Front. Agr. Sci. Eng.
https://doi.org/10.15302/J-FASE-2023531

RESEARCH ARTICLE

Proline-2′-deoxymugineic acid, a phytosiderophore analog, drives beneficial rhizobacterial community formation to promote peanut micronutrition

Tianqi WANG*[1,2], Nanqi WANG*[2], Kunguang WANG[2], Qiaofang LU[2], Zhechao DOU[2], Zhiguang CHI[2], Dongming CUI[2], Motofumi SUZUKI[3], Yuanmei ZUO (✉)[2]

1 Root Biology Center, State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, College of Natural Resources and Environment, South China Agricultural University, Guangzhou 510642, China.
2 College of Resources and Environmental Sciences; State Key Laboratory of Nutrient Use and Management; National Academy of Agriculture Green Development, China Agricultural University, Beijing 100193, China.
3 Aichi Steel Corporation, Wanowari, Arao-machi, Tokai-shi, Aichi-ken, 476-8666, Tokai, Japan.

*These authors contribute equally to the work

KEYWORDS

Beneficial rhizobacteria recruitment, peanut, plant-soil micronutrition enhancement, proline-2′-deoxymugineic acid, stable microbial network

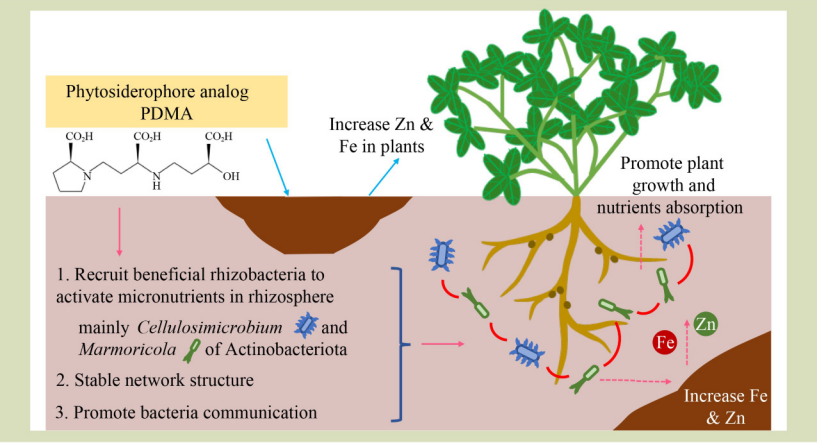
HIGHLIGHTS

- Proline-2′-deoxymugineic (PDMA) significantly altered the bacterial community in the peanut rhizosphere.
- PDMA resulting in a substantial increase of beneficial bacteria related with micronutrition in plant and soil, especially Actinobacteriota.
- PDMA application led to the development of a tight, stable microbial network and fosters microbial communication in the rhizosphere.

Received August 3, 2023;
Accepted November 6, 2023.

Correspondence: zuoym@cau.edu.cn

GRAPHICAL ABSTRACT



ABSTRACT

2′-Deoxymugineic (DMA), a phytosiderophore secreted by Poaceae species, can improve iron nutrition in plants. However, little is known about how DMA influences beneficial bacteria in rhizosphere microecosystem. To address this gap, the DMA analog proline-2′-deoxymugineic (PDMA) was used to evaluate its positive effect on peanut rhizobacterial communities and network structure. This study demonstrated that PDMA can promote the absorption of several mineral nutrients in plants and activate micronutrients in the rhizosphere. Specifically, PDMA led to significant impact on the bacterial community structure in the peanut rhizosphere, resulting in a substantial increase in the relative abundance of Actinobacteriota with six beneficial rhizobacterial genera in this phylum. The *Cellulosimicrobium* and *Marmoricola* of Actinobacteriota recruited by PDMA may enhance micronutrient availability

both to peanut plants and in soil. PDMA application led to the development of a tight, stable microbial network, as indicated by higher topological parameters and a greater variety of keystone genera. Functional prediction revealed that PDMA fosters microbial communication in the rhizosphere. Overall, PDMA was shown to recruit beneficial bacteria and to modulate bacterial network structure in the peanut rhizosphere. It is concluded that these findings demonstrate that phytosiderophore might promote plant growth and nutrition absorption by regulating plant–soil microecosystem.

1 Introduction

Root exudates released by plants into the rhizosphere alter the plant growth environment and promote nutrient absorption. Rhizosphere microbial community structure is also affected by root exudates through the recruitment of beneficial microorganisms and improvement of microbial function[1]. For example, flavonoids from legume roots activate rhizobium colonization, thereby improving nitrogen fixation[2]. Coumarin released by *Arabidopsis* alters the rhizosphere microorganism community to improve iron absorption by plants[3]. However, the effects of numerous root exudates on rhizobacteria remain unknown.

Plant adaptations to Fe deficiency are divided between a reduction mechanism in Strategy I plants (mostly the dicots) and a chelate mechanism in Strategy II plants (mostly poaceous plants)[4]. The main siderophore exuded by Strategy II plants, 2′-deoxymugineic (DMA), can activate and chelate insoluble Fe in soil[5]. In a previous study, it was shown that DMA released by maize was directly absorbed by intercropped peanut plants, leading to improved plant Fe nutrition[6]. Although study has demonstrated that DMA supplies resources for and enhances the physiologic functions of beneficial microorganisms[7], the contribution of DMA to the formation of rhizosphere microbial communities requires further investigation.

DMA is an effective Fe fertilizer; however, external DMA application is limited because of its instability and high cost[8]. To address these problems, the DMA analog proline-2′-deoxymugineic (PDMA) was recently synthesized[8]. Pot and field experiments have revealed that PDMA significantly promotes Fe uptake and plant growth in both peanut (Strategy I) and rice (Strategy II) plants, thereby improving crop yield and fostering biofortification[8–10]. The similar advantages of PDMA and DMA in terms of fostering Fe nutrition and plant growth suggest that both compounds might share a mechanism that influences the regulation of rhizobacteria. Despite the effects of PDMA on Fe nutrition have been well established, further research is needed to fully understand its impact on the absorption of other mineral nutrients in plants.

The purpose of this study was to assess the influences of external PDMA on the composition and function of peanut rhizobacteria. We first investigated the impact of PDMA on mineral nutrients in plants and soil. Then, we evaluated the effects of PDMA application to peanut plants on the community composition, bacteria-plant interaction in mineral nutrition and network structure of rhizosphere bacteria. Our results provide theoretical evidence that should help elucidate the mechanism of rhizobacterial regulation by phytosiderophores.

