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Effects of different concentrations of chlormequat chloride on bacterial community composition and diversity in peanut soil

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Abstract

The application of pesticides may have significant impacts on soil environment and communities. In order to understand the deep relationship between the application of chlormequat chloride (CC) and the bacterial community in peanut soil, high-resolution characterization was performed using peanut soil samples (12 points; 0-20 cm rhizosphere soil) from untreated and sprayed with different concentrations of CC. Experimental data showed that with the increase of concentration, operational taxonomic units (OTUs) richness showed a decreasing tendency. The OTUs richness at low concentration (D, 50% CC diluted 5000 times, 45 g ai/ha), medium concentration (M, 50% CC diluted 300 times, 75 g ai/ha), and high concentration (G, 50% CC diluted 1000 times, 225 g ai/ha) were 5583, 5430, and 3910, respectively. Low concentrations increased the composition and relative abundance of soil bacterial communities. In contrast, high concentrations significantly reduced bacterial diversity. As the concentration of CC increases, the abundance of Proteobacteria decreases, while the abundance of Firmicutes and Bacteroidetes increases. The number of Acidobacterium and Bacteroidetes increased in groups D and M, while it decreased in group G. D, M and G groups showed a decrease in the abundance of *Pseudomonas*, *polaromonas*, and *Azovibrio* compared to CK, while the abundance of *Flavobacterium* increased. In addition, the abundance of *Rahnella1* decreased in groups D and M, while the abundance increased in group G. The main metabolic pathways included the metabolisms of nucleotides, terpenoids, polyketides, other amino acids, cofactors, vitamins, lipids, glycan biosynthesis, energy, carbohydrates, xenobiotics, amino acids, and other secondary metabolites.

Keywords Chlormequat chloride, Peanuts, Soil bacterial diversity, Environmental ecology

Introduction

Peanuts, a significant source of plant-based oils and proteins, are among the important oil crops in China [1]. Peanut overgrowth is a frequent phenomenon during cultivation, which is primarily characterized by enlarged leaves, shading in the field, and a corresponding increase in the distance between the fruit needle and the ground, which delays needle placement, thereby significantly affecting peanut yield and quality [2]. Therefore, appropriate and effective measures are required to control excessive growth, which can help peanut plants maintain good morphology, optimize nutrient allocation, and promote reproductive growth, thereby improving peanut fruiting rate and plumpness [1, 3]. It has been observed that plant growth regulators can modulate flowering, improve peanut plant morphology and structure, as well as enhance peanut seed quantity and quality [4].

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Chlormequat chloride (CC) is a common inhibitory regulator of plant growth, which not only regulates plant growth but also resists lodging. Therefore, it is widely used in the production process of grain crops, vegetables, fruits, medicinal plants, etc. [5–9]. Recently, it has been reported to be used in peanut production [10–12]. With increasing studies on sustainable agricultural development and ecological health, the potential impact of CC use is becoming a research hotspot [13]. CC exhibits toxicological properties and has developmental and reproductive toxicity [14–16]. In the United States CC was detected in urine collected from 2017 to 2022, with a significant increase in urine levels in 2023 [17].

The foundation of agricultural ecosystems is soil biodiversity, which is increasingly recognized as beneficial to human health as it can inhibit pathogenic soil organisms and provide clean air, water, and food [18]. Soil biodiversity is significantly and positively correlated with various ecosystem functions. These functions include nutrient cycling, decomposition, plant production, and reducing the potential for pathogens and underground biological warfare [19]. During peanut cultivation, the application of CC may directly or indirectly affect the structure of soil microbial communities and lead to complex changes in soil biodiversity.

Several studies have investigated the effects of the exogenous application of plant growth regulators on soil microorganisms [20–23]; however, there are only a few studies on the impact of CC on soil microbial communities. Therefore, this study aimed to elucidate the association between CC and peanut soil diversity, providing an important reference for achieving high peanut yield and quality as well as harmonious coexistence with the ecological environment.

Materials and methods

Field experiments

In 2023, field experiments were conducted in Xiaochengzi Town, Kangping County, Shenyang City, Liaoning Province, China (E123.35446, N 42.75081). Kangping Xiaochengzi Town belongs to a temperate continental monsoon climate, with distinct four seasons and year-round peanut cultivation. The soil is sandy loam, with an average annual precipitation of about 456.3 mm, an average annual sunshine hours of about 2584.4 h, and an average annual temperature of about 8.1 ℃. Set up a total of 4 processes (3 repetitions/processes). Using the random plot selection method, select 5×6 m plot areas with a spacing of 50 cm for each treatment. Peanuts were sown on May 10th, with the tested peanut variety being Baisha.

The commercially available 50% CC was prepared in the following concentrations: low concentration (D, 50%CC diluted 5000 times, 45 g ai/ha), medium concentration (M, 50%CC diluted 3000 times, 75 g ai/ha), and high concentration (G, 50%CC diluted 1000 times, 225 g ai/ha). The CK group was not sprayed with CC. On July 5th, foliar sprayed CC. Soil Samples were taken on August 5th. Used the five point sampling method within the field, the peanut rhizosphere soil was collected using the shaking method, and fine roots, plant residues, and stones were removed using a 20 mesh sieve. Soil samples were treated with liquid nitrogen and stored at −80 ℃ for DNA extraction.

Soil DNA extraction and sequencing

Soil DNA was extracted using the BayBiopure Magnetic Bead Soil DNA Extraction Kit (Guangzhou Bay Area Biotechnology Co., Ltd.), and bacterial PCR amplification was performed using primers F (ACTCCTACGGGAGGCAGCA) and R (CCGTCAATTCMTTRAGTT) in the V3-V4 variable region. The PCR amplification system and amplification conditions were described in reference to the literature [24]. The PCR reaction consisted of 13 μL MOBIO PCR water, 10 μL 5 Prime HotMasterMix, 0.5 μL forward and reverse primers (final concentration of 10 μM), and 1.0 μL genomic DNA. Maintained DNA denaturation at 94℃ for 3 min, amplified at 94℃, 45 s for 35 cycles, 50℃ for 60 s, and 72℃ for 90 s; extended at 72℃ for 10 min to ensure complete amplification. After PCR quality verification, the soil DNA samples were sent to Nanjing Paiseno Biotechnology Co., Ltd. for paired end sequencing of community DNA fragments using the Illumina platform.

