

EFFECT OF CHICKEN MANURE-BASED FERTILISER ON BACTERIAL COMMUNITIES AND DIVERSITY OF TOMATO ENDOSPHERE MICROBIOTA

HAFEEZ UL HAQ, YE LI, LINGYUE JIN, TING ZHANG, LINJIE CHENG, ZHE LI, BAOYU TIAN*

College of Life Sciences, Fujian Normal University, Fuzhou, China

Haq, H.U., Li, Y., Jin, L., Zhang, T., Cheng, L., Li, Z., and Tian, B. 2021: Effect of chicken manure-based fertiliser on bacterial communities and diversity of tomato endosphere microbiota. *Agriculture (Pol'nohospodárstvo)*, 67(3), 144–154.

The frequent use of chemical fertiliser produces harmful effects on the soil ecosystem. Therefore, biocompatible methods are used to improve plant health and production through natural fertiliser or plant beneficial microorganisms. This study aims to investigate the effect of amended chicken manure-based natural fertiliser on bacterial communities and plant beneficial bacteria of tomato endosphere microbiome using the high throughput 16S rRNA gene amplicon sequencing. The results showed *Proteobacteria* ($89.4 \pm 4.7\%$ to $86.7 \pm 3.9\%$), *Actinobacteria* ($6.03 \pm 2.9\%$ to $3.56 \pm 2.1\%$), and *Firmicutes* ($3.34 \pm 1.3\%$ to $0.59 \pm 0.3\%$) as the dominant bacterial phyla of tomato endophytic microbiome. *Pseudomonas* and *Bacillus* were the most abundant identifiable genera in the chicken manure root (CMR) (amended manure treatments) sample. There was no significant difference in alpha bacterial diversity (Shannon index: $p = 0.48$) and species richness (Chao 1: $p = 0.43$) between control original root (OR) and CMR. However, the distribution of the dominant phyla was mainly affected by manure fertilisation. The non-metric multidimensional scaling (NMDS) and PCoA of beta diversity analysis suggested a significant separation in bacterial communities of tomato endophytes between CMR and OR. The most differently abundant bacterial taxa belong to *Bacteroidetes* in the OR group, whereas the beneficial bacterial communities of *Actinobacteria* and *Firmicutes* were more abundant in the CMR group. Therefore, the chicken manure application can significantly affect bacterial communities of tomatoes' root endophytic microbiome and effectively improve the abundance of the beneficial microbes as biofertilisers.

Key words: manure addition, endosphere microbiome, 16S rRNA gene amplicon sequencing, tomato

With an increase in the world population and the need for food, agricultural systems have become more productive and sustainable (Hartmann *et al.* 2015). The frequent use of chemical fertiliser can produce harmful effects on the soil ecosystem (Bebber & Richards 2020). Organic fertiliser, especially animal manure or plant residue-based natural fertiliser can be considered an important measure for improving soil quality and efficient ecological agricultural production (Li *et al.* 2014). Besides natural fertilisers, endophytes-based biofertiliser can

be alternative tools to chemical fertiliser (Hallmann *et al.* 1997; Xu *et al.* 2019; Sturz *et al.* 2000). It is widely accepted that as ubiquitous colonizers of plants, endophytes improve plant health and productivity (Eo & Park 2016). Endophytes may have beneficial effects on host plants by protecting the host from pathogen or pests, providing the nutrients, increasing competition for space, producing phytohormones to promote plant growth, etc. (Bulgarelli *et al.* 2013; Turner *et al.* 2013; Tian *et al.* 2017).

Extensive research has been carried out to as-

Hafeez Ul Haq, Ye Li, Lingyue Jin, Ting Zhang, Linjie Cheng, Zhe Li, Baoyu Tian (*Corresponding author), College of Life Sciences, Fujian Normal University, No.8, Shangsang Road, Cangshan District, Fuzhou, 350108, Fujian, China. E-mail: tianby@fjnu.edu.cn

sess the potential of endophytes as biological control agents and biofertilisers (Tian *et al.* 2017; Sturz *et al.* 2000). Recently, high throughput sequencing-based metagenomic analysis has revealed that *Actinobacteria* and *Proteobacteria* were present in the endosphere of Arabidopsis, rice, and tomatoes (Bulgarelli *et al.* 2013). Endophytic bacteria from genera of *Streptomyces*, *Pseudomonas*, and *Bacillus* have been demonstrated as beneficial microbes with remarkable activities in different studies (Eo & Park 2016). Several *Pseudomonas* have shown antagonism against pathogen bacteria, fungi, and nematodes. *Pseudomonas* also played important role in promoting plant growth through the production of secondary metabolites, such as phenazine derivatives, and hydrogen cyanide (Weller *et al.* 2002; Loper *et al.* 2012; Lundberg *et al.* 2012). Recent studies have revealed that *Pseudomonas* and *Bacillus* have a significant probiotic effect on the expression of Indole-3-acetic acid (IAA) in tomatoes, weakening the abscisic acid (ABA), the expression of ethylene, and also provides protection to plants from phytopathogens through preproduction clottase, antibiotics, and antibacterial peptides substances (Lin *et al.* 2012; Dombrowski *et al.* 2017; Tian *et al.* 2017; Xu *et al.* 2019).

The diversity of endosphere bacterial communities is critical to the sustainability, stability, and integrity of endosphere ecosystems (Wang *et al.* 2015). The use of natural organic fertilisers significantly impacts the bacterial structure, diversity of soil, and plant microbiome, from soil-plant systems to the ecological environment (Beecher 1998). Bacterial diversity significantly decreases in the field with continuous input of chemical nitrogen content, which may cause stress conditions and inhibit bacterial growth (Yang *et al.* 2017). In contrast, bacterial diversity in the organic fertilised soil is significantly higher than that of chemically fertilised soils in the legume and tomato system (Manching *et al.* 2014). Field experiments in tomato gardens to study the effects of different fertilisation on the soil microbial community indicate that organic fertiliser treatment has a higher diversity of soil bacterial communities (van Elsas *et al.* 2012; Xiao *et al.* 2018). However, previous studies only focused on the impact of different organic matter on bacterial diversity of endophytic microbiota in the plant, and little work

has been done to evaluate the contribution of biofertiliser on plant beneficial bacteria of plant root endophytic microbiota. In this paper, the influence of manure addition was investigated by using 16S rRNA gene amplicon sequencing. Community structure and composition, bacterial diversity of tomato root endosphere microbiome, and plant beneficial bacteria between control (OR) and manure treatments (CMR) were compared in detail. Therefore, based on the results, we are convinced that our study will open a new way for the development of the environment-friendly practice in agriculture with fertiliser additions in the future.