2 Materials and methods

2.1 Plant culture conditions

The chemical compound PDMA, which was previously described by Suzuki et al.[8], was obtained from Aichi Steel Corp. in Tokai, Japan. A pot experiment was conducted in a greenhouse located at China Agricultural University in Beijing, China. The soil properties and sowing methods for peanut plants in this experiment were uniform as described by Wang et al.[10]. The PDMA treatment involved irrigating 40 μmol·L^{-1} of the compound into the rhizosphere of each peanut plant, using 300 mL per pot, at four intervals: 30, 48, 65 and 83 days post-sowing (dps), which corresponded to the flowering, early pod-bearing, late pod-bearing, and fruit-ripening stages of the plants. The control pots were irrigated with an equal volume of water. The plants were harvested at 90 dps, and the rhizosphere soil was collected following the destruction of the pots. The plant samples were subjected to oven drying at 60 °C until a constant weight was achieved, after which the mineral nutrient concentration was determined. To obtain the rhizosphere soil,

soil particles adhering to the roots were gently removed with a brush, after which the soil on the roots was shaken off. Rhizosphere soil was collected from six individual plants from each pot and combined into a single sample. The samples were then stored on ice until microbial composition analysis could be conducted. Each treatment had four replicates.

2.2 Determination of plant and soil nutrition

Following the grinding of plant, 0.5 g of the sample was placed into digestion tube, then 5 mL of concentrated H_2SO_4 was added and set overnight. The solution was heated using a digestion heater (Hanon, Jinan, Shandong, China) and 2 mL of H_2O_2 was added twice, with thorough shaking after each addition. The solution changed from yellow to brown to colorless, indicating the completion of digestion. After cooling, the solution was diluted to a final volume of 100 mL. The nitrogen concentration of solution was measured by Semi micro Kjeldahl method with an automatic Kjeldahl apparatus (Hanon, Jinan, Shandong, China). The grinding plant samples were digested using a microwave digestion instrument (MARS 6 Classic, Cem Corp. Matthews, NC, USA) and calcium, boron and zinc concentrations were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES) using a 7300DV system (Perkin Elmer, Shelton, CT, USA). Soil Zn and Cu were determined after DTPA extraction by ICP-OES using air-dried rhizosphere soil as described in previous work[10].

2.3 DNA extraction and polymerase chain reaction amplification

To obtain microbial genomic DNA from peanut rhizosphere soil samples, the E.Z.N.A. soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) was used following the manufacturer's instructions. The extracted DNA quality and concentration were assessed by 1% agarose gel electrophoresis and a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), respectively. The DNA samples were stored at −80 °C for microbiome analysis, as described below. To amplify the hypervariable region (V3–V4) of the bacterial 16S rRNA gene, the primer pairs 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGA CTACHVGGGTWTCTAAT-3′) were employed, using an ABI GeneAmp 9700 polymerase chain reaction (PCR) thermocycler (Applied Biosystems, Waltham, MA, USA) as described by Liu et al.[11]. The PCR reaction mixture comprised of 4 μL 5× Fast Pfu buffer, 2 μL 2.5 mmol·L^{-1} dNTPs, 0.8 μL of each primer (5 μmol·L^{-1}), 0.4 μL Fast Pfu polymerase, 10 ng of template DNA, and ddH_2O to make up a final volume of 20 μL. The PCR amplification process involved an initial denaturation step at 95 °C for 3 min. Subsequently, 27 cycles were conducted, consisting of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s. A single extension was performed at 72 °C for 10 min, followed by a final incubation at 4 °C until retrieval of the samples. Triplicate samples were amplified during the process. To purify the PCR product, it was extracted from a 2% agarose gel and purified with the AxyPrep DNA Gel Extraction Kit (Axygen, Corning, NY, USA), which followed the manufacturer's instructions. Thereafter, the PCR product was quantified using the Quantus Fluorometer (Promega, Madison, WI, USA).

2.4 Illumina miniature sequencing analysis

Equimolar amounts of purified amplicons were collected and underwent paired-end sequencing on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) following standard protocols developed by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). The raw sequencing reads were uploaded into the National Center for Biotechnology Information (NCBI) Sequence Read Archive database with the accession number PRJNA906269.

2.5 Data processing

A custom Perl script was used to demultiplex the Raw FASTQ files. The resulting sequences were subjected to quality control and merging using fastp v0.19.6[12] and FLASH v1.2.7[13] software. The following criteria were used to filter the sequences: reads longer than 400-bp were truncated if they had an average quality score of less than 20 over a 50-bp sliding window, reads with ambiguous characters or shorter than 50 bp were discarded. Only overlapping sequences of more than 10 bp were assembled based on their overlapped sequence, with a maximum mismatch ratio of the overlap region that was limited to 0.2. Reads that could not be assembled were discarded. The resulting sequences were clustered into amplicon sequence variants (ASV) using UPARSE v7.1 software[14] using a sequence similarity level of 97%. This generated optimized sequences that could subsequently be used for further analysis. The most prevalent sequence was chosen as a representative for each ASV. After manual filtering of the ASV feature table, all chloroplast sequences were removed from the samples, as indicated in Table S1. The taxonomic classification of each ASV representative sequence was determined through RDP Classifier v2.2[15] with a confidence threshold of 0.7 against the 16S rRNA gene database (e.g., Silva v138), as shown in Table S2.

2.6 Statistical analyses

We utilized R v4.2.0 software[16] for bioinformatics analysis of soil microbiota. The library size of each sample was normalized to 32,541 through scaling with ranked subsampling. Using Mothur v1.30.1 software[17], rarefaction curves as well as α-diversity indices such as observed species, abundance-based coverage estimator (ACE) richness, Chao1 richness, and Shannon's diversity index were computed based on ASV data. To visualize Bray-Curtis dissimilarity, non-metric multidimensional scaling (NMDS) was performed utilizing the R package "vegan"[18] as the ordination method for 16S rRNA-based bacterial community structure variations, and the Bray-Curtis dissimilarity was performed by distance between points[19]. We used linear discriminant analysis (LDA) effect size (LEfSe)[20] to determine the abundance of bacterial taxa (ranging from phylum to genus) that differed significantly among various groups (LDA score > 2.5, $P < 0.05$). The R package "psych"[21] was used to perform correlation analysis between bacteria and plant–soil phenotypes with Spearman method, and the P values were adjusted by Holm method, which was then visualized by the R package "pheatmap"[22]. The significance of key nodes was evaluated based on their intra-module connectivity (Zi) or inter-module connectivity (Pi) value (Zi > 2.5 or Pi $\leqslant$ 0.62). Also, co-occurrence networks were constructed to study the relationships between microbiomes within the samples, as described previously[23]. A P-value of less than 0.05 and Spearman's correlation coefficient ranging from −0.85 to 0.85 were used to determine the statistical significance of correlations between two nodes. Finally, the PICRUSt2 software[24] was used at genus level to provide predictions of the ecological functions of the microbial communities in the rhizosphere.

3 Results

3.1 PDMA improved plant–soil nutrition

The results showed that the concentrations of N, Ca, B, and Zn in peanut plants were significantly increased by PDMA treatment. Among these nutrients, N showed the greatest improvement, with a 2.4-times increase in PDMA-treated peanut plants, while Ca, B, and Zn were enhanced by 32.7%, 15.5% and 20.5%, respectively (Fig. 1(a–d)). In terms of soil micronutrition, PDMA was found to significantly increase the Zn and Cu concentration in the peanut rhizosphere by 8.6% and 49.7%, respectively (Fig. 1(e–f)). These findings suggest that PDMA has the ability to activate other micronutrients in soil and promote plant nutrient absorption in addition to Fe, which also opens up avenues for further investigation into the influence of PDMA on rhizobacterial communities and their role in micronutrient cycling (Fig. 1(g)).

