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Effects of microalgal biomass as biofertilizer on the growth of cucumber and microbial communities in the cucumber rhizosphere

Junping LV[1], Shufang LIU[1], Jia FENG[1], Qi LIU[1], Jun GUO[2], Lige WANG[2], Xiaoyan JIAO[2], Shulian XIE[1,*]

[1]School of Life Science, Shanxi University, Taiyuan, China

[2]Institute of Agricultural Environment and Resource, Shanxi Academy of Agricultural Sciences, Taiyuan, China

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Abstract: In the present study, microalgal biomass (*Anabeana circinalis* and *Scenedesmus quadricauda*) was used as biofertilizer in cucumber cultivation. The response of cucumber growth characters to microalgal biofertilizer was evaluated. Moreover, rhizosphere microbial diversity and community composition of cucumber was analyzed through 16S rRNA high-throughput Illumina sequencing data. The results showed that microalgal biomass as biofertilizer significantly promoted height, number of leaves and flower buds, and stem diameter of cucumber. The application of a high concentration of *S. quadricauda* to soil raised the diversity of rhizosphere fungi of cucumber. The rhizosphere microbial community of cucumber also responded to microalgal biofertilizer. The most abundant bacterial phylum in all samples was *Proteobacteria*. *Acidobacteria*, *Actinobacteria*, and *Gemmatimonadetes* were also highly abundant in all samples. *Ascomycota* was the most predominant fungal phylum in all samples. *Zygomycota* was the dominant phylum in the control, whereas *Basidiomycota* was dominant in all treatments. At the genus level, both growth-promoting bacteria and fungi (*Azotobacter*, *Bacillus*, *Pseudomonas*, *Cryptococcus*, *Fusarium*, *Penicillium*, and *Trichoderma*) from the rhizosphere of cucumber were enhanced in all treatments after the application of microalgae fertilizer. All results indicate a significant impact of microalgal biofertilizer on the growth of cucumber and microbial abundance in the cucumber rhizosphere.

Key words: Rhizosphere microorganisms, Illumina MiSeq sequencing, microalgal biofertilizer, cucumber

1. Introduction

Soil microorganisms are important components of the soil ecosystem and play important roles in biogeochemical cycling such as organic decomposition, mineral release, and energy conversion (Zhou and Lei, 2007). The growth of most terrestrial plants is closely related to complex microbial communities, which can promote plant growth by producing plant-growth regulators, fixing nutrients from soils, and protecting plants from phytopathogens (Ahemad and Kibret, 2014). The rhizosphere is the direct environment of plants in a soil ecosystem. It is a dynamic interface that supports nutrient exchange between plants and their surrounding soils (Peiffer et al., 2013). Rhizosphere microorganisms have been regarded as the second genome of plants, and plant roots affect each other through these microorganisms (Berendsen et al., 2012; Philippot et al., 2013). Rhizosphere microorganisms have been of wide concern due to their great impact on plant life (Pii et al., 2015a).

Microalgae are a highly diverse group of microorganisms and have gained great notice in recent decades. They can be used to produce high-value products such as astaxanthin, β-carotene, phycobilin pigments, and polyunsaturated fatty acids (Borowitzka, 2013). Moreover, they have been widely used in sewage treatment and in the production of biodiesel (Maity et al., 2014; Chen et al., 2015). Interestingly, cyanobacteria can be directly applied to soil to increase crop productivity. The principle of cyanobacteria as biofertilizer is related to nitrogen fixation of filamentous cyanobacteria, which converts inorganic nitrogen into organic nitrogen. As reported by Tripathi et al. (2008), the mineral composition, yield, and growth of rice plants were improved when soils were amended with cyanobacteria as biofertilizer. The growth and yields in chickpea (*Cicer arietinum* L.) were enhanced through novel cyanobacterial and biofilmed inoculants (Bidyarani et al., 2016). Moreover, Mulbry et al. (2005) found that microalgal biomass produced by the treatment of anaerobically digested dairy manure could be used as fertilizer. In the study, microalgal biomass was obtained from the growth of green algal species. Renuka et al. (2016) also reported that wastewater-grown green algae biomass as biofertilizer improved yields in wheat. Therefore, green algae can also be used as fertilizer because

* Correspondence: xiesl@sxu.edu.cn

they contain a large amount of nutrients. However, the response of plant growth to green algae, as compared to cyanobacteria, has rarely been reported. Moreover, rhizosphere microorganisms, as described above, are an important component of plant roots and the two can affect each other. Therefore, biofertilizer may also affect rhizosphere microbial communities. However, the response of rhizosphere microorganisms to microalgal biomass as biofertilizer has not yet been reported.

Second-generation high-throughput sequencing-based new technologies have been developing rapidly. The major advantage of these technologies is their ability to produce large amounts of DNA reads from which to analyze microbial community structures in detail. Illumina MiSeq sequencing is one of the popular high-throughput sequencing systems and has been widely used to analyze soil microbial communities (Zhang et al., 2016; Gu et al., 2017).

In the present study, the growth response of cucumber to two microalgae (*Anabeana circinalis* and *Scenedesmus quadricauda*) used as biofertilizer is evaluated. Moreover, the composition of rhizosphere microorganisms of cucumber is analyzed by Illumina MiSeq sequencing. The main aim is to evaluate the fertilizer value of microalgal biomass and make an effort to characterize the diversity and abundance of rhizosphere microorganisms in cucumber after application of microalgae fertilizer, which is the novel approach of our research.

2. Materials and methods

2.1. Experimental materials

In the present study, soil belonging to the 10–20 cm plow layer of farmland from Hongsi Village, Taiyuan City, Shanxi Province, China was taken to cultivate cucumber. The soil pH was 6.8. The soil contained 8.93% (w/v) organic carbon, 3 mg/kg NH+ 4, 1 mg/kg NO− 3, 234 mg/kg extractable P, and 1117 mg/kg K. The cucumber seeds (Chinese line "Shengyou") were obtained from Shanxi Yongshengchang Seed Industry Co., Ltd. Both *S. quadricauda* (FACHB-1468) and *A. circinalis* (FACHB-606) were purchased from the Institute of Hydrobiology of the Chinese Academy of Sciences (FACHB-Collection, Wuhan, China) and were used to produce the microalgal biomass.

2.2. Experimental design

Both microalgae strains were precultivated with BG11 medium at 25 °C under incident light intensity of 3000 lux and a 12 h:12 h (light:dark) cycle, and shaken at 160 rpm/min. Then, 300 mL of microalgal solutions were collected and centrifuged at 6000 rpm for 8 min while they were in the logarithmic growth phase (*S. quadricauda* was 4.0×10^6 cells/mL, and *A. circinalis* was 7.8×10^6 cells/mL). The precipitate was washed with deionized water and centrifuged at 6000 rpm for 8 min. Afterwards, the precipitate was suspended with tap water as biofertilizer.

