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Effects of wheat root exudates on bacterial communities in the rhizosphere of watermelon

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Abstract: In this study, we investigated the effects of wheat root exudates on soil bacterial communities in the watermelon rhizosphere using quantitative PCR and Illumina MiSeq sequencing. The qPCR results showed that wheat root exudates significantly increased the abundance of total bacteria, *Pseudomonas*, *Bacillus* and *Streptomyces* spp. Illumina MiSeq sequencing results showed that wheat root exudates significantly changed the bacterial community structure and composition. These results indicated that plant root exudates play a role in plant-to-plant signalling, strongly affect the microbial community composition.

Keywords: root exudates; plant-microbe interaction; plant growth-promoting rhizobacteria; soil microbial community

Plant-plant interaction is the process by which plants recognise their neighbouring species and interact according to their genetic identity (Rahman et al. 2019). The bioactive substances of root exudates play an important role in plant-microbe interaction (Ankati and Podile 2019). The rice root exudates can directly inhibit pathogens *Fusarium oxysporum* f. sp *niveum* spore germination and sporulation (Ren et al. 2016). The root exudates can indirectly inhibit pathogens by reshaping the soil microbial community and recruiting plant growth-promoting rhizobacteria (PGPR) (Ankati and Podile 2019). Specific components, such as coumaric acid from cucumber, can change the bacteria community structure in the cucumber rhizosphere (Zhou et al. 2018). Therefore, the root exudates are one of the important bases in rhizosphere talk between plant and microbe.

Differences in root exudates play a selective cultural role in affecting rhizosphere microorganisms growth. Hao et al. (2010) performed high-performance liquid chromatography (HPLC) on rice and watermelon found that *p*-coumaric acid was only detected in rice and ferulic acid only in watermelon. The *p*-coumaric acid has the ability to inhibit spore germination and sporulation of *Fusarium oxysporum* f. sp. *niveum*, alter the compositions of microbial and changes the plant-soil microbial interaction (Zhou et al. 2018). However, ferulic acid can stimulate the growth of *Fusarium oxysporum* f. sp. *niveum* (Lv et al. 2018). Therefore, it is of great significance to further study the influence of root exudates from one plant species on the microbial community in the rhizosphere of another plant.

Beneficial microorganisms can promote agricultural production. Most of the beneficial microorganisms are bacteria such as *Bacillus*, *Pseudomonas* and *Streptomyces* spp. These beneficial bacteria can interfere with and inhibit pathogenic ones by secreting toxins, antibiotics, etc. (Couillerot et al. 2009)

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and promote plant growth by inducing systemic resistance (Zamioudis and Pieterse 2012).

Previous studies have shown that wheat intercropping can affect the rhizosphere bacterial community of watermelon (Xu et al. 2015). In this study, we exogenously applied wheat root exudates to bare soil or watermelon rhizosphere soil to study their effects on soil bacterial community composition using quantitative PCR and Illumina MiSeq sequencing; and hypothesised that wheat root exudates (*i*) effects on the beneficial bacterial abundance of bare and rhizosphere soil would be different, and (*ii*) alter bacterial community diversity, structure and composition of watermelon rhizosphere soil.

MATERIAL AND METHODS

Collection of wheat root exudates. The collection method is described by Ren et al. (2008). In briefly, the 105 wheat seeds (cv. D123) were planted in a pot (0.135 × 0.15 m) with 900 g soil. When the aboveground seedling length reached about 0.2 m, the root was taken out of and rinsed with tap water and deionised water. Then, seedlings were transferred to a 500 mL beaker full with 0.5 mmol $CaCl_2$ solutions. The solution initial volume was replenished every 2 h. After 6 h, the roots were taken out and wiped water for weighting. According to the weight of the fresh root, the concentration of wheat root exudates was 1 g fresh weight (FW)/10 mL water solution (1 g FW/10 mL). Finally, the root exudate was filtered through a 0.45 μm microporous membrane and stored at –80 °C for use.

Pot experiment

Effect of wheat root exudates on watermelon continuous cropping soil. The experiment was conducted from April to July 2019 in a greenhouse of the Horticultural Station of Northeast Agricultural University, Harbin, China (45°41'N, 126°37'E). The watermelon continuous cropping soil was collected from the upper layer (0–0.15 m) of a greenhouse and sieved. The soil was sandy loam, contained inorganic N 56.19 mg/kg, available P 377.26 mg/kg, available K 364.00 mg/kg, organic matter 30.60 mg/kg, pH 7.21 (1:2.5, *w*/*v*), electrical conductivity 1.08 mS/cm (1:2.5, *w*/*v*).

