than those in BT2, BT4, HT2, and HT4 treated soils. Moreover, Venn diagrams revealed that 1 508 and 1 488 OTUs were commonly detected in soils receiving four HT and BT treatments, respectively. For the Huayu 22 cultivar, whereas we detected unique 16S OTUs in response to the HT2 and HT4 treatments (HT2: 1 OTU; HT4: OTUs), there were no unique OTUs in the HT1 and HT3 treated soils (Figure 3C). For the NN-1 cultivar, we detected 6, 1, 4 and 2 unique 16S OUTs in response to the BT1, BT2, BT3, and BT4 treatments, respectively (Figure 3D).

Beta diversity-based comparison of peanut rhizosphere and geocarposphere microbial communities. PCA and cluster analyses were used to compare the peanut rhizosphere's and geocarposphere's bacterial composition. The first principal component (PC1, 54.77%)

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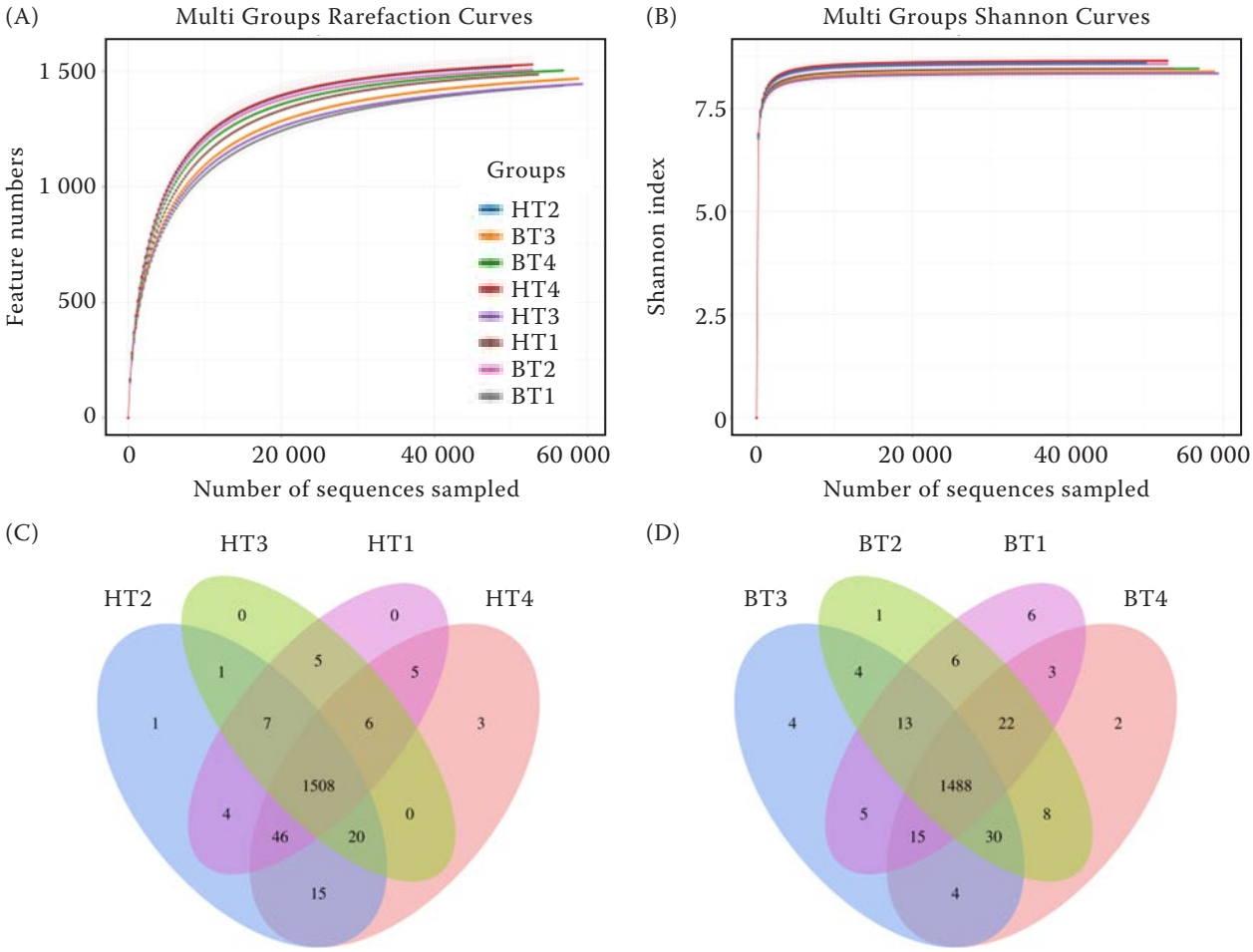