MATERIAL AND METHODS

Experimental setup and sample collection

In this experiment, the soil was collected from lawns without a known history of organic fertiliser on the campus of Fujian Normal University. Chicken manure was collected as a nutrient from the chicken farmhouse near campus because of its high concentration of micronutrients (Duncan 2005). The physicochemical properties of the soil and manure were examined with an inductively coupled plasma atomic emission spectrometer (Thermo Scientific, Waltham, MA, USA) (Peiffer *et al.* 2013). The soil and manure properties were as follows: pH 6.7, total carbon (2.9%; 35.0%), total nitrogen (0.35%; 4.7%), (16; 3,700 mg/kg) NH_4^+ , and (255.0; 57 mg/kg) NO_3^- , respectively. A pot experiment was performed in flower pots with 25 cm in height and 15 cm in diameter. The first treatment contained a total of 1.5 kg of soil with chicken manure (1 kg soil + 0.5 kg chicken manure) as a treated sample (CMR), and the other contained 1.5 kg soil without chicken manure as a control (OR) (Zhang *et al.* 2020b). Each treatment was performed with three replicates. 3–5 tomato seeds were planted in each flower pot under 25°C in daylight (14 h) and 20°C in dark (10 h) (70% humidity) in a climate-controlled glasshouse (Table S1). After 45 days of planting, tomato plants were pulled from every flower pot (Dong *et al.* 2019). The roots were shaken to remove soil particles completely and followed by washing with water. After that, root samples were separately immersed in 30% hydrogen peroxide for 30 min, and 70% ethanol for

1 min to remove the bacterial community residing on the surface of roots, and then washed with sterilized water up to three times. Finally, 0.5 g of the lateral root samples were chosen and cut into 0.1 to 0.5 mm sections using sterile scissors and placed in a 1.5 mL Eppendorf tube for further DNA extraction process (Zhang *et al.* 2019).

DNA extraction

Total genomic DNA was extracted with Power Soil® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA), following the manufacturer's protocols. The extracted DNA was run on 1% agarose gels at 90 V for 45 min in 1× TAE (Tris-acetate-EDTA) buffer to examine the DNA size and integrity. The concentration and purity of the extracted DNA were assessed using an ND2000c spectrophotometer (Thermo Scientific, Dreieich, Germany). Extracted DNA was stored at –80°C for high-throughput sequencing analysis.

PCR amplification and high throughput sequencing

The V5-V7 hypervariable regions of bacterial 16S rRNA gene were amplified using primer pair 799F (5' AACMGGATTAGATACCKG-3') and 1193R (5'-ACGTCATCCCCACCTTCC-3') fused with the appropriate Illumina adapters and a 12-bp index sequence unique to each sample (Cheng *et al.* 2019). All the Polymerase chain reaction (PCR) amplification reactions were carried out in 10 ng metagenomic DNA per 50 µL reaction. DNA amplicon was subsequently purified, combined in equimolar ratios, and sent to Sangon Biotech (Shanghai, China) for producing paired 300-nucleotide reads at an Illumina Miseq platform.

Data processing and analysis

The raw sequence data generated from a paired-end library was first linked using FLASH (version 1.2.3) (Magoč & Salzberg 2011). Cutadapt (version 1.9.1) (Martin 2011) was used to remove adapters, barcodes, and primers sequences. Low-quality sequence, lengths shorter than 200 bp, or sequences with ambiguous bases were removed to control sequence quality using USEARCH (version 8.1.1861) (Edgar 2010). Chimeric sequences were identified and removed with a *de novo* method using USEARCH (version 8.1.1861) (Edgar 2010), finally resulted in high-quality and effective sequences

for subsequent analysis. A summary of the obtained data used for processing steps is provided in Table S2. To correct the difference in sequencing depth, the number of bacterial reads per sample was separately elevated to the minimum number of reads. For individual samples, effective bacterial sequences were separately subsampled for the subsequent statistical analysis.

After subsampling, the data were processed according to a modified standard operating procedure (SOP) pipeline based on software package QIIME and USEARCH (Caporaso *et al.* 2010; Edgar 2010). Briefly, the effective sequence was clustered to operational taxonomic units (OTUs) using USEARCH at 97% sequence similarity (Edgar 2010). Each OTU's representative sequence was aligned to the 16S rDNA SILVA database (Release 128) (Yilmaz *et al.* 2014). Taxonomy was subsequently assigned to each representative sequence with 90% confidence using the ribosomal database project (RDP) classifier. The community richness and diversity index (α -diversity) within the sample were measured (Chao1 Index, ACE Index, Shannon diversity, and Simpson diversity). The community richness, chao1 index, and abundance-based coverage (ACE). ACE index were used to assess the abundance of bacterial taxa, while the Shannon and Simpson indices were used to assess alpha diversity. Statistical analysis was carried out using an analysis of variance (ANOVA) and $\alpha = 0.05$ to determine whether the diversity index or species richness estimator was statistically significant among root samples. To estimate the beta diversity, the weighted UniFrac distance was used to calculate the similarities of the membership and structures at the OTUs levels found in both groups. Principal coordinate analysis (PCoA) and non-metric multidimensional scaling (NMDS) plots using the Vegan package in R (version 3.3.1) were used to observe bacterial community differences and composition between individuals or in groups. The significance of the effects of manure addition on beta diversity was statistically measured using permutational multivariate analysis of variance (PERMANOVA), Adonis, or analysis of similarities (ANOSIM) performed using the Vegan package in R (version 3.3.1). Linear discriminant analysis effect size (LEfse) is an algorithm that can identify the characteristics of microbial communi-

ties in different treatment methods as well as emphasizes the statistical significance and biological relevance at the same time (<http://huttenhower.sph.harvard.edu/lefse/>). The non-parametric factorial Kruskal-Wallis sum rank test for LEfse was performed to identify significant differences in bacterial taxa among the groups and then estimate each feature's effect on differentially abundant features through linear discriminant analysis size. Functional annotation of protein was searched against the evolutionary genealogy of genes: non-supervised Orthologous groups (egglog, version 3.0) databases using basic local alignment search tool program (BLASTP) (version 2.2.26) (Altschul *et al.* 1990) with the E-value of 105 cutoffs. The abundance of a certain egglog entry in each sample was calculated by the total found genes and weighted by their coverage. The egglog database provides multiple sequence alignments and broad term functional annotation. The raw sequencing data were deposited in the National Center for Biotechnology Information (NCBI) sequence read archive (SRA) under accession number PRJNA593402.

RESULTS

Data processing

A total of 255,551 original sequences were obtained from both groups through high-throughput sequencing. After quality control filtration (removal of primer sequences, low-quality fraction sequences, and chimeric sequences, etc.), 234,813 high-quality, effective sequences were obtained, with an average of 39,135 sequences per sample (Table S2). The resultant filtered sequences were classified as 5,963 operational taxonomic units (OTUs) at a similarity level of 97%. A certain number of sequences were randomly selected from the sequence as horizontal coordinates. The number of OTUs represented by these sequences is shown in the OTUs accumulation curve (Figure S1). It showed that as the sample size increased, the OTUs accumulation curve tended to flatten, indicating that most of the OTUs in the samples were detected. The average coverage of 6 samples was 0.98 (min-0.96, max-0.99), which supported the above observation that sequencing had covered the vast majority of the species in each sample

to meet the further analysis requirements.

