Response of soil bacterial community to bioorganic fertilizer application as a weed management strategy in rice paddy

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Abstract

Background: The growth of weed is common problem in rice cropping, leading to the application of herbicidal substances to suppress weed growth. Weed biocontrol through novel bioorganic fertilizer (BIO) has been established in rice cultivation, however, its main herbicidal components and influence on soil bacterial community are unknown.

Results: We identified three herbicidal components, hexadecanoic, isovaleric, and 2-methylbutyric acids, in BIO extract. We conducted 16S rRNA sequencing to identify changes in soil bacterial community in response to BIO treatments and performed a RDA analysis with soil chemical properties and weed-control effect. The OTU, Chao1 and Shannon indices did not differ substantially among the BIO treatments, and the bacterial diversity was not significantly affected by BIO. As result from PCA analysis, we discovered that soil bacterial community was not significantly influenced by BIO. We identified six dominant phyla (*Proteobacteria*, *Acidobacteria*, *Chloroflexi*, *Nitrospirae*, and *Verrucomicrobia*) in BIO-treated paddies. The five most abundant genera were *Anaeromyxobacter*, *Candidatus Nitrosotalea*, *Clostridum sensustricto1*, *Haliangium* and *Candidatus Nitrotoga*. From the RDA analysis, the highest correlations were obtained for soil pH, total K, and *Pseudomonas*. The weed-control effect mostly correlated with the abundance of *Candidatus Koribacter*, *Clostridium sebsustricto 9*, and *Nonomuraea*. Rice yield had a distinct relationship with *Nonomuraea*, *Nitrospira*, and *Candidatus Koribacter*.

Conclusions: With the changes in soil pH and total K, BIO could impact bacterial communities and weed control, and in turn affect rice yield. This foundation can be helpful that application BIO is a “not harmful” and feasibility weed biocontrol strategy.

Background

Weeds have negative effects on the germination and yield of crop plants; however, their eradication from agricultural lands is often labor intensive and expensive (Chauhan BS et al. 2012). Currently, there are several strategies for weed control (mechanical, chemical, and biological), and the application of synthetic herbicides is by far the most common method. Chemical herbicide is extensive and effective for weed management that can save labor and time. However, the excessive use of weed killers pollutes the environment and negatively affects the agricultural ecosystems. A recent study reported that less than 0.1% of the applied herbicides could be absorbed by their target weeds and that most of them move into the environment (Duhan A et al. 2019). Hence, weed control policies should not only focus on yield and economic benefits, but also pay attention and provide to ecological security, with safer and more environment-friendly methods for the agricultural ecosystems (Gnanavel I et al. 2014; Zhang Y. et al. 2018).

In general, microorganisms are highly sensitive to changes in their environments (Ma ZW et al. 2017). Recent studies reported significant alterations in the microbial biomass in response to altered aboveground plant productivity, chemical fertilizers, and pesticides (Dai XQ et al. 2017; Mahmoud E et al. 2019). Furthermore, soil bacterial communities are documented to be sensitive to physical and chemical variations of the soil environment as early indicators of soil health trends (induced by natural and anthropogenic disturbances) (Gupta VK et al. 2018; Cagnini CZ et al. 2019). Biochar application (as a soil amendment), combined with NPK fertilizers, strongly affected the composition and functions of the microbial community in a 30-day incubation study, by increasing gram-positive bacteria, which consequently influenced organic matter cycling in the rice paddy soil (Tian J et al. 2016). In a 4-year rice-rice-crop paddy soil, organic manure partial replacement of inorganic fertilization could

slightly increase the abundance of *pqqC* gene and significantly enhance *phoD*-harboring bacteria (*Bradyrhizobium* and *Methylobacterium*) than whole inorganic fertilization (Bi QF et al. 2020). The tricyclazole did not influnce on the structure and diversity of soil microbial communities though PCR-DGGE as to its low bioavailability in rice paddy (Jaramillo GM et al. 2016). The 35 g/ha and 70 g/ha of bispyribac sodium (inhibit rice weeds) impacted on the soil microbial population, enzyme activities and functional microbial diversity in paddy soil (Kumar U et al. 2020). Thus, soil bacterial community is influenced by agricultural practices in farmland ecosystems.

In our previous study, we developed a novel bioorganic weeding fertilizer (BIO) by fermenting mature compost with kitchen garbage, maize straw, wood-destroying fungal dregs, rice straw, tobacco straw, plant ash, chicken, and sheep manure. The novel BIO was found to be effective in controlling grass and broad-leaved weeds in three rice fields (Huanan, Hainan, and Heilongjang, in china) for two years (2014 and 2015) with an average rate of more than 80% weed suppression. In addition, the BIO treatments significantly increased rice yield (16.3%-29.8% relative to the control) and yield components (e.g., number of spikes per square meter, plant height, and number of kernels per spike) (Li ZR et al. 2018). However, the BIO effects on soil bacteria in rice paddy are not wellknown. In the present study, we analyzed the weed-control effect of BIO in rice paddy, and its herbicidal compounds by GC-MS. We also evaluated BIO-affected soil chemical properties and soil bacterial community composition by16S rRNA-sequencing. These results might lay atheoretical foundation for BIO applications.

Materials And Methods

Bio-organic fertilizer (BIO) manufacturing

The organic substrates in the BIO were composed of kitchen garbage, maize straw, wood-destroying fungal dregs, rice straw, tobacco straw, plant ash, and chicken and sheep manure. The physical and chemical properties of the compost material measured were provided in our previous study (Li ZR et al. 2018). The combined process of ZF-5.5 mechanical fertilizer preparation and pile fermentation was used to produce composting manure at a temperature range of 40°C-80°C for 15 days. Man-made heating and cooling was used to control temperature on the first day. The compost was moved out and piled fermentation began later one day. After 15 days, the compost turned taupe gray, exhibited threadiness and had a slightly sour fragrance. This compost contained 53.4% organic matter, 2.0% N, 3.7% P_2O_5, and 1.1% K_2O.

Herbicidal chemical component analysis

Two kilograms of BIO was immersed in 8 L of distilled water for 24 h and stirred every 4 h. The material was then filtered through vacuum (SHZ-DIII; Shanghai Giangqiang Industrial Development Co., Ltd., Shanghai, China), and the filtrate was collected and stored at 4°C. The leftover BIO was re-immersed twice in the same way. All these aqueous extracts were pooled and evaporated on a rotary evaporator (IKA RV10; Germany). The aqueous extract residues were stored at 4°C.