Figure 3. Rarefaction curves and Shannon curves for the operational taxonomic units (OTUs) of rhizosphere and geocarposphere bacterial communities of peanut under partitioned fertilisation at 97% sequence similarity (A, B). Venn diagrams showing the unique and shared OTUs detected in the samples collected from the soil with (A) different partitioned fertilisation (C, D). B – peanut cultivar NN-1; H – peanut cultivar Huayu 22; BT1 and HT1 – no fertiliser applied to cultivars B and H, respectively; BT2 and HT2 – fertiliser applied to the geocarposphere; BT3 and HT3 – fertiliser applied to the rhizosphere; BT4 and HT4 – fertiliser applied to both the rhizosphere and geocarposphere

revealed a considerable difference between the rhizosphere and geocarposphere bacterial communities and inter-sample variance in response to N application to different regions, with PC2 accounting for a further 15.28% of the inter-sample variance (Figure 4A). Thus, these findings indicated that the bacterial communities' structure in BT1 and BT3 and HT1 and HT3 soils differed significantly from those in soils receiving the BT2 and BT4 and HT2 and HT4 treatments, respectively. In addition, the UPGMA clustering results revealed that samples from the eight treatments were clustered into two groups, with the BT1 and BT3 and HT1 and HT3 samples distinct from the BT2 and BT4 and HT2 and HT4 samples (Figure 4).

Composition of soil bacterial communities. The relative abundance of soil bacteria in the eight treatments was analysed at the phylum (Figure 5A), class (Figure 5B), order (Figure 5C), and family (Figure 5D) levels. At the phylum level, Proteobacteria, Acidobacteria, Actinobacteria, Gemmatimonadetes, Chloroflexi, and Bacteroidetes were identified as predominant bacterial groups, among which Proteobacteria were found to have a higher relative abundance in the HT2 (39.52%), HT4 (39.27%), and BT2 (39.84%), BT4 (41.35%) soils than in soils receiving other treatments. In response to the HT2 and HT4 treatments, we detected significant increases in the relative abundances of Proteobacteria and Gemmatimonadetes compared with those in the other

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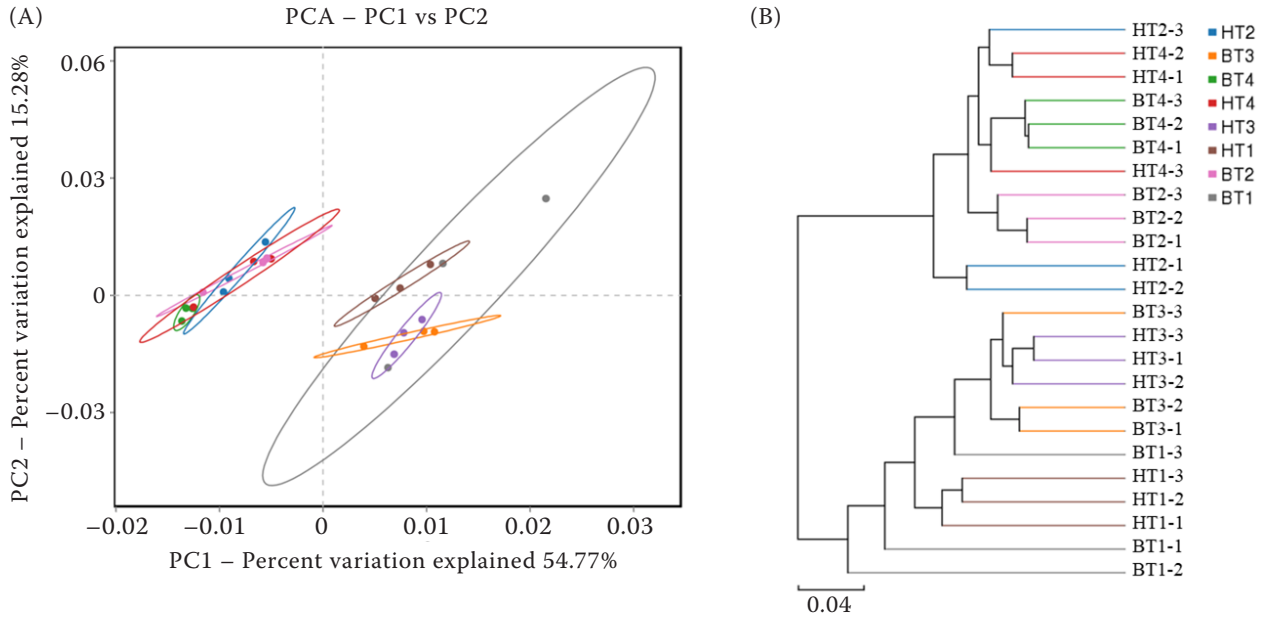