Estimating the bacterial diversity and richness of the endosphere microbiota

To quantify the diversity (Shannon diversity and Simpson diversity) and richness (ACE Index and Chao1 Index) of the bacterial community of root samples among both groups, we analysed the α -diversity index using the OTU-based analysis method (Figure S2). The results showed that the manure addition did not affect the diversity of bacterial communities. No significant differential OTU richness (ACE index: $P = 0.4252$, and Chao1 index: $P = 0.4304$) and bacterial diversity (Shannon diversity: $P = 0.4843$, and Simpson diversity: $P = 0.3716$) were observed between CMR and OR group.

Community structures and compositions of the endosphere microbiota

The sequences of both group samples were classified at the phylum, class, order, family, and genus levels. 27 phylum, 52 classes, 110 orders, 189 families, and 448 genera were identified in the root samples. *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Bacteroidetes* were the dominant phyla while the remaining phyla were in low abundance in tomato root samples (Figure 1A). Only the two most abundant phyla *Proteobacteria* and *Firmicutes* were significantly abundant in CMR compared to that of the OR sample (Figure 2). The relative abundances of *Proteobacteria* were $89.4 \pm 4.7\%$ in CMR samples. In comparison, $86.7 \pm 3.9\%$ to the OR samples their abundances were significantly lower than those in the CMR sample. Top fifty genera with sequence abundances $> 1\%$ were identified, including other, and unclassified (Figure 1B). The most abundant genera in both groups were *Pseudomonas*, *Bacillus*, *Herminiimonas*, *Propionibacterium*, *Staphylococcus*, *Corynebacterium*, *Sphingomonas*, *Flavobacterium*, *Gaiella*, and *Variovorax Ramlibacter*. The unclassified genera were the second most abundant in the CMR and OR. Among identifiable genera, *Pseudomonas* ($81.61 \pm 7.36\%$), *Bacillus* ($1.71 \pm 1.15\%$), *Herminiimonas* ($0.76 \pm 0.14\%$), *Propionibacterium* ($0.71 \pm 0.48\%$), and *Staphylococcus* ($0.55 \pm 0.45\%$) were the most abundant in the CMR sample (Figure 1B). While the most abundant identifiable genera in the OR sample were *Pseudomonas* ($66.6 \pm 12.4\%$),

Sphingomonas ($0.65 \pm 1.1\%$), *Herminiimonas* ($0.61 \pm 0.37\%$), *Ramlibacter* (0.53 ± 0.28) and *Gaiella* ($0.50 \pm 0.64\%$). The results indicated that the manure addition had a significant effect on the structure of the tomato roots' endogenous bacterial communities and with a higher abundance of the beneficial microbes, such as *Pseudomonas* and *Bacillus* (Figure 1B). Bacterial taxa with significantly differential abundances were detected by using linear discriminant analysis effect size (LEfSe) between the CMR and OR groups (Figure 3). The most differently abundant bacterial taxa belong to *Proteobacteria* Phylum in the OR group, whereas the phylum *Actinobacteria*, *Firmicutes*, and *Proteobacteria* were more abundant in the CMR group. Moreover, at the genus level, 9 genera showed significantly enriched between the CMR and OR groups. Specifically, at the order level, *Burkholderiales* were enriched in

OR group ($p = 0.03$) (Figure 3). In addition, *Microbacteriaceae*, *Rhizobiaceae*, *Bacillus*, *Bacillaceae*, *Bacillales* *Bacilli*, and *Actinomycetales* were most abundant in CMR groups (Figure 3).

Estimating the dissimilarity and similarities of the endosphere microbiota using the beta diversity

The principal coordinate analysis (PCoA) based on the Bray-Curtis algorithm clearly shows that the endosphere microbial community structure differed between the CMR and OR groups (Figure 4A). CMR and OR were separated from each other. In the total variance of the data set, the two principal components explain 59% of the total bacterial community. Furthermore, the first principal coordinate (PCoA1) is the most important, which displayed a 40% difference in bacterial community's variation, and the principal coordinate (PCoA2) explained 19%

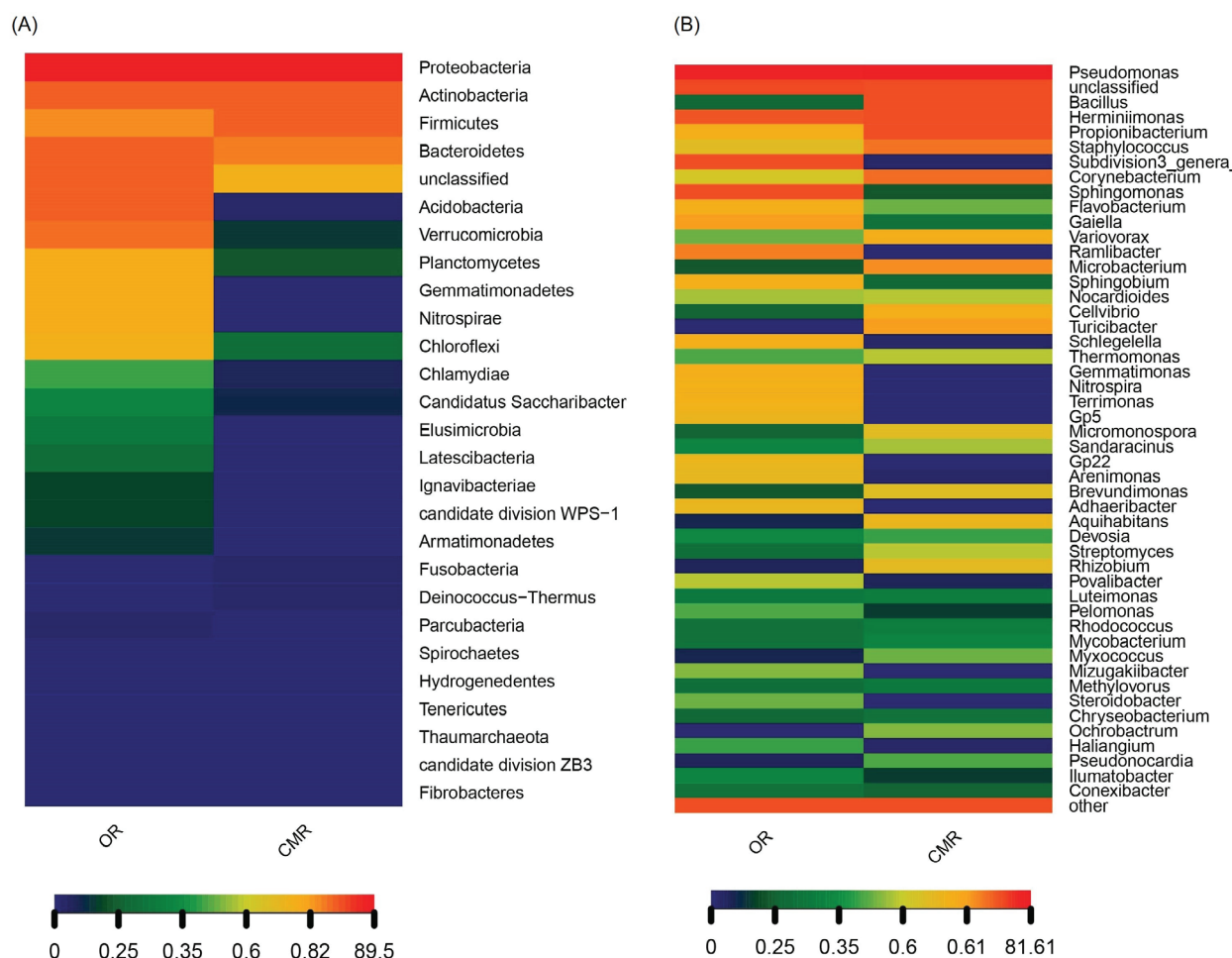


Figure 1. The composition and relative abundance of major bacterial taxa in tomato endosphere sample between CMR and OR while the bar represent the relative abundance of bacterial taxa. Heatmap was drawn using the average value of three replicates of each sample group. A – the composition and relative abundance of major bacterial Phylum; B – the composition and relative abundance of major bacterial genera.