The chemical compounds in BIO extracts were identified by GC-MS-ITS-40 (GC-ITMS; Finnigan MAT, San Jose, CA) as reported previously (Sivankalyani V et al. 2017). The qualitative analysis was performed by matching the MS spectra with the spectra in NIST library and Wiley GC-MS Library. The relative percentage of extracts depended on the peak acreage of the total ion chromatograms (TIC) (Preisler AC et al. 2020).

Hexadecanoic, isovaleric, and 2-methylbutyric acidswere purchased from Adamas Changsa company. Barnyard grass seedlings in the three-leaf-stage were sprayed with 10 mL of water or the test compounds (hexadecanoic,

isovaleric, and 2-methylbutyric acids) at 0.1, 0.5, 1, and 2 mg/L concentrations. The barnyard grass seedling were grown under 12 h photoperiod (100–150 μmol.m^{-2}.s^{-1}) at 25°C/20°C during the light/dark cycle in a growth chamber. After 7 days, fresh weight of barnyard grass seedling was recorded. Based on the fresh weight data, herbicidal effect was calculated as previously reported (Li ZR et al. 2018).

Field experiment and soil sample collection

Our study site is in a field, Gaoqiao, Changsha, Hunan Province, China (N28°28′20", E113°4′51"), that has been cropped rice-rice per year (from April to October, mean annual precipitation and temperature in the last three years were 1427.41mm and 18°C) and had already been carry out for 30 years. The field trial started by the arrangement of eight 40m×20m plots on April 26, 2018. The rice plants were transplanted as 25 plants/m^2 and the rice varity was Longxiang 32. Three days after transplantation (on April 29), BIO and common fertilizer (CBF, contained 54.4% organic matter, 1.8% N, 3.5% P_2O_5, and 1.2% K_2O, Changsha Beye Agricultural Ltd., Changsha, China) were spread over the plots. The experimental treatments were carried out three times with five application rates of BIO (750 kg/ha (BIO-50), 1500 kg/ha (BIO-100), 3000 kg/ha (BIO-200), 6000 kg/ha (BIO-400), and 12000 kg/ha (BIO-800); 25g/L of herbicide Penoxsulam OD (HP, Dow AgroSciences); 1500kg/ha CBF; hand weeded; and an untreated control (CK, without weed management strategy). All field management practices were in line with the local, traditional ways, except for the irrigation during BIO application, as a 3-5cm water layer had to be maintained for 7 days. After this period, irrigation was conducted traditionally. No topdressing and other weed management practices were carried out in these plots. The traditional rice agronomical management strategies were used as Zou YB described (1999). Soil samples were collected from all plots on May 27, 2018, at one month after BIO application. One hundred grams of surface soil (0–15 cm) was collected from 30 points in each plot and then 3 points samples were mixed together in 10 plastic bags from each treatment. Soil samples were sieved at 2 mm and then divided into two parts, one part was frozen and stored at -80°C, and the other part was air dried for one week and stored at 25°C.

Soil chemical properties and weed control effect measurements

Weeds in fields were investigated on May 27, 2018. Three points (1 m^2) were randomly chosen in each plot and the number of grass and broad-leaf weed species were recorded separately. Aboveground fresh weed biomass was measured at 30 days after BIO application. Control effect (%)= (CK-Tt)/ CK× 100; CK: untreat control plots weeds number or fresh weight, Tt: BIO, CBF and HP plots weeds plants number or fresh weight.

Soil sample pH was measured in soil-water solution (W/V 1:5). Total N and K content was measured using an elemental analyzer (Carlo Erba, Milan, Italy) and total P content was measured calorimetrically using the molybdate method. Hydrolytic N, extractable P, exchangeable K, and organic matter content was measured as described previously (Tao JM et al. 2018).

DNA extraction and MiSeq sequencing

Sample soil DNA was extracted using the MoBioPower Soil DNA Isolation Kit (MO BIO, San Diego) according to the manufacturer's protocol. After quantification using Nanodrop (ND-1000 Spectrophotometer; Nanodrop Products, Wilmington, USA), the V4 hyper variableregion of the 16S rRNA gene was amplified with the following primer pair: 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). Amplicon quality was visualized by agarose gel electrophoresis. The amplicons were purified using the AMPure XP beads (Agencourt), and then amplified in a second round of PCR. The PCR process was carry out by 20 μL mixture containing 5 μL FastPfu buffer, 2 μL dNTPs, 1 μL primer, 0.5 μL FastPfu Polymerase, 2μL DNA and 8μL water. Following another

round of purification using the AMPure XP beads, the final amplicons were quantified using the Qubit dsDNA assay kit. Equal amounts of purified amplicons (200 ng) were pooled for library construction and the subsequent sequencing using the Illumina MiSeq platform (Illumina, San Diego, CA) with the MiSeq 500 cycles kit.

Sequence preprocessing and statistical analysis

Sequence processing was conducted using Galaxy pipeline (http://zhoulab5.rccc.ou.edu: 8080/ root) according to a previous study (Bolger AM et al. 2014). Briefly, the raw sequences were assigned to samples by “Detect barcodes” script, and ambiguous bases (N) were detected and cut off using Trimmomatic software (Wang Q et al. 2007). The cut off values for the low-quality sequences were set at an average quality score of 20, and these sequences were eliminated using the sliding window trimming approach. Forward and reverse reads, with at least 10-bp overlap and less than 5% mismatch, were then combined using Flash. The shorter sequences and chimeras were removed from the combined sequences using QIIME software (version 1.8.0). Operational taxonomic units (OTUs) clustering was performed using UCLUST at the 97% similarity level, and taxonomic assignment was conducted using the Ribosomal Database Project (RDP) classifier, with a minimal of 50% confidence estimate. Samples were rarefied at 19600 sequences per sample, and these were classified into 15706 OTUs. All data were translated into OTU relative abundance table for the subsequent analysis.

Alpha diversity indices of the microbial community, including Shannon-Weiner’s and Chao1 indices, were calculated using “Vegan” package. The Chao1 diversity index was calculated as reported in a previous study (Qin C et al. 2019). Beta diversity was analyzed using the principal coordinates analysis (PCA), carried out using “Vegan” package based on shared branches of weighted unique fraction (UniFrac) distances. The redundancy analysis (RDA) was performed using “Vegan” package to investigate the relationships between the soil chemical properties, microbial properties, weed control efficiency and rice yield. The weed control efficiency and soil chemical properties data (Three replicates) were performed ANOVA with Post-Hoc test using SSPS software. The results with a p value < 0.05 were considered statistically significant.