Soil diversity analysis

DADA2 sequence denoising method [25], used QIIME2 analysis software, first called qiime cutadapt trim paired to remove primer fragments from the sequence and discard sequences with unmatched primers; Then, called DADA2 for quality control, denoising, splicing, and de chimerism through qiime dada2 denoise paired. Merged OTU feature sequences and OTU tables, and removed singletons OTUs. Used R language scripts to statistically analyze the length distribution of high-quality sequences contained in all samples.The final obtainedvalid data was submitted to the Paisenno Cloud platform for data processing and analysis.

Sequence processing and analysis

The Alpha diversity index was calculated using Mothur software, including Chao1 index and Shannon index. The Chao1 index reflects the richness of the community, while the Shannon index reflects the diversity of the community. Beta diversity analysis was conducted using R3.5.1 language, namely principal component analysis

(PCoA). The microbial community structure bar chart was drawn using QIIME software to present the composition and abundance distribution of soil microbial communities at different taxonomic levels [26].

Results

Analysis of richness and diversity of soil bacterial and microbial communities

Alpha diversity analysis

Statistical analysis of sequencing data revealed that after 30 days of drug application, a total of 847,671 raw sequences were obtained from 12 samples. After filtering, a total of 794,468 valid sequences were generated, with at least 66,206 valid sequences produced per sample, resulting in an average of 66,206 valid sequences (Supplementary Table 1). According to the dilution curves of the samples (Supplementary Fig. 1), it can be seen that the curves of all four groups of samples tend to flatten, indicating that the sequencing quantity of the samples is reasonable and the sequencing depth can meet the experimental requirements.

Soil bacterial and microbial community OTUs clustering analysis

The OTUs of D, M, G, and CK were 5583, 5430, 3910, and 4740, respectively (Fig. 1) and the order of OTUs from high to low was D > M > CK > G. The number of OTUs decreased with the increase of CC concentration, indicating that low and medium CC concentrations can increase OTUs values in peanut microbial community. Moreover, the D treatment had the highest OTUs values, whereas the G treatment reduced OTUs of peanut microbial communities.

Alpha diversity refers to the indicators of richness, diversity, and evenness of species in a locally uniform habitat, also known as intra habitat diversity. In order to comprehensively evaluate the alpha diversity of microbial communities, richness was characterized by Chao1 [27] and Observed species indices, diversity was characterized by Shannon [28, 29] and Simpson indices [30], evolutionary diversity was characterized by Faith index [31], evenness was characterized by Pielou index [32], and coverage was characterized by coverage index [33]. From Table 1, it can be seen that there was no significant difference in coverage between the different concentration treatments, indicating that the coverage of the soil sample bank for each treatment was high. The Chao1, Shannon, Simpson, and Pielou indices of low concentration CC treatment were significantly higher than those of other concentration treatments. As the concentration of CC increased, the Chao1, Shannon, Simpson, and Pielou indices showed a decreasing trend. After applyed low concentrations of CC to peanuts, the bacterial diversity and richness of the soil significantly increased. As the CC concentration increased, the bacterial diversity and richness of the soil significantly decreased (Table 2).

Beta diversity analysis

The Beta Diversity Index focuses on the comparison of diversity between different habitats, that is, the differences between samples. By using the Bray Curtis distance [34] method, analyzing the OTU composition of different

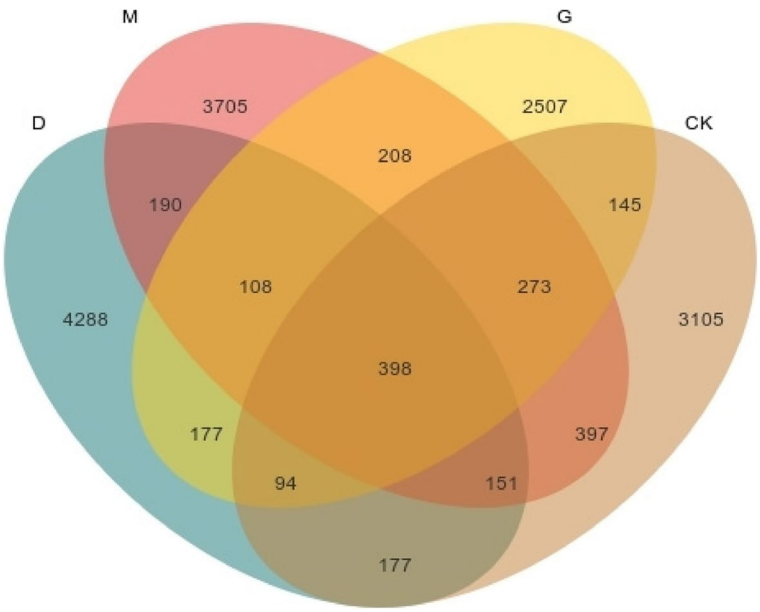

Fig. 1 Venn diagram of bacterial community in peanut rhizosphere soil

samples can reflect the differences and distances between samples, such as the closer the sample community structure, the closer the distance reflected in the PCA graph (Fig. 2). Inter group difference analysis was conducted using the Python scikit bio package for "permenova" inter group difference analysis, with a permutation test count set to 999. As shown in Fig. 2a, the distance between soils treated with different concentrations of CC was relatively far, the contribution rates of the two principal components extracted by bacteria were 31.5% and 27.4%, respectively; According to 2b, all treatment groups were greater than 0, indicating significant changes in soil bacterial community structure after applyed different concentrations of CC.