To elucidate the effect of microalgal biomass as biofertilizer on the growth of cucumber and microbial communities in the cucumber rhizosphere, the farmland soil was placed in plastic pots (21 cm diameter × 18 cm) and amended with a microalgal suspension before sowing cucumber seeds. The soil mass in each plastic pot was approximately 3 kg. After sowing, the microalgal suspension was also applied to the soil every 7 days. In the present study, soil amended with 200 mL of microalgal suspension (about 4.0×10^8 cells) at each application was the low dosage group (low dosage of *A. circinalis*: AC_L; low dosage of *S. quadricauda*: SQ_L). The soil amended with 400 mL (about 8.0×10^8 cells) of microalgal suspension at each application was the high dosage group (high dosage of *A. circinalis*: AC_H; high dosage of *S. quadricauda*: SQ_H). For plant cultivation, four cucumber seeds were evenly placed into the middle of each pot. When all of the cucumber seedlings grew to about 4–5 cm, the best seedling was retained. Control group cucumber seeds were planted without amending with microalgal suspension and then irrigated with tap water that did not contain microalgal biomass every 7 days. All pots were placed in a glasshouse at 25 ± 2 °C, and the source of light was natural. All these experiments were performed in 6 replicates.

2.3. Growth status and soil collection

On the 45th day, plant height, stem diameter, leaf number, and flower bud number of the cucumbers were measured. Loosely adhered soil of cucumber roots was removed by vigorously shaking roots. After the aforementioned processing, the soil tightly adhered to the roots was considered the rhizosphere soil. The rhizosphere soil was collected with a sterile brush, and large inorganic impurities were removed. The soil from the 6 replicates was mixed, and the mixed sample was put into a 10 mL sterile tube and stored at −80 °C for microbial community structure analysis.

2.4. DNA extraction and PCR amplification

Genomic DNA of soil samples (0.25 g) was extracted by using PowerSoil® DNA Isolation kit (Mobio, USA) according to manufacturer protocol. To test DNA integrity, the extracted DNA was analyzed by agarose gel (1%, w/v) electrophoresis.

For amplification of the V4–V5 region of the bacterial 16S rRNA gene, universal primers 515F (GTGCCAGCMGCCGCGGTAA) and 926R (CCGTCAATTCMTTTRAGTTT) were used. For two-step amplicon library building, the Illumina 5' overhang adapter sequences were contained in primers according to manufacturer protocol. Bacteria 16S rRNA gene fragments were amplified via two PCR steps. PCR reactions were done in a 25 μL reaction system which contained 20 ng

DNA template, 0.25 μM of each primer, 0.5 U Phusion DNA polymerase (New England Biolabs, USA), 1X reaction buffer, and 250 μM dNTPs. The reaction was performed in a thermal cycler (GeneAmp 9700, Applied Biosystems, USA). Initial denaturation (94 °C for 2 min) was followed by denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s with 25 cycles. Afterwards, a final extension was performed at 72 °C for 5 min. For multiplexing, the Illumina Nextera XT Index kit (Illumina Inc., USA) was used. To incorporate two unique barcodes on either end of 16S amplicons, eight cycles of PCR amplifications were conducted. This included an initial cycle at 94 °C for 3 min, which was followed by 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s with 8 cycles. Then, a final extension was done at 72 °C for 5 min. The barcoded PCR products were purified and quantified via DNA gel extraction kit (Axygen, China) and a Qubit dsDNA HS assay kit (Life Technologies, USA), respectively, before building libraries. Finally, libraries were sequenced on the MiSeq platform by Tiny Gene Bio-Tech Co., Ltd. (Shanghai, China).

Primers ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS2 (GCTGCGTTCTTCATCGATGC) were used to amplify the ITS1 region of the fungal rRNA gene. These primer pairs were modified before pyrosequencing according to manufacturer protocol. Then, PCR reactions were performed in a 25 μL reaction system, which contained 40 ng DNA template, 10 μM of each primer, 0.5 U DNA polymerase (Takara Biotech. Co., Ltd., Japan), 10X reaction buffer, and 2.5 mM dNTPs. The reaction contained an initial denaturation at 94 °C for 4 min, which was followed by 94 °C for 30 s, 50 °C for 45 s, and 72 °C for 1 min with 35 cycles. Afterwards, a final extension was done at 72 °C for 7 min. The barcoded PCR products were purified via a commercial kit (Axygen Bio, USA). Finally, sequencing was achieved on the MiSeq platform by Tiny Gene Bio-Tech Co., Ltd. (Shanghai, China).

2.5. Biological information analysis

Ambiguous reads were removed by barcode and filtration. Reads shorter than 200 bp or longer than 600 bp were also removed. To check for chimeric sequences, the UCHIME algorithm included in USEARCH was used. All sequences were processed using Mothur (version 1.35.1) according to previous reports (Kozich et al., 2013). Silva 119, Greengenes, and RDP were used for 16S rRNA gene annotation. UNITE was used for ITS gene annotation. Potential sequences from mitochondrial and chloroplast DNA of *Cucumis sativus* L were rejected during data analysis and processing. The effective sequences were classified as operational taxonomic units (OTUs) based on more than 97% sequence similarity. Parameters on species richness and diversity [such as abundance-based coverage estimator (ACE), Chao 1 richness, and Shannon–Wiener index] were obtained by Mothur. Principal coordinate analysis (PCoA) was done by Mothur, which evaluated similarities of different samples using an unweighted UniFrac approach. All 16S rRNA and ITS sequences were available through GenBank BioProject PRJNA390673.

2.6. Statistical analysis

The results of *C. sativus* growth were expressed as mean ± standard deviation. The data was analyzed by SPSS 17.0 (one-way ANOVA) and Duncan's method ($P < 0.05$).

3. Results

3.1. Effects of microalgal biomass on the growth of cucumber

Figure 1 illustrated the effect of microalgal biomass on the growth of cucumber. The height of cucumber cultivated in soil enriched with *S. quadricauda* and *A. circinalis* significantly increased ($P < 0.05$). Similarly, the application of *S. quadricauda* and *A. circinalis* to soil significantly increased the number of leaves and flower buds in cucumber ($P < 0.05$). The application of *S. quadricauda* significantly increased the stem diameter of cucumber ($P < 0.05$). In general, the application of the two microalgae to soil significantly promoted the growth of cucumber.

3.2. Rhizosphere microbial diversity and richness analysis

In the present study, 213,636 and 350,530 sequences were generated for rhizosphere bacteria and fungi from 5 samples (Table S1). Average sequences of rhizosphere bacteria and fungi for the samples were 42,727 and 70,106, respectively. After filtering, 165,454 and 294,871 effective sequences remained for rhizosphere bacteria and fungi from 5 samples (Table S1), which occupied 77% and 84% of the total sequences, respectively. Average effective sequences of rhizosphere bacteria and fungi for the samples were 33,090 and 58,974, respectively. These sequences could be clustered into 1241, 1270, 1228, 1300, and 1285 bacterial OTUs in control, AC_H, AC_L, SQ_H, and SQ_L, respectively, at a cut-off of 97% sequence similarity (Table). In fungi there were 250, 253, 272, 386, and 369 OTUs in control, AC_H, AC_L, SQ_H, and SQ_L, respectively, at a cut-off of 97% sequence similarity (Table). Although there was almost no response of bacterial OTUs to microalgal biomass, the application of microalgal fertilizer to soil increased the number of rhizosphere fungal OTUs. In particular, the application of *S. quadricauda* increased the number of rhizosphere fungal OTUs. In this study, the rarefaction curves of these 5 samples were also drawn (Figure S1). Results showed that the rarefaction curves of these samples almost reached a plateau, reflecting the fact that each sample had been sampled to saturation with respect to species diversity.