The watermelon seeds (cv. Black beauty) were first washed soaked in the water bath at constant temperature (55 °C) for 30 min, then the seeds were incubated in an incubator at 28 °C until germinated. Each pot (0.15 × 0.135 m) contained 4 kg watermelon continuous cropping soil and a germinated seed. The seedling was treated with wheat root exudates when growing to three leaves stage. To clarify the role of wheat root exudates four treatments were set up as follow: watermelon-rhizosphere soil treated with wheat root exudates 5 mL (WR) or without wheat root exudates (WC), bare soil treated with wheat root exudates (NR) or without wheat root exudates (NC). In WR and NR, 5 mL wheat root exudates were added to the soil through a sterile syringe. 5 mL of deionised water containing 0.5 mmol $CaCl_2$ was added to the NC and WC treatments. Root exudates and deionised water containing 0.5 mmol $CaCl_2$ was added 1 cm from the watermelon root. They were added at the same position in the pot without watermelon seedlings. Each treatment contained three replications. And five pots were planted in each replication. The wheat root exudates or deionised water containing 0.5 mmol $CaCl_2$ were added to the watermelon-rhizosphere soil and bare soil at 0, 5, 10, 15, 20, 25, 30 and 35 days. The soil samples in each treatment were collected 40 days after the first application of wheat root exudates or deionised water containing 0.5 mmol $CaCl_2$. All samples were sieved (2 mm) and stored at –80 °C.

DNA extraction and quantitative PCR assay. According to the manufacturer's instructions, PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, USA) was used to extract the total soil DNA.

Abundances of total bacteria and *Bacillus*, *Pseudomonas*, *Actinomycetes* and *Streptomyces* spp. communities were estimated by quantitative PCR assays with primer sets of F338/R518 (Ling et al. 2011), BacF/BacR (Ghorbani et al. 2008), PSF/PSR (Levy et al. 2018), F243/R518 (Derakhshani et al. 2016), and Sm5R/Sm6F (Levy et al. 2018), respectively.

Illumina MiSeq sequencing and data processing. The Illumina MiSeq platform was used to estimate the rhizosphere bacterial community composition with high-throughput sequencing. The V3–V4 hypervariable regions of the bacterial 16S rRNA gene were amplified with primers 338F/806R (Derakhshani et al. 2016). The resulted PCR products were extracted from a 2% agarose gel and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, USA) and quantified using QuantiFluor™-ST (Promega, Madison, USA) according to the manufacturer's protocol.

Raw fastq files were quality-filtered by trimmomatic and merged by Flash as previously described (Zhou et al. 2017). Operational taxonomic units (OTUs) were delineated at 97% sequence similarity with Uparse using an agglomerative clustering algorithm (Edgar 2013). After that, a sequence from each OTU was classified taxonomically through BLAST in the RDP database (Wang et al. 2007). All sequences have been deposited in the NCBI-Sequence Read Archive (Accession Number SRP336327).

Statistical analysis. The compilation of the test raw data was done using Microsoft Excel (Office 2010) software (Redmond, USA). For the analysis of beta diversity, the "Vegan" package in "R" was used as the PCoA diagram based on Bray-Curtis distance to study the similarity or difference of sample community composition (Oksanen et al. 2014). The abundance of soil total bacterial and beneficial bacteria while bacterial α-diversity index and taxa were analysed using Welch's *t*-test method.

RESULTS AND DISCUSSION

Effects of wheat root exudates on bacterial community abundance in the rhizosphere of watermelon. In watermelon-rhizosphere soil, wheat root exudates increased the abundances of total bacteria, *Bacillus*, *Pseudomonas*, *Actinomycetes* and *Streptomyces* spp. (Figure 1) ($P < 0.05$). In bare soil, wheat root exudates decrease increased the abundance of *Pseudomonas* spp. but increased that of *Actinomycetes* spp. (Figure 1C, D) ($P < 0.05$). Wheat root exudates could increase the total bacterial community abundance in watermelon-rhizosphere soil, which is consistent with the previous studies (Xu et al. 2015). The input of the root exudates provides carbon, which promotes microbial biomass and microbial activity (Fang et al. 2021). Research has shown that root exudates can stimulate the growth of rhizosphere microorganisms (Sun et al. 2015) and attract specific microbial populations (Feng et al. 2021). These results supported our first hypothesis.