Analysis of soil bacterial and microbial community composition

The top 5 dominant bacterial phyla in the bacterial community were Proteobacteria (36.67%~56.22%), Firmicutes (16.38%~38.16%), Acidobacteriota (4.05%~8.77%), Bacteroidetes (4.19%~6.99%), and Gemmatimonadota (3.23%~7.39%) (Fig. 3A). As the concentration of CC increased, the abundance of Proteobacteria decreased compared to CK, and the G group decreased by 24.26%. The abundance of Firmicutes and Bacteroidota increased

Table 1 Alpha diversity index of soil samples

Group	Goods_coverage	Chao1	Observed species	Shannon	Simpson	Faith_pd	Pielou-e
D	0.99283a	2512.39a	2450.9a	9.27990a	0.98565a	200.600ab	0.824196a
M	0.98960a	2502.79a	2375.2a	8.52057b	0.97555ab	225.711a	0.760328b
G	0.99288a	1709.12c	1633.5c	6.81162c	0.94544c	170.004b	0.638321d
CK	0.99251a	2118.82ab	2055.1b	7.83026b	0.96499b	187.206b	0.711616c

Different lowercase letters indicate significant differences between treatments at the 5% level

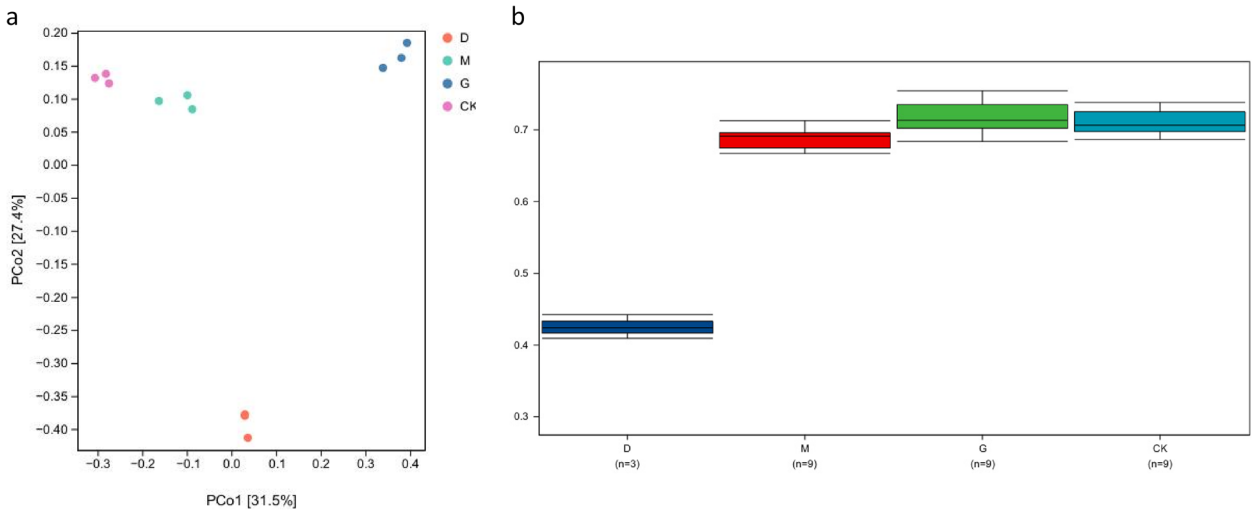

Fig. 2 Analysis of intergroup differences in peanut soil bacterial communities based on distance matrix and PCoA

Table 2 SD of Alafa diversity index

Group	Chao1	Faith_pd	Goods_coverage	Observed_species	Pielou_e	Shannon	Simpson
D	83.7823633	13.64166714	0.001887852	89.34601278	0.021548099	0.284703853	0.003543613
M	35.05043842	31.14852697	0.004981056	83.4130885	0.021528278	0.397748991	0.003300758
G	74.33758067	4.502500898	0.002397299	48.508178	0.038658032	0.55612862	0.009211182
CK	94.69218078	15.83879437	0.001479634	82.68270678	0.013834012	0.113040673	0.00707468

