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Which of soil microbes is in positive correlation to yields of maize (*Zea mays* L.)?

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ABSTRACT

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Soil microorganisms are critical to maintain soil function, enhance plant health and increase crop yields. This study investigated the effects of organic matter on soil microbial community and assessed which of soil microbes were in positive correlation to maize yields. The results showed that different fertilizer treatments shaped specific microbial communities in the same soils. The most abundant beneficial soil microbes were found in treatments with organic fertilizer produced from cattle manure, return of wheat straw and 70% NPK admixture fertilizers treatment. The correlation analysis revealed that maize yields were in no correlation both to the shifts of soil microbial community structure and to the number of sequences or operational taxonomic units (OTUs) in soil microbes. However, maize yields were in positive correlation to microbial community structure shifts at the species level. 35 bacteria OTUs from 19 orders in 14 classes in 9 phyla were in positive correlation to yields of maize, while in fungi only one OTU_{25} belonging to Sordariales was in positive correlation. Our results indicate that the long-term application of organic and inorganic amendments could enrich the soil bacterial and fungal community and promote its diversity.

Keywords: soil microbiome; plant-growth-promoting rhizobacteria; corn field; mineral fertilizer

Soil microorganisms are critical to maintain soil function because of their contributions to soil structure formation, decomposition of organic matter, toxin removal, and the biogeochemical cycling of carbon, nitrogen, phosphorus and sulphur (Doran and Zeiss 2000, Zhao et al. 2014). Inoculation with plant-growth-promoting rhizobacteria (*Azospirillum* s-21) in the corn field increases dry weights of leaf, stem and grain (Gholami et al. 2012). Organic inputs have a positive effect on both bacterial and fungal diversity with or without chemical fertilizers. Total diversity of

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bacterial and fungal communities is closely related to agro-ecosystem management practices and may partially explain the yield differences observed between different treatments (Kamaa et al. 2011).

Many studies have been focused on exploring the effects of long-term fertilizer applications and return of plant residues to agricultural fields on soil physical properties (Pernes-Debuyser and Tessier 2004), soil fertility (Mallarino and Borges 2006), soil organic matter and crop yields (Li et al. 2008, Cao et al. 2011). Mineral fertilizer and organic manure addition can maintain or improve crop yields, and affect the soil microbial characteristics under different fertilizer treatments. Structure of maize (*Zea mays* L.) rhizosphere bacteria is evaluated to explore the feasibility of identifying novel rhizosphere bacteria using culture-independent method based on direct amplification and analysis of 16S rRNA gene sequences and especially to obtain a better understanding of bacterial community structure and diversity from maize (Chauhan et al. 2011, Vega-Avila et al. 2015).

The functional soil microbiome can provide a sustainable and effective strategy to alter plant morphology, enhance plant growth and increase crop yields. However, little is known about which of soil microbes are related to yields of maize under long-term fertilization and wheat-maize rotation agro-ecosystem. The aim of this study was to investigate the relationship between soil microbes and yields of maize by high-throughput sequencing of soil microbial metagenomic analyses, and to find out the key microbes that influence maize yields.

MATERIAL AND METHODS

Experimental design. The field experiment was established in 2011 to investigate the effects of continuous application of farmyard manure, crop residues, and chemical fertilizer (NPK) on soil microbial communities in a winter wheat-summer maize rotation. Micro plot trial for maize (*Zea mays* L.) was performed at the farm of the Linquan Agriculture Research Institute (32°55'N, 115°06'E), Anhui province, China as a randomized complete block design with 17 (Table 1) treatments replicated three times (three blocks). Each plot was a rectangle with an area of 50 m^2 (5 × 10 m^2). The averages of the soils characteristics were as follows: pH 5.88, organic matter (12.64 g/kg), total N (1.21 g/kg), total P (0.36 g/kg), and total K (115.48 mg/kg). Soil samples for isolating DNA were collected at the summer maize harvest time in 2014.