3.2 PDMA alters the rhizosphere microbial communities of peanut plants

High-throughput sequencing revealed a substantial influence of PDMA on the rhizobacterial community; after quality control, we obtained 32,541–35,529 high-quality sequences from each sample. To eliminate bias in subsequent analyses related to variations in sequencing depth, we normalized all data to a sequencing depth of 32,541. The Good's coverage values ranged from 99.95% to 99.99%, demonstrating adequate sequencing depth to capture diversity in the results. No significant differences were detected in bacterial community richness (Chao1 and ACE richness) or Shannon diversity (Table 1) in response to PDMA treatment. The NMDS results revealed that four PDMA-treated and four control soil samples were clearly clustered into separate groups, with a stress value of 0.046 (Fig. 2(a)). Thus, PDMA significantly altered microbial composition, rather than microbial species richness, in the peanut rhizosphere.

3.3 PDMA recruits *Actinobacteria* to enhance micronutrients in the peanut rhizosphere

LEfSe analysis detected the responses of bacterial community biomarkers to PDMA (LDA > 2.0 and $P < 0.05$). The PDMA treatments had 24 enriched biomarkers, whereas seven in the control treatments (Table S3). Significant enrichment of the phylum Actinobacteriota was observed in PDMA-treated soils (relative abundance: control, 19.3% and PDMA, 21.0%); no phyla showed significantly lower abundance in the PDMA treatments than in the control treatments (Fig. 2(b)). At genus level, JG30_KF_CM45, an uncultured Geminicoccaceae, *Solirubrobacter*, *Tumebacillus*, *Micromonospora*, an unclassified Propionibacteriaceae, *Actinoplanes*, an unclassified Rhizobiales, *Marmoricola*, *Cellulosimicrobium*, and an unclassified Caulobacteraceae were enriched after PDMA application (Fig. 2(b) and Table S4). Of the 11 enriched genera in the PDMA group, six belonged to the Actinobacteriota. In contrast, PDMA appeared to repel AKYG1722, an uncultured Saprospiraceae, *Thermincola*, and *Caenimonas* (Fig. 2(b) and Table S4). Variation in microbial composition between treatments was regarded as the effect of PDMA on peanut rhizobacteria.

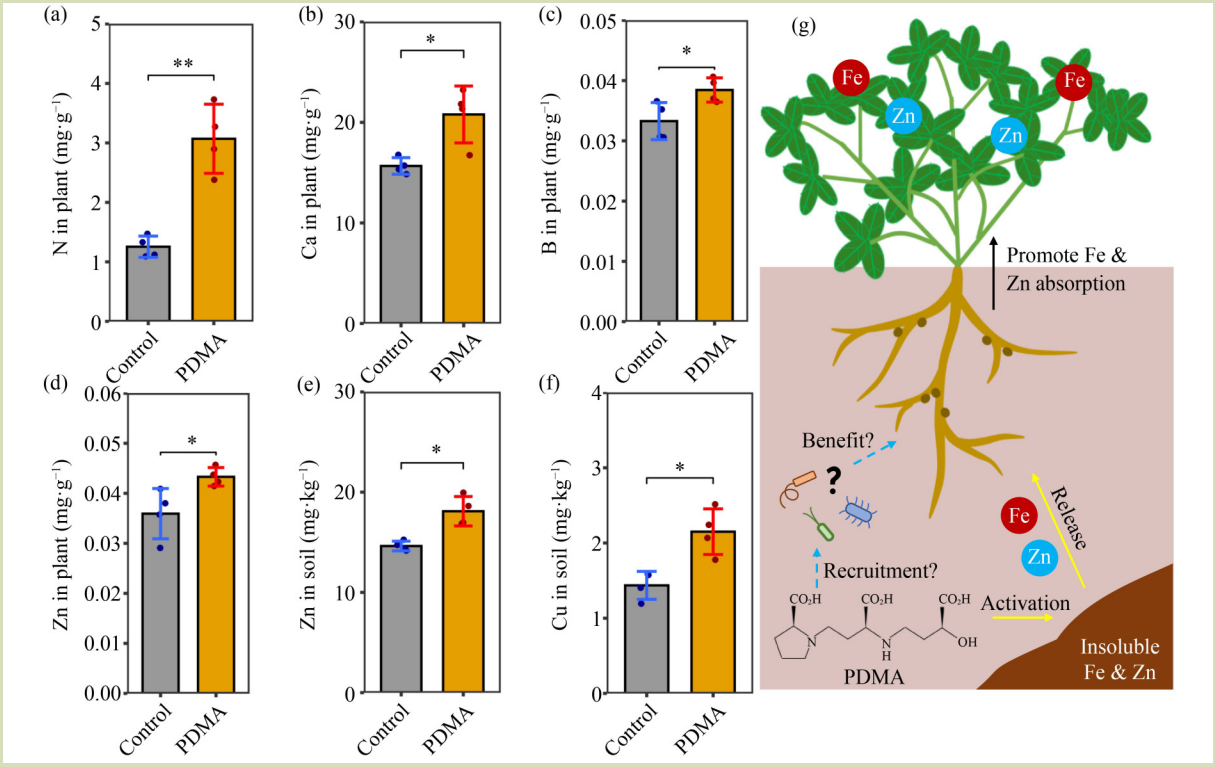


Fig. 1 Promotion of mineral nutrition in peanut plant and soil under PDMA treatment. (a) Nitrogen (N), (b) calcium (Ca), (c) boron (B), and (d) zinc (Zn) concentration in peanut plants with external PDMA. (e) Zn and (f) copper (Cu) concentration in peanut rhizosphere under PDMA treatment. Data are means ± SD of four biological replicates. Asterisks indicate significant differences between control and PDMA treated peanuts: *, $P < 0.05$; and **, $P < 0.01$ (Student's *t*-test). PDMA, peanut irrigated with PDMA; and control, peanut irrigated with water. (g) Model of PDMA application on improving Fe and Zn nutrition peanut. The insoluble Fe and Zn in peanut rhizosphere was significantly activated by PDMA, resulting in Fe and Zn nutrition promotion in peanut. However, whether PDMA influences the composition of rhizobacteria, further beneficial to micronutrition of peanut is still unknown.

Table 1 Effects of PDMA on the *α*-diversity of rhizosphere microbial community

α-Diversity	Control	PDMA
Observed species	1330 ± 48	1340 ± 50
ACE	1330 ± 49	1340 ± 51
Chao1	1330 ± 49	1340 ± 56
Shannon	2.25 ± 0.01	2.25 ± 0.02

Note: Data are means ± SD ($n = 4$). ACE means the abundance-based coverage estimator; Chao1 means the Chao1 estimator; Shannon means the Shannon index.