Figure 4. Principal component analysis of the bacterial community structure based on Bray-Curtis analysis (A). Ellipses represent the 95% confidence intervals. Cluster analysis of bacterial communities based on the unweighted UniFrac distance matrices (B). B – peanut cultivar NN-1; H – peanut cultivar Huayu 22; BT1 and HT1 – no fertiliser applied to cultivars B and H, respectively; BT2 and HT2 – fertiliser applied to the geocarposphere; BT3 and HT3 – fertiliser applied to the rhizosphere; BT4 and HT4 – fertiliser applied to both the rhizosphere and geocarposphere

treatments of the cultivar Huayu 22. In contrast, the highest relative abundances of Acidobacteria and Actinobacteria were detected in soil receiving the HT3 treatment. For the NN-1 cultivar, there were increases in the relative abundances of Bacteroidetes and Gemmatimonadetes in response to treatments BT2 and BT4 and in that of Actinobacteria following the BT3 treatment.

At the class level, Alphaproteobacteria, Gammaproteobacteria, Gemmatimonadetes, and Subgroup_6 were identified as the predominant bacterial groups (Figure 5B), among which the highest relative abundances of Alphaproteobacteria were detected in response to treatments T2 and T4. Betaproteobacteriales, Acidobacteriales, C0119, and Bacillales were found to be the bacterial orders predominating analysed rhizosphere and geocarposphere soils (Figure 5C), with higher relative abundances of Betaproteobacteriales, and Acidobacteriales being detected in response to the T1 and T3 treatments of the two cultivars compared with those in the T2 and T4 treated soils. At the family level (Figure 5D), compared with no N addition, the application of N was found to promote increases in the relative abundance of Gemmatimonadaceae for both peanut cultivars. In contrast, Sphingomonadaceae was identified as the family with the highest relative abundances in response to the T2 and T4 treatments, with increases of 32.81, 28.42, 51.62 and 60.71% being recorded for the Huayu 22 and NN-1 cultivars, respectively. In addition, we detected higher relative abundances of uncultured_bacterium_c_Subgroup_6 and SC-I-84 in response to the T1 and T3 treatments, although lower abundances in the T2 and T4 treated soil.

The hierarchical heat map depicted in Figure 6 revealed that bacterial community compositions in soils receiving the BT2 and BT4 and HT2 and HT4 treatments differed significantly from those in BT1 and BT3 and HT1 and HT3 treated soils. Moreover, at the genus level, the bacterial community composition in HT2 soil was similar to that in HT4 soil and the composition in BT2 soil was similar to that in BT4. Similarities were likewise detected among the bacterial community compositions of BT1, BT3, HT1, and HT3 treated soils.