bacterial variation. A non-metric multidimensional scale (NMDS) was obtained to compare and analyse the differences between and within groups, directly reflecting the differences in bacterial community structure. The NMDS analysis results were in alignment with these results. However, these results indicate significant differences in endosphere bacterial communities between CMR and OR (Figure 4B). The PERMANOVA test (ADONIS) revealed that the CMR group accounted for high differences (ADONIS: $R = 0.2471$, $p = 0.0015$), while it was notable that ANOSIM tests between the two group bacterial communities were more similar ($R = 0.530$, $p = 0.001$).

Functional annotation of protein

For functional annotations, the read was annotated with the eggNOG database. Most functions

based on the eggNOG database were shared by both group samples (Figure 5). Functional gene annotations based on COG classes showed that the relative distribution of the 26 basic metabolic categories of CMR and OR was similar. Additionally, in general functional prediction, two groups contain more genes related to energy production and transformation, amino acid transport and metabolism, transcription, carbohydrate transport and metabolism, signal transduction mechanisms, transcription, inorganic ion transport, and metabolism.

DISCUSSION

The diversity of the soil microbial population is critical to the stability, integrity, and survival of

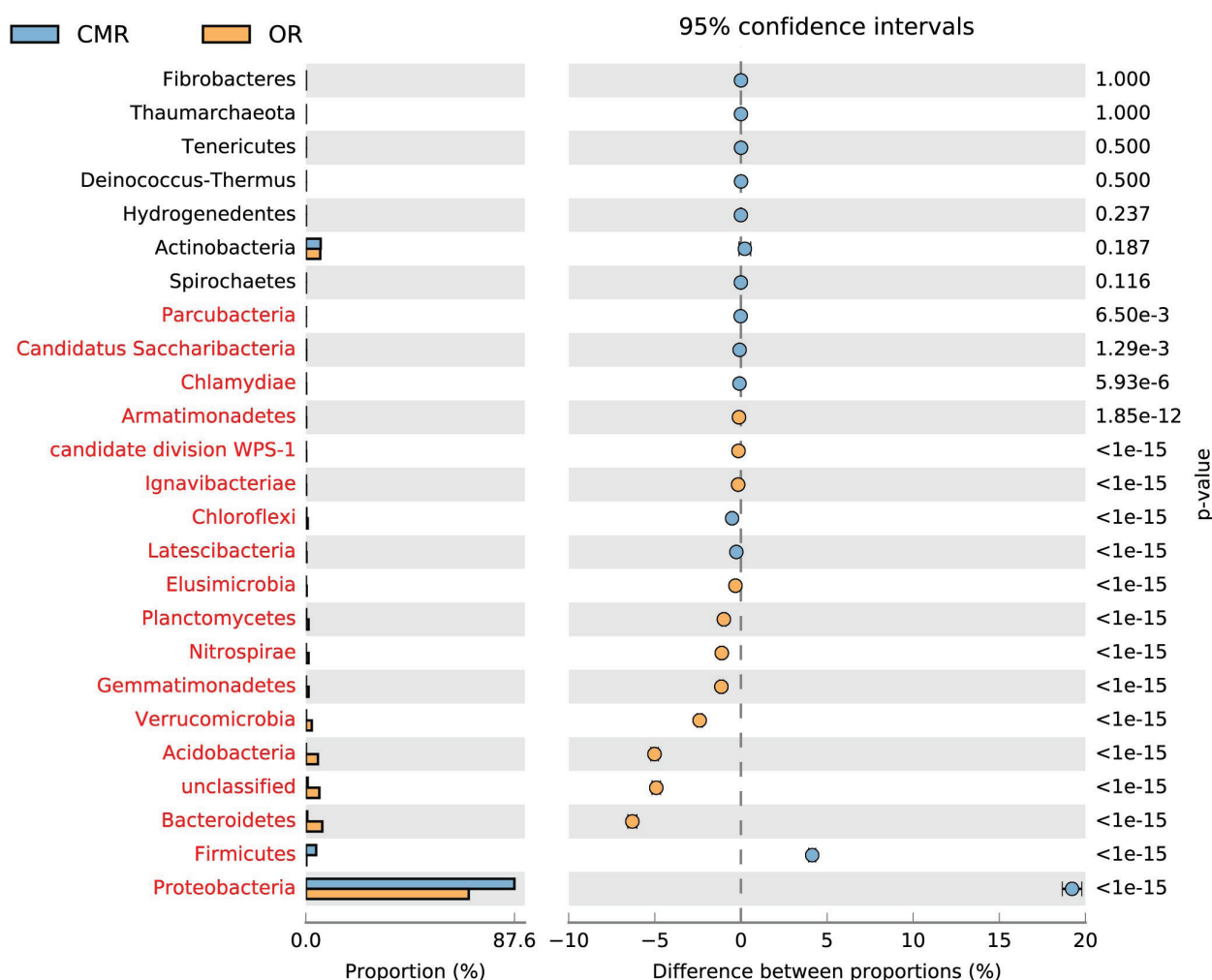


Figure 2. Fisher exact test plot of bacterial taxa abundance (means percent) in the CMR and OR samples at the phylum levels. The plot was drawn using the average value of three replicates of each sample group.

the soil environment (Cheng *et al.* 2019). In agro-ecosystems, different fertilisation applications can affect the soil microenvironment. Chemical fertilisation has been shown to harm soil bacterial diversity in a variety of agricultural ecosystems (Turner *et al.* 2013). A substantial decrease in bacterial diversity was found at high nitrogen levels, which might produce severe situations and inhibit bacte-

rial growth (Lee *et al.* 2019). In contrast, bacterial diversity was substantially higher in soils with organic fertilisation than in soils with chemical fertilisation in wheat and the legume system (Allard *et al.* 2016). Similarly, a field study in tomato cultivation was performed to investigate the impact of various fertilisation on soil bacterial communities, showing that organic manure application had the most