Results

GC-MS analysis: herbicidal activity of major compounds in BIO

Total ion chromatograph of BIO aqueous extracts was obtained from the GC/MS chromatogram (Fig. 1). GC-MS revealed three main herbicidal components (hexadecanoic, isovaleric, and 2-methylbutyric acids) in crude extract. The IC_{50} values of hexadecanoic acid, isovaleric, and 2-methylbutyric acids were 22.81, 8.34, and 12.48 mg/L, respectively. The inhibitory effects of the three compounds decreased in the following order: isovaleric acid > 2-methylbutyric acid > hexadecanoic acid. With an increase in dosage, the inhibitory effects of isovaleric and 2-methylbutyric acids increased (Fig. 2).

Main bacteria in BIO

The top five genera in the BIO samples were *Pseudomonas*, *Anaeromyxobacter*, *Haliangium*, *Candidatus Nitrosotalea*, and *Geobacter*, whereas those in the CBF samples were *Cercisgigantea*, *Halocella*, *Pseudomonas*, *Actinomadura*, and *Nonomuraea* (Fig. 3a). The five phyla with the highest relative abundance in the BIO samples were *Proteobacteria*, *Acidobacteria*, *Chloroflexi*, *Nitrospirae*, and *Verrucomicrobia*, whereas those in the CBF samples were *Firmicutes*, *Cyanobacteria*, *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* (Fig. 3b).

Effect of BIO on the soil chemical properties

There was no significant difference in the extractable P content (values ranged from 0.21 to 0.45 mg/kg in all samples) between BIO treatments and untreated control (CK) samples, and between the HPS and CBFS treatments. Soil pH values under the BIO-50 and BIO-100 treatments were close to that of the untreated control, but an increased dosage of BIO (BIO-200 to BIO-800) increased these values by 3.36%-4.46% compared with the untreated soil. There were marginal differences in the recorded pH values among HPS, CBFS, and CK. Similar results were obtained for the total K, total N, total P, exchangeable K, hydrolytic N, and organic matter content. These results demonstrate that applying BIO in paddy fields will only result in minor changes in the soil chemical properties.

Effect of BIO on soil bacterial communities

Sequencing the full-length 16S rRNA genes revealed a diverse bacterial community composition and dynamics. The number of OTUs in all samples was 405–1633, and 47 OTUs were common in all samples, as shown by the flower plot (Fig. 4). The analysis results of alpha diversity indices (Chao1and Shannon) of the different soil treatments (BIO and CBF) are shown in Fig. 5. These indices displayed uniform species abundance among BIO-treated soil samples, and even the highest dose of BIO had negligible effect on the diversity index. Furthermore, there were no significant differences among the CBF, BIO, and HP soil samples. However, the alpha diversity of CBF samples was significantly lower ($p < 0.05$) than that of the other (BIO treatment, CBF, BIO, and HP) soil samples.

Beta diversity is commonly used to compare differences among a set of samples. Our PCA analysis of beta diversity indicated that all replicates of treated soils (CBF soil samples, HP soil samples, untreated soil samples, and BIO samples) clustered together (Fig. 6).The first and second axes showed 2.78% and 2.59% of the variance and 5.37% in total of the cumulative variance, which indicating that the soil bacterial community was no significantly differences among the CBF, BIO, and HP soil samples. Bacterial community structure at the phylum level in the BIO-treated soil samples, CBF soil samples, and HP soil samples is shown in Fig. 7a. The five most dominant phyla in both BIO samples and BIO-treated, CBF, and HP soil samples were *Proteobacteria*, *Acidobacteria*, *Chloroflexi*, *Nitrospirae*, and *Verrucomicrobia*. Bacterial community structure (at the genus level)in the BIO- treated, CBF, and HP soil samples is shown in Fig. 7b. The five most representative genera among the BIO-treated soil samples were *Anaeromyxobacter*, *Candidatus Nitrosotalea*, *Clostridium sensustricto1*, *Haliangium*, *Candidatus*, and *Nitrotoga*.

Soil chemical properties, bacterial composition, and weed control effect

The RDA analysis among microbial community, soil chemical properties, and weed control effect was illustrated in Fig. 8. We observed significant positive correlations among soil pH, total K, and bacterial abundance in our study. For instance, soil organic matter with *Anaerolinea* ($p = 0.81$) and *Sorangium* ($p = -0.74$). In addition, the total K content had a distinct relationship with *Spirochaeta2* ($p = 0.74$), whereas the abundance of *Sphingomonas* ($p = -0.74$) had an obvious effect on the total K content. Interestingly, *Candidatus Koribacter* was the most correlated genus ($p=-0.67$) with the weed control effect, followed by *Clostridium sebsustricto9* ($p=-0.62$) and *Nonomuraea* ($p = 0.54$). The rice yield had a distinct relationship with *Nonomuraea* ($p = 0.83$), *Nitrospira* ($p = 0.79$), and *Candidatus Koribacter* ($p=-0.76$).

Discussion

It is known that some weed management strategies (especially, herbicide application) can have knock-on effects on soil and its microorganisms (Parra B et al. 2019). In the present study, HPS had significant effects on the abundance of soil bacteria, including *Firmicutes* and *Proteobacteria*, compared with the untreated soil. The bacterial diversity in the BIO-treated soil samples was not significantly different from the untreated soil samples at the phyla level, but four genera (*Clostridum sensu stricto*, *Pseudomonas*, *Haliangium*, and *Sorangium*) were significantly affected by BIO application (Fig. 6). In some cases, the total abundance of soil bacteria in vineyard soil was on an average 260% higher under herbicide application than under mechanical weeding (Mandl K et al. 2018). Penoxsulam, a sulfonylurea herbicide, has been reported to show toxicity against no-target organisms and soil bacterial population (Sondhia S et al. 2016; Rajuput S et al 2016). Another study reported a considerable reduction in the abundance of Ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) in 5.0 mg/kg mesotrione-treated soil (Du ZK et al. 2018). AOA and AOB communities were significantly disturbed by atrazine (Parada J et al. 2019).

The novel BIO also have effectively manages weeds in rice paddies from our report. In this study, we also observed that the weed control effect and rice yield of BIO-200 treatment was equivalent to that of 25g/L Penoxsulam OD. The GC-MS analysis and bioassay revealed three main herbicidal components (isovaleric, 2-methylbutyric,and hexadecanoic acids) in BIO aqueous extract (Fig. 2). *Candidatus Koribacter*,*Clostridium sensustricto9*, and *Nonomuraea* correlated the most with weed control effect (Fig. 7b and Fig. 8). Some bacterial species are known to produce inhibitory products such as phenol, organic compounds, and acetone to disturb the emergence of plant weeds (Cray J et al. 2013).For example the natural compound 2,4-diacetyl-phloroglucinol, produced by *Pseudomonas* spp., is an effective biocontrol agent with the ability to inhibit plant primary root growth and seed germination (Andreolli M et al. 2019). It has been reported that the proportion of *Haliangium* in the soil microbial community decreased by the integrated use of chicken manure and legume straw with chemical fertilizers (Wang L 2019). Several *Clostridium* species are known to produce organic acids, such as butyric, acetic, and lactic acids, and these are reported to reduce weed seed germination (Dolejs I et al. 2014; Rao V et al. 2018). As our knowledge, the organic acids is a important natural compound to inhibited weed, which may be a key reason to weeds control by BIO.