Microbial community functional prediction. The findings of functional predictions based on FAPROTAX analysis revealed that in all assessed samples, the functional groups with the highest relative abundances were those associated with chemoheterotrophy (including aerobic_chemoheterotrophy), nitrification, aerobic_ammonia_oxi-

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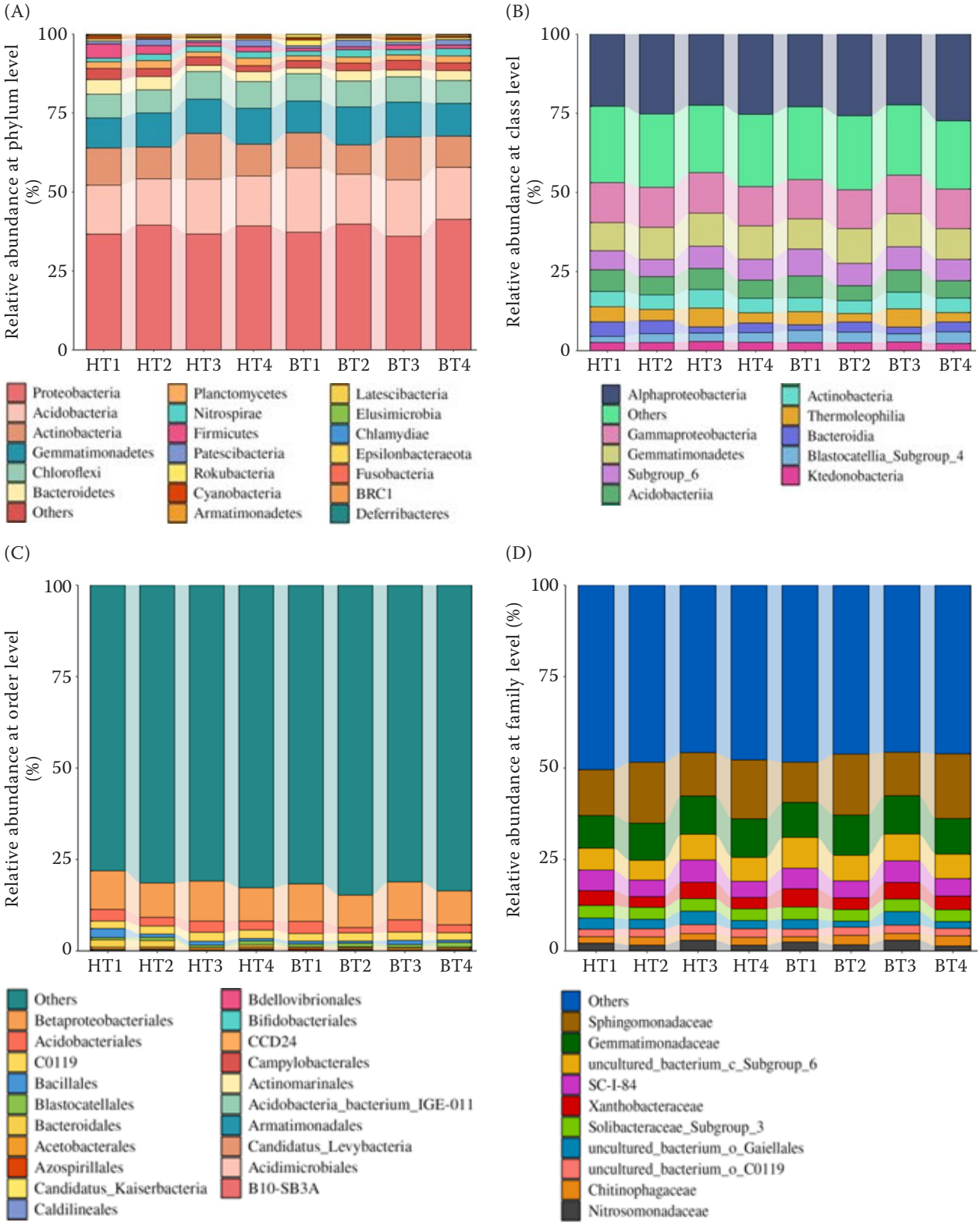

Figure 5. Relative abundance of soil bacteria at (A) the phylum; (B) class; (C) order, and (D) family levels in soil subjected to partition fertilisation. B – peanut cultivar NN-1; H – peanut cultivar Huayu 22; BT1 and HT1 – no fertiliser applied to cultivars B and H, respectively; BT2 and HT2 – fertiliser applied to the geocarposphere; BT3 and HT3 – fertiliser applied to the rhizosphere; BT4 and HT4 – fertiliser applied to both the rhizosphere and geocarposphere