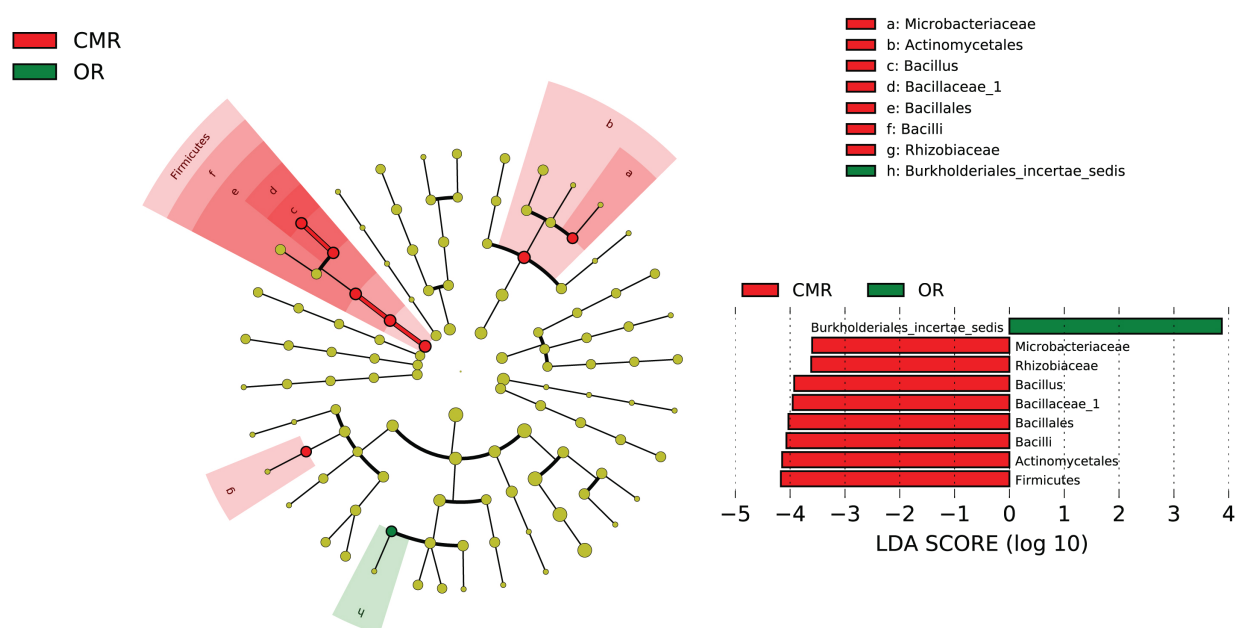


Figure 3. Linear discrimination analysis (LEfse) shows statistically different bacterial communities in CMR and OR. The LEfse and linear discriminant analysis (LDA) were drawn using the average value of three replicates of each sample group.

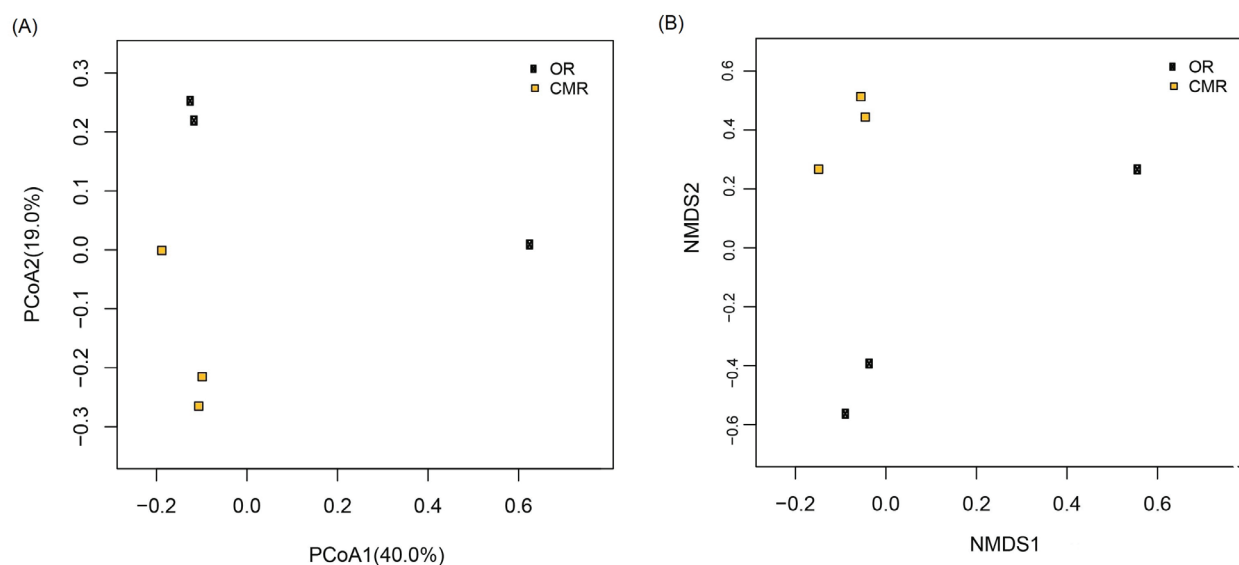


Figure 4. Microbial community differentiation between all endosphere samples. A – principal coordinate analysis (PCoA); B – non-metric multidimensional scaling (NMDS) analysis of bacterial communities based on Bray-Curtis similarity matrices.

diverse soil bacterial population (Lee *et al.* 2019). However, prior research only examined the effect of various organic matter on bacterial diversity but didn't address the effect of endosphere bacterial diversity in tomato cultivation. In our recent study, we focused on the complex changes in root bacterial diversity in tomato cultivation that had been fertilised with chicken manure in a pot experiment. The result showed that the manure application had no significant effect on bacterial diversity in term of OTUs richness and alpha-diversity (Figure S2), however, led to a significant effect on bacterial community's diversity (Figure 4) a similar trend presented in the previous study which described that fertilisation has a positive impact on beta-diversity on a tomato plantation (Luo *et al.* 2019). The complete utilization of nutrients in chicken manure by root bacteria may be the possible reason for high diversity in the tomato endosphere according to prior research (Ye *et al.* 2021). Therefore, we strongly believe that seasonal changes such as fertilisation would affect root bacterial diversity in tomato cultivation. Season and fertilisation can have a synergistic impact

on soil microorganism diversity. The composition of endosphere communities was susceptible to various fertilisation applications in agriculture practices, *Proteobacteria*, *Chloroflexi*, *Acidobacteria*, *Actinobacteria*, and *Bacteroidetes* were the most abundant phyla in agricultural soils (Berg *et al.* 2005). *Proteobacteria*, *Actinobacteria*, and *Acidobacteria* were the most abundant phyla in soils with various fertilisation levels in tomato cultivations (Green *et al.* 2012). Here, in the endosphere community composition was examined and the result revealed that *Proteobacteria*, *Actinobacteria*, *Firmicutes*, and *Bacteroidetes* were the most prevalent phyla in tomato cultivation (Figure 1). Recognizing variations in endosphere bacteria between fertilised and unfertilised soils can provide more insight to determine soil fertility and the impact of manure fertilisers. *Proteobacteria* and *Actinobacteria* are the two most common phyla in the bacterial community (Xu *et al.* 2019). A significant proportion of *Proteobacteria* and *Actinobacteria* can enhance important nutrients cycles, resulting in sustainable utilization and increased soil fertility. *Proteobacteria* have evolved