Due to their pivotal roles in soil ecosystems and their ability to reveal changes in soil status, the effects of bio-fertilizers on soil microorganisms have received considerable attention (Welbaum G et al. 2014; Llewellyn D 2018).We evaluated BIO-treated soil chemical properties and soil bacterial community composition to verify BIO application for weed control effect and crop yield. The RDA analysis showed that soil pH and total K significantly correlated with *Spirochaeta2*, and rice yield had a distinct relationship with *Nonomuraea*, *Nitrospira*, and *Candidatus Koribacter. Candidatus Koribacter*, *Clostridium sensustricto9*, and *Nonomuraea* were the genera that correlated the most with weed-control effect (Fig. 8). Previous studies have also suggested that bacterial abundance is affected by changes in soil pH and total K, supporting our results for several phyla (e.g.,*Verrucomicrobia* and *Acidobacteria* members were positively correlated with the soil pH) (Shen C et al. 2017).It has been reported that *Candidatus Koribacter* and *Nitrospira* have a beneficial effect on the yield of crop (Zhou LJ et al. 2019).Therefore, the application of BIO not only effectively manages weeds in rice paddies, but also has significative effects on the rice yield. However, to elucidate this, further long-term field studies at multiple sites on rice paddies are necessary.

Conclusions

To verify BIO security for soil health, its main herbicidal components and influence on soil bacterial community were evaluated. It has been identified three herbicidal components, hexadecanoic, isovaleric, and 2-methylbutyric acids in BIO extract and six dominant phyla (*Proteobacteria*, *Acidobacteria*, *Chloroflexi*, *Nitrospirae*, and *Verrucomicrobia*) in BIO-treated paddies. The five most abundant genera were *Anaeromyxobacter*, *Candidatus Nitrosotalea*, *Clostridum sensustricto1*, *Haliangium*, and *Candidatus Nitrotoga*. BIO treatment may affect soil pH and total K content, thereby affecting bacterial communities, which in turn could affect weed control, and result in the yield improvement (Fig. 9). The application of BIO not only effectively manages weeds and yield in rice paddies, but also has no negative effects on the soil microbial content.

Declarations

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Lianyang Bai and Xueduan Liu conceived and designed the experiments. Huidang Jiang and Ducai Liu performed experiments. Zuren Li and Huidang Jiang analyzed the data. Zuren Li wrote the article. All authors commented on the manuscript.

Abbreviations

BIO: novel bioorganic fertilizer; CBF: common fertilizer; RDA: redundancy analysis; PCA: principal coordinates analysis ; EF: control effect;

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Tables

Table 1

Chemical properties of the surface soil layer (0–15cm) from the BIO-treated and untreated sites.

Variable source	pH	Total K mg/kg	Total N mg/kg	Total P mg/kg	Exchangeable K mg/kg	ExtractableP mg/kg	Hydrolytic N mg/kg	Organic matter mg/kg
BIO-50	5.25c	18.6c	1.4ef	0.45d	38c	0.21a	133.67c	19.90d
BIO-100	5.17c	18.53c	1.44def	0.50b	45.67c	0.36a	147.67bc	21.63bc
BIO-200	5.54ab	19.77a	1.84a	0.58a	80.67ab	0.36a	220a	25.47a
BIO-400	5.36bc	19.1abc	1.53c	0.46cd	76b	0.42a	177.67b	21.57bc
BIO-800	5.61a	19.67ab	1.62b	0.50bc	108.33a	0.45a	219.67a	22.07b
CK	5.36bc	18.63c	1.51cd	0.49bc	56bc	0.22a	165.33bc	21.97b
HPS	5.22c	18.90bc	1.48cde	0.50bc	42c	0.25a	142.67bc	20.67cd
CBFS	5.32c	19.13abc	1.36f	0.51b	37.67c	0.45a	146.33bc	21.1bc

Values shown here represent the average of three repetitions (n = 3). Means with different letters represent significant differences at $p < 0.05$.BIO-50: 750 kg/ha BIO-treated soil; BIO-100: 1500 kg/ha BIO-treated soil; BIO-200: 3000 kg/ha BIO-treated soil; BIO-400: 6000 kg/ha BIO-treated soil; BIO-800: 12000 kg/ha BIO-treated soil; HPS: herbicide-treated soil; CBFS: common bio-fertilizer-treated soil, and CK: untreated control soil.