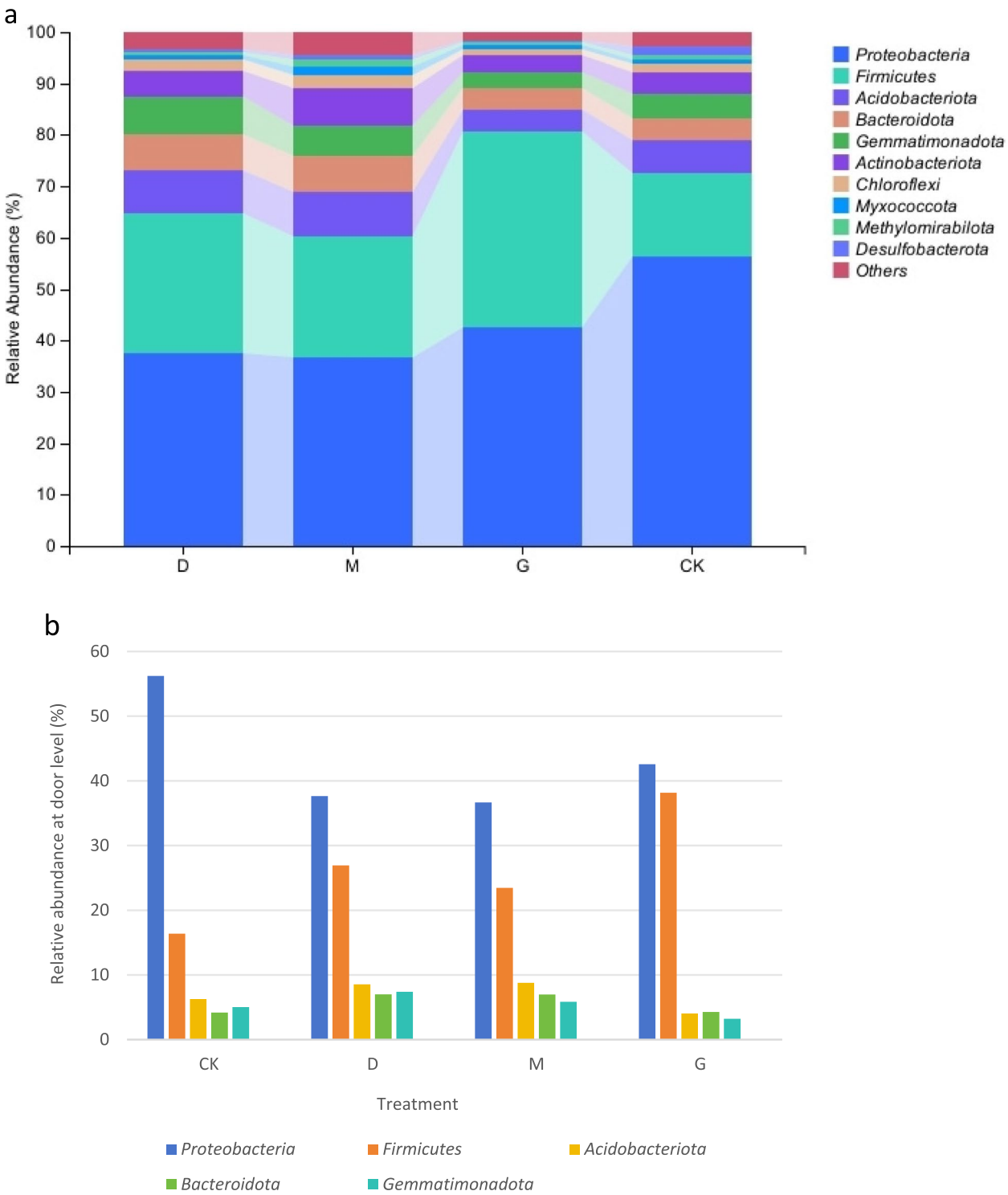

Fig. 3 Bacterial structure of the phylum

compared to CK. The abundance of Firmicutes in Group G reached 132.97%, while the abundance of Bacteroidota in Group D was 66.83%. The abundance of Acidobacteriota and Bacteroidota in groups D and M increased compared to CK, with a significant increase under D group, at 36.36% and 46.92%, respectively. In G

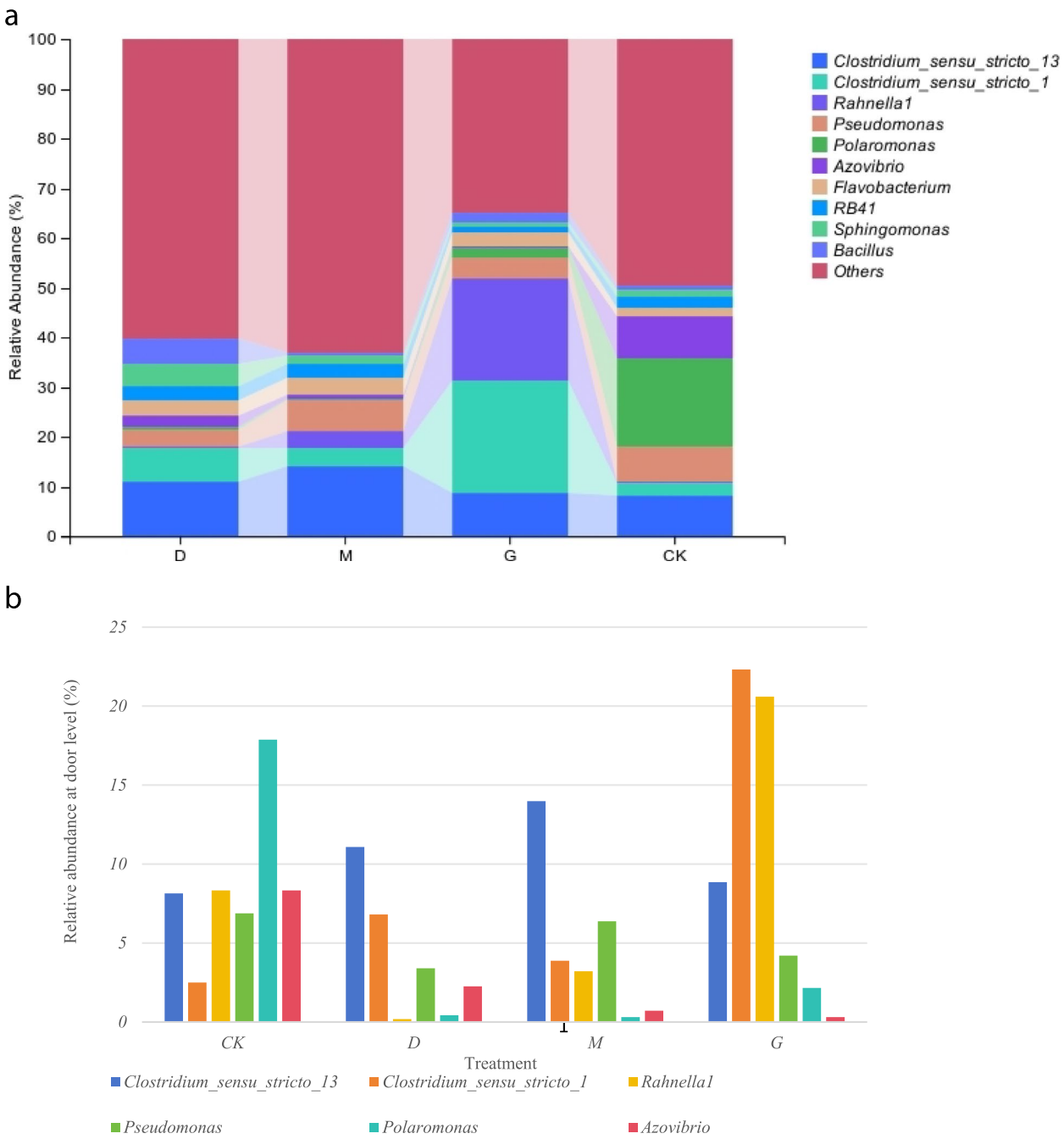

Fig. 4 Bacterial structure of the genus

group, the abundance of Acidobacteriota and Bacteroidetesota decreased compared to CK, reaching 35.40% and 35.79%, respectively (Fig. 3B).

At the genus level, the proportion of bacterial communities without identified genera was relatively high (Fig. 4). The dominant bacterial genera in the rhizosphere soil of each group were *Clostridium_sensu*_stricto_*1, Rahnella1, Pseudomonas, Clostridium* sensu-*stricto_13, Azovibrio, Polaromonas, RB41, Flavobacterium,* and *Sphingomonas, Bacillus* (Fig. 4A). Compared with CK, the D, M, and G groups had reduced abundance of *Pseudomonas* (50.58, 7.27, and 38.95%), *Polarimonas* (97.59, 98.27, and 87.9%), and *Azovibrio* (72.87, 91.36, and 96.28%), whereas the abundance of *Clostridium-sensu_stricito-13* (36.12, 71.74, and 8.72%) and *Clostridium-sensu_stricito-1* (172.4, 55.2, and 792.8%) was increased.

Moreover, compared to the CK, *Rahnella1* abundance in the D and M groups were decreased (97.83% and 61.5%, respectively), while increased in the G group (147.30%) (Fig. 4B).