DNA extraction and PCR amplification. Microbial DNA was extracted from soil samples using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, USA) according to the manufacturer's protocols. The V_4–V_5 region of the bacteria 16S rRNA gene was amplified by PCR (95°C for 2 min, followed by 25 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s and a final extension at 72°C for 5 min) using primers 515F 5'-barcode-GTGCCAGCMGCCGCGG)-3' and 907R 5'-CCGTCAATTCMTTTRAGTTT-3', where barcode is an eight-base sequence unique to each sample. The fungi 18S rRNA gene were amplified by PCR using primers 817F 5'-TAGCATGGAATAATRRAATAGGA-3' and 1196R 5'-TCTGGACCTGGTGAGTTTCC-3'.

Illumina MiSeq sequencing. Amplicons were extracted using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, USA) according to the manufacturer's instructions and quantified using QuantiFluor™-ST (Promega, Madison, USA). Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 250) on an Illumina MiSeq platform. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SRR3099036-3099047, SRR3099069, SRR3099089, SRR3099110, SRR3099129, SRR3099146, and SRR3100852).

Processing of sequencing data. Raw fastq files were demultiplexed, quality-filtered using QIIME (version 1.17, GitHub, San Francisco, USA). Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (http://drive5.com/uparse/) and chimeric sequences were identified and removed using UCHIME. The OTUs counts were normalized into relative abundances (%). These software programmes were carried out with the SPSS version 20.0 (IBM, Armonk, USA) for statistical analysis, and R version 3.2.2 for drafts. Unless stated, $P < 0.05$ were considered significant.

RESULTS AND DISCUSSIONS

This study characterizes the soil microbial community composition of maize planted in randomized and replicated plots at harvest time in

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Table 1. Experimental design of maize fertilization in different treatments

Treatment	Types and quantities of fertilizers (kg/ha)							Fertilizer ingredients (kg/ha)			
	NPK	POF	COF	wheat straw	BDA	PIOF	CIOF	organic matter	N	P	K
W_1	–	–	–	–	–	–	–	0	0	0	0
W_2	307	–	–	–	–	–	–	0	250.0	19.6	37.3
W_3	215	3000	–	–	–	–	–	1362	244.0	42.1	56.0
W_4	215	–	3000	–	–	–	–	1524	244.3	32.5	86.4
W_5	154	6000	–	–	–	–	–	2724	263.0	66.5	78.4
W_6	154	–	6000	–	–	–	–	3048	263.6	47.4	139.2
W_7	307	–	–	3000	–	–	–	2700	268.9	22.0	57.5
W_8	307	–	–	3000	150	–	–	2835	269.8	22.1	58.5
W_9	307	–	–	3000	300	–	–	2970	270.8	22.2	59.5
W_{10}	215	3000	–	3000	–	–	–	4062	262.9	44.5	76.2
W_{11}	215	–	3000	3000	–	–	–	4224	263.2	34.9	106.6
W_{12}	154	6000	–	3000	–	–	–	5424	281.9	68.9	98.6
W_{13}	154	–	6000	3000	–	–	–	5748	282.5	49.7	159.3
W_{14}	–	–	–	–	–	1800	–	396	219.6	24.1	61.3
W_{15}	–	–	–	–	–	–	1800	450	217.6	22.4	59.4
W_{16}	–	–	–	–	–	3600	–	792	439.2	48.1	122.6
W_{17}	–	–	–	–	–	–	3600	900	435.2	44.8	118.8

NPK – chemical fertilizer (250 kg/ha N + 20 kg/ha P + 37 kg/ha K); POF – kind of organic fertilizer produced from pig manure; COF – kind of organic fertilizer produced from cattle manure; BDA – biological decomposing agent, kind of microbial inoculum which can accelerate the decomposition and degradation of straw; PIOF – compound fertilizer was composed of chemical fertilizer and organic fertilizer produced from pig manure as the main raw material; CIOF – compound fertilizer was composed of chemical fertilizer and organic fertilizer produced from cattle manure as the main raw material; – indicates no addition