Abundance and diversity of microbial communities were also evaluated. The number of bacterial OTUs was

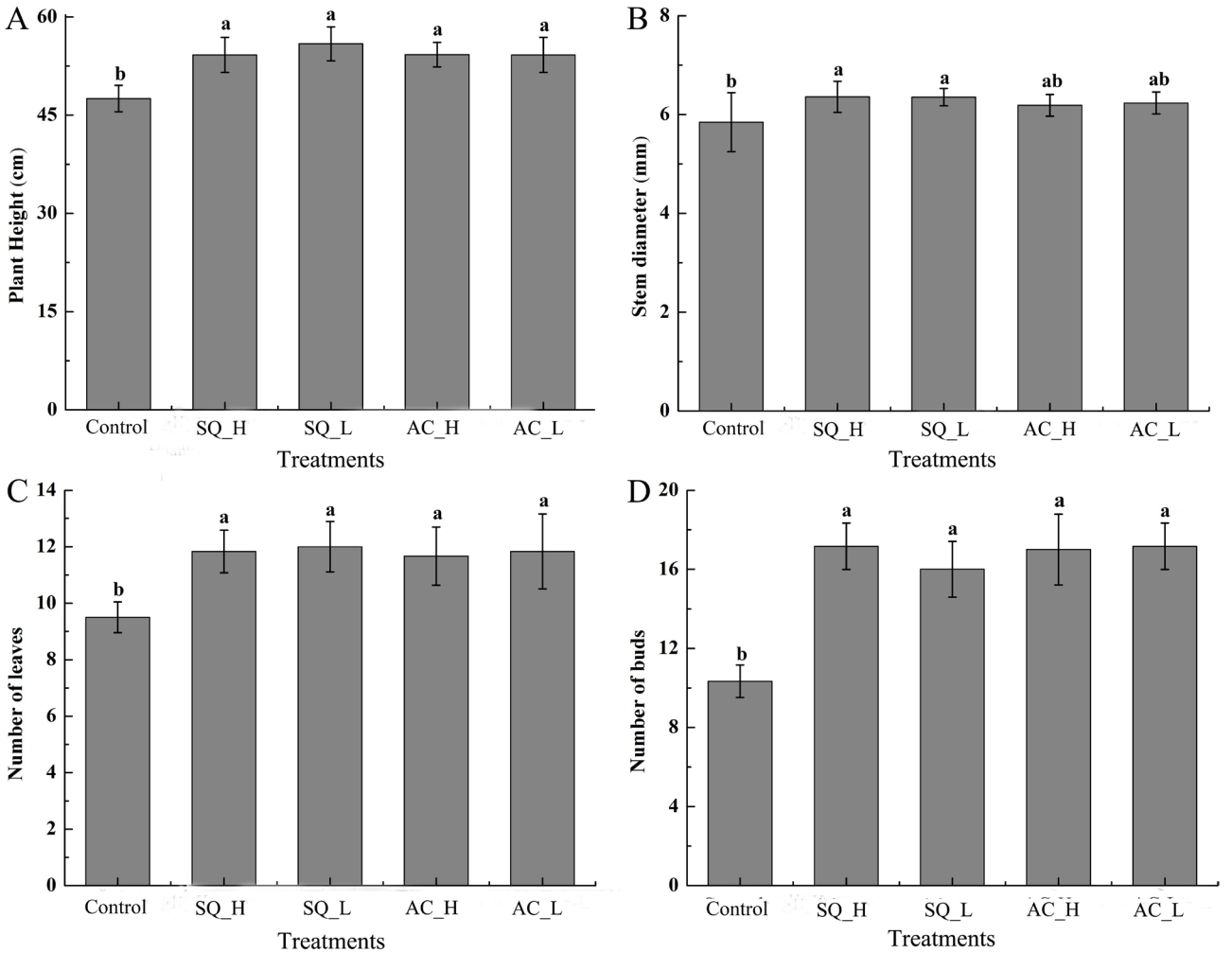

Figure 1. Effect of microalgal biofertilizer on plant height (A), stem diameter (B), number of leaves (C), and number of buds (D) in cucumber. Values are represented by mean ± standard deviation. Different letters indicate significant differences ($P < 0.05$). SQ_H: high dosage of *S. quadricauda*; SQ_L: low dosage of *S. quadricauda*; AC_H: high dosage of *A. circinalis*; AC_L: low dosage of *A. circinalis*.

Table. The estimated OTU richness and diversity indices of the 16S rDNA and ITS genes obtained from the Illumina MiSeq analysis in soils with different treatments.

Treatment	Bacteria					Fungi				
	OTUs	ACE	Chao	Shannon	Simpson	OTUs	ACE	Chao	Shannon	Simpson
Control	1241	1305	1304	5.87	0.0067	241	250	256	3.53	0.0598
AC_H	1270	1348	1378	5.83	0.0079	250	253	253	4.07	0.0383
AC_L	1228	1360	1364	5.76	0.0091	266	272	272	3.52	0.0671
SQ_H	1300	1349	1347	6.07	0.0050	382	386	386	3.95	0.0402
SQ_L	1285	1366	1390	6.02	0.0053	345	369	369	2.82	0.1678

estimated by Chao estimator, as shown in Table. There was no obvious response of bacterial richness to microalgal fertilizers. The abundance-based coverage estimator (ACE) showed trends similar to the Chao estimator. To assess the diversity and evenness of the bacterial community among these treatments, the Shannon and Simpson indexes were analyzed. There was no difference in bacterial diversity among different treatments.

Nevertheless, fungi OTUs of SQ_H and SQ_L were higher than those of the control, AC_H, and AC_L. Obviously, the application of *S. quadricauda* to soil increased the number of rhizosphere fungi in cucumber. Similarly, the ACE and Chao estimators demonstrated again that the richness of rhizosphere fungi associated with cucumber increased through irrigating *S. quadricauda* into the soil. Interestingly, although the richness of rhizosphere fungi of SQ_H and SQ_L was almost identical according to the Chao and ACE estimators, the Shannon index found that SQ_H was higher than SQ_L. It indicated that the application of a high concentration of *S. quadricauda* to the soil was beneficial for raising the diversity of the rhizosphere fungi of cucumber.

Principal coordinate analysis of the rhizosphere bacterial community showed that the control did not group together with treatment groups (Figure 2a), and the sample AC_L was also different from the others (AC_H, SQ_H, and SQ_L). The results indicated that microalgal biomass as fertilizer had some effects on bacterial community composition. Similarly, microalgal biomass as fertilizer also had some effects on fugal community composition (Figure 2b).

3.3. Microbial community analysis

To gain more insight into the response of rhizosphere microbial communities of cucumber to microalgal biomass, the microbial community was analyzed at the phylum level. As shown in Figure 3a, there were 19 bacterial phyla in each sample. *Proteobacteria* was the most abundant phylum in all samples. It had the highest proportion and ranged from 35% to 42%. Moreover, *Acidobacteria* (12%–15%, averaging 13%), *Actinobacteria* (11%–13%, averaging 12%), and *Gemmatimonadetes* (8%–12%, averaging 10%) were also rich in all samples, followed by other abundant phyla (average abundance: >1%) including *Bacteroidetes* (8%–10%, averaging 9%), *Chloroflexi* (6%–9%, averaging 7%), and *Planctomycetes* (5%–6%, averaging 6%). Compared to control, the relative abundances of *Gemmatimonadetes* decreased from 12% to 8%, 9%, and 10% in AC_L, SQ_H, and SQ_L, respectively. Relative abundances of *Chloroflexi* increased from 6% to 9% and 8% in SQ_H and SQ_L, respectively. All of the results above indicated that microalgal biomass as fertilizer greatly influenced the abundance of some bacteria at the phylum level.