Figures

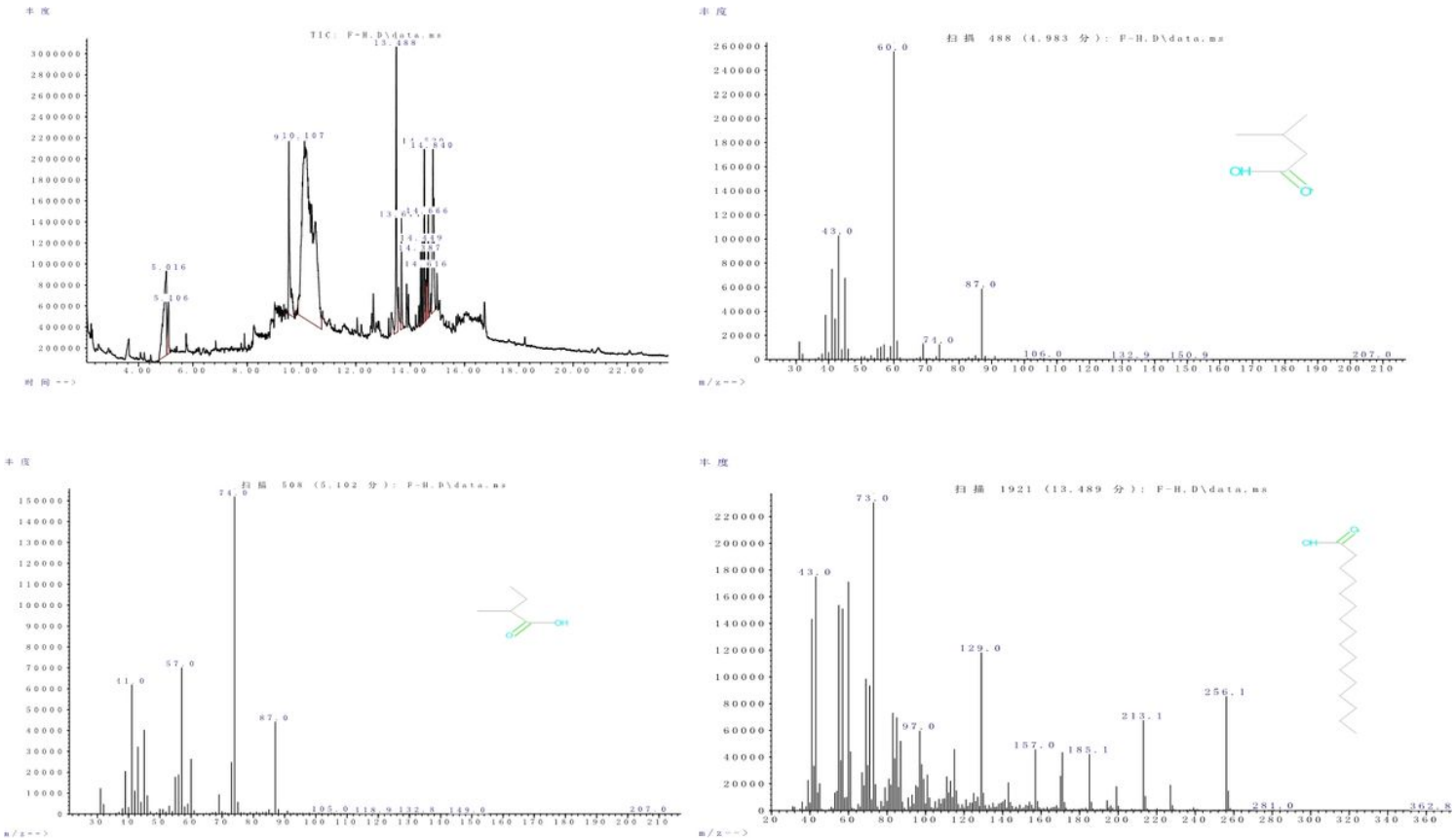

Figure 1

Total ion chromatograph of the aqueous extracts of BIO from GC/MS chromatograms.

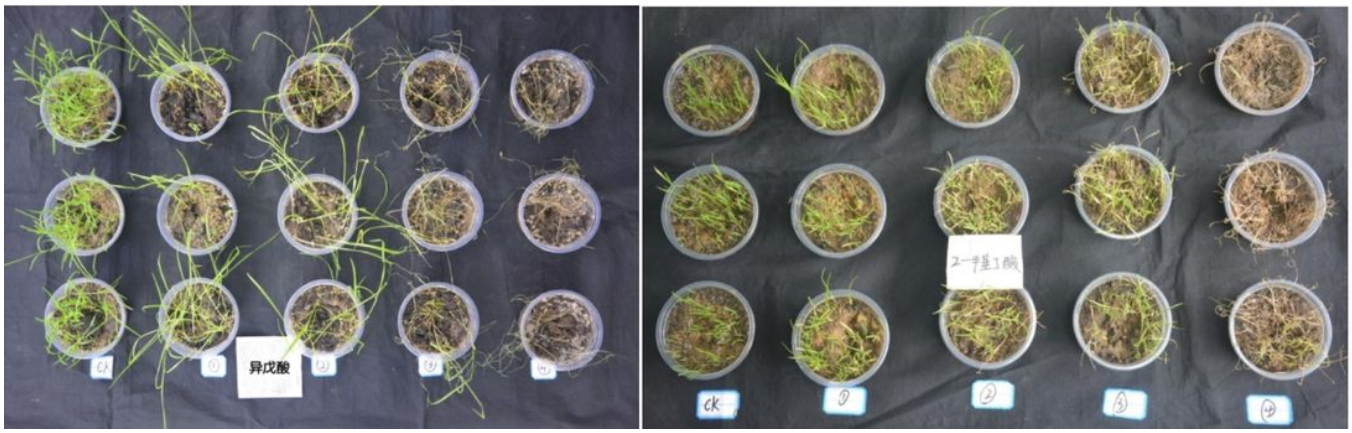

Figure 2

Toxicity test of aqueous extract of BIO using barnyard grass (after 7 d). A. Determination of toxicity of isovaleric acid with different concentration gradients to barnyard grass seedlings (0, 0.1, 0.5, 1, 2 mg/L); B. determination of toxicity of 2-Methylbutyric acid with different concentration gradients to barnyard grass seedlings (0, 0.1, 0.5, 1, 2 mg/L).