17 fertilizer treatments. The highest yield of maize was 10 037 kg/ha in W_{11}, and the lowest yield was 3 672 kg/ha in W_1. The variance of maize yields was significant in these different fertilizer treatments. The most abundant phyla found in all fertilizer treatments were Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, Firmicutes and Gemmatimonadetes. Distinctions in the composition of bacterial communities were also observed at the phylum level. The composition clustering tree of bacterial communities was mainly clustered into three clades (Clade I, II, and III) (Figure 1). The organic fertilizer content was the highest in the fertilization treatments of Clade I, and was lower in the fertilization treatments of Clade II, but no organic fertilizer was observed in the fertilization treatments of Clade III. The results indicated that organic manure affected the abundance and diversity of soil microflora. It has recently been indicated that organic inputs had a positive effect on both bacterial and fungal diversity with or without chemical fertilizers (Kamaa et al. 2011). Organic fertilization regime can maintain a stable tomato yield with reduced inputs of chemical fertilizers, which could be attributed to its beneficial effects on improving soil microflora and soil nutrient availability (Cai et al. 2015). Our results strongly indicated that soil microbial community structure and diversity can be improved by adopting suitable agricultural practices with organic fertilizer.

By sequencing of soil microbial communities, there were 24 174 fungi sequences, 15 911 bacteria sequences, 117 fungi operational taxonomic units, and 1373 bacteria OTUs in W_{11}, while 18 905 fungi sequences, 13 876 bacteria sequences, 121 fungi OTUs, and 1374 bacteria OTUs in W_1. At the phylum level, only Gemmatimonadetes and Proteobacteria displayed higher relative abundance in W_{11}, but the highest relative abundances did not exist in W_{11}. Furthermore, maize yields were in no correlation to both the shifts of soil microbial

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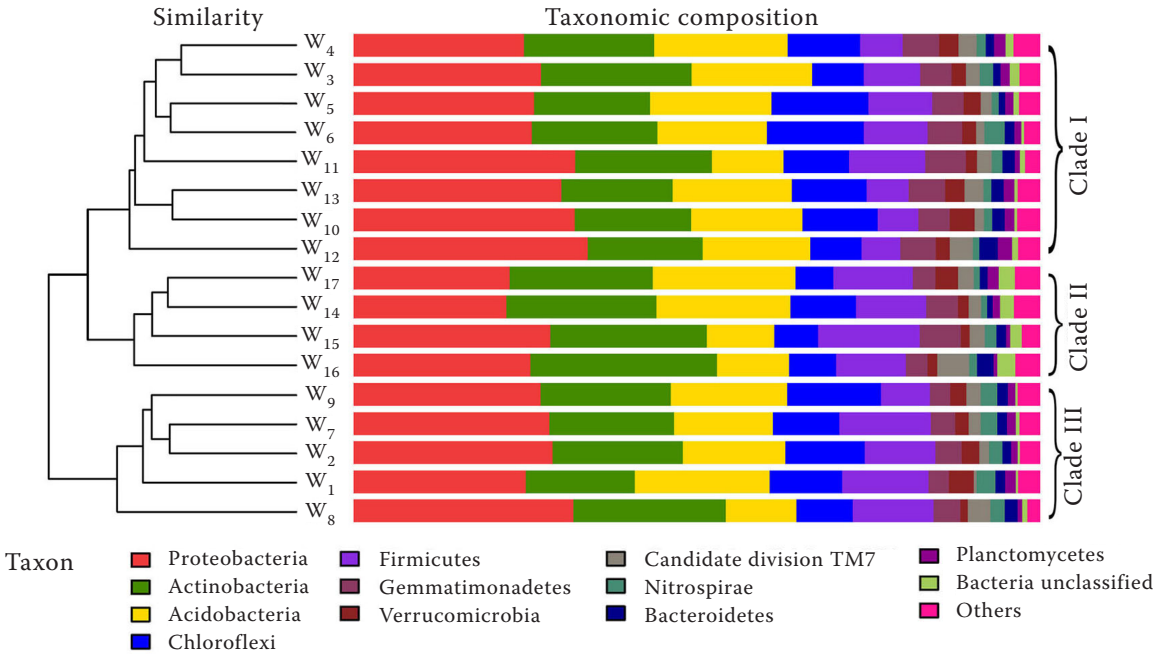