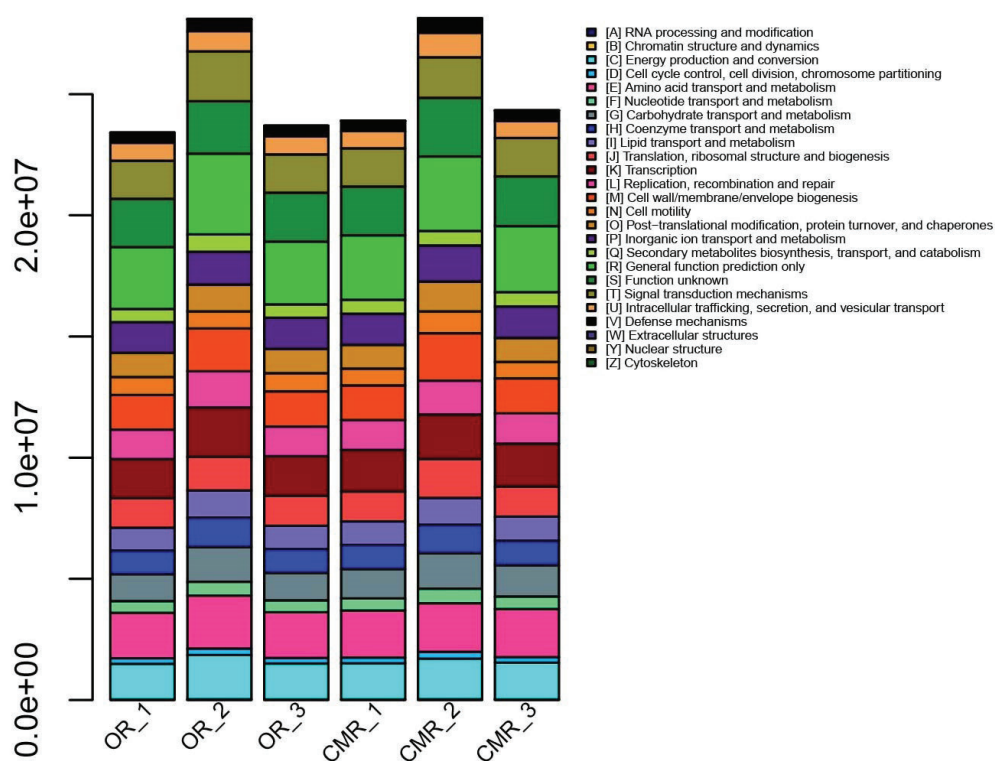


Figure 5. Functional protein annotation of CMR and OR samples against eggNOG database.

to survive in habitats that are rich in resources (Haas & Défago 2005; Zhen *et al.* 2014). *Actinobacteria* were formerly assumed to prefer oligotrophic environments which resource-limited. According to previous research, the proportion of *Proteobacteria* to *Actinobacteria* can represent soil nutrition status: a higher ratio indicates that more organic matter is being added to the soil (Zhang *et al.* 2020a; Ye *et al.* 2021). A previous study identified that a relatively higher abundance of *Proteobacteria* significantly correlated with the organic substitution ratio in tomato cultivation, but the abundance of *Actinobacteria* was negatively correlated with this ratio (Schreiter *et al.* 2018). Although, there is no evidence about the features of *Proteobacteria* and *Actinobacteria* in tomato cultivation with chicken manure. In the current research, the relative abundance of *Proteobacteria* increased significantly in soils with chicken manure application, suggesting that soils containing chicken manure may provide a suitable environment for soil bacteria that is intermediate between oligotrophic and copiotrophic conditions. *Bacteroidetes* and *Firmicutes* are the two main phyla of bacteria present in soil and manure (Guron *et al.* 2019). *Firmicutes* are known to have a wide range of functions and to aid in the degradation of organic materials (Smit *et al.* 2001; Torsvik & Øvreås 2002). They were comparatively inactive opponents under high nutrient environments compared to other microbes, occupying a lower role than *Proteobacteria* or *Actinobacteria*. However, they had a competitive advantage over other bacteria due to their slow growth and ability to decompose complex substrates (Ji *et al.* 2018). The relative abundance of *Firmicutes* in chicken manure-treated soils was significantly higher than in the control soils, suggesting that manure application produced a nutrient-rich condition that favoured *Firmicutes*. These findings were consistent with previous research (Kamau *et al.* 2008). *Bacteroidetes* are the most common microbes in manure (Zhong *et al.* 2018). The prior analysis revealed that *Bacteroidetes* were identified to be the most abundant bacteria in livestock manure and they had a high cellulose decomposition capacity in the thermophilic process of composting (Geisseler & Scow 2014). However, the *Bacteroidetes* were not found to be the dominant bacteria in soils that applied organic fertiliser. In the current research, the relative abundance of *Bacteroidetes* in control soil was significantly higher than the manure treated soil (Fig-

ure 2) suggesting that the soil in tomato cultivation without chicken manure was good for the growth of *Bacteroidetes*. They may actively participate in a series of soil activities and make important contributions to soil nutrient conversion and material recycling. *Flavobacterium* belonged to the *Bacteroidetes*, and its relative abundance was higher in soils without manure (Figure 1). It was reported to be capable of degrading macromolecular organic matter such as lipid and protein, as well as potential denitrification and nitrification ability (Zhang *et al.* 2017). The bacteria in the genus *Pseudomonas* were aerobic denitrifiers and could participate in the action of aerobic denitrification which was the main process of natural nitrogen cycling (Zhang *et al.* 2017). A relatively high abundance of *Pseudomonas* in soils with manure application suggested that organic fertilisation can encourage bacteria to participate in the nitrogen cycle and enhanced the biochemical cycle in tomato cultivation.

CONCLUSIONS

We concluded that as a biofertiliser, chicken manure application significantly improved the abundance of the beneficial microbes and the effects on community structure and bacterial diversity of the endosphere in tomato cultivation. In addition, our study provides a principled approach to the rational use of chicken manure for tomato cultivation, which is crucial to reducing the consumption of chemical fertilizers.

Acknowledgments. This work was supported by grants from the Department of Science and Technology of Fujian Province, China (Nos. 2020J01173; 2017J01625) and the National Natural Science Foundation of China (No. Nos. 31670125; 31660544).

Conflict of interest. The authors declare that they have no conflict of interest.