3.4 Rhizobacteria recruited by PDMA correlated with plant–soil nutrition

The relationship between phylum-level bacteria and plant–soil phenotype were revealed by our heatmap analysis. The 10 most abundant phyla in the peanut rhizosphere had varying correlations with plant growth, nutrition, and soil micronutrient status. Notably, our results indicated a significant positive correlation between Actinobacteriota and micronutrient concentrations in both peanut plants and rhizosphere soil. Specifically, this correlation was observed in terms of active Fe in young leaves, Zn in plants, and available Fe and Zn in the rhizosphere (Fig. 3(a) and Tables S5–S6). These results indicate that in addition to its primary function, PDMA may also activate soil micronutrients and promote the absorption of plant micronutrients by recruiting Actinobacteriota.

The selected genus-level biomarkers identified through LEfSe analysis were used to explore the relationship between plant–soil response and these biomarkers. The correlation heatmap revealed a significant association between the Fe status in plants and soil and the abundance of specific rhizobacterial genera belonging to Actinobacteriota, namely *Micromonospora*, *Cellulosimicrobium*, and *Marmoricola*

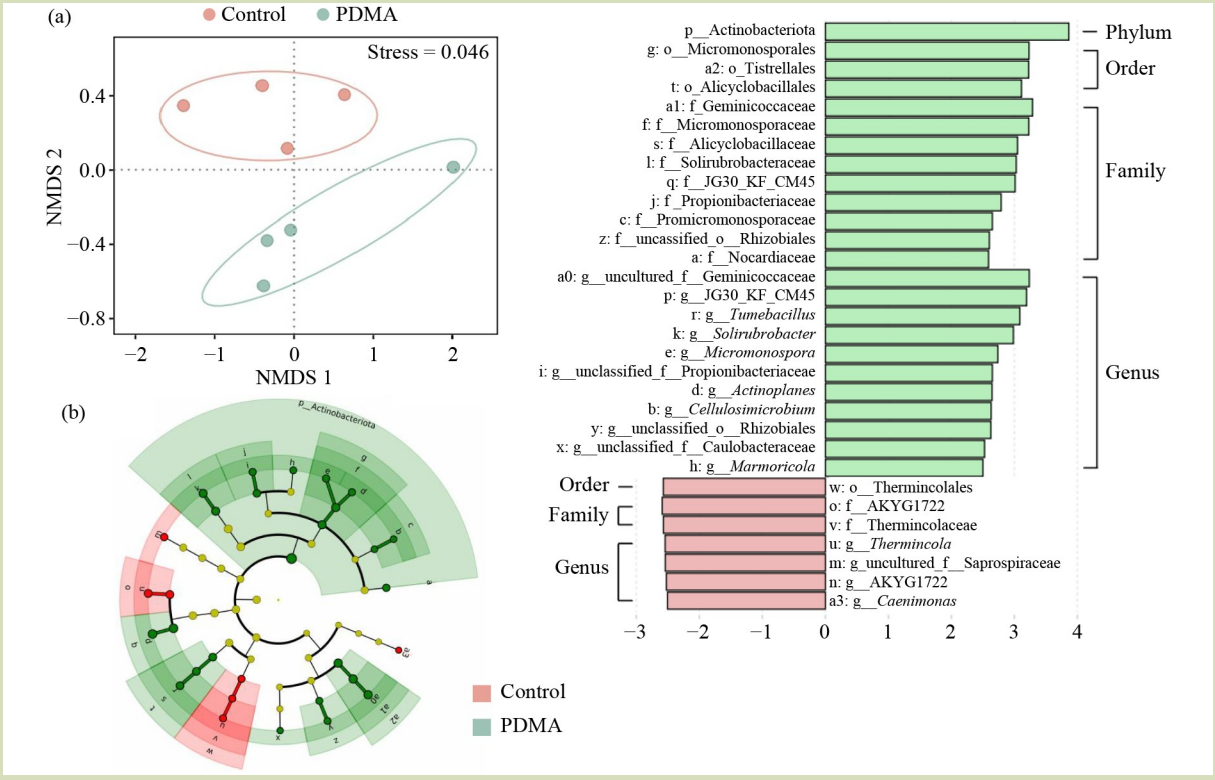


Fig. 2 Regulation of PDMA on microbial communities and bacteria biomarkers in peanut rhizosphere. (a) Non-metric multidimensional scaling (NMDS) analysis diagram was used to represent β-diversity of rhizosphere microbial communities. NMDS was based on the Bray-Curtis dissimilarity matrix; stress = 0.046, assuring the reliability of ordinations. Green points and ellipse represent bacterial community structures of four replicates of soil from PDMA group. Red points and ellipse represent bacterial community structures of four replicates of soil from control group. (b) Bacterial taxa with linear discriminant analysis (LDA) values greater than 2.5 are displayed ($P < 0.05$) by linear discriminant analysis effect size (LEfSe) analysis. Nodes represent bacterial phylogenetic levels from phylum to genus from the inside outwards. Red nodes and columns represent bacteria biomarkers in control group; green nodes and columns represent bacteria biomarkers in PDMA group. PDMA, peanut irrigated with PDMA; and control, peanut irrigated with water.

(Fig. 3(b), Tables S7–S8). Additionally, the heatmap analysis demonstrated a strong positive correlation between zinc nutrition in plants and soil and the relative abundance of specific Actinobacteriota, particularly an unclassified Propionibacteriaceae, *Cellulosimicrobium*, and *Marmoricola* (Fig. 3(b), Tables S7–S8). These findings indicate that *Cellulosimicrobium* and *Marmoricola* may serve as vital Actinobacteriota-biomarkers involved in the rhizosphere-mediated activation of Fe and Zn and their subsequent absorption in peanut plants.

3.5 PDMA stabilizes the network architecture of bacterial communities

The application of microbial network analysis to the 200 most abundant genera with significant correlations ($R > 0.85$ and $P < 0.05$) revealed variations in topological features between empirical and related random networks according to modularity. Visualization of the control and PDMA treatment networks using network parameters revealed distinct network architectures, with significantly higher edge numbers (positive and negative), connectance, average degree, and centralization closeness values under PDMA treatment than under control treatment (Table 2). These results indicate that all nodes within microbial networks under PDMA application were highly interconnected; the inner network was tightly connected.