The fungal community structures at the phylum level were depicted in Figure 3b. The number of phyla detected in the samples ranged between 5 and 6. This indicated that fungal diversity was lower than bacterial diversity at the phylum level. The most predominant phylum was *Ascomycota* in all five samples. Compared to a low abundance of *Basidiomycota* in the control, relative abundance of the phylum significantly increased in AC_H, AC_L, SQ_H, and SQ_L. On the other hand, relative abundances of *Zygomycota* decreased from 19% to 6%, 13%, 6%, and 5%, respectively. It was clear that microalgal

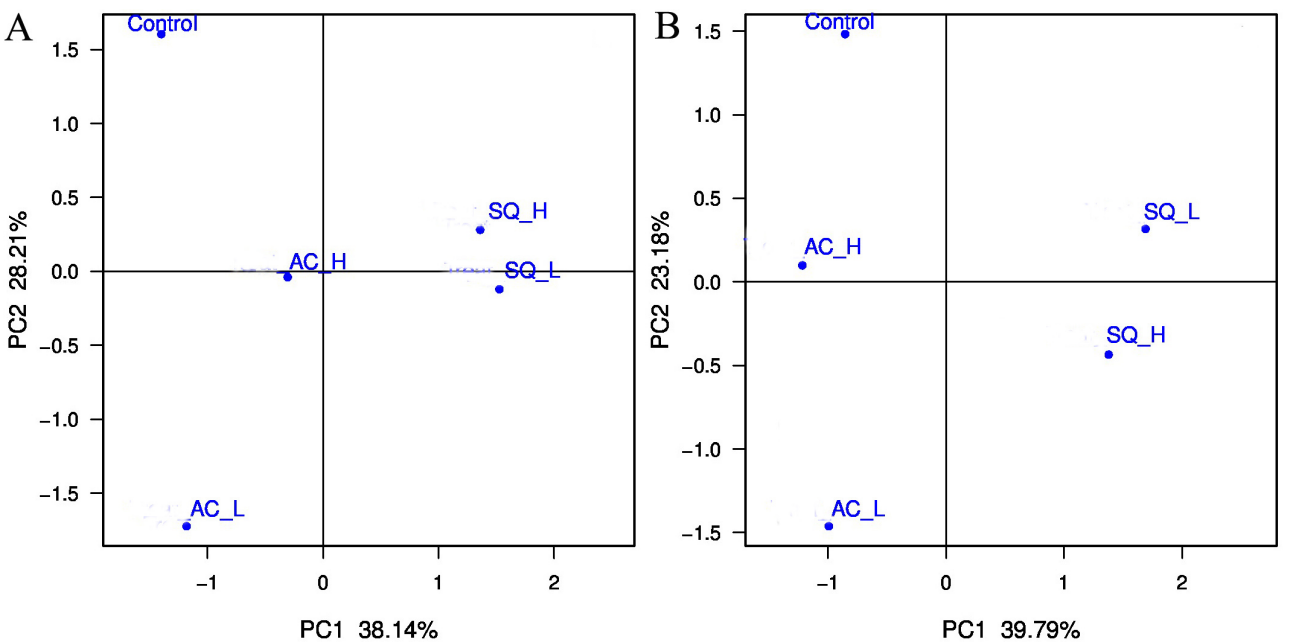

Figure 2. Principal coordinate analysis (PCoA) of five samples based on the composition of bacterial (A) and fungal (B) communities based on OTUs. SQ_H: high dosage of *S. quadricauda*; SQ_L: low dosage of *S. quadricauda*; AC_H: high dosage of *A. circinalis*; AC_L: low dosage of *A. circinalis*.

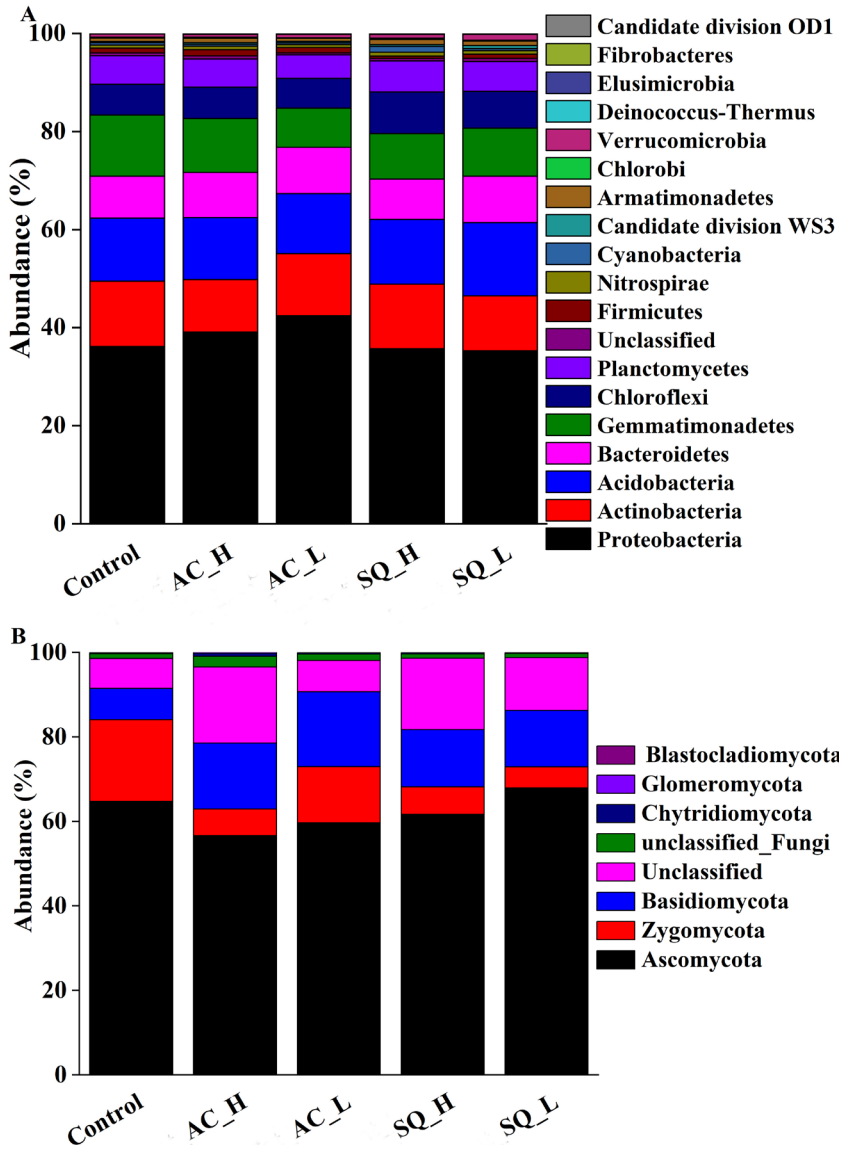

Figure 3. Bacterial (A) and fungal (B) community structure of five samples at the phylum level. SQ_H: high dosage of *S. quadricauda*; SQ_L: low dosage of *S. quadricauda*; AC_H: high dosage of *A. circinalis*; AC_L: low dosage of *A. circinalis*.

biomass as fertilizer greatly influenced the abundance of *Basidiomycota* and *Zygomycota*. Among all samples, 7%–18% of species were unclassified at the phylum level.