Effects of wheat root exudates on bacterial community structure and diversity in the rhizosphere of watermelon. PCoA analysis showed that samples from the same treatment were grouped together, while samples from different treatments were separated from each other (Figure 2) (ANOSIM, $R = 1$, $P = 0.001$; ADONIS, $R^2 = 0.605$, $P = 0.003$). Wheat root exudates significantly changed the bacterial community structure in watermelon rhizosphere soil and bare soil. Wheat root exudates decreased the bacterial community richness index in bare soil but had no effect in watermelon-rhizosphere soil (Figure 3). It does not fit with our second hypothesis. The time-lag response of soil communities to changes in plant

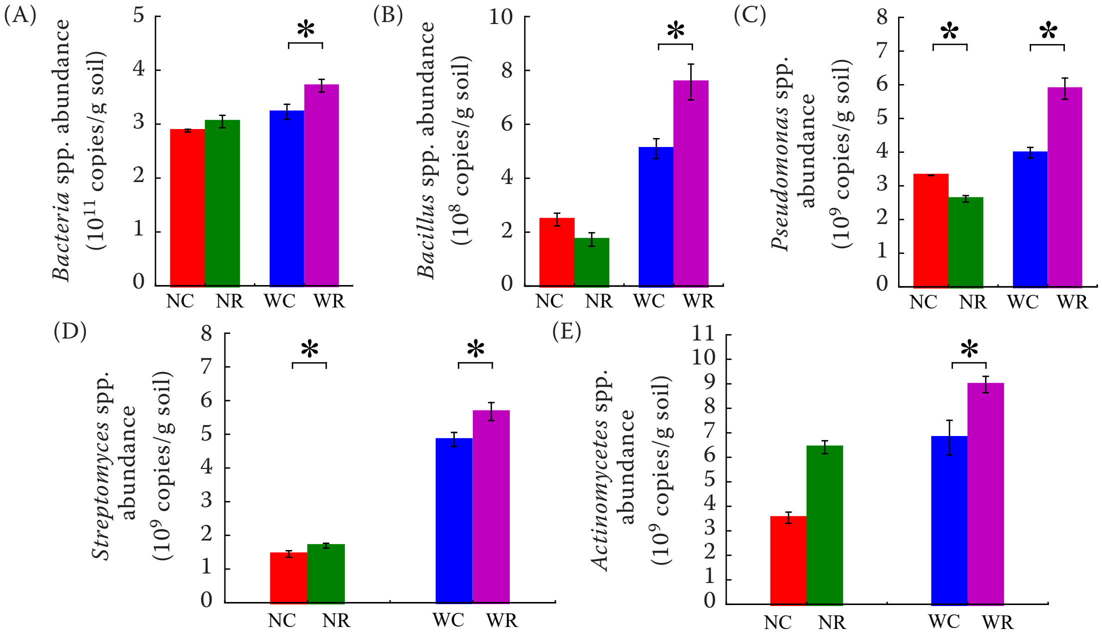

Figure 1. Abundances of (A) total bacteria; (B) *Bacillus*; (C) *Pseudomonas*; (D) *Streptomyces* and (E) *Actinomycetes* spp. based on analysis ($P < 0.05$, Welch's *t*-test). Bare soil treated without or with wheat root exudates was represented by NC or NR; watermelon-rhizosphere soil treated without or with wheat root exudates was represented by WC or WR