Figure 1. The composition and cluster analysis of bacterial communities at the phylum level in 17 fertilizer treatments

community structure in the classification level from phylum to genus and the number of sequences or OTUs in soil microbe by the correlation analysis (Figure 2). However, the relative abundance of some OTUs in different fertilizer treatments was significant in positive correlation to yields of maize by the correlation analysis. Relative abundance of soil microbes which was in a positive correlation

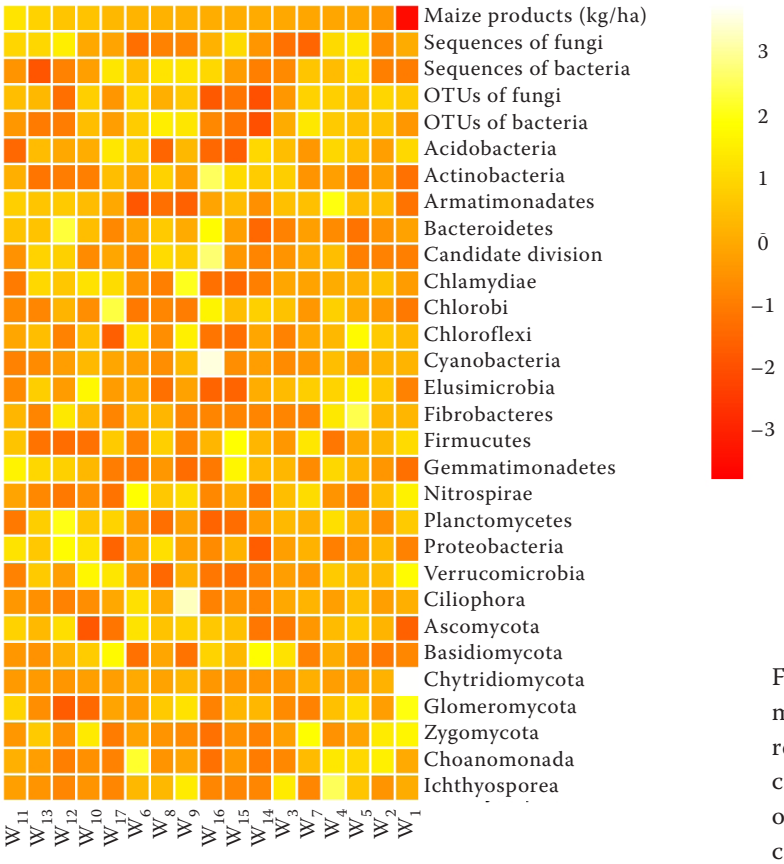

Figure 2. Maize yields and soil microbial metagenomic analyses. The heatmap on the relation of maize yields and soil microbial communities of continuous application of farmyard manure, crop residues, and chemical fertilizer

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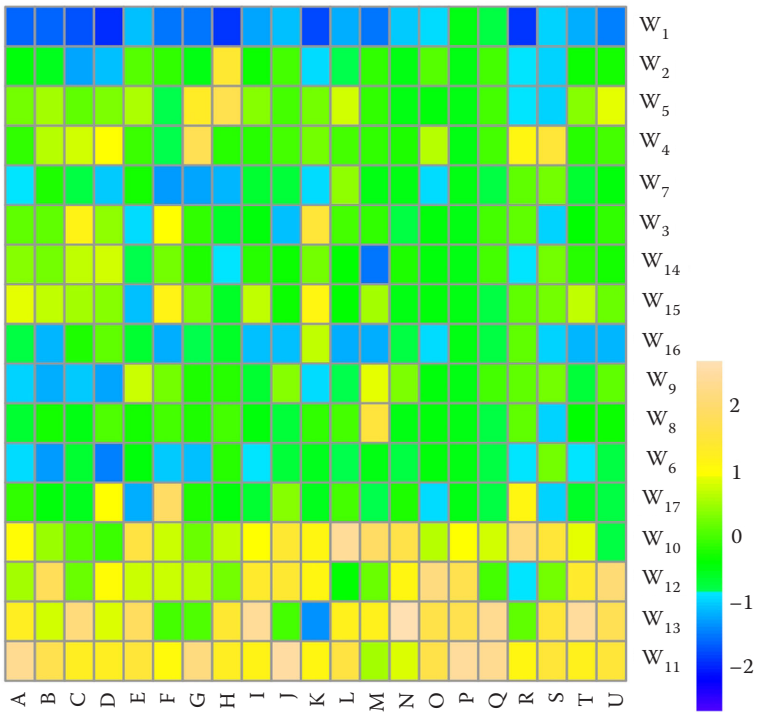
to maize yields was 41.86% of fungi and 10.21% of bacteria in W_{11}, while 14.39% of fungi and 1.48% of bacteria in W_1. Our results indicated that different fertilizer treatments could shape specific microbial communities, inhibit spoilage organisms, and increase beneficial microbes when grown in the same soils.