In order to obtain more comprehensive information about rhizosphere microbial communities of cucumber, the microbial community was also analyzed at the genus level. As shown in Figure 4, there was a low heterogeneity in the top 20 bacterial genera among these samples (total: 31 genera for 5 samples). *Lysobacter* was the most abundant genus in the control. However, its abundance decreased in SQ_H and SQ_L. In AC_H and AC_L, the most abundant genus was *Novosphingobium*. The abundance of the genus in the two samples was higher than in the control, SQ_H, and SQ_L. In SQ_H and SQ_L, the most abundant genus was *Skermanella* and *Novosphingobium*, respectively. It was noted that the distribution of some genera was related to treatments. For instance, the abundance of *Erythrobacter* and *Pseudoxanthomonas* increased in AC_H, AC_L, SQ_H, and SQ_L, compared to the control. *Arenimonas* and *Azotobacter* were more abundant in SQ_H and SQ_L. *Bacillus* was more abundant in AC_H. *Thermomonas* was rich in AC_H and AC_L. *Sandarakinorhabdus* was dominant in the control, whereas its abundance was very low in AC_H, AC_L, SQ_H, and SQ_L. *Pseudomonas*, described as the plant-growth–promoting bacteria (Khalid et al., 2004; Herman et al., 2008), was rich in all

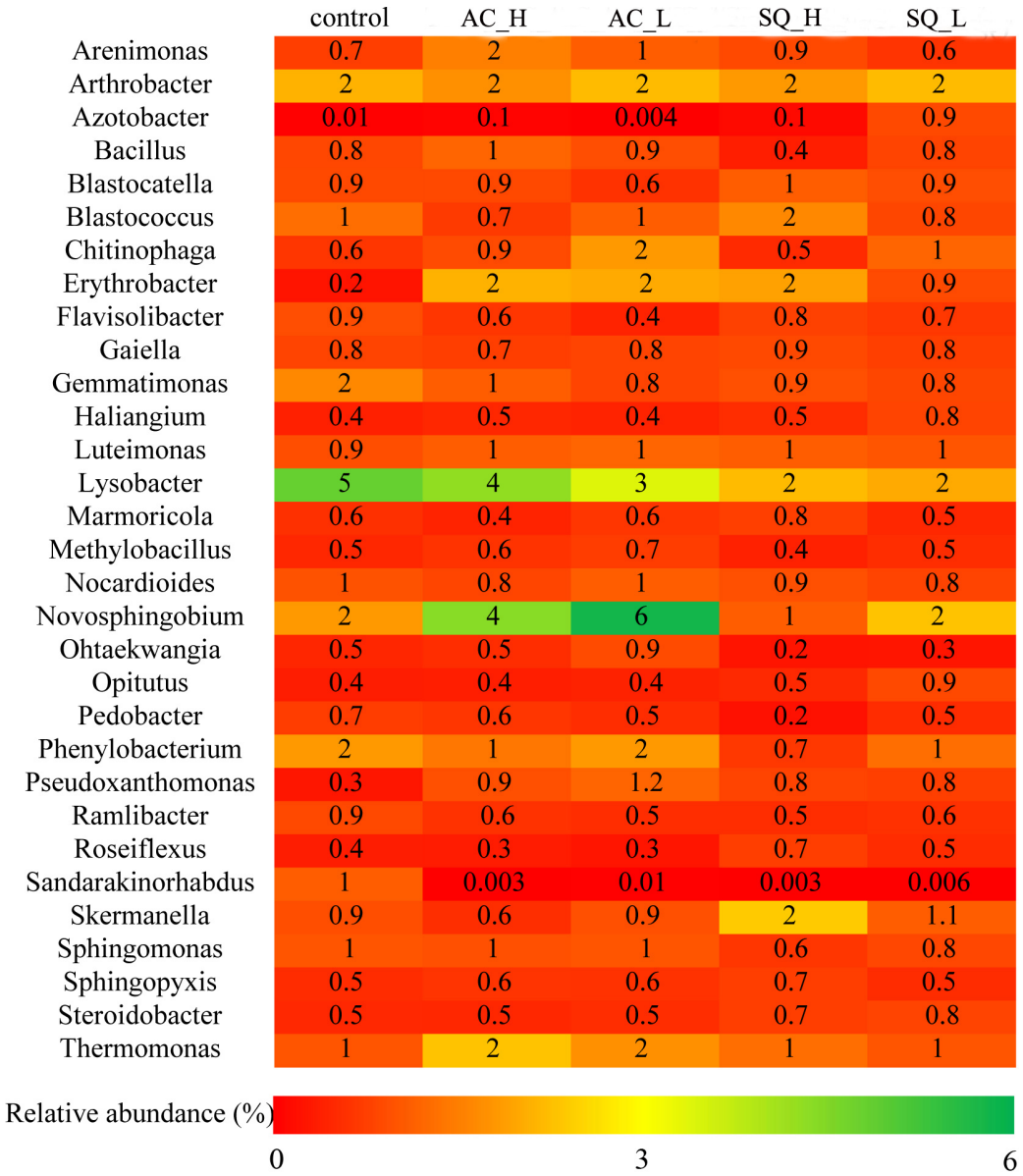

Figure 4. Heatmap analysis of the top 20 genera of the bacterial community from different samples. SQ_H: high dosage of *S. quadricauda*; SQ_L: low dosage of *S. quadricauda*; AC_H: high dosage of *A. circinalis*; AC_L: low dosage of *A. circinalis*.

treatments, compared to the control. Moreover, there were 167 identified genera in the control, fewer than those identified in the treatments (181, 177, 179, and 182 genera in AC_H, AC_L, SQ_H, and SQ_L, respectively). These results strongly implied that microalgal biomass as fertilizer had some effects on bacterial abundances at the genus level. In the present study, 51%–60% of sequences were unclassified at the genus level. Therefore, much effort would be needed to analyze these unknown sequences and clarify the relationship between these sequences and fertilization in the future.