Analysis of species differences in soil bacterial microbial communities

The LEfSe analysis (Fig. 5) identified 100 bacterial biomarkers at the phylum, class, order, family, genus, and species levels. Of these, 9 were identified at the genus level (based on LDA log scores > 4), included *Azovibrio*, *Bacillus*, *Clostridium*-sensu-*stricto-1*, *Polaromonas*, 3.4 *Sphingobium*, *Clostridium*-sensu-*stricto-13*, *Sphingomonas*, *Massilia*, and *Rahnella1.*

The heatmap of species composition (Fig. 6) indicated that the most abundant genus in the CK group were *Polarimonas* and *Azovibrio;* in the D group, were *Bacillus* and *Sphingomonas;* in the M group were *Clostridium_sensu_stricto_13;* in the G group were *Clostridium_sensu_stricto_1* and *Rahnella1.* D, M and G groups showed a decrease in the abundance of *Pseudomonas*, *polaromonas*, and *Azovibrio* compared to CK, while the abundance of *Flavobacterium* increased.

Functional prediction of metabolites

The metabolic pathway (Fig. 7) included 4 cellular processing pathways, 2 environmental information processing pathways, 4 genetic information processing pathways, 11 metabolism pathways. The main concentrated metabolic pathways included the metabolisms of nucleotides, terpenoids, polyketides, other amino acids, cofactors, vitamins, lipids, glycan biosynthesis, energy, carbohydrates, xenobiotics, amino acids, and other secondary metabolites.

Compared to the CK group, the positive regulatory pathways were significantly different ($p < 0.001$, LogFC > 1) in the D group and included naphthalene degradation and spliceosome, whereas the negative regulatory pathways involved the degradation of polycyclic aromatic hydrocarbons (Fig. 8). Furthermore, the markedly different positive regulatory pathways observed in the M group included other polysaccharide degradation pathways, whereas the negative regulatory pathways involved cell apoptosis and vasopressin-modulating water reabsorption pathways. G treatment was related to the following positive regulatory pathways: phosphotransferase system, bacterial invasion of epithelial cells, bacterial invasion of epidermal cells, and bacterial

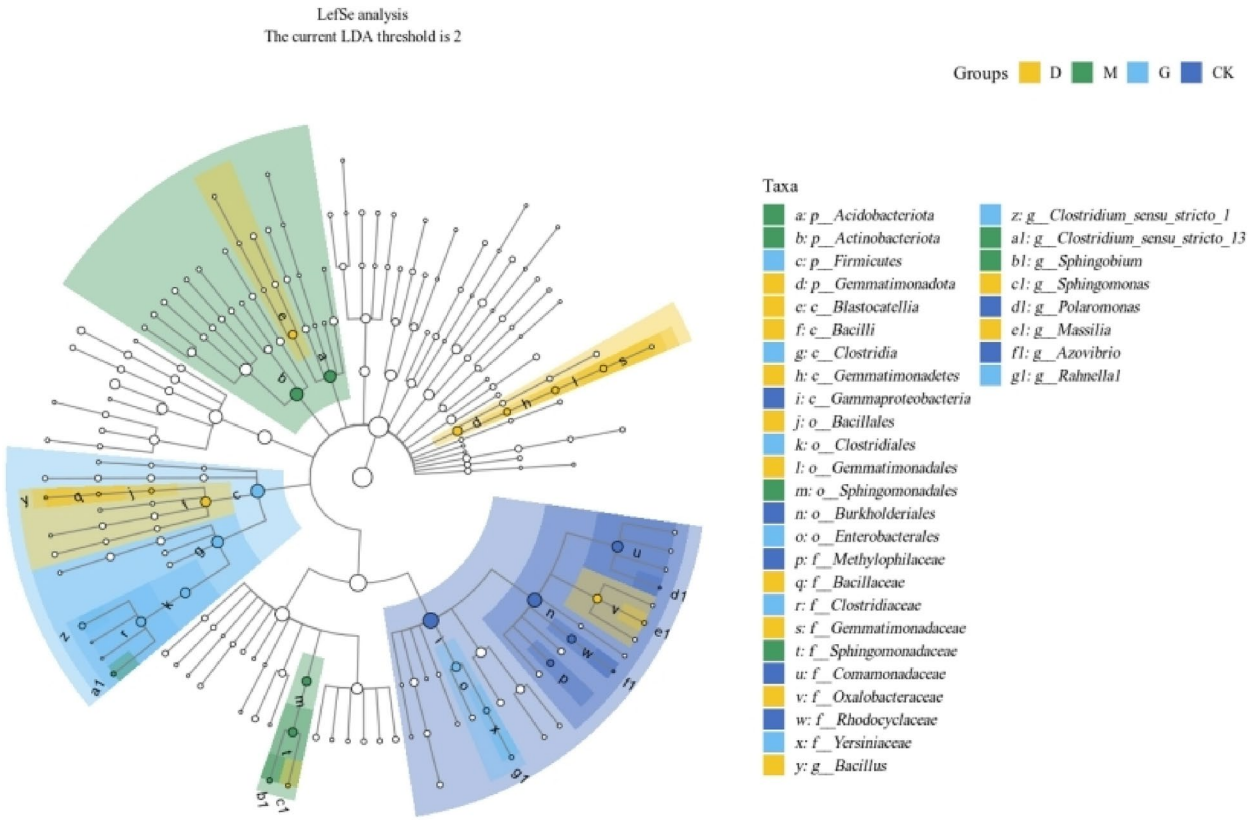

Fig. 5 LEfSe differential analysis of soil microbial community

Note: p, c, o, f, g, and s represent phylum, class, order, family, genus, and species, respectively

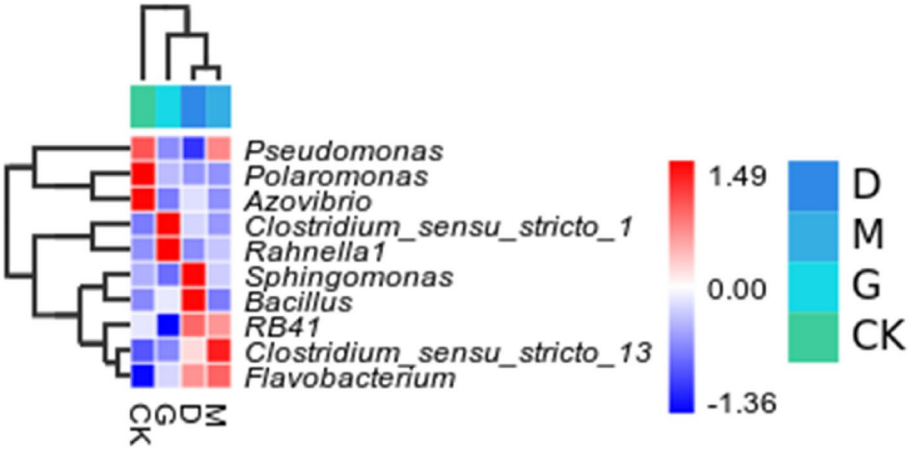

Fig. 6 Genus composition heatmap

invasion of epithelial cells. The negative regulatory pathways of G treatment included D-arginine and D-ornithine metabolism, cell apoptosis, and styrene degradation pathways.