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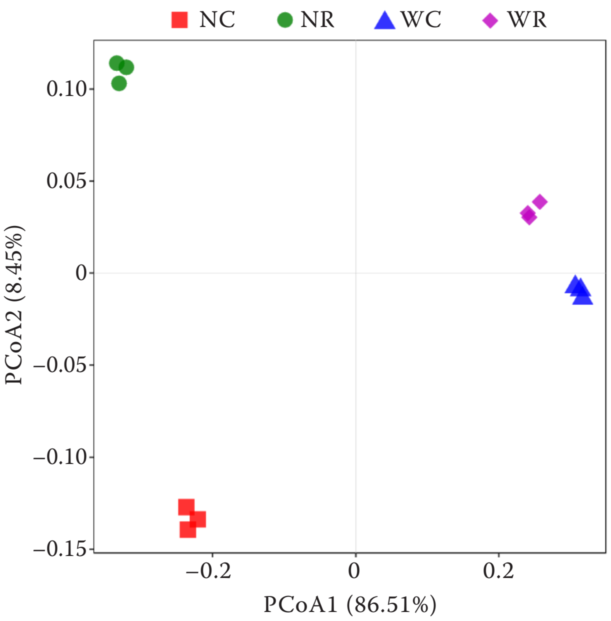

Figure 2. The PCoA plot is based on the Bray-Curtis distance at the operational taxonomic unit level (97% sequence similarity). Red square and green circle were represented bare soil treated without wheat root exudates (NC) and with wheat root exudates (NR); blue triangle and purple diamond were represented watermelon-rhizosphere soil treated without root exudates (WC) and with wheat root exudates (WR)

diversity can partly explain this controversial issue (Eisenhauer et al. 2011). For example, the effects of plant diversity on soil organisms were not significant until 4–6 years after the experiment was established (Eisenhauer et al. 2011). Therefore, in order to improve our understanding of the effect of wheat root exudates on the diversity of rhizosphere bacterial community in watermelon, the sampling time should be prolonged in the future.

Effects of wheat root exudates on bacterial community composition in the rhizosphere of watermelon. 32 bacterial phyla were detected. The main bacterial phylum in the watermelon soil was Proteobacteria, Pseudobacteria, Acidobacteria, and Actinomycetes. Actinomycetes were more abundant in NR than in NC (Figure 4A). Parcubacteria was more abundant in WC than in WR (Figure 4B). The bacterial genus, *Blastococcus* and *Arenimonas* spp. were more abundant in NR than in NC. *Parasegetibacter*, *Rhodobacter*, *Agromyces*, *Flavisolibacter*, *Porphyrobacter* and *Rubellimicrobium* spp. were more abundant in NC than in NR (Figure 4C). In addition, seven bacterial genera with relative abundance > 0.3% were more prevalent in WC samples, including *Fluviicola*, *Rhizobium*, *Cellvibrio*, *Flavobacterium*, *Actinoplanes* and *Shinella* spp.