Figure 5 exhibited the top 20 genera of rhizosphere fungi from all samples. *Mortierella* was the most abundant genus in the control. However, its abundance decreased in AC_H, AC_L, SQ_H, and SQ_L, respectively. *Cryptococcus* was the most abundant genus in AC_H, AC_L, SQ_H, and SQ_L. Its proportion was higher in treatments than that in the control. The abundance of *Chaetomium* was relatively high in the control. Nevertheless, its abundance decreased in AC_H, AC_L, SQ_H, and SQ_L. Similarly, the abundance of *Stachybotrys* decreased in all treatments. AC_L had high abundance of *Monographella*, and levels

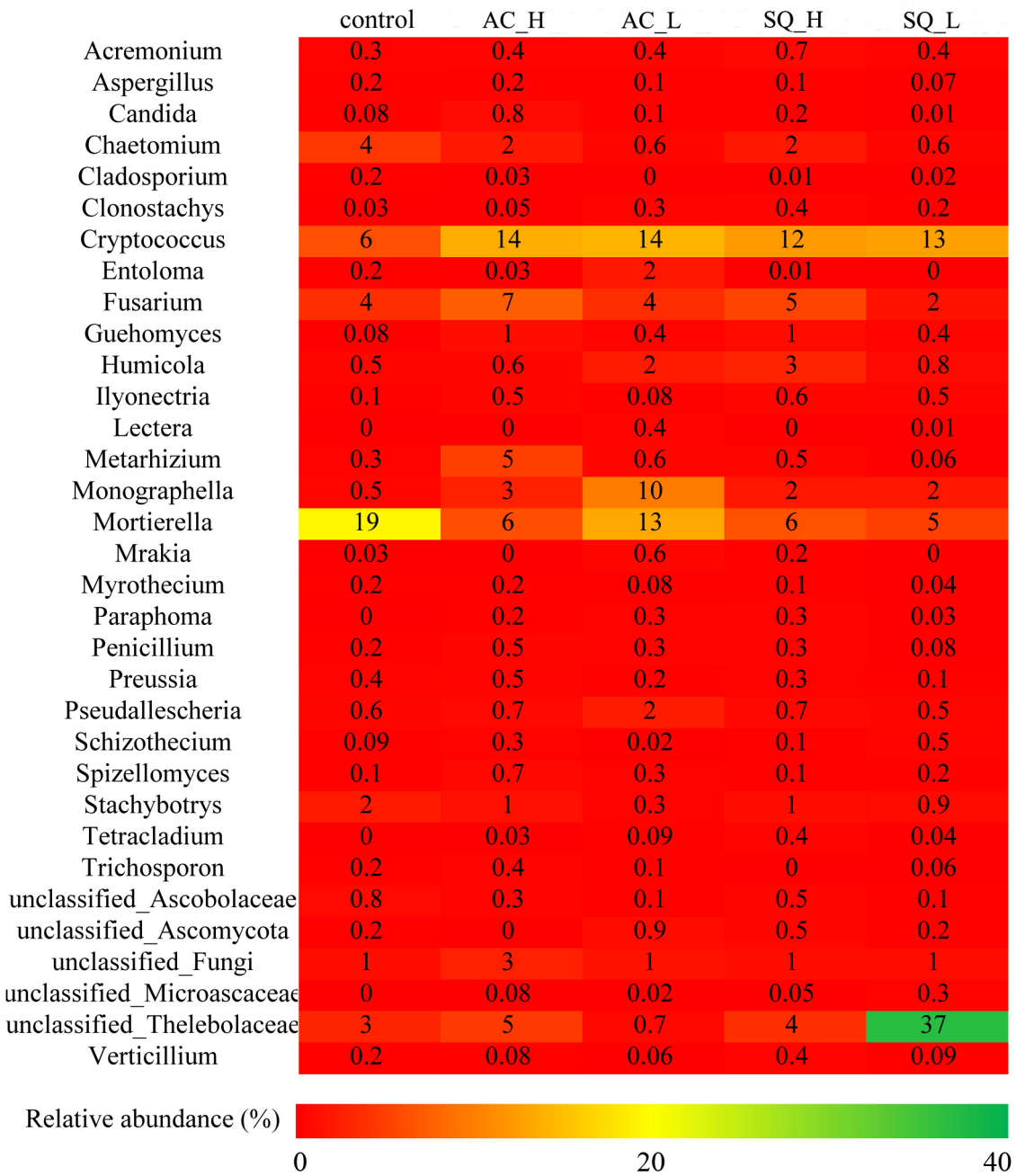

Figure 5. Heatmap analysis of the top 20 genera of the fungal community from different samples. SQ_H: high dosage of *S. quadricauda*; SQ_L: low dosage of *S. quadricauda*; AC_H: high dosage of *A. circinalis*; AC_L: low dosage of *A. circinalis*.

were higher than in the other samples. Moreover, the abundance of other genera, such as *Fusarium*, *Humicola*, and *Metarhizium* also responded to microalgal biomass as fertilizer. As described by Meera et al. (1994), species of the genera *Penicillium*, *Phoma*, and *Trichoderma* could promote plant growth. Although the abundance of these genera was relatively low, they also responded to microalgal biomass, as shown in Figure 5. Interestingly, 37% of unclassified *Thelebolaceae* was in SQ_L, a higher percentage than in other samples. All of the results above indicated that microalgal biomass as fertilizer also had an effect on rhizosphere fungal abundances at the genus level.

4. Discussion

The present study found that microalgal biomass as biofertilizer significantly promoted height, number of

leaves and flower buds, and stem diameter of cucumber. This was comparable to the plant height and number of leaves in cucumber cultivated with farmyard manure fertilizer and inorganic fertilizer (Eifediyi and Remison, 2009; Eifediyi and Remison, 2010). The result was also consistent with previous reports showing that *Spirulina platensis* enhanced plant growth in the aspects of leaf number, plant height, and root length (Wuang et al., 2016). All results indicated that microalgal biomass was a good fertilizer. Cyanobacterial inoculation was known to enhance crop yields and the availability of soil nitrogen via nitrogen fixation (Prasanna et al., 2012; Dey et al., 2017). Nevertheless, a small fluctuation in cyanobacteria abundance in soil was observed in the study via high-throughput sequencing (except in SQ_H), and no *Anabeana* was detected at the genus and species levels. Therefore, *A. circinalis* might not be customized to the soil to promote plant growth through nitrogen fixation. It was speculated that it affected the growth of cucumber through the presence of micro- and macronutrients of *A. circinalis*. Some researchers reported that the extraction of green algae or algal cells enhanced the growth of willow and superior grapevines (Abd and Abd-Allah, 2008; Grzesik et al., 2017), which verified again that green algae *S. quadricauda* used as a biofertilizer promoted the growth of cucumber. In this study, microalgal cells were obtained in the logarithmic growth phase and were made into biofertilizer. It was very convenient for microalgae to grow into the logarithmic growth stage having obtained enough biomass, whether a synthetic medium or wastewater was used (Kong et al., 2010; Li et al., 2010). Therefore, our applied concentrations were suitable for field applications.

As reviewed by Vessey (2003), the plant rhizosphere was regarded as the preferred ecological niche for soil microorganisms due to availability of nutrients. Rhizosphere microorganisms were also closely related to the successful production of crops and maintenance of soil fertility (Vessey, 2003). In this study, the response of the microbial richness and diversity of the cucumber rhizosphere to microalgal biomass as biofertilizer was first viewed through Illumina MiSeq sequencing. The number of rhizosphere bacterial OTUs ranged from 1228 to 1300, and the number of rhizosphere fungal OTUs ranged from 241 to 382. The number of rhizosphere bacterial and fungal OTUs was comparable to those of cucumber plants in earlier investigations (Qiu et al., 2012; Tian and Gao, 2014). It was also found that the application of *S. quadricauda* greatly increased the number of rhizosphere fungal OTUs. *Scenedesmus quadricauda* had high protein content, which reached 50% of its dry weight (data not shown). Therefore, high quality nitrogen sources from *S. quadricauda* might be one of the important reasons to improve fungal diversity.