Discussion

From an environmental perspective, before agricultural chemicals were widely used in agriculture, their toxicological or ecological impacts should be evaluated [35]. It has been observed that the application of plant growth regulators can increase plant biomass [36]. The use of EDTA and CA reduced the diversity and richness of soil bacterial communities, while the combination of DA-6 and EDTA or CA foliar spraying increased the diversity and richness of soil bacterial communities [37]. Most articles have reported that pesticides can reduce the diversity and richness of microbial communities [38, 39]; But there was no significant difference in the alpha diversity of bacterial communities between the pesticide/fertilizer mixed and single fertilization treatments in sugarcane fields without the addition of pesticides or fertilizers [37](Huang et al., 2021). However, our research indicated that as the concentration of CC increases, the Chao1, Shannon, Simpson, and Pielou indices showed a decreasing trend, while the low concentration CC increased compared to CK, indicated that the application of low concentration CC on peanuts significantly increased soil bacterial diversity and richness.

The results of this study indicated that the dominant bacterial phyla in soil are Proteobacteria, Firmicutes, Acinetobacteria, Bacteroidetes, and Gemmatimonadota, which was consistent with previous research findings [40–42]. In some studies, it has been found that low application can increased the number of certain beneficial bacteria in soil [43], such as *Azovibrio*, *Bacillus*, *Sphingobium* and *Sphingomonas*, which was consistent with the results of our study. *Azovibrio* can help fix nitrogen in crops and reduce nitrogen fertilizer application [44]. *Bacillus* can inhibit pathogens, induce systemic resistance, promote growth, and thus promote its widespread use as a biological control bacterium [45]. In addition, *sphingolipids* and *sphingomonas* were beneficial rhizosphere microorganisms in plants that can degrade organic pollutants such as chlorpyrifos [46], thereby inducing plant growth and inhibiting pathogens, thereby enhancing plant disease resistance [47, 48]. In addition, a significant positive correlation has been observed between members of the North Star genus and the concentration of vanadium components in tailings, indicating that the North Star genus has high vanadium resistance or can utilize vanadium for energy metabolism processes [49]. As the concentration increased, it may caused changes in the community structure of soil microorganisms. High concentration application may have serious toxic effects on soil microorganisms. It may damage the cell membrane structure of microorganisms or interfere with their metabolic processes. In this study, high concentrations of CC can reduce the number of bacteria in the soil, leading to interference in processes such as cell apoptosis, and styrene degradation pathways in the soil.

The degradation process of pesticides in soil was largely attributed to microorganisms, but there were also other influencing factors [50]. Although our data was only for one year and few monitoring indicators, we have also obtained some preliminary results. In the future, we will further improved it, not limited to planting peanuts in one season, but can conduct research on continuous

Fig. 7 Metabolic pathway statistics

multi season peanut planting or rotation with other crops. Observed the effects of different concentrations of CC on soil diversity in different seasons and crop rotation, and understanded its variation patterns under different planting systems. At the same time, increased research indicators to enrich the results, such as soil enzyme activity determination, soil physicochemical property analysis, and soil nematode and actinomycete community research.

Conclusion

As the concentration of CC increases, the Chao1, Shannon, Simpson, and Pielou indices showed a decreasing trend. This indicates that the application of low concentration CC to peanuts significantly increased soil bacterial diversity and richness. As the concentration of CC increased, the abundance of Proteobacteria decreased, while Firmicutes and Bacteroidota increased. The abundance of Acidobacteriota and Bacteroidota in groups D

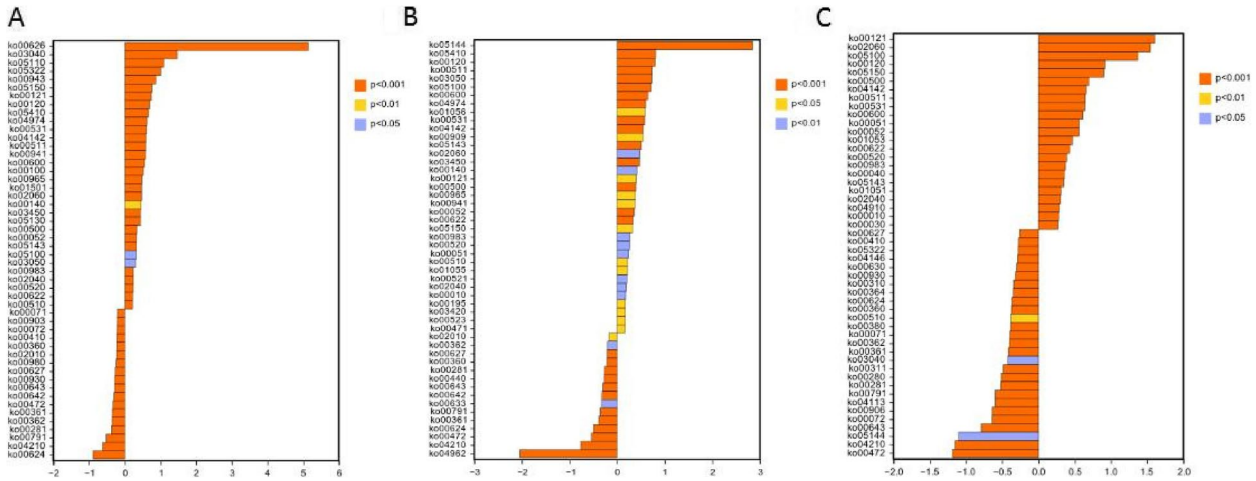

Fig. 8 Differences in metabolic pathways under different CC concentrations. **A** D *vs.* CK. **B** M *vs.* CK. **C** G *vs.* CK

and M increased, while in G group decreased. The D, M and G groups showed a decrease in the abundance of *Pseudomonas*, *polaromonas*, and *Azovibrio* compared to CK, while the abundance of *Flavobacterium* increased. Moreover, *Rahnella1* abundance in the D and M groups was decreased, while increased in the G group.