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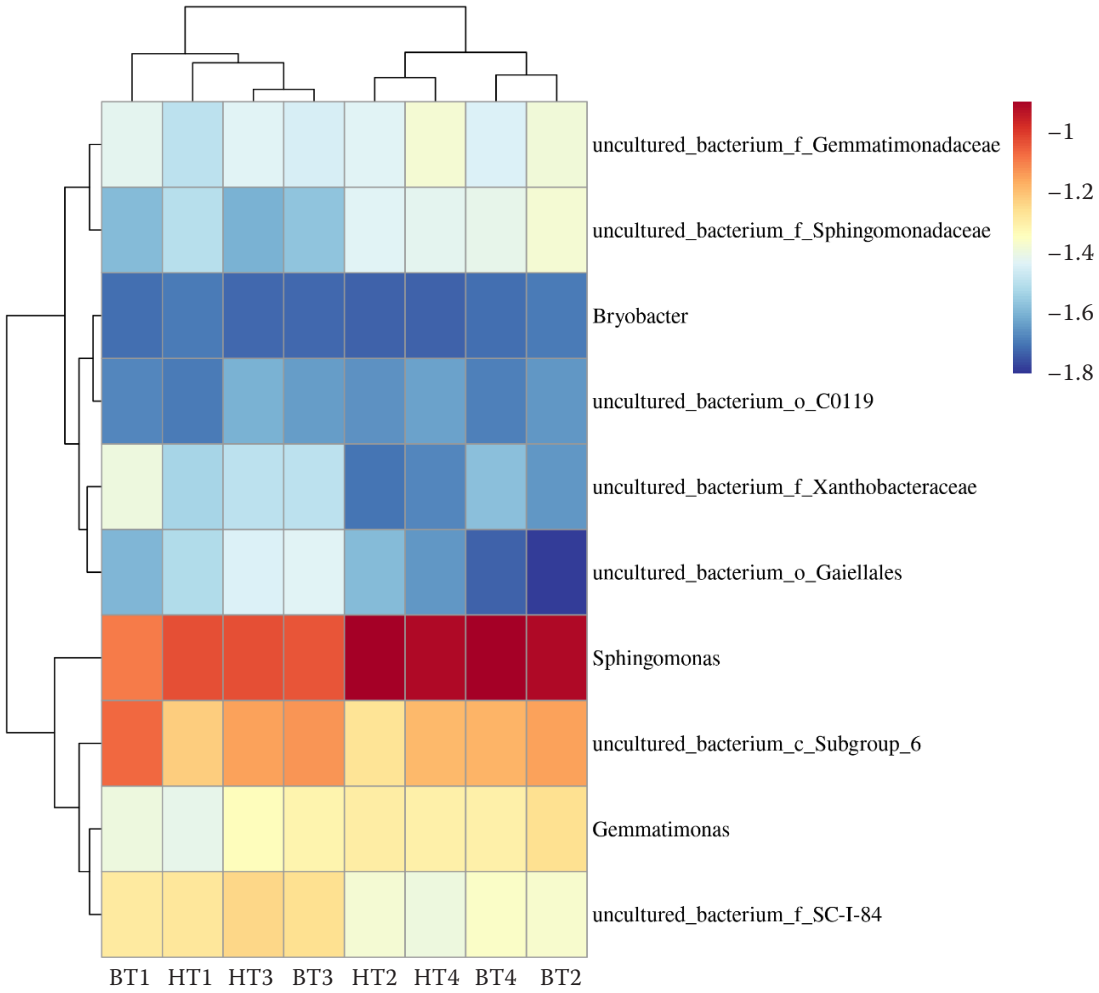

Figure 6. Distribution of the top 10 bacterial genera in terms of abundance in rhizosphere and geocarposphere soils. B – peanut cultivar NN-1; H – peanut cultivar Huayu 22; BT1 and HT1 – no fertiliser applied to cultivars B and H, respectively; BT2 and HT2 – fertiliser applied to the geocarposphere; BT3 and HT3 – fertiliser applied to the rhizosphere; BT4 and HT4 – fertiliser applied to both the rhizosphere and geocarposphere

dation, aerobic_nitrite_oxidation, and ureolysis, accounting for 70.62, 6.61, 3.55, 3.07, and 2.39% of the total bacterial community, respectively (Figure 7). The mean proportions of bacteria associated with chemo heterotrophy were found to be higher in the HT2 and HT4 and BT2 and BT4 treated soils than in soils receiving the HT1 and HT3 and BT1 and BT3 treatments, respectively, whereas the proportions of bacteria associated with aerobic_ammonia_oxidation and phototrophy where found be higher in BT3 and HT3 soils than those in BT2 and HT2 soils, respectively (Figure 8).