Rhizosphere microorganisms were important for plant growth. However, the response of rhizosphere microorganisms to microalgal biofertilizer is still unclear. In this experiment, it was found that *Proteobacteria*, *Acidobacteria*, and *Actinobacteria* were the most abundant phyla in all samples, which was consistent with previous reports on cucumber rhizosphere microbial communities (Qiu et al., 2012; Zhou et al., 2018). The abundance of some phyla in the study (such as *Bacteroidetes*, *Chloroflexi*, *Gemmatimonadetes*, and *Planctomycetes*) was higher than in other reports on cucumber rhizosphere microbial communities (Qiu et al., 2012; Tian and Gao, 2014). As reported in the literature (Khalid et al., 2004; Herman et al., 2008), genera such as *Acetobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, and *Pseudomonas* had plant-growth–promoting activity. As shown in this study, the abundance of *Azotobacter*, *Bacillus*, and *Pseudomonas* increased in some or all treatments. Bacteria of the three genera were also isolated and identified as plant-growth–promoting rhizobacteria (PGPR) from the cucumber rhizosphere (El-Borollosy et al., 2012; Islam et al., 2016). This indicated that microalgal biomass as biofertilizer enhanced the abundance of PGPR. *Azospirillum* and *Stenotrophomonas*, also reported as PGPR from the cucumber rhizosphere (Pii et al., 2015b; Islam et al., 2016), were not found in this study. Although there were some differences in abundance at the genus level, a low heterogeneity was observed in the top 20 bacterial genera among these samples (total: 31 genera for 5 samples). Generally, only minor differences were found in the bacterial community compositions associated with the cucumber rhizosphere between soils amended with microalgal biomass and corresponding untreated soils. The response of rhizosphere bacteria to microalgal biomass was mainly reflected through differential bacterial abundances.

Fertilization also affected the composition of rhizosphere fungi (Viaud et al., 2000). In the present study, the rhizosphere fungi were mainly composed of *Ascomycota*, *Basidiomycota*, and *Zygomycota*, which is consistent with the literature (Viaud et al., 2000). In all samples, the presence of *Ascomycota* was much higher than *Basidiomycota*, which was consistent with some reports (Qiu et al., 2012) but contradicted other reports (Lim et al., 2010). At the genus level, *Cryptococcus* was most abundant in the fungal communities associated with cucumber rhizosphere amended with microalgal biomass. *Cryptococcus* was the dominant fungal genus in the soil (Wuczkowski and Prillinger, 2004; Vishniac, 2006; Connell et al., 2008). The competitive advantage of *Cryptococcus* was closely related to its polysaccharide capsules (McFadden et al., 2006), which helped it to absorb nutrients and compete with other microbes (Vishniac, 2006). In this study, the

abundance of *Cryptococcu*s increased in the microalgal manure groups. It suggested that microalgal biomass as a biofertilizer affected the nutrition of rhizosphere soil and promoted the metabolism of polysaccharide capsules, which changed the number of *Cryptococcus*. From zoysiagrass Meera et al. (1994) isolated rhizosphere fungi belonging to the genera *Fusarium*, *Penicillium*, *Phoma*, and *Trichoderma*, which could enhance the growth of various crop plants. In this study, abundances of *Fusarium*, *Penicillium*, and *Trichoderma* were enhanced in some treatments. It was speculated that microalgal biomass as biofertilizer also raised the abundance of plant-growth–promoting fungi. Generally, the abundance of rhizosphere fungi in cucumber had been changed due to the application of microalgal biofertilizer.

In the present study, the response of cucumber to microalgal biomass from *A. circinalis* and *S. quadricauda* as biofertilizer was investigated. The application of the two microalgae to soil significantly promoted the growth of cucumber. Moreover, the rhizosphere microbial community of cucumber also responded to microalgal biofertilizer. The abundance of growth-promoting bacteria and fungi (*Azotobacter*, *Bacillus*, *Pseudomonas, Cryptococcus*, *Fusarium*, *Penicillium*, and *Trichoderma*) from the rhizosphere of cucumber increased after application of microalgae fertilizer. This study evidenced the applicability and microbial basis of *A. circinalis* and *S. quadricauda* as biofertilizer for plant growth.

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Conflict of interest
The authors declare no conflict of interest.

References

Abd EA, Abd-Allah ASE (2008). Effect of green alga cells extract as foliar spray on vegetative growth, yield and berries quality of superior grapevines. American-Eurasian Journal of Agricultural and Environmental Sciences 4: 427-433.

Ahemad M, Kibret M (2014). Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. Journal of King Saud University, Science 26: 1-20.

Berendsen RL, Pieterse CM, Bakker PA (2012). The rhizosphere microbiome and plant health. Trends in Plant Science 17: 478-486.

Bidyarani N, Prasanna R, Babu S, Hossain F, Saxena AK (2016). Enhancement of plant growth and yields in chickpea (*Cicer arietinum* L.) through novel cyanobacterial and biofilmed inoculants. Microbiological Research 188: 97-105.

Borowitzka MA (2013). High-value products from microalgae—their development and commercialisation. Journal of Applied Phycology 25: 743-756.

Chen G, Zhao L, Qi Y (2015). Enhancing the productivity of microalgae cultivated in wastewater toward biofuel production: a critical review. Applied Energy 137: 282-291.

Connell L, Redman R, Craig S, Scorzetti G, Iszard M et al. (2008). Diversity of soil yeasts isolated from South Victoria Land, Antarctica. Microbial Ecology 56: 448-459.

Dey SK, Chakrabarti B, Prasanna R, Pratap D, Singh SD et al. (2017). Elevated carbon dioxide level along with phosphorus application and cyanobacterial inoculation enhances nitrogen fixation and uptake in cowpea crop. Archives of Agronomy and Soil Science 63: 1-11.

El-Borollosy AM, Oraby MM (2012). Induced systemic resistance against *Cucumber mosaic cucumovirus* and promotion of cucumber growth by some plant growth-promoting rhizobacteria. Annals of Agricultural Sciences 57: 91-97.

Eifediyi EK, Remison SU (2009). The effects of inorganic fertilizer on the yield of two varieties of cucumber (*Cucumis sativus* L.). Report and Opinion 1: 74-80.

Eifediyi EK, Remison SU (2010). Growth and yield of cucumber (*Cucumis sativus* L.) as influenced by farmyard manure and inorganic fertilizer. Journal of Plant Breeding and Crop Science 2: 216-220.

Grzesik M, Romanowska-Duda Z, Kalaji HM (2017). Effectiveness of cyanobacteria and green algae in enhancing the photosynthetic performance and growth of willow (*Salix viminalis* L.) plants under limited synthetic fertilizers application. Photosynthetica 55: 510-521.

Gu Y, Van Nostrand JD, Wu L, He Z, Qin Y et al. (2017). Bacterial community and arsenic functional genes diversity in arsenic contaminated soils from different geographic locations. PloS One 12: e0176696.

Herman MAB, Nault BA, Smart CD (2008). Effects of plant growth-promoting rhizobacteria on bell pepper production and green peach aphid infestations in New York. Crop Protection 27: 996-1002.

Islam S, Akanda AM, Prova A, Islam MT, Hossain MM (2016). Isolation and identification of plant growth promoting rhizobacteria from cucumber rhizosphere and their effect on plant growth promotion and disease suppression. Frontiers in Microbiology 6: 1360.

Khalid A, Arshad M, Zahir ZA (2004). Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. Journal of Applied Microbiology 96: 473-480.