Abbreviations

CC	Chlormequat chloride
OTUs	Operational taxonomic units
PCoA	Principal component analysis

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-03828-5.

Supplementary Material 1

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Authors' contributions
QL and JW conceived and coordinated this study, and wrote this paper. XW, CG, LL, and TP designed, executed, and analyzed experiments and graphs. XZ and GL provided technical assistance and sampling. All authors reviewed the results and approved the final version of the manuscript.

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Data availability
The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive in National Genomics Data Center, China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA022691) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Zhou XJ, Ren XP, Luo HY, Huang L, Liu N, Chen WG, et al. Safe conservation and utilization of peanut germplasm resources in the Oil Crops Middle-term Genebank of China. Oil Crop Sci. 2022;7(1):9–13. https://doi.org/10.1016/j.ocsci.2021.12.001.
2. Lu Q, Huang L, Liu H, Hao L, Vanika G, Sunil SG, et al. A genomic variation map provides insights into peanut diversity in China and associations with 28 agronomic traits. Nat Genet. 2024;56:530–40. https://doi.org/10.1038/s41588-024-01660-7.
3. Wei SC, Li KW, Yang YT, Wang CY, Liu C, Zhang JQ. Comprehensive climatic suitability evaluation of peanut in Huang-Huai-Hai region under the background of climate change. Sci Rep. 2022;12:11350. https://doi.org/10.1038/s41598-022-15465-3.