To gain a greater understanding of the interactions between rhizobacteria and plants, genus Zi and Pi values, which indicate within- and between-module connectivity, respectively, were calculated to identify keystone genera. The networks of the two treatment groups had significantly different architectures (Fig. 4(b,c), Tables S9–S13). Five keystone genera, which connected the network modules, were identified in PDMA-

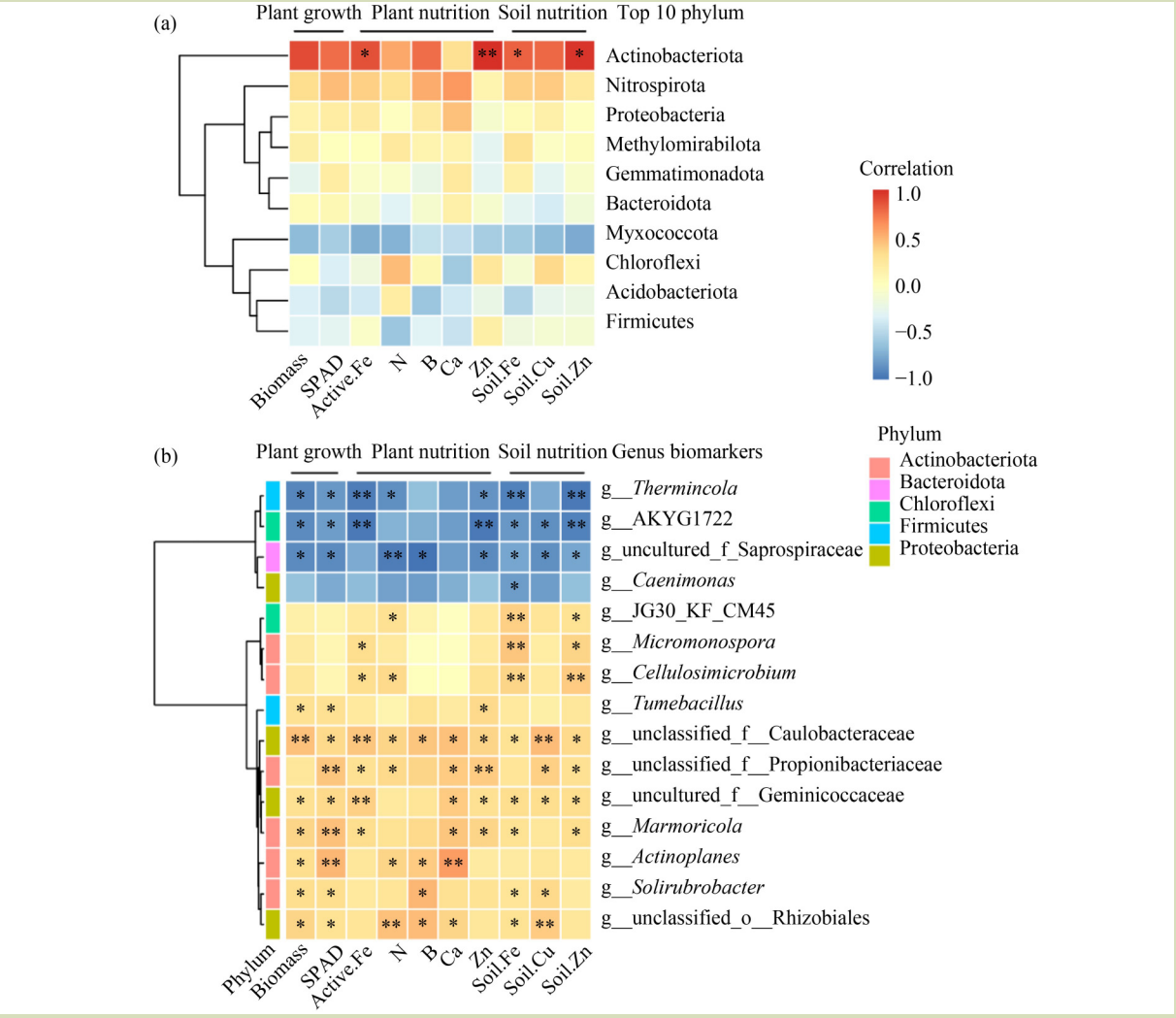


Fig. 3 The correlation between peanut phenotype and bacteria at phylum and genus level. (a) Heatmap of correlation for bacteria at phylum level with top 10 abundance with peanut biomass, SPAD value and active Fe concentration in young leaves, N, B, Ca and Zn concentration in peanut plants, and Fe, Cu and Zn concentration in rhizosphere soil. (b) Heatmap of correlation for biomarkers from both treatments at genus level with the peanut phenotype showed above. The correlation ranges from 1 to 0 to −1 (red to yellow to blue). Asterisks indicate significant correlations between bacteria at different phylum levels and plant growth and plant–soil nutrients status: *, $P < 0.05$; and **, $P < 0.01$.

Table 2 Effects of PDMA on the topological parameters of microbial network

Parameters	Control	PDMA
Edge number	2110	2670
Positive edge number	1240	1390
Negative edge number	868	1280
Connectance	0.106	0.134
Average degree	21.1	26.7
Centralization closeness	1.12	1.38

treated peanut bacterial communities: an uncultured Caldilineaceae, a Dadabacteriales, an uncultured Microscillaceae, *Rubrobacter*, and an unassigned Comamonadaceae (Fig. 4(a,b), Tables S14 and S16). In contrast, three Proteobacteria module hubs were observed in rhizosphere bacterial communities in the control group: an uncultured Rhizobiales, an unassigned Beijerinckiaceae, and *Reyranella* (Fig. 4(a,c), Tables S15–S16). These results indicate that PDMA increased the number of key nodes that connect modules, drastically altering keystone genera in the network.