Kong QX, Li L, Martinez B, Chen P, Ruan R (2010). Culture of microalgae *Chlamydomonas reinhardtii* in wastewater for biomass feedstock production. Applied Biochemistry and Biotechnology 160: 9-18.

Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Applied and Environmental Microbiology 79: 5112-5120.

Li X, Hu H, Gan K, Yang J (2010). Growth and nutrient removal properties of a freshwater microalga *Scenedesmus* sp. LX1 under different kinds of nitrogen sources. Ecological Engineering 36: 379-381.

Lim YW, Kim BK, Kim C, Jung HS, Kim BS et al. (2010). Assessment of soil fungal communities using pyrosequencing. Journal of Microbiology 48: 284-289.

Maity JP, Bundschuh J, Chen CY, Bhattacharya P (2014). Microalgae for third generation biofuel production, mitigation of greenhouse gas emissions and wastewater treatment: present and future perspectives—a mini review. Energy 78: 104-113.

McFadden D, Zaragoza O, Casadevall A (2006). The capsular dynamics of *Cryptococcus neoformans*. Trends in Microbiology 14: 497-505.

Meera MS, Shivanna MB, Kageyama K, Hyakumachi M (1994). Plant growth promoting fungi from zoysiagrass rhizosphere as potential inducers of systemic resistance in cucumbers. Phytopathology 84: 1399-1406.

Mulbry W, Westhead EK, Pizarro C, Sikora L (2005). Recycling of manure nutrients: use of algal biomass from dairy manure treatment as a slow release fertilizer. Bioresource Technology 96: 451-458.

Peiffer JA, Spor A, Koren O, Jin Z, Tringe SG et al. (2013). Diversity and heritability of the maize rhizosphere microbiome under field conditions. Proceedings of the National Academy of Sciences of the United States of America 110: 6548-6553.

Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH (2013). Going back to the roots: the microbial ecology of the rhizosphere. Nature Reviews Microbiology 11: 789-799.

Pii Y, Mimmo T, Tomasi N, Terzano R, Cesco S et al. (2015a). Microbial interactions in the rhizosphere: beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. Biology and Fertility of Soils 51: 403-415.

Pii Y, Penn A, Terzano R, Crecchio C, Mimmo T et al. (2015b). Plant-microorganism-soil interactions influence the Fe availability in the rhizosphere of cucumber plants. Plant Physiology and Biochemistry 87: 45-52.

Prasanna R, Joshi M, Rana A, Shivay YS, Nain L (2012). Influence of co-inoculation of bacteria-cyanobacteria on crop yield and C–N sequestration in soil under rice crop. World Journal of Microbiology and Biotechnology 28: 1223-1235.

Qiu M, Zhang R, Xue C, Zhang S, Li S et al. (2012). Application of bio-organic fertilizer can control Fusarium wilt of cucumber plants by regulating microbial community of rhizosphere soil. Biology and Fertility of Soils 48: 807-816.

Renuka N, Prasanna R, Sood A, Ahluwalia AS, Bansal R et al. (2016). Exploring the efficacy of wastewater-grown microalgal biomass as a biofertilizer for wheat. Environmental Science and Pollution Research 23: 6608-6620.

Tian Y, Gao L (2014). Bacterial diversity in the rhizosphere of cucumbers grown in soils covering a wide range of cucumber cropping histories and environmental conditions. Microbial Ecology 68: 794-806.

Tripathi RD, Dwivedi S, Shukla MK, Mishra S, Srivastava S et al. (2008). Role of blue green algae biofertilizer in ameliorating the nitrogen demand and fly-ash stress to the growth and yield of rice (*Oryza sativa* L.) plants. Chemosphere 70: 1919-1929.

Vessey JK (2003). Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil 255: 571-586.

Viaud M, Pasquier A, Brygoo Y (2000). Diversity of soil fungi studied by PCR-RFLP of ITS. Mycological Research 104: 1027-1032.

Vishniac HS (2006). A multivariate analysis of soil yeasts isolated from a latitudinal gradient. Microbial Ecology 52: 90-103.

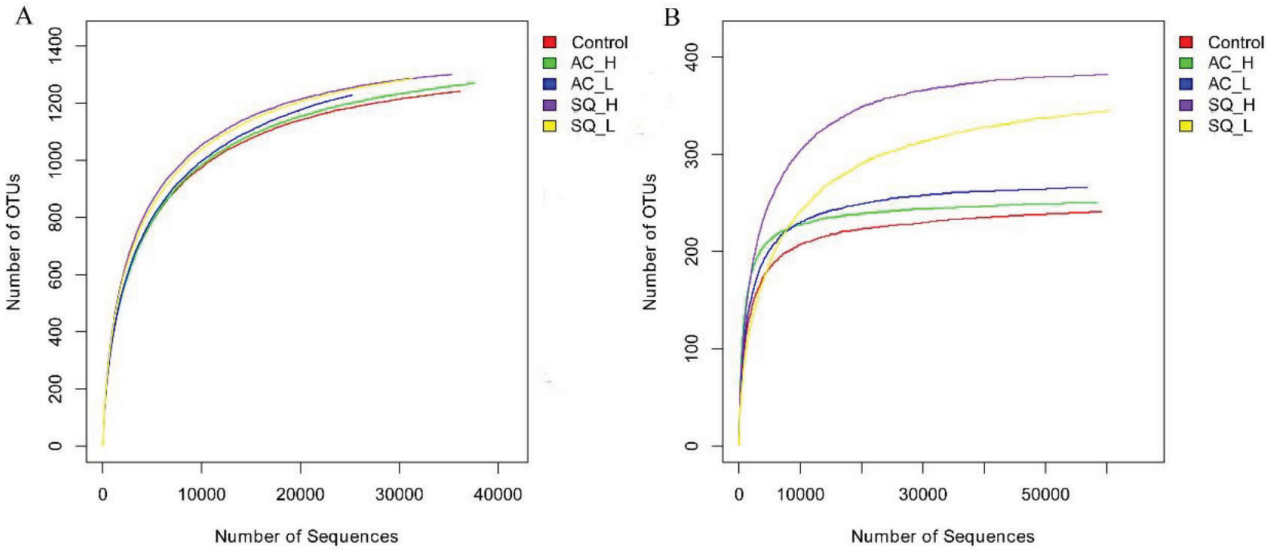
Wuang SC, Khin MC, Chua PQD, Luo YD (2016). Use of *Spirulina* biomass produced from treatment of aquaculture wastewater as agricultural fertilizers. Algal Research 15: 59-64.

Wuczkowski M, Prillinger H (2004). Molecular identification of yeasts from soils of the alluvial forest national park along the river Danube downstream of Vienna, Austria (Nationalpark Donau-Auen). Microbiological Research 159: 263-275.

Zhang W, Chen L, Zhang R, Lin K (2016). High throughput sequencing analysis of the joint effects of BDE209-Pb on soil bacterial community structure. Journal of Hazardous Materials 301: 1-7.

Zhou J, Lei T (2007). Review and prospects on methodology and affecting factors of soil microbial diversity. Biodiversity Science 15: 306-311 (in Chinese).

Zhou X, Zhang J, Pan D, Ge X, Jin X et al. (2018). p-Coumaric can alter the composition of cucumber rhizosphere microbial communities and induce negative plant-microbial interactions. Biology and Fertility of Soils 54: 363-372.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