DISCUSSION

Effects of partitioned fertilisation on plant biomass and nitrogen accumulation. Nitrogen is an essential element, the provision of which is necessary to ensure high crop yields, and is also a limiting nutrient for microorganisms (Lu et al. 2011, Geisseler and Scow 2014). In the present study, among the assessed treatments, we found that N fertilisation of the geocarposphere soil and the rhizosphere and geocarposphere soil of the Huayu 22 cultivar (treatments HT2 and HT4, respectively) contributed to promoting the highest total plant dry weight (Figure 1A). Notably, compared with Huayu 22, for which we detected no significant differences among the treatments concerning plant biomass, for the NN-1 cultivar, application N to the rhizosphere was found to have a clear beneficial effect in enhancing plant total dry weight. Values obtained for pod dry weight were found to show a trend similar to that recorded for plant dry weight. Moreover, we established that

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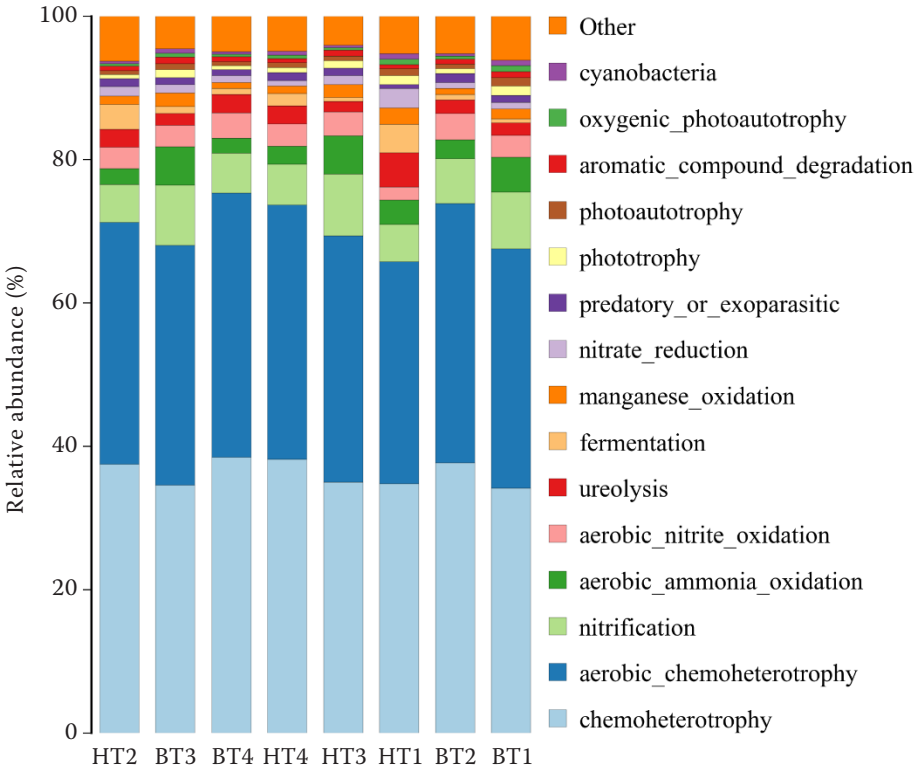

Figure 7. Functional prediction of microbial communities colonising the rhizosphere and geocarposphere soils of two peanut cultivars under different partitioned fertilisation conditions. B – peanut cultivar NN-1; H – peanut cultivar Huayu 22; BT1 and HT1 – no fertiliser applied to cultivars B and H, respectively; BT2 and HT2 – fertiliser applied to the geocarposphere; BT3 and HT3 – fertiliser applied to the rhizosphere; BT4 and HT4 – fertiliser applied to both the rhizosphere and geocarposphere

application to the rhizosphere soil combined with geocarposphere N can significantly increase total plant and pod N accumulation (Figure 2). These findings accordingly provide evidence that the two assessed peanut cultivars are characterised by differential responses to the application of N, with the NN-1 cultivar being the more sensitive of the two. This difference can be ascribed to the fact that. In