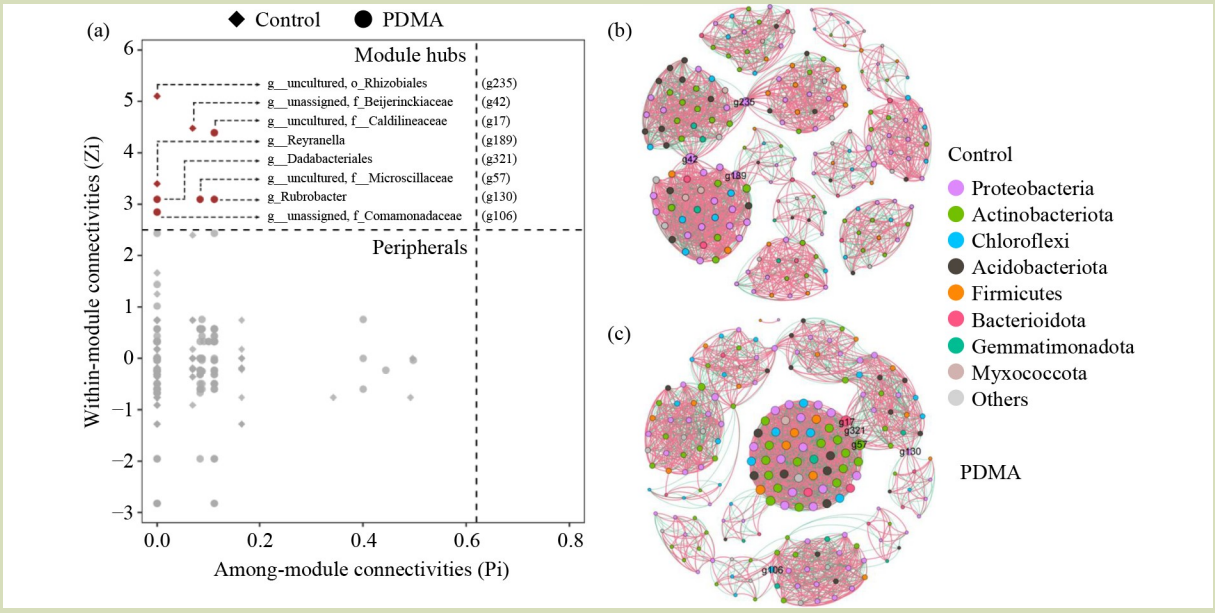


Fig. 4 Effects of PDMA on microbial network structure. (a) Effects of PDMA on Zi-Pi values of microbial network. Diamonds represent genus from control group, circles represent genus from PDMA group. Gray points indicate insignificant genera (peripherals), and red points indicate key genera in the microbial network (module hubs). (b) The co-occurrences network of rhizosphere microbial community structure in control treatment. (c) The co-occurrences network of rhizosphere microbial community structure in PDMA treatment. The nodes with different colors in the co-occurrences network represent different genus; the node size represents the abundance of the genus in the co-occurrences network; the edge between nodes indicates a correlation between the genus; the color of the edge represents a positive correlation (red) and a negative correlation (green). The key genera are marked in co-occurrences network. PDMA, peanut irrigated with PDMA; and control, peanut irrigated with water.

3.6 PDMA influences rhizosphere microorganism function

By assigning bacterial sequences from both treatment groups to functional groups according to the PICRUSt2 database, bacterial functions were divided into Level 1 and 2 pathways, where the Level 1 pathway consists of basic cell functions and the Level 2 pathway consists of higher functions. Our Level 1 pathway results indicated that PDMA enhanced environmental information processing, cellular processes, and genetic information processing; it suppressed genetic information processing (Table S17). These findings suggest that PDMA promotes bacterial communication with the environment and other bacteria in the rhizosphere (Fig. 5). The Level 2 pathway analysis results indicated that PDMA treatment enhanced xenobiotics biodegradation and metabolism, signaling and cellular processes, membrane transport, and cellular community; in contrast, genetic information processing, protein metabolism, and translation were enhanced in the control group (Fig. 5, Table S18). Overall, PDMA had a substantial effect on the functions of peanut rhizobacteria, mainly in terms of bacterial communication.

4 Discussion

PDMA is a new synthetic biofertilizer analogous to DMA, which is secreted by Poaceae species under Fe-limited conditions. Studies have demonstrated that PDMA can enhance plant growth, crop production and biofortification[8–10]. The present work further supports these findings by demonstrating that PDMA can also promote the uptake of other mineral nutrients, including N, Ca and B in plants (Fig. 1(a–c)), possibly because the improved nutrition absorption capacity in peanuts after diminishing Fe deficiency symptoms. Also, these nutrients are essential for plant growth and photosynthesis, which may explain the promotion in growth and yield of peanut observed in previous studies[10]. Additionally, our results showed an increase in Zn concentrations in both plants and soil, which is consistent with the biofortification of peanut kernels proved in previous work (Fig. 1(d–e))[10]. Overall, the promotion of various nutrients in both plants and soil is a novel advantage of PDMA in promoting plant growth and improving crop yield.

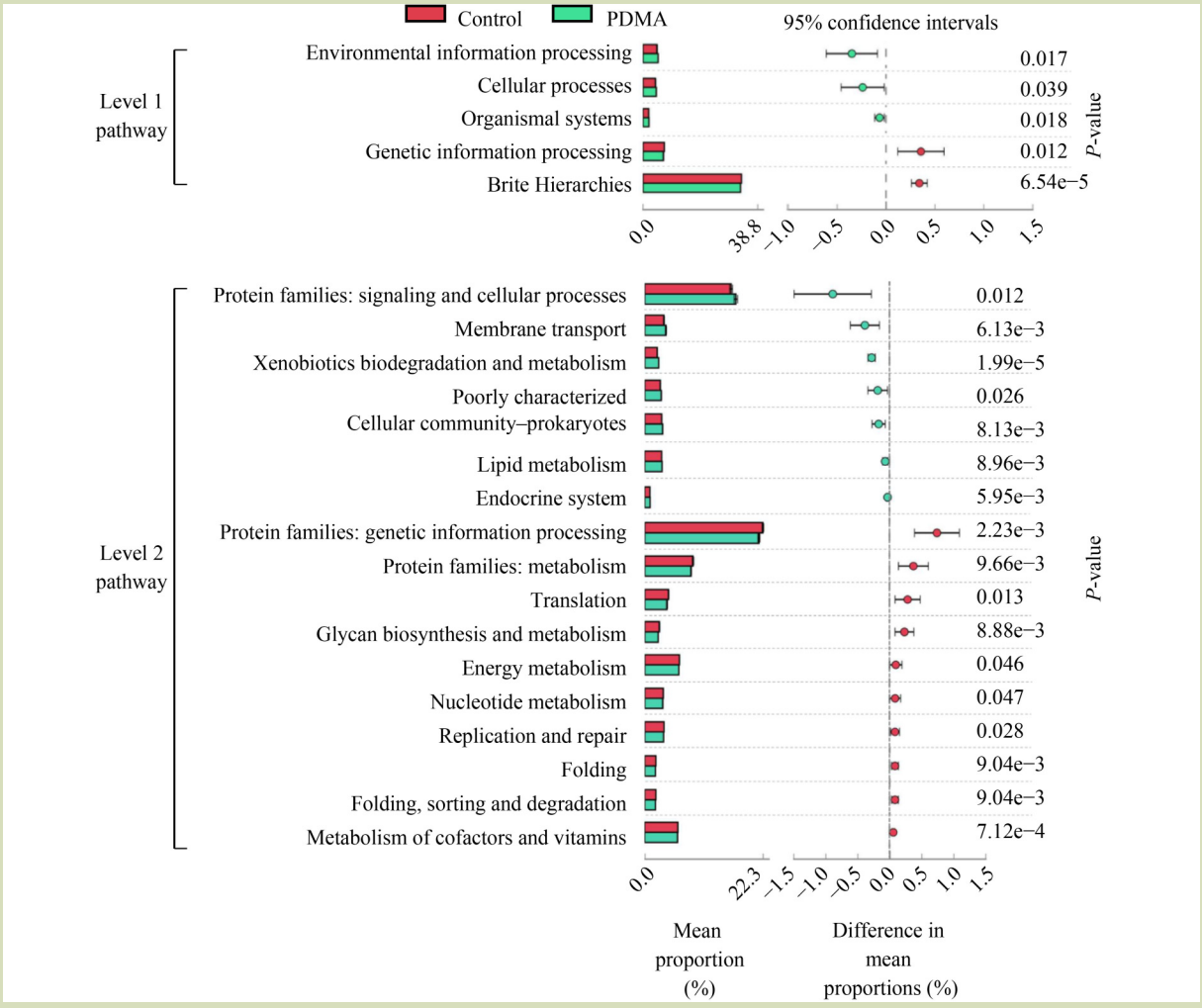


Fig. 5 Functional prediction in PDMA and control. The functions at Level 1 and Level 2 pathway which exhibit significant differences between PDMA and control treatments are presented ($P < 0.05$). *P*-values are calculated with Spearman correlation index. Red columns represent abundance of predicted function in control group; green columns represent abundance of predicted function in PDMA group. The color of the nodes represents the function is significantly higher in PDMA (green) or control (red). PDMA, peanut irrigated with PDMA; and control, peanut irrigated with water.