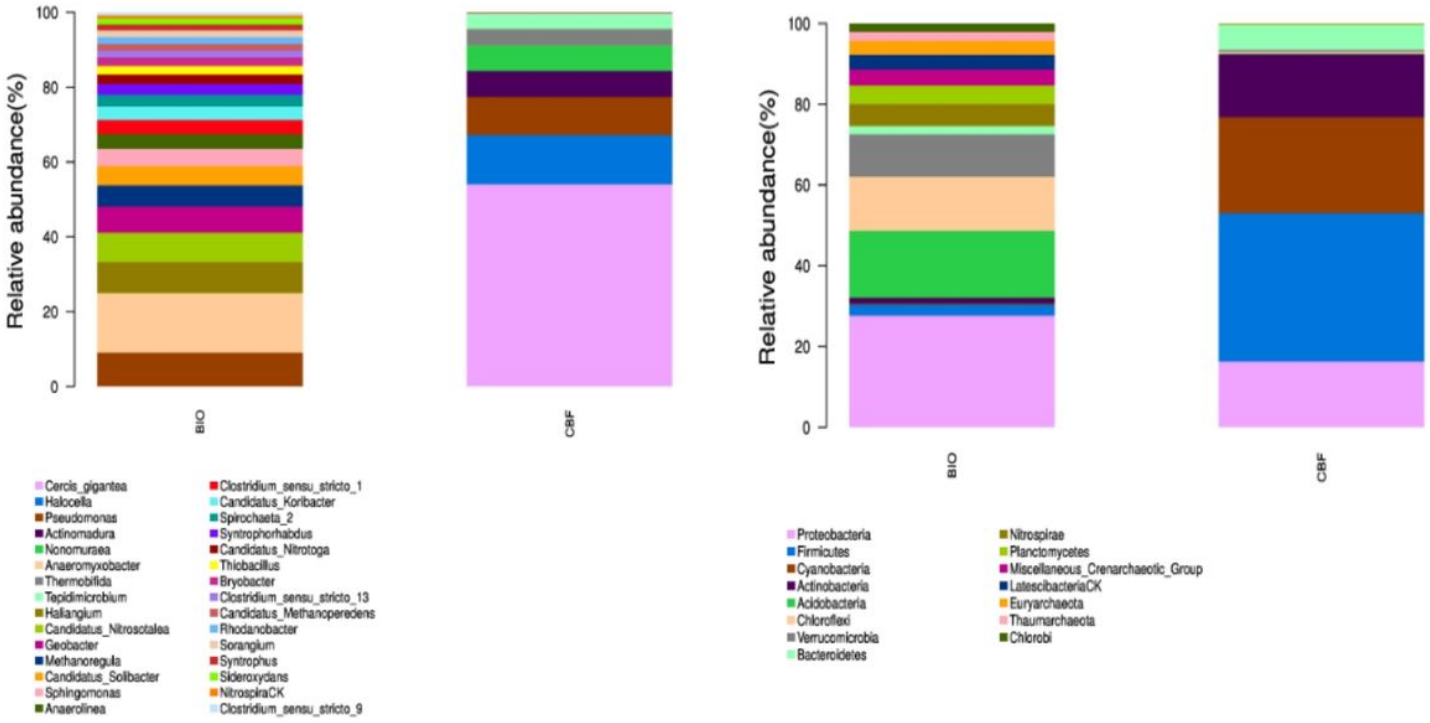

Figure 3

(a) Relative abundance of the top30 genera among the BIO-treated soil samples.(b) Relative abundance of the top15 phyla among the BIO-treated soil samples.BIO: BIO sample; CBF: common bio-fertilizer.

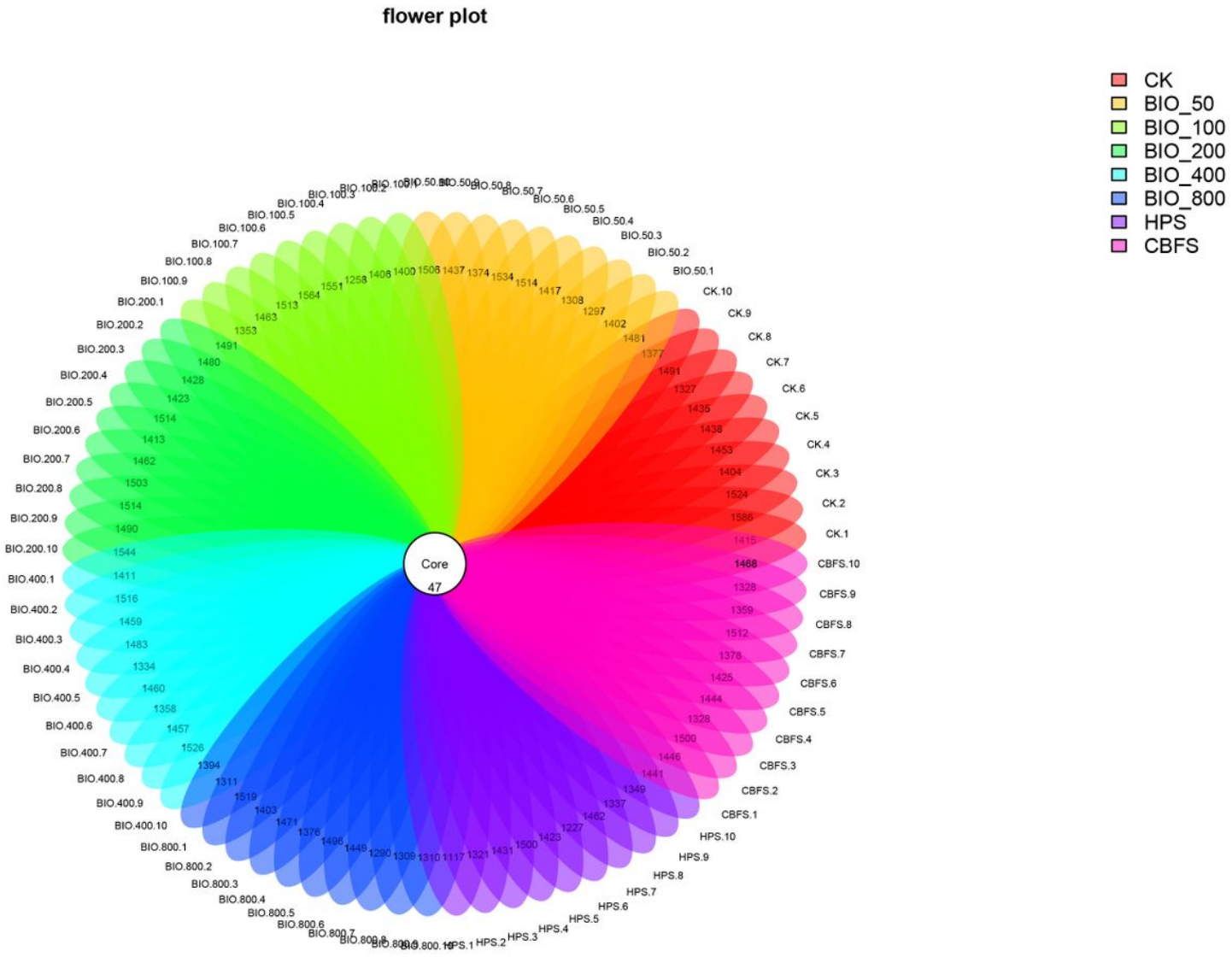

Figure 4

Operational taxonomic units (OTU) based petal map (flower plot). Description: Different colors in the diagram represent a (group) sample. Middle core numbers represent the number of OTUs common in all samples and numbers on the petals represent the number of OTU unique to this sample. Samples in the flower plots are as follows: BIO-treated soil samples, CBF soil samples, HP soil samples and CK. The acronyms denote the following: BIO-50: 750 kg/ha BIO-treated soil; BIO-100: 1500 kg/ha BIO-treated soil; BIO-200: 3000 kg/ha BIO-treated soil; BIO-400: 6000 kg/ha BIO-treated soil; BIO-800: 12000 kg/ha BIO-treated soil; HPS: herbicide-treated soil; CBFS: common bio-fertilizer-treated soil, and CK: untreated control soil.