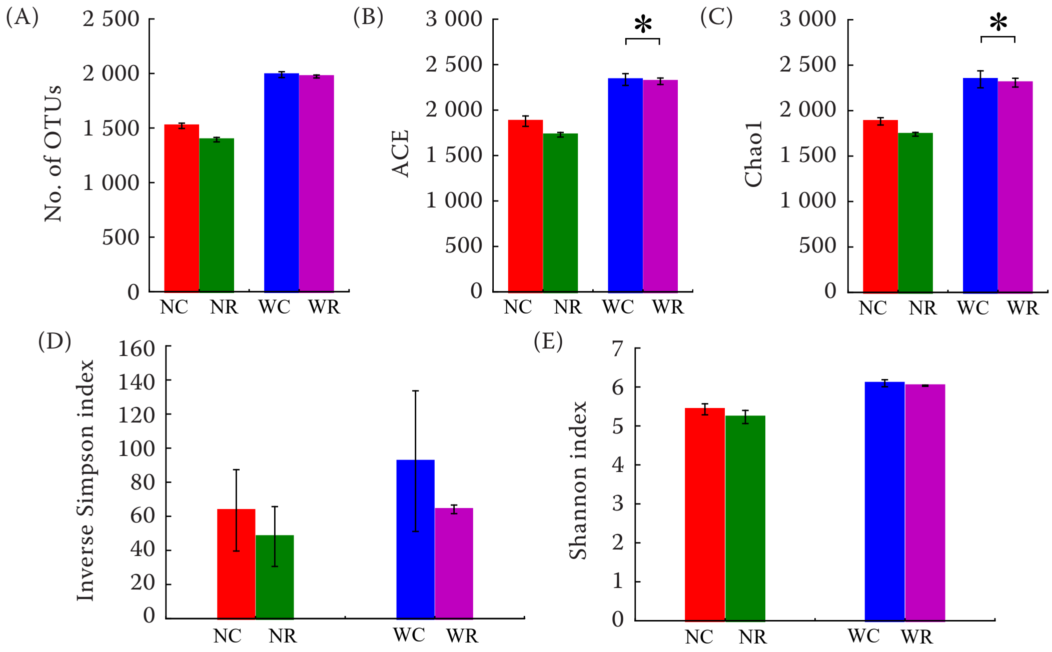

Figure 3. Bacterial alpha diversity in bare soil soil and watermelon rhizosphere ($P < 0.05$, Welch's t-test). Operational taxonomic units (OTUs) were delineated at 97% sequence similarity. Bare soil treated without or with wheat root exudates was represented by NC or NR; watermelon-rhizosphere soil treated without or with wheat root exudates was represented by WC or WR. Chao1 and ACE index represented microbial community richness, Shannon and Inverse Simpson index represented microbial community diversity