4.1 Better rhizobacterial structure was achieved through PDMA modulation

To gain a better understanding of the impact of PDMA on plant–soil-microbe interaction, we conducted further investigations into the bacterial composition of the peanut rhizosphere. The β-diversity results clearly indicated that PDMA altered the rhizosphere microbial communities of peanut plants (Fig. 2(a)). This finding is consistent with a previous report that the loss of a plant phytosiderophore Fe transporter impacted microbial community composition[25]; however, the present study is the first to observe the direct effects of phytosiderophore application on rhizosphere microorganisms.

Plants alter the composition of rhizosphere microorganisms by releasing root exudates; subsequently, recruited microorganisms improve plant growth, nutrient absorption and pathogen resistance[1]. Our results demonstrated that PDMA, an analog of maize root exudates, enriched several peanut rhizobacteria at the genus to phylum levels, especially Actinobacteriota (Fig. 2(b)). In many studies, Actinobacteriota is described as plant growth-promoting rhizobacteria, and species in Actinobacteriota are primarily related to N fixation, phosphorus solubilization, siderophore release, and phytohormone generation[26,27]. Present results also indicate that Actinobacteriota might foster micronutrients in soil and peanut (Fig. 3(a)). The genera from Actinobacteriota enriched in the PDMA-treated peanut rhizosphere have been intensively

studied to elucidate their plant growth-promoting effects[28,29]. Previous study has demonstrated the potential capability of *Cellulosimicrobium* and *Marmoricola*, both in the Actinobacteriota, to activate Fe and Zn in soil[30,31]. These findings align with the observed relationship between these two genera and the increased of Fe and Zn in both plants and soil in current study (Fig. 3(b)). These findings indicate that PDMA can promote plant growth and nutrition absorption through the enhancement of rhizosphere colonization by beneficial bacteria, mostly within *Cellulosimicrobium* and *Marmoricola* in the Actinobacteriota. Plant survival in the presence of external stress is aided by a robust rhizosphere microbial network[32]. Our results revealed that peanut rhizobacteria interacted more frequently after PDMA treatment (Fig. 4, Table 2); thus, internal connections within microbial groups were closer than the connections exhibited by untreated peanut plants[33]. Microbial networks under PDMA treatment had more key nodes from different phyla (Fig. 4), which indicated a complex, stable microbial community in the peanut rhizosphere[34]. Our results indicated that PDMA enhances the relationship between bacteria and the environment (Fig. 5); positive regulation of microbe-microbe interactions by PDMA may provide a healthier environment for peanut plants to tolerate biotic and abiotic stresses.

4.2 Potential mechanisms drive rhizobacteria variation

Root exudates regulate the growth and physiologic functions of rhizosphere microorganisms via multiple pathways[32]. As an analog of organic acid root exudates, PDMA provides C and N sources for microorganisms, thereby ensuring the growth of beneficial bacteria[27]. However, plant-derived Fe-DMA complexes appear to be effective Fe sources for bacteria[35]; this potentially constitutes another microbial regulation effect of PDMA. Although microorganisms may compete with plants for PDMA utilization, our results demonstrate that PDMA synergistically promotes plant nutrition and the recruitment of beneficial bacteria. This finding may be related to the greater stability and slow degradation of PDMA, compared with DMA[8]. Peanut root exudates may also change under PDMA application. For example, the secretion of coumarin, riboflavin, and protons under Fe deficiency may decrease because of improved Fe nutrition[3]. However, further investigation is needed to determine whether PDMA-induced variation in root exudates contributes to the enrichment of beneficial bacteria.

Previous studies have undertaken in-depth investigations of the mechanism underlying DMA-mediated Fe nutrition enhancement in legumes during intercropping with cereals, although we solely focused on the plant-plant interaction level[6]. To our knowledge, the present study is the first to show that a phytosiderophore analog can regulate plant growth by altering the rhizosphere microorganism composition, providing a theoretical basis for elucidation of belowground interactions between cereals and legumes. Collectively, these data provide a powerful body of evidence to clarify the mechanism by which PDMA can serve as a new bacterial fertilizer to influence plant–soil microecosystem, further promote plant growth.

5 Conclusions

Our results provide insights into the roles of PDMA in the recruitment of beneficial bacteria (mostly Actinobacteriota) in the peanut rhizosphere, and the recruited *Cellulosimicrobium* and *Marmoricola* might assist in the activation of micronutrients in the rhizosphere and further promote their absorption in plants. Also, we found that a stable, complex microbial network was formed after PDMA treatment, and microbe-microbe interactions were promoted. Overall, our findings indicate that PDMA can mediate the dynamic association between plants and beneficial rhizobacteria, further illuminating its capacity to serve as a new functional fertilizer.

Supplementary materials

The online version of this article at https://doi.org/10.15302/J-FASE-2023531 contains supplementary materials (Tables S1–S18).

Acknowledgements

The authors thank Prof. Kosuke Namba in Tokushima University for the development of PDMA, and express our profound gratefulness to researchers from our laboratory for the support given. This work was supported by the National Key Research and Development Program of China (2022YFD1901500/2022YFD1901501) and National Natural Science Foundation of China (32372810, 31872183).

Compliance with ethics guidelines

Tianqi Wang, Nanqi Wang, Kunguang Wang, Qiaofang Lu, Zhechao Dou, Zhiguang Chi, Dongming Cui, Motofumi Suzuki, and Yuanmei Zuo declare that they have no conflicts of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors.