In soil bacteria community, yields of maize were in positive correlation to 35 OTUs, which in taxonomic classification belong to 19 orders within 14 classes within 9 phyla. There were 10.21% relative abundance of soil bacteria, which positively correlated to maize yields in W_{11}, and more than 9.21% relative abundance belonged to Rhizobiales (5.56%, Alphaproteobacteria, 9 OTUs), subgroup 3 (1.27%, Acidobacteria, 2 OTUs), Rhodospirillales (0.79%, Alphaproteobacteria, 4 OTUs), Gaiellales (0.72%, Actinobacteria, 4 OTUs), Xanthomonadales (0.31%, Gammaproteobacteria, 1 OTU), Micromonosporales (0.30%, Actinobacteria, 1 OTU), and Burkholderiales (0.27%, Betaproteobacteria, 2 OTUs) in W_{11}.

In fungi, only OTU_{25} belonging to Sordariales within the phylum Ascomycota was significant in positive correlation to maize yields. Indeed, the more relative abundance of these OTUs with positive correlation distributed from W_{10} to W_{13}, not in other fertilizer treatments (Figure 3).

In all 2477 bacteria OTUs, there were 65 OTUs belonging to the order Rhizobiales in which maize yields were in no correlation to 51 OTUs, positive correlation to 9 OTUs (Figure 4a), and negative correlation to 5 OTUs (Figure 4b). Relative abundance of no correlation OTUs was 4.21% in W_{11}, and relative abundance of negative correlation OTUs was 0.03%. Maize yields were significant in positive correlation to 9 OTUs in which there were 3 OTUs belonging to the family Xanthobacteraceae (3.57%) and 6 OTUs belonging to the genus *Rhizomicrobium* (1.99%, Rhizobiales incertae sedis). Although OTU_{337} and OTU_{758} also belonged to the family Xanthobacteraceae, they were in no correlation to maize yields.

In soil fungi communities, there were 49 OTUs that negatively correlated to yields of maize and 179 OTUs that were in no correlation to yields of maize. OTU_{178} belonged to the order Sordariales, but was in no correlation to maize yields. Only the fungus OTU_{25} was significant in positive correlation to maize yields, and Pearson's correlation was 0.558 (Figure 5a). OTU_{25} was an uncultured microorganism, and belonged to Sordariales within the class Sordariomycetes, phylum Ascomycota, kingdom Fungi. In the quantum of OTUs of soil fungi, there were 13 OTUs within the order Sordariales, in