REFERENCES

- Allard, S.M., Walsh, C.S., Wallis, A.E., Ottesen, A.R., Brown, E.W., and Micallef, S.A. 2016. *Solanum lycopersicum* (tomato) hosts robust phyllosphere and rhizosphere bacterial communities when grown in soil amended with various organic and synthetic fertilizers. *Science of*

- the *Total Environment*, 573, 555–563. DOI:10.1016/j.scitotenv.2016.08.157.
- Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, 215(3), 403–410. DOI:10.1016/S0022-2836(05)80360-2.
- Bebber, D.P. and Richards, V.R. (2020). A meta-analysis of the effect of organic and mineral fertilizers on soil microbial diversity. *bioRxiv*, DOI:2020.10.04.325373.
- Beecher, G.R. (1998). Nutrient content of tomatoes and tomato products. *Experimental Biology and Medicine*, 218(2), 98–100. DOI:10.3181/00379727-218-44282a.
- Berg, G., Eberl, L., and Hartmann, A. (2005). The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. *Environmental microbiology*, 7(11), 1673–1685. DOI:10.1111/j.1462-2920.2005.00891.x.
- Bulgarelli, D., Schlaeppli, K., Spaepen, S., Ver Loren van Themaat, E., and Schulze-Lefert, P. (2013). Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology*, 64, 807–38. DOI:10.1146/annurev-arplant-050312-120106.
- Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.L., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., and Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, 7(5), 335–336. DOI:10.1038/nmeth.f.303.
- Cheng, D., Tian, Z., Feng, L., Xu, L., and Wang, H. (2019). Diversity analysis of the rhizospheric and endophytic bacterial communities of *Senecio vulgaris* L. (Asteraceae) in an invasive range. *PeerJ*, 6, e6162. DOI:10.7717/peerj.6162.
- Dombrowski, N., Schlaeppli, K., Agler, M.T., Hacquard, S., Kemen, E., Garrido-Oter, R., Wunder, J., Coupland, G., and Schulze-Lefert, P. (2017). Root microbiota dynamics of perennial *Arabis alpina* are dependent on soil residence time but independent of flowering time. *The ISME Journal*, 11(1), 43–55. DOI:10.1038/ismej.2016.109.
- Dong, C.-J., Wang, L.-L., Li, Q., and Shang, Q.-M. (2019). Bacterial communities in the rhizosphere, phyllosphere and endosphere of tomato plants. *PloS one*, 14(11), e0223847–e0223847. DOI:10.1371/journal.pone.0223847.
- Duncan, J. (2005). *Composting chicken manure*. WSU cooperative extension. King County Master Gardner and Cooperative Extension Livestock Advisor, Washington State University, Pullman. Retrieved 11 Nov 2017.
- Edgar, R.C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, 26(19), 2460–1. DOI:10.1093/bioinformatics/btq461.
- Eo, J. and Park, K.-C. (2016). Long-term effects of imbalanced fertilization on the composition and diversity of soil bacterial community. *Agriculture, Ecosystems & Environment*, 231, 176–182. DOI: org/10.1016/j.agee.2016.06.039.
- Geisseler, D. and Scow, K.M. (2014). Long-term effects of mineral fertilizers on soil microorganisms—A review. *Soil Biology and Biochemistry*, 75, 54–63. DOI:10.1016/j.soilbio.2014.03.023.
- Green, S.J., Prakash, O., Jasrotia, P., Overholt, W.A., Cardenas, E., Hubbard, D., Tiedje, J.M., Watson, D.B., Schadt, C.W., and Brooks, S.C. (2012). Denitrifying bacteria from the genus *Rhodanobacter* dominate bacterial communities in the highly contaminated subsurface of a nuclear legacy waste site. *Applied and Environmental Microbiology*, 78(4), 1039–1047. DOI:10.1128/AEM.06435-11.
- Guron, G.K., Arango-Argoty, G., Zhang, L., Pruden, A., and Ponder, M.A. (2019). Effects of dairy manure-based amendments and soil texture on lettuce-and radish-associated microbiota and resistomes. *Mosphere*, 4(3), 13–19. DOI:10.1128/mSphere.00239-19.
- Haas, D. and Défago, G. (2005). Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature Reviews Microbiology*, 3(4), 307–319. DOI:10.1038/nrmicro1129.
- Hallmann, J., Quadt-Hallmann, A., Mahaffee, W., and Kloepper, J. (1997). Bacterial endophytes in agricultural crops. *Canadian Journal of Microbiology*, 43(10), 895–914. DOI: 10.1139/M97-131.
- Hartmann, M., Frey, B., Mayer, J., Mäder, P., and Widmer, F. (2015). Distinct soil microbial diversity under long-term organic and conventional farming. *The ISME Journal*, 9(5), 1177–1194. DOI:10.1038/ismej.2014.210.
- Ji, L., Wu, Z., You, Z., Yi, X., Ni, K., Guo, S., and Ruan, J. (2018). Effects of organic substitution for synthetic N fertilizer on soil bacterial diversity and community composition: A 10-year field trial in a tea plantation. *Agriculture, Ecosystems & Environment*, 268, 124–132. DOI:10.1007/s00374-020-01439-y.
- Kamau, D., Spiertz, J., and Oenema, O. (2008). Carbon and nutrient stocks of tea plantations differing in age, genotype and plant population density. *Plant and Soil*, 307(1), 29–39. DOI:10.1007/s11104-008-9576-6.
- Lee, S.A., Kim, Y., Kim, J.M., Chu, B., Jo, J.-H., Sang, M.K., Song, J. and Weon, H.-Y. (2019). A preliminary examination of bacterial, archaeal, and fungal communities inhabiting different rhizocompartments of tomato plants under real-world environments. *Scientific Reports*, 9(1), 1–15. DOI: org/10.1038/s41598-019-45660-8.
- Li, C., Yan, K., Tang, L., Jia, Z., and Li, Y. (2014). Change in deep soil microbial communities due to long-term fertilization. *Soil Biology and Biochemistry*, 75, 264–272. DOI:10.1016/j.soilbio.2014.04.023.
- Lin, L., Ge, H.M., Yan, T., Qin, Y.H., and Tan, R.X. (2012). Thaxtomin A-deficient endophytic *Streptomyces* sp. enhances plant disease resistance to pathogenic *Streptomyces scabies*. *Planta*, 236(6), 1849–1861. DOI:10.1007/s00425-012-1741-8.
- Loper, J.E., Hassan, K.A., Mavrodi, D.V., Davis II, E.W., Lim, C.K., Shaffer, B.T., Elbourne, L.D., Stockwell, V.O., Hartney, S.L., and Breakwell, K. (2012). Comparative genomics of plant-associated *Pseudomonas* spp.: insights into diversity and inheritance of traits involved in multitrophic interactions. *PLoS Genet*, 8(7), e1002784. DOI:10.1371/journal.pgen.1002784.
- Lundberg, D.S., Lebeis, S.L., Paredes, S.H., Yourstone, S., Gehring, J., Malfatti, S., Tremblay, J., Engelbrektson, A., Kunin, V., and Del Rio, T.G. (2012). Defining the core *Arabidopsis thaliana* root microbiome. *Nature*, 488(7409), 86–90. DOI:10.1038/nature11237.
- Luo, L., Wang, P., Zhai, Z., Su, P., Tan, X., Zhang, D., Zhang, Z., and Liu, Y. (2019). The effects of *Rhodopseudomonas palustris* PSB06 and CGA009 with different agricultural applications on rice growth and rhizosphere bacterial communities. *AMB Express*, 9(1), 1–10. DOI:10.1186/s13568-019-0897-z.
- Magoč, T. and Salzberg, S.L. (2011). FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, 27(21), 2957–63. DOI:10.1093/bioinformatics/btr507.
- Manching, H.C., Balint-Kurti, P.J., and Stapleton, A.E. (2014). Southern leaf blight disease severity is correlated with decreased maize leaf epiphytic bacterial species richness and the phyllosphere bacterial diversity decline is enhanced by