REFERENCES

1. Bastías D A, Balestrini R, Pollmann S, Gundel P E. Environmental interference of plant–microbe interactions. *Plant, Cell & Environment*, 2022, **45**(12): 3387–3398
2. Dong W, Song Y. The significance of flavonoids in the process of biological nitrogen fixation. *International Journal of Molecular Sciences*, 2020, **21**(16): 5926
3. Stassen M J J, Hsu S H, Pieterse C M J, Stringlis I A. Coumarin communication along the microbiome–root–shoot axis. *Trends in Plant Science*, 2021, **26**(2): 169–183
4. Marschner H, Römheld V, Kissel M. Different strategies in higher plants in mobilization and uptake of iron. *Journal of Plant Nutrition*, 1986, **9**(3): 695–713
5. Clemens S. Metal ligands in micronutrient acquisition and homeostasis. *Plant, Cell & Environment*, 2019, **42**(10): 2902–2912
6. Xiong H, Kakei Y, Kobayashi T, Guo X, Nakazono M, Takahashi H, Nakanishi H, Shen H, Zhang F, Nishizawa N K, Zuo Y. Molecular evidence for phytosiderophore-induced improvement of iron nutrition of peanut intercropped with maize in calcareous soil. *Plant, Cell & Environment*, 2013, **36**(10): 1888–1902
7. Mimmo T, Del Buono D, Terzano R, Tomasi N, Vigani G, Crecchio C, Pinton R, Zocchi G, Cesco S. Rhizospheric organic compounds in the soil–microorganism–plant system: their role in iron availability. *European Journal of Soil Science*, 2014, **65**(5): 629–642
8. Suzuki M, Urabe A, Sasaki S, Tsugawa R, Nishio S, Mukaiyama H, Murata Y, Masuda H, Aung M S, Mera A, Takeuchi M, Fukushima K, Kanaki M, Kobayashi K, Chiba Y, Shrestha B B, Nakanishi H, Watanabe T, Nakayama A, Fujino H, Kobayashi T, Tanino K, Nishizawa N K, Namba K. Development of a mugineic acid family phytosiderophore analog as an iron fertilizer. *Nature Communications*, 2021, **12**(1): 1558
9. Ueno D, Ito Y, Ohnishi M, Miyake C, Sohtome T, Suzuki M. A synthetic phytosiderophore analog, proline-2′-deoxymugineic acid, is efficiently utilized by dicots. *Plant and Soil*, 2021, **469**(1–2): 123–134
10. Wang T, Wang N, Lu Q, Lang S, Wang K, Niu L, Suzuki M, Zuo Y. The active Fe chelator proline-2′-deoxymugineic acid enhances peanut yield by improving soil Fe availability and plant Fe status. *Plant, Cell & Environment*, 2023, **46**(1): 239–250
11. Liu C, Zhao D, Ma W, Guo Y, Wang A, Wang Q, Lee D J. Denitrifying sulfide removal process on high-salinity wastewaters in the presence of Halomonas sp. *Applied Microbiology and Biotechnology*, 2016, **100**(3): 1421–1426
12. Chen S, Zhou Y, Chen Y, Gu J. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, 2018, **34**(17): i884–i890
13. Magoč T, Salzberg S L. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, 2011, **27**(21): 2957–2963
14. Edgar R C. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods*, 2013, **10**(10): 996–998
15. Wang Q, Garrity G M, Tiedje J M, Cole J R. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, 2007, **73**(16): 5261–5267
16. R core team. R: a language and environment for statistical computing. *R Foundation for Statistical Computing,* 2022
17. Schloss P D, Westcott S L, Ryabin T, Hall J R, Hartmann M, Hollister E B, Lesniewski R A, Oakley B B, Parks D H, Robinson C J, Sahl J W, Stres B, Thallinger G G, Van Horn D J, Weber C F. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, 2009, **75**(23): 7537–7541
18. Oksanen J, Simpson G, Blanchet F G, Kindt R, Legendre P, Minchin P R, O'Hara R B, Solymos P, Stevens M H H, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H B A, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M O, Lahti L, McGlinn D, Ouellette M H, Ribeiro Cunha E, Smith T, Stier A, Ter Braak C J F, Weedon J. Vegan: Community Ecology Package. R Package Version 2.6-4, 2022
19. Bray J R, Curtis J T. An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs*, 1957, **27**(4): 326–349
20. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett W S, Huttenhower C. Metagenomic biomarker discovery and explanation. *Genome Biology*, 2011, **12**(6): R60
21. Revelle W. Psych: Procedures for Psychological, Psychometric, and Personality Research. R Package Version 2.3.3, 2023
22. Kolde R. Pheatmap: Pretty Heatmaps. R Package Version 1.0.12, 2019
23. Barberán A, Bates S T, Casamayor E O, Fierer N. Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME Journal*, 2012, **6**(2): 343–351
24. Douglas G M, Maffei V J, Zaneveld J R, Yurgel S N, Brown J R, Taylor C M, Huttenhower C, Langille M G I. PICRUSt2 for prediction of metagenome functions. *Nature Biotechnology*, 2020, **38**(6): 685–688
25. Xu L, Dong Z, Chiniquy D, Pierroz G, Deng S, Gao C, Diamond S, Simmons T, Wipf H M L, Caddell D, Varoquaux N, Madera M A, Hutmacher R, Deutschbauer A, Dahlberg J A, Guerinot M L, Purdom E, Banfield J F, Taylor J W, Lemaux P G, Coleman-Derr D. Genome-resolved metagenomics reveals role of iron metabolism in drought-induced rhizosphere microbiome dynamics. *Nature Communications*, 2021, **12**(1): 3209
26. Dahal B, NandaKafle G, Perkins L, Brözel V S. Diversity of free-living nitrogen fixing *Streptomyces* in soils of the badlands

of South Dakota. *Microbiological Research*, 2017, **195**: 31–39

27. Anwar S, Ali B, Sajid I. Screening of rhizospheric *Actinomycetes* for various *in-vitro* and *in-vivo* plant growth promoting (PGP) traits and for agroactive compounds. *Frontiers in Microbiology*, 2016, **7**: 1334
28. El-Tarabily K A, AlKhajeh A S, Ayyash M M, Alnuaimi L H, Sham A, ElBaghdady K Z, Tariq S, AbuQamar S F. Growth promotion of *Salicornia bigelovii* by *Micromonospora chalcea* UAE1, an endophytic 1-aminocyclopropane-1-carboxylic acid deaminase-producing actinobacterial isolate. *Frontiers in Microbiology*, 2019, **10**: 1694
29. Franke-Whittle I H, Manici L M, Insam H, Stres B. Rhizosphere bacteria and fungi associated with plant growth in soils of three replanted apple orchards. *Plant and Soil*, 2015, **395**(1–2): 317–333
30. Chatterjee S, Sau G B, Sinha S, Mukherjee S K. Effect of co-inoculation of plant growth-promoting rhizobacteria on the growth of amaranth plants. *Archives of Agronomy and Soil Science*, 2012, **58**(12): 1387–1397
31. Pan X, Zhang S, Zhong Q, Gong G, Wang G, Guo X, Xu X. Effects of soil chemical properties and fractions of Pb, Cd, and Zn on bacterial and fungal communities. *Science of the Total Environment*, 2020, **715**: 136904
32. Trivedi P, Leach J E, Tringe S G, Sa T, Singh B K. Plant-microbiome interactions: from community assembly to plant health. *Nature Reviews. Microbiology*, 2020, **18**(11): 607–621
33. Chaney L, Baucom R S. The soil microbial community alters patterns of selection on flowering time and fitness-related traits in *Ipomoea purpurea*. *American Journal of Botany*, 2020, **107**(2): 186–194
34. Shakir S, Zaidi S S, de Vries F T, Mansoor S. Plant genetic networks shaping phyllosphere microbial community. *Trends in Genetics*, 2021, **37**(4): 306–316
35. Boiteau R M, Markillie L M, Hoyt D W, Hu D, Chu R K, Mitchell H D, Pasa-Tolic L, Jansson J K, Jansson C. Metabolic interactions between *Brachypodium* and *Pseudomonas fluorescens* under controlled iron-limited conditions. *mSystems*, 2021, **6**(1): e00580–e20