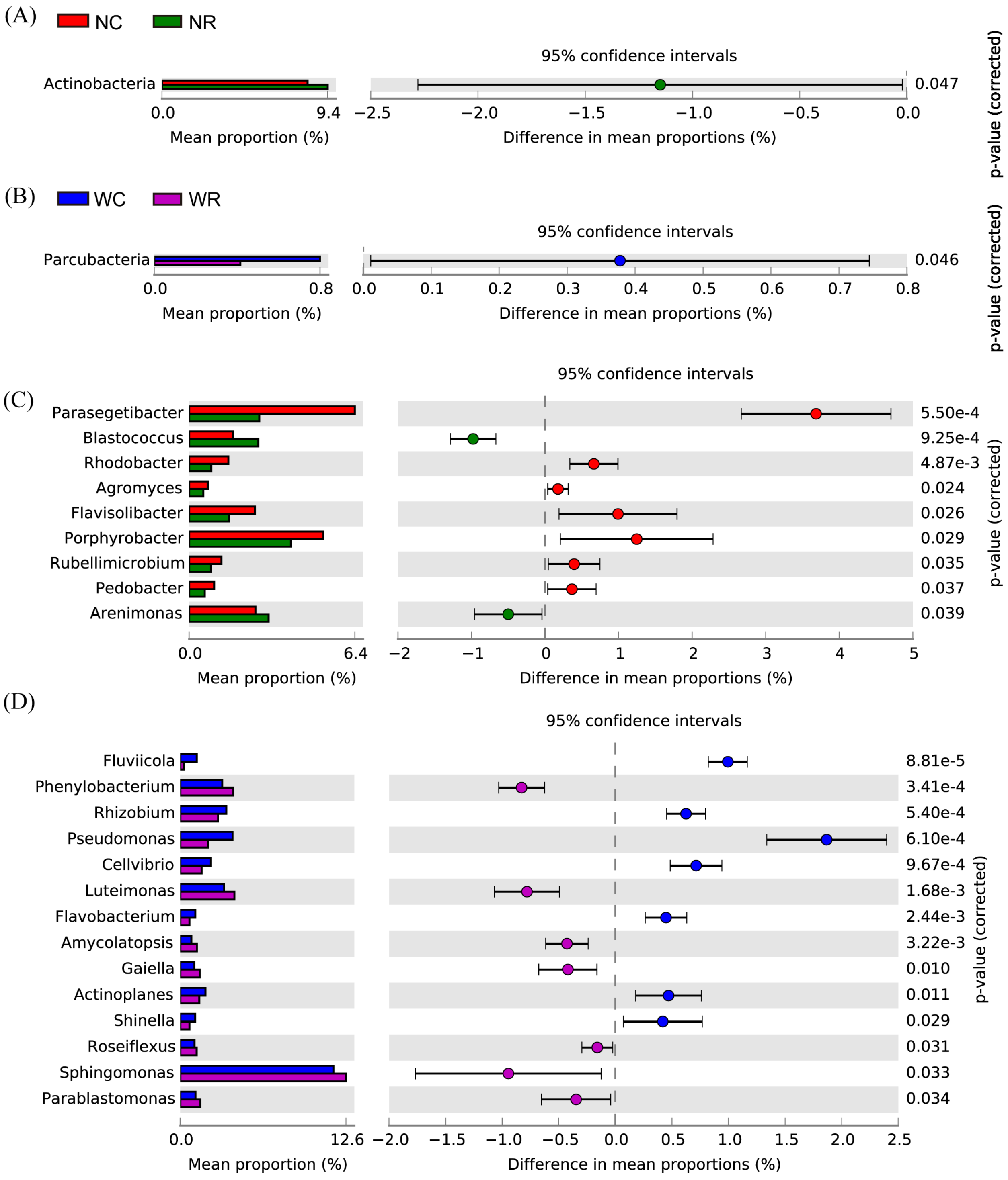

Figure 4. Relative abundances of main bacterial phyla (A, B) and genus (C, D) in bare soil and watermelon rhizosphere soil. Bacterial phyla and genus with average relative abundances > 0.5% and 0.3% were shown in at least one treatment, respectively. Data are represented as the means of three independent replicates. Values in the bar plot are expressed as mean ± standard error. The coloured circles represent the 95% confidence intervals. Only indicate significant difference between treatments based on Welch's t-test ($P < 0.05$) Red and green were represented bare soil treated without wheat root exudates (NC) and with wheat root exudates (NR); blue and purple were represented watermelon-rhizosphere soil treated without root exudates (WC) and with wheat root exudates (WR)

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In contrast, the *Phenylobacterium*, *Luteimonas*, *Amycolatopsis*, *Gaiella*, *Roseiflexus*, *Shingomonas* and *Parablastomonas* spp. were more abundant in the WR soil than in WC (Figure 4D). The effect of watermelon rhizosphere and bare soil treated with or without wheat root exudates on microbial community composition had significantly different. Root exudates play a role in transmitting information in the interaction between plant rhizosphere and microbial environment. Root exudates as chemoattractants are capable of directing bacteria chemotaxis. It can also through mediate the root-specific signal molecules that caused rhizosphere priming effects then change the microbial community composition (Fontaine et al. 2003). The study indicated that the root exudates of *Zea mays* interaction with or without root exudates of *Cajanus cajan* will change the content of root exudates and effect the chemotaxis of bacteria (Vora et al. 2021). Compared with bare soil, watermelon roots can sense the addition of wheat root exudates and secrete some