- nitrogen fertilization. *Frontiers in Plant Science*, 5, 403. DOI:10.3389/fpls.2014.00403.
- Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet Journal*, 17(1), 10. DOI:10.14806/ej.17.1.200.
- Peiffer, J.A., Spor, A., Koren, O., Jin, Z., Tringe, S.G., Dangl, J.L., Buckler, E.S., and Ley, R.E. (2013). Diversity and heritability of the maize rhizosphere microbiome under field conditions. *Proceedings of the National Academy of Sciences*, 110(16), 65486553. DOI:10.1073/pnas.1302837110.
- Schreiter, S., Babin, D., Smalla, K., and Grosch, R. (2018). Rhizosphere competence and biocontrol effect of *Pseudomonas* sp. RU47 independent from plant species and soil type at the field scale. *Frontiers in Microbiology*, 9, 97. DOI:10.3389/fmicb.2018.00097.
- Smit, E., Leeflang, P., Gommans, S., van den Broek, J., van Mil, S., and Wernars, K. (2001). Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods. *Applied and Environmental Microbiology*, 67(5), 2284–2291. DOI:10.1128/AEM.67.5.2284-2291.2001.
- Sturz, A.V., Christie, B.R., and Nowak, J. (2000). Bacterial endophytes: potential role in developing sustainable systems of crop production. *Critical Reviews in Plant Sciences*, 19(1), 1–30. DOI:10.1080/07352680091139169.
- Tian, B., Zhang, C., Ye, Y., Wen, J., Wu, Y., Wang, H., Li, H., Cai, S., Cai, W., and Cheng, Z. (2017). Beneficial traits of bacterial endophytes belonging to the core communities of the tomato root microbiome. *Agriculture, Ecosystems & Environment*, 247, 149–156. DOI:10.1016/j.agee.2017.06.041.
- Torsvik, V. and Øvreås, L. (2002). Microbial diversity and function in soil: from genes to ecosystems. *Current Opinion in Microbiology*, 5(3), 240–245. DOI:10.1016/s1369-5274(02)00324-7.
- Turner, T.R., James, E.K., and Poole, P.S. (2013). The plant microbiome. *Genome Biol*, 14(6), 209. DOI:10.1186/gb-2013-14-6-209.
- van Elsas, J.D., Chiurazzi, M., Mallon, C.A., Elhottova, D., Kristufek, V., and Salles, J.F. (2012). Microbial diversity determines the invasion of soil by a bacterial pathogen. *Proceedings of the National Academy of Sciences of the USA*, 109(4), 1159–64. DOI:10.1073/pnas.1109326109.
- Wang, X., Van Nostrand, J.D., Deng, Y., Lü, X., Wang, C., Zhou, J., and Han, X. (2015). Scale-dependent effects of climate and geographic distance on bacterial diversity patterns across northern China's grasslands. *FEMS Microbiology Ecology*, 91(12). DOI:10.1093/femsec/fiv133.
- Weller, D.M., Raaijmakers, J.M., Gardener, B.B.M., and Thomashow, L.S. (2002). Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annual Review of Phytopathology*, 40(1), 309–348. DOI:10.1146/annurev.phyto.40.030402.110010.
- Xiao, Y., Liu, X., Meng, D., Tao, J., Gu, Y., Yin, H., and Li, J. (2018). The role of soil bacterial community during winter fallow period in the incidence of tobacco bacterial wilt disease. *Applied Microbiology and Biotechnology*, 102(5), 2399–2412. DOI:10.1007/s00253-018-8757-3.
- Xu, W., Wang, F., Zhang, M., Ou, T., Wang, R., Strobel, G., Xiang, Z., Zhou, Z., and Xie, J. (2019). Diversity of cultivable endophytic bacteria in mulberry and their potential for antimicrobial and plant growth-promoting activities. *Microbiological Research*, 229, 126328. DOI:10.1016/j.micres.2019.126328.
- Yang, H., Li, J., Xiao, Y., Gu, Y., Liu, H., Liang, Y., Liu, X., Hu, J., Meng, D., and Yin, H. (2017). An integrated insight into the relationship between soil microbial community and tobacco bacterial wilt disease. *Frontiers in Microbiology*, 8, 2179. DOI:10.3389/fmicb.2017.02179.
- Ye, G., Banerjee, S., He, J.-Z., Fan, J., Wang, Z., Wei, X., Hu, H., Zheng, Y., Duan, C., Wan, S., Chen, J., and Yongxin, L. (2021). Manure application increases microbiome complexity in soil aggregate fractions: Results of an 18-year field experiment. *Agriculture Ecosystems & Environment*, 307, 107249. DOI:10.1016/j.agee.2020.107249.
- Yilmaz, P., Parfrey, L.W., Yarza, P., Gerken, J., Priesse, E., Quast, C., Schweer, T., Peplies, J., Ludwig, W., and Glöckner, F.O. (2014). The SILVA and “All-species Living Tree Project (LTP)” taxonomic frameworks. *Nucleic Acids Research*, 42(D1), D643–648. DOI:10.1093/nar/gkt1209.
- Zhang, S., Sun, L., Wang, Y., Fan, K., Xu, Q., Li, Y., Ma, Q., Wang, J., Ren, W., and Ding, Z. (2020a). Cow manure application effectively regulates the soil bacterial community in tea plantation. *BMC Microbiology*, 20(1), 190. DOI:10.1186/s12866-020-01871-y.
- Zhang, X., Zhang, Q., Liang, B., and Li, J. (2017). Changes in the abundance and structure of bacterial communities in the greenhouse tomato cultivation system under long-term fertilization treatments. *Applied Soil Ecology*, 121, 82–89. DOI:10.1016/j.apsoil.2017.08.016.
- Zhang, Y.J., Hu, H.W., Chen, Q.L., Singh, B.K., Yan, H., Chen, D., and He, J.Z. (2019). Transfer of antibiotic resistance from manure-amended soils to vegetable microbiomes. *Environment International*, 130, 104912. DOI:10.1016/j.envint.2019.104912.
- Zhang, Y.J., Hu, H.W., Chen, Q.L., Yan, H., Wang, J.T., Chen, D., and He, J.Z. (2020b). Manure application did not enrich antibiotic resistance genes in root endophytic bacterial microbiota of cherry radish plants. *Applied and Environmental Microbiology*, 86(2). DOI:10.1128/AEM.02106-19.
- Zhen, Z., Liu, H., Wang, N., Guo, L., Meng, J., Ding, N., Wu, G., and Jiang, G. (2014). Effects of manure compost application on soil microbial community diversity and soil microenvironments in a temperate cropland in China. *PLOS ONE*, 9(10), e108555. DOI:10.1371/journal.pone.0108555.
- Zhong, X.-Z., Ma, S.-C., Wang, S.-P., Wang, T.-T., Sun, Z.-Y., Tang, Y.-Q., Deng, Y., and Kida, K. (2018). A comparative study of composting the solid fraction of dairy manure with or without bulking material: performance and microbial community dynamics. *Bioresource Technology*, 247, 443–452. DOI:10.1016/j.biortech.2017.09.

Received: April 20, 2021

Accepted: August 31, 2021