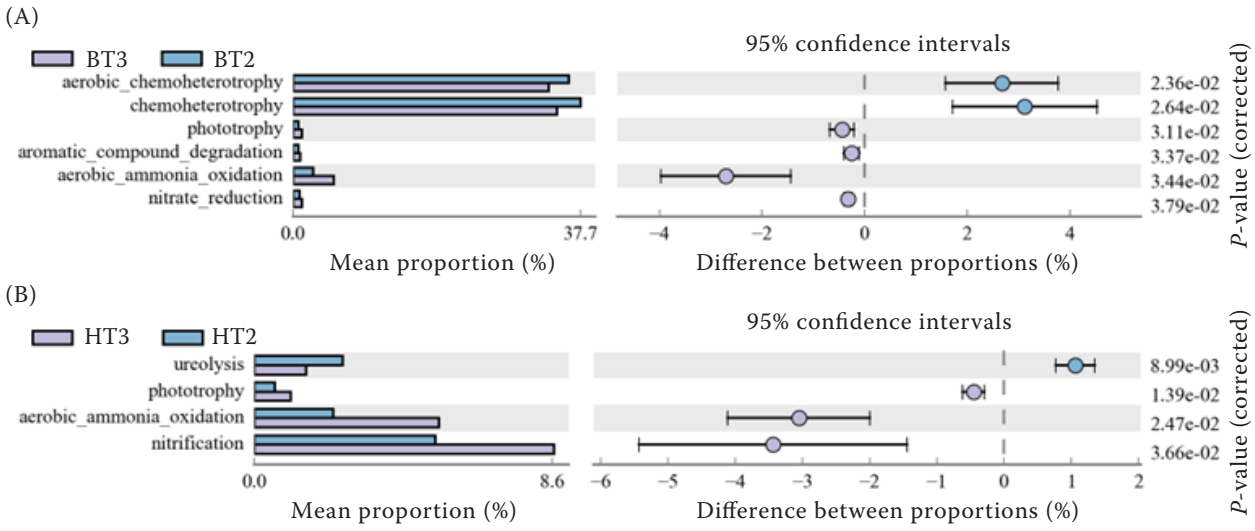

Figure 8. Functional prediction of the microbial communities colonising the rhizosphere and geocarposphere soils of two peanut cultivars under single rhizosphere N and geocarposphere N conditions. Functional differences in bacterial communities in response to BT2 and BT3 treatments (A). Functional differences in bacterial communities in response to HT2 and HT3 treatments (B). B – peanut cultivar NN-1; H – peanut cultivar Huayu 22; BT1 and HT1 – no fertiliser applied to cultivars B and H, respectively; BT2 and HT2 – fertiliser applied to the geocarposphere; BT3 and HT3 – fertiliser applied to the rhizosphere; BT4 and HT4 – fertiliser applied to both the rhizosphere and geocarposphere

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contrast, the nodulated Huayu 22 cultivar can fix N, and NN-1 plants are unable to form nodules for biological N fixation. Consequently, this cultivar is dependent on soil and fertiliser N as sources of this element. These findings are consistent with those reported in previous studies examining N application's effects on N uptake and distribution in nodulated and non-nodulated peanut cultivars (Selamat and Gardner 1985, Shi et al. 2012). Moreover, our finding that simultaneous rhizosphere and geocarposphere fertilisation was the most effective in terms of promoting crop biomass and N accumulation reflects the fact that peanut pod can directly absorb nutrients from the soil, which is consistent with the findings of Hou et al. (2022), who revealed that the pod zone application of N can significantly enhance both the pod yield and N content.