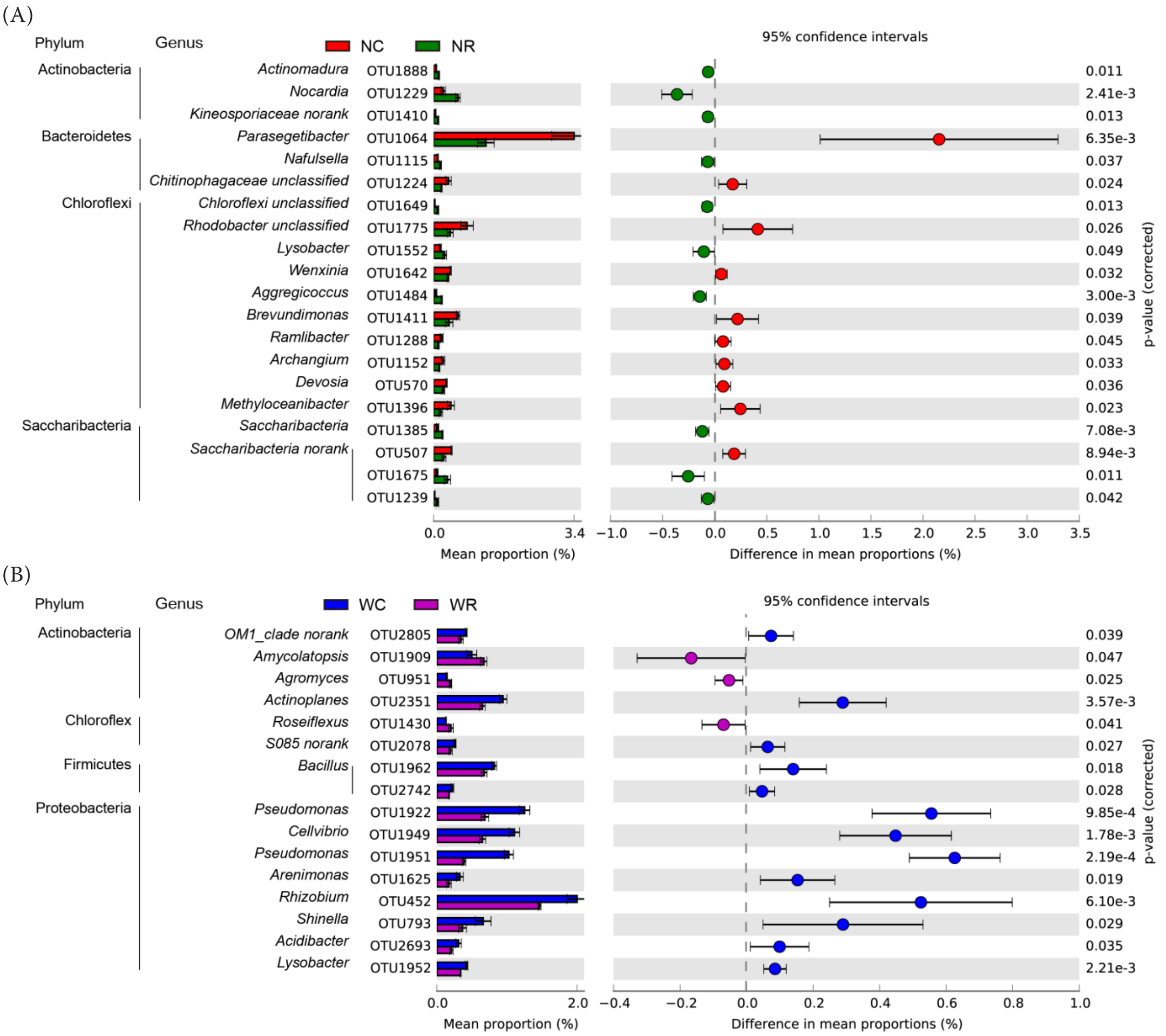

Figure 5. The bacterial operational taxonomic units (OTUs) in (A) bare soil and (B) watermelons rhizosphere soils. OTUs were delineated at 97% sequence similarity. Bacterial OTUs with average relative abundances > 0.1% were shown in at least one treatment (showing significant discrepancy). Values in the bar plot are expressed as mean ± standard error. The coloured circles represent the 95% confidence intervals. Only indicate a significant difference between treatments based on Welch's t-test ($P < 0.05$). Red and green were represented bare soil treated without wheat root exudates (NC) and with wheat root exudates (NR); blue and purple were represented watermelon-rhizosphere soil treated without root exudates (WC) and with wheat root exudates (WR)

bioactive substances in response. Besides, after adding wheat root exudates, the abundances of *Nocardia* (Azadi and Shojaei 2020) and *Blastococcus* (Yue et al. 2021) were increased in bare soil and *Gaiella* (Zhao et al. 2021) was increased in watermelon rhizosphere soil that has the ability to degrade polycyclic aromatic hydrocarbons. The reason may be that these microbial tend to be in an environment where wheat root exudates exist. The influence of root exudates may largely be due to the combined effect of its degradation products. Therefore, microorganisms with the ability to degrade compounds are very important in determining the role of root exudates.

A total of 2 735 OTUs were identified in all samples, with a 97% similarity. In bare soil, the relative abundances of OTU1229, OTU1484, OTU1385, OTU1675, OTU1239, OTU1888, OTU1410, OTU1649, OTU1115, and OTU1552 increased significantly with the addition of wheat root exudates (Figure 5A). However, watermelon-rhizosphere soil treated with wheat root exudates demonstrated higher relative abundances of OTU1951, OTU1922, OTU1949, OTU1952, OTU2351, OTU452, OTU1962, OTU1625, OTU2078, OTU2742, OTU793, OTU2693, OTU2805 and lower relative abundances of OTU951, OTU1430, OTU1909 (Figure 5B). The watermelon-rhizosphere soil treated with wheat root exudates had the most unique operational taxonomic units changes. The effect of wheat root exudates on the distribution of microorganisms in the watermelon-rhizosphere is more significant. The effects of wheat root exudates on the microbial composition of bare soil and watermelon rhizosphere soil were different. It should be taken seriously that the implications of rhizosphere bacterial distribution trends for the development of effective strategies to manage beneficial plant-microorganism interactions. The assembly of rhizosphere bacteria is related to the resources around it (Stringlis et al. 2018). However, the nature of root exuded components and specific bacterial strains involved in this mechanism should be identified in future work.

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