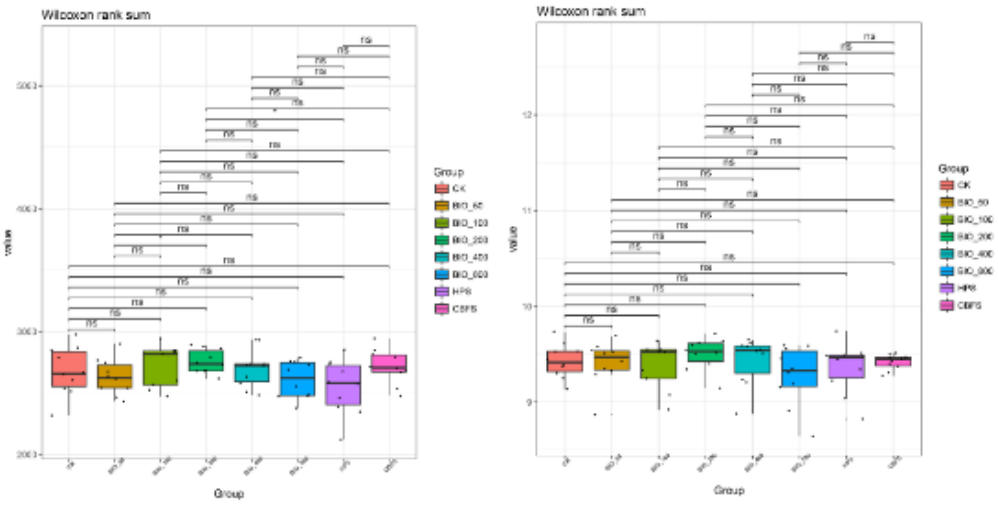

Figure 5

Alpha-diversity indices, (a) Chao1 and (b) Shannon indices, of the bacterial community structure in the following samples: BIO application soil samples, CBF soil samples, HP soil samples, CBF samples, and BIO samples. The acronyms denote the following: BIO: BIO sample; BIO-50: 750 kg/ha BIO-treated soil; BIO-100: 1500 kg/ha BIO-treated soil;BIO-200: 3000 kg/ha BIO-treated soil; BIO-400: 6000 kg/ha BIO-treated soil; BIO-800: 12000 kg/ha BIO-treated soil; HPS: herbicide-treated soil; CBF: common bio-fertilizer; CBFS: common bio-fertilizer-treated soil, and CK: untreated control soil.

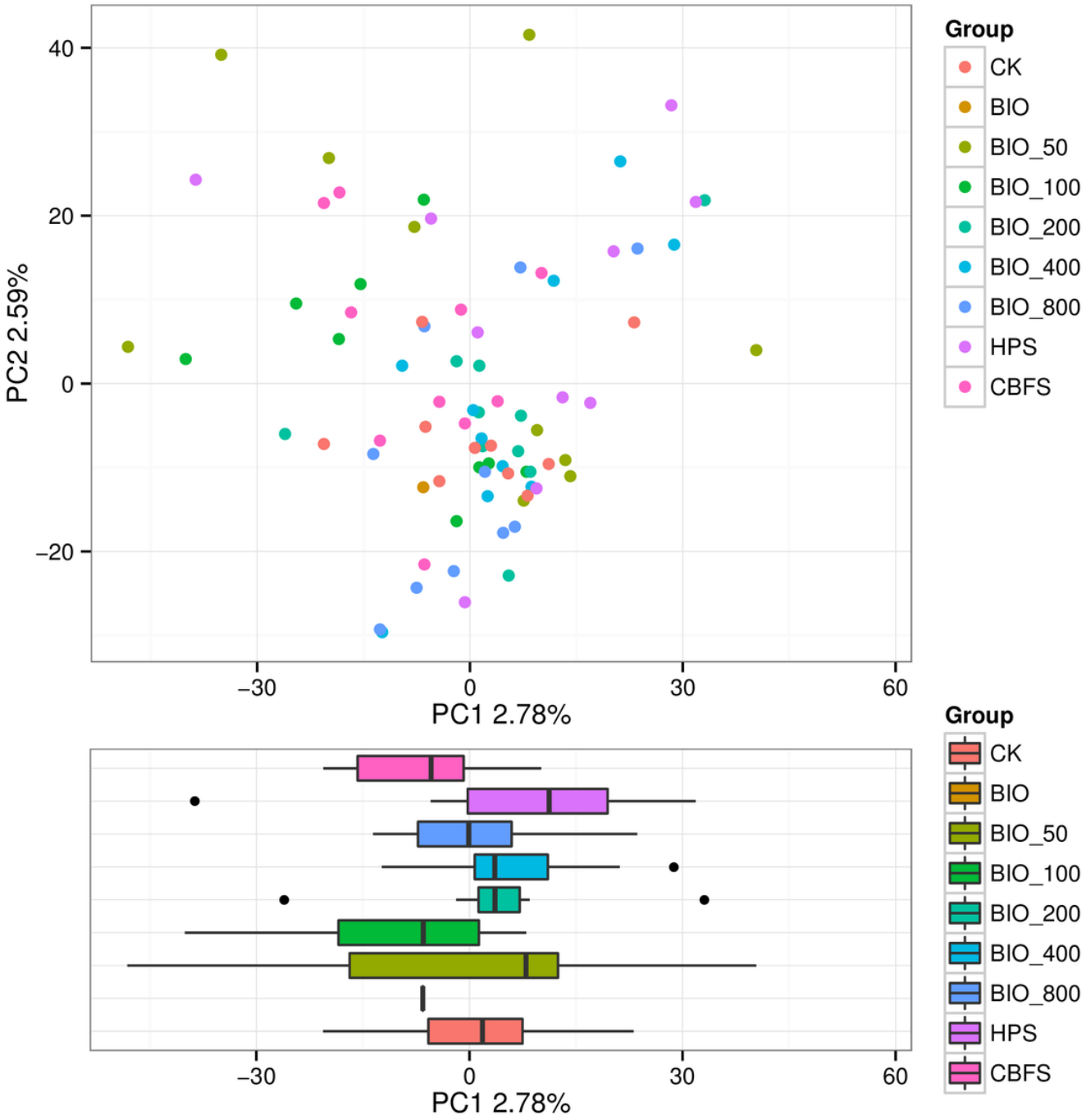

Figure 6

Beta-diversity indices of bacterial community structure in BIO-treated soil samples, CBF soil samples, HP soil samples, CBF samples, and BIO samples (PCA plot) , BIO: BIO sample; BIO-50: 750 kg/ha BIO-treated soil; BIO-100: 1500 kg/ha BIO-treated soil;BIO-200: 3000 kg/ha BIO-treated soil; BIO-400: 6000 kg/ha BIO-treated soil; BIO-800: 12000 kg/ha BIO-treated soil; HPS: herbicide-treated soil; CBF: common bio-fertilizer; CBFS: common bio-fertilizer-treated soil, and CK: untreated control soil.