Effects of partitioned fertilisation on two peanut cultivars' microbial populations in the rhizosphere and geocarposphere. The findings of numerous studies have indicated that N fertilisation can have a pronounced influence on the composition of soil microbial communities (Treseder 2008, Geisseler and Scow 2014). The rhizosphere microbiota plays important roles in plant growth and development, with the activities of these microbes being influenced to differing extents by biological and abiotic factors, and community structures have been observed to differ among different plant species and cultivars (Arafa et al. 2010, Qu et al. 2020). In the present study, for both the assessed peanut cultivars, compared to T1 and T3, we detected higher relative abundances of *Proteobacteria* and *Gemmatimonadetes* in soil receiving the T2 and T4 treatments at both the phylum and class levels. Figures 5A, B, which would tend to indicate that the pod zone application of N can promote an increase in bacterial community abundance. Similar findings have been reported by Bai et al. (2015) and Xu et al. (2021). Bacteria in the phylum *Proteobacteria* include many N-fixing species that could enhance the biological N-fixation capacity of legumes (Gyaneshwar et al. 2011). We thus assume that pod zone application N promotes the recruitment of a larger number of beneficial microbial flora, thereby potentially contributing to an elevation in soil N levels and a corresponding enhancement of plant N uptake (Figure 1). Furthermore, the findings of our PCA and UPGMA clustering analyses revealed that there were significant differences between the bacterial communities colonising the rhizosphere and geocarposphere zones of peanut subjected to partitioned N application, the diversity of geocarposphere bacteria being found to be significantly higher than that of bacteria colonising the rhizosphere. These findings are consistent with those reported by Xu et al. (2021) and Kloepper et al. (1992), and we speculate that these differences could be attributed to the fact that in response to the application of N fertiliser, plant roots, and pods will release different suites of exudates, thereby attracting different populations of microbial flora. We assume that the relatively lower abundance of bacterial communities in the rhizosphere reflects a competitive exclusion caused by an increase in the availability of soil N. In this regard, by promoting the proliferation of symbiotic N-fixing bacteria and the competitive exclusion of other species, N fixation in peanut nodules may result in a reduction in local biodiversity (Bobbink et al. 2010, Reinhold-Hurek et al. 2015, Li et al. 2019).

Effects of portioned N application in determining the potential functions of microbial communities. Generally, changes in the soil environment and remodelling of the microbial community can influence the potential functions of microbial communities. The findings of our microbial functional predictions provided evidence to indicate that the bacterial community plays a role in N cycling by mediating the processes in interconnected biogeochemical pathways (including aerobic_nitrite_oxidation, nitrification, and nitrate reduction). Moreover, the abundance of these aerobic_nitrite_oxidation bacteria was higher in HT3 and BT3 soils than in soils receiving the HT2 and BT2 treatments. HT3 and BT3 soils were also characterised by a similar enrichment of phototrophic bacteria (Figure 8). Thus, these findings would indicate that N input may be a key factor in promoting the proliferation of aerobic_nitrite_oxidation and phototrophic bacteria in rhizosphere soils. In contrast to a significant enhancement in the activities of nitrification bacteria following application of N to the rhizosphere zone of Huayu 22 peanuts (HT3), we detected no comparable effects in response to application to the rhizosphere zone of the non-nodulated cultivar NN-1 (BT3) (Figure 8), thereby highlighting the differential effects of partitioned fertilisation on these peanut cultivars. It is well established that plants are dependent upon thriving soil populations of beneficial microbes to sustain nutrient acquisition (Shi et al. 2022), and consequently, promoting positive changes in the ecological function of microbial community and sustaining the balance of beneficial microbial com-

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munities play pivotal roles in enabling the plant to adapt appropriately to different environments (Weiss and Shaw 2015). Moreover, besides nitrogen, other physical and chemical properties of soil, such as pH and soil organic carbon content, have prominent effects on soil microbial communities. Accordingly, it is reasonable to assume that such properties may contribute to determining the observed enrichment of certain microbial taxa in the rhizosphere and geocarposphere soil environments.

Collectively, our findings in this study indicate that partitioned N application to the roots or pods, combined with root N potential, can enhance the accumulation of N in plants of the nodulated Huayu 22 cultivar of peanuts, although no significant effects were detected concerning the non-nodulated NN-1 cultivar. In addition, we established that N input to the rhizosphere and geocarposphere had a significant influence on the composition of soil microbial communities, with significant differences between rhizosphere and geocarposphere soils being detected concerning bacterial communities composition and functions, despite the proximity of these soils in subterranean environments. Additionally, in response to the N application, we detected increases in the relative abundance of beneficial microbes in geocarposphere soil compared with the root zone. Such bacterial community analyses may thus contribute to the identification of beneficial bacteria that play key roles in N cycling, as well as in improving pod N absorption. Accordingly, our findings will provide theoretical guidance for screening strains for establishing repositories of beneficial rhizosphere and geocarposphere bacteria.

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