Lanes A: Rhizobiales (OTU_{1002}, OTU_{1052}, OTU_{1075}, OTU_{2050}, OTU1694, OTU_{350}, OTU_{2121}, OTU_{1480}, OTU_{15}); lanes B: Subgroup_3 (OTU_{1963}, OTU_{186}); Lanes C: Rhodospirillales (OTU_{1136}, OTU_{1904}, OTU_{31}, OTU_{803}); Lanes D: Gaiellales (OTU_{140}, OTU_{2161}, OTU_{1507}, OTU_{1921}); Lanes E: Xanthomonadales (OTU_{2449}); Lanes F: Micromonosporales (OTU_{937}); Lanes G: Burkholderiales (OTU_{2475}, OTU_{1396}); Lanes H: C0119 (OTU_{28}); Lanes I: SC-I-84 (OTU_{989}); Lanes J: JG37-AG-4 (OTU_{1724}); Lanes K: Alphaproteobacteria (OTU_{947}); Lanes L: Subgroup_7 (OTU_{169}); Lanes M: Acidimicrobiales (OTU_{1004}); Lanes N: Candidate_divisioin TM7 (OTU_{1096}); Lanes O: Gemmatimonadales (OTU_{2232}); Lanes P: Cytophagales (OTU_{2064}); Lanes Q: Sphingobacteriales (OTU_{1461}); Lanes R: JG30-KF-CM66 (OTU_{599}); Lanes S: OPB35_soil_group (OTU_{880}); Lanes T; SC-I-84 (OTU_{989}); Lanes U: Sordariales (OTU_{25})

Figure 3. The distribution of some bacteria operational taxonomic units (OTUs) with positive correlation to maize yields in 17 fertilizer treatments

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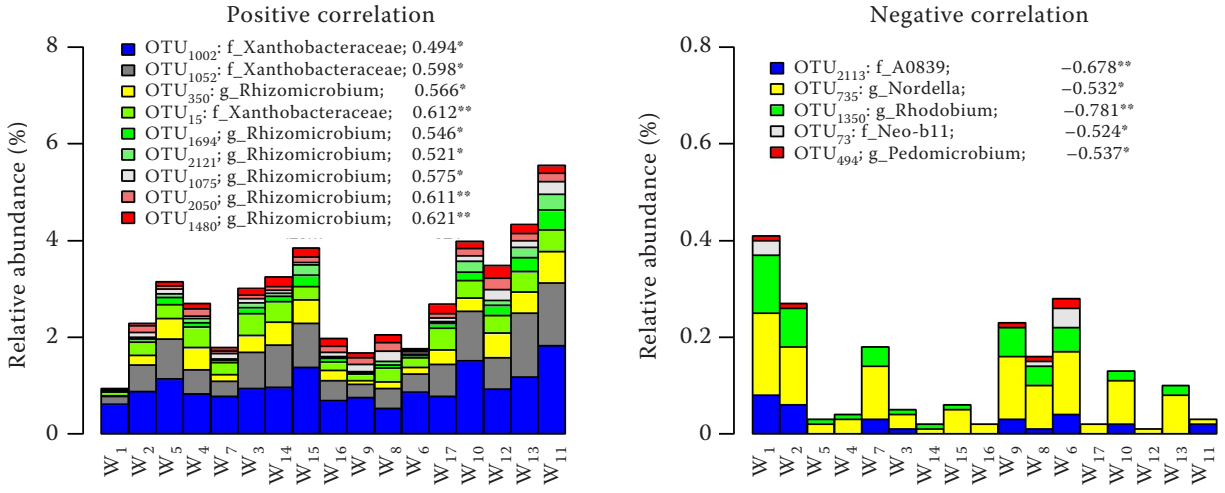

Figure 4. Relative abundances and Pearson's correlation of bacteria operational taxonomic units (OTUs) belonging to Rhizobiales in 17 fertilizer treatments, positive correlation and negative correlation. $^{**}P < 0.01$ (2-tailed), $n = 17$; $^{*}P < 0.05$ (2-tailed), $n = 17$

which OTU_{84} and OTU_{126} were in negative correlation to yields of maize (Figure 5b), and other 10 OTUs were in no correlation. However, OTU_{131} and OTU_{178} were in positive correlation to OTU_{25}.