4. Zhang YQ, Chen CX, Nie SH, Xu QJ, Sai SLH, Lei JJ. Analysis of the regulation of winter wheat stem lodging resistance during the drip application period of Chlormequat chloride. Xinjiang Agric Sci. 2023;60(08):1873–8.
5. Zhou X, Xu Y, Xu Q. Effects of different concentrations of chlormequat chloride treatment on excessive growth and post planting growth of cucumber seedlings in high-temperature environments. Vegetables. 2024;04:30–5.
6. Cui D, Han J, Xu Y, Wang J, Li S. Effects of different concentrations of Chlormequat chloride, naphthylacetic acid, and uniconazole on the growth of scallion seedlings. China Vegetables. 2024;01:90–5.
7. Li J, Yuan YJ, Yin LY, Yin SH. Effects of chlormequat chloride on strawberry plug cutting seedlings and after planting. Chin Fruit Tree. 2024;02:63–7.
8. Han J, Luo Z, Ma X, Zang Y, Xie L, Li D. Study on the Effect of Atractylodes macrocephala on the Content of 13 Effective Ingredients in Astragalus membranaceus. Lishizhen Med Materia Medica. 2023;34:987–91.
9. Ma Y, Cao Y, Su L, Wang H, Xin S, Jiang B, et al. The effect of spraying chlormequat chloride on tomato plant and fruit quality. Shandong Agric Sci. 2023;55:71–8.
10. Li T, Xue R, Feng M, Wang Y, Zhuang Y, Ge W, et al. The effects of four chemical control agents on the main traits and yield of peanuts. Liaoning Agric Sci. 2018;03:87–8.
11. Li X. The effect of dwarfism on peanut plant growth. Modern Rural Sci Technol 2013;(15):59+45.
12. Zhong R, Chen Y, Tang X, Jiang J, Han Z, He L, et al. The effects of three plant growth regulators on the photosynthetic physiology, yield, and quality of peanuts. J Food Composition Anal Chin Agric Sci Bull. 2013;29:112–6.
13. Lin QJ, Wu XX, Zou X, Li G, Wang JZ, Guo CJ. Application and safety analysis of plant growth regulators in peanuts. J Agronomy. 2024;:1–6.
14. Sorensen MT, Danielsen V. Effects of the plant growth regulator, chlormequat, on mammalian fertility. Int J Androl. 2006;29:129–33. https://doi.org/10.1111/j.1365-2605.2005.00629.x.
15. Hou X, Hu H, Xiagedeer B, Wang P, Kang C, Zhang Q, et al. Effects of chlorocholine chloride on pubertal development and reproductive functions in male rats. Toxicol Lett. 2020;319:1–10. https://doi.org/10.1016/j.toxlet.2019.10.024.
16. Xiao Q, Hou X, Kang C, Xiagedeer B, Hu H, Meng Q, Jiang J, Hao W. Effects of prenatal chlorocholine chloride exposure on pubertal development and reproduction of male offspring in rats. Toxicol Lett. 2021;351:28–36. https://doi.org/10.1016/j.toxlet.2021.08.005.
17. Temkin AM, Evans S, Spyropoulos DD, Naidenko OV. A pilot study of chlormequat in food and urine from adults in the United States from 2017 to 2023. J Expo Sci Environ Epidemiol. 2024;34(2):317–21. https://doi.org/10.1038/s41370-024-00643-4.
18. Wall D, Nielsen U, Six J. Soil biodiversity and human health. Nature. 2015;528:69–76. https://doi.org/10.1038/nature15744.
19. Delgado-Baquerizo M, Reich PB, Trivedi C, David JE, Sebastián A, Fernando DA, et al. Multiple elements of soil biodiversity drive ecosystem functions across biomes. Nat Ecol Evol. 2020;4:210–20. https://doi.org/10.1038/s41559-019-1084-y.
20. Zhao H, Li Q, Jin X, Li D, Zhu Z, Li Q. Chiral enantiomers of the plant growth regulator paclobutrazol selectively affect community structure and diversity of soil microorganisms. Sci Total Environ. 2021;797:148942. https://doi.org/10.1016/j.scitotenv.2021.148942.
21. Guo T, Wang F, Tahmasbian I, Wang Y, Zhou T, Pan X, et al. Core soil microorganisms and abiotic properties as key mechanisms of complementary nanoscale zerovalent iron and nitrification inhibitors in decreasing paclobutrazol residues and nitrous oxide emissions. J Agric Food Chem. 2024;72:7672–83. https://doi.org/10.1021/acs.jafc.3c06972.
22. Lin CH, Kuo J, Wang YW, Chen M, Lin CH. Bacterial diversity in paclobutrazol applied agricultural soils. J Environ Sci Health Part B. 2010;45(7):711–8. https://doi.org/10.1080/03601234.2010.502464.
23. Huang R, Cui X, Luo X, Mao P, Zhuang P, Li Y, et al. Effects of plant growth regulator and chelating agent on the phytoextraction of heavy metals by Pfaffia glomerata and on the soil microbial community. Environ Pollut. 2021;283:117159. https://doi.org/10.1016/j.envpol.2021.117159.
24. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, et al. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci U S A. 2011;108(supplement_1):4516–22. https://doi.org/10.1073/pnas.1000080107.
25. Callahan BJ, Mcmurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13:581–3. https://doi.org/10.1038/nmeth.3869.
26. Zhou CF, Heal K, Tigabu M, Xia LD, Hu HY, Yin DY, et al. Biochar addition to forest plantation soil enhances phosphorus availability and soil bacterial community diversity. Forest Ecol Manag. 2020;455(C):117635–117635. https://doi.org/10.1016/j.foreco.2019.117635.
27. Chao A. Nonparametric Estimation of the Number of Classes in a Population. Scand J Stat. 1984;11:265–70.
28. Shannon CE. A mathematical theory of communication. Bell Syst Tech J. 1948;27:379–423.
29. Shannon CE. A mathematical theory of communication. Bell Syst Tech J. 1948;27:623–56.
30. Simpson EH. Measurement of diversity. Nature. 1949;163:688.
31. Faith DP. Conservation evaluation and phylogenetic diversity. Biol Cons. 1992;61:1–10.
32. Pielou EC. The measurement of diversity in different types of biological collections. J Theor Biol. 1966;13:131–44.
33. Good IJ. The population frequency of species and the estimation of the population parameters. Biometrics. 1953;40:237–46.
34. Bray JR, Curtis JT. An ordination of the upland forest communities of southern wisconsin. Ecological Monographs, 1957, 27.
35. Schmitz J, Hahn M, Brühl CA. Agrochemicals in field margins-an experimental field study to assess the impacts of pesticides and fertilizers on a natural plant community. Agr Ecosyst Environ. 2014;193:60–9. https://doi.org/10.1016/j.agee.
36. Sun X, Qiu L, Kolton M, HäggblomRui M, Xu R, Kong T, et al. VV Reduction by Polaromonas spp. in Vanadium Mine Tailings. Environ Sci Technol. 2020;54:14442–54. https://doi.org/10.1021/acs.est.0c05328.
37. Huang W, Lu Y, Chen L, Sun D, An Y. Impact of pesticide/fertilizer mixtures on the rhizosphere microbial community of field-grown sugarcane. 3 Biotech. 2021;11(5):210. https://doi.org/10.1007/s13205-021-02770-3.
38. Elżbieta W, Agata J, Urszula W, Andrzej B, Bożena Ł. Soil biological activity as an indicator of soil pollution with pesticides – A review. Appl Soil Ecol. 2020;147:103356. https://doi.org/10.1016/j.apsoil.2019.09.006.
39. Avinash SH, Nagaraj MN, Mahadev S, Bheemanna M, Harishchandra RN, Gundappagol RC. Analysis of soil microbial activity and population in rhizosphere soil exposed to chlorpyrifos. Int J Environ Climate Change. 2023;13(11):3096–103. https://doi.org/10.9734/ijecc/2023/v13i113480.
40. Wang RB, Wang ZM, Wang HY, Yu JL. Landward distribution and association of the soil stable organic carbon fractions and dominant bacterial phyla. Acta Microbiol Sin. 2022;62(6):2389–402.
41. Han SK, Sang HL, Ho YJ, Kevin TF, Man JK. Diversity and composition of soil acidobacteria and proteobacteria communities as a bacterial indicator of past land-use change from forest to farmland. Sci Total Environ. 2021;797:148944. https://doi.org/10.1016/j.scitotenv.2021.148944.
42. Morais Farias J, Krepsky N. Bacterial degradation of bisphenol analogues: an overview. Environ Sci Pollut Res. 2022;29:76543–64. https://doi.org/10.1007/s11356-022-23035-3.
43. Kuo J, Wang YW, Chen M, Fuh G, Lin CH. The effect of paclobutrazol on soil bacterial composition across three consecutive flowering stages of mung bean. Folia Microbiol (Praha). 2019;64(2):197–205. https://doi.org/10.1007/s12223-018-0644-x.
44. Chen L, Long C, Wang D, Yang J. Phytoremediation of cadmium (Cd) and uranium (U) contaminated soils by Brassica juncea L. enhanced with exogenous application of plant growth regulators. Chemosphere. 2020;242:125112. https://doi.org/10.1016/j.chemosphere.2019.125112.
45. Poulaki EG, Tjamos SE. Bacillus species: factories of plant protective volatile organic compounds. J Appl Microbiol. 2023;134(3):lxad037. https://doi.org/10.1093/jambio/lxad037.
46. Li Y, Feng FY, Mu QE, Li M, Ma LY, Wan Q, et al. Foliar spraying of chlorpyrifos triggers plant production of linolenic acid recruiting rhizosphere Bacterial *Sphingomonas* sp. Environ Sci Technol. 2023;57(45):17312–23. https://doi.org/10.1021/acs.est.3c04593.
47. Liu B, Wang H, Zhang K, Zhu J, He Q, He J. Improved Herbicide Resistance of 4-Hydroxyphenylpyruvate Dioxygenase from Sphingobium sp. TPM-19 through Directed Evolution. J Agric Food Chem. 2020;68:12365–74. https://doi.org/10.1021/acs.jafc.0c05785.
48. Shen J, Wang M, Wang E. Exploitation of the microbiome for crop breeding. Nat Plants. 2024;10:533–4. https://doi.org/10.1038/s41477-024-01657-4.

49. Sun S, Zhou X, Cui X, Liu C, Fan Y, McBride MB, et al. Exogenous plant growth regulators improved phytoextraction efficiency by Amaranths hypochondriacus L. in cadmium contaminated soil. Plant Growth Regul. 2020;90:29–40. https://doi.org/10.1007/s10725-019-00548-5.
50. Doolotkeldieva T, Konurbaeva M, Bobusheva S. Microbial communities in pesticide-contaminated soils in Kyrgyzstan and bioremediation possibilities. Environ Sci Pollut Res. 2018;25:31848–62. https://doi.org/10.1007/s11356-017-0048-5.

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