Most Sordariales are saprobic, producing solitary perithecial ascomata and commonly found on dung or decaying plant matter. They are beneficial to decompose organic matter because of more microbial β-glucosidase and phenol oxidase activity with a high C/N ratio and low N fertilizer (Carreiro et al. 2000, Sinsabaugh and Follstad 2011). This could explain that the relative abundance of Sordariales OTU_{25} was higher in W_{11}, W_{12} and W_{13} fertilizer treatments which contained farmyard manure and return of wheat straw than in others. It was possible that OTU_{25} significantly increased crop yields because of available P and C source from decomposed organic matter. Further analyses at the species level of bacteria revealed that population shifts of the Rhizobiales were mainly responsible for the yields of maize, and Xanthobacteraceae (3.56%) and Rhizomicrobium in Rhizobiales incertae sedis (1.99%) of the Rhizobiales (9.80%) were in positive correlation to yields of maize in W_{11}. Some species of the family Xanthobacteraceae are able to fix dinitrogen (Reding et al. 1991, Arun et

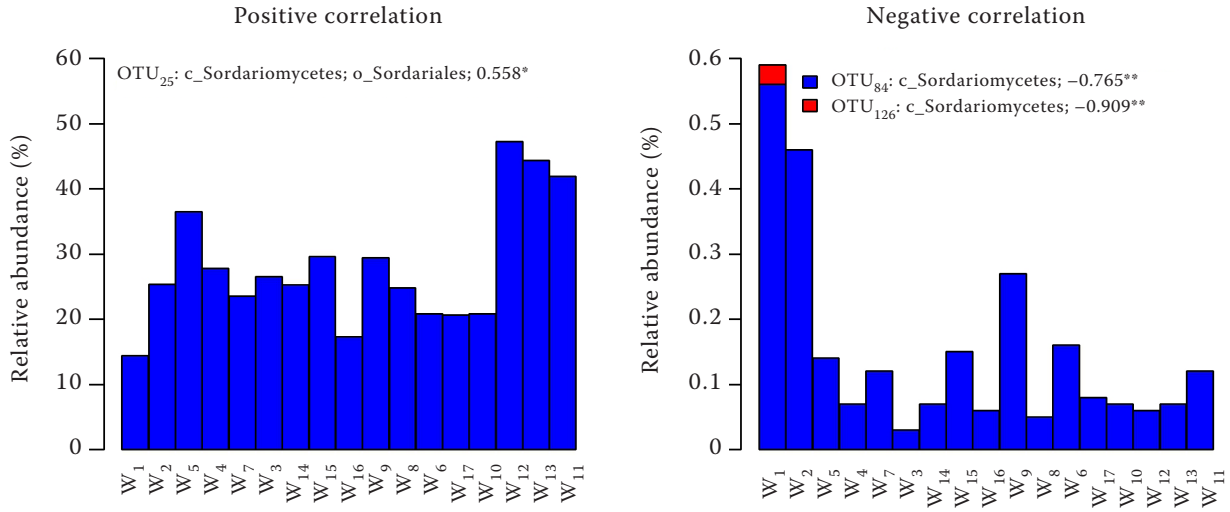

Figure 5. Relative abundances and Pearson's correlation of fungus operational taxonomic units (OTUs) belonging to Sordariales in 17 fertilizer treatments, positive correlation and negative correlation. $^{**}P < 0.01$ level (2-tailed), $n = 17$; $^{*}P < 0.05$ level (2-tailed), $n = 17$

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al. 2008). Bacteria of the genus *Rhizomicrobium* are facultative autotrophic, fixing dinitrogen, and reducing Fe (III) to Fe (II) in the presence of glucose (Ueki et al. 2010, Kodama and Watanabe 2011). Therefore, these strains could promote development of maize because of available N and Fe source and other metabolic products (Reding et al. 1991).

The complex soil microbial community is crucial for plant health (Lundberg et al. 2012). Our results confirmed that organic manure had a positive effect on microbial communities when grown in the same soils. However, microbial community structure shifts at the phylum level were not significant in positive correlation to yields of maize. OTU_{1002} was in positive correlation to yields of maize whereas OTU_{735} was in negative correlation although both OTU_{1002} and OTU_{735} belonged to the same order Rhizobiales. Thus, the microbial community structure shift at the species level was responsible for the yields of corp. Soil microbial communities and their functional attributes need further investigation by the functional microbial metagenomic analyses.

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