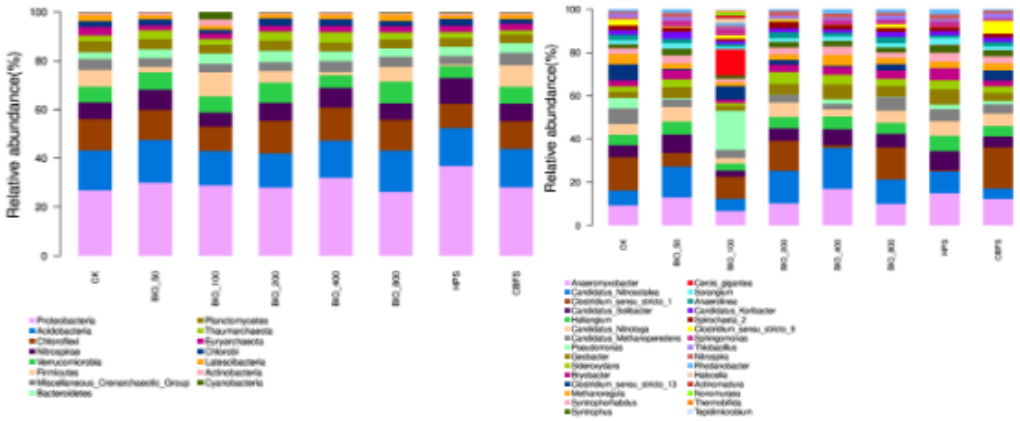

Figure 7

Bacterial diversity, as represented by the relative abundances (%) of different phyla and genera in BIO-treated, CBF, and HP soil samples, and CBF and BIO samples, respectively.(a) Relative abundance of the top15 phyla among the BIO-treated soil samples.(b) Relative abundance of the top15 genera among the BIO-treated soil samples. Abbreviations denote the following:BIO-50: 750 kg/ha BIO-treated soil; BIO-100: 1500 kg/ha BIO-treated soil;BIO-200: 3000 kg/ha BIO-treated soil; BIO-400: 6000 kg/ha BIO-treated soil; BIO-800: 12000 kg/ha BIO-treated soil; HPS: herbicide-treated soil; CBFS: common bio-fertilizer-treated soil, and CK: untreated control soil.

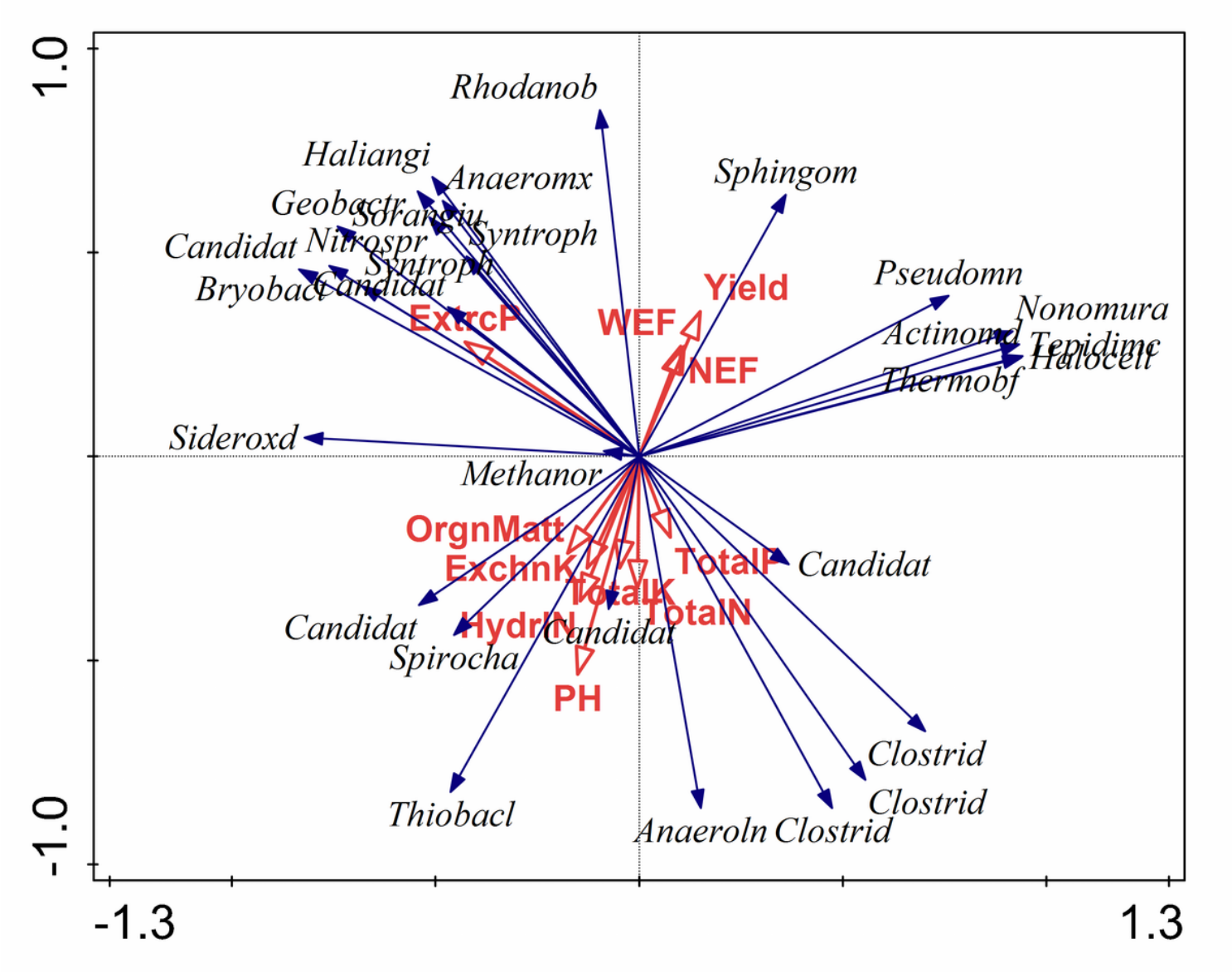

Figure 8

Redundancy analysis (RDA) of microbial community and soil chemical properties (p value=0.02). NEF: weed number control effect; WEF: weed fresh weight control effect. Sample names denote the following: BIO: BIO sample; BIO-50: 750 kg/ha BIO-treated soil; BIO-100: 1500 kg/ha BIO-treated soil;BIO-200: 3000 kg/ha BIO-treated soil; BIO-400: 6000 kg/ha BIO-treated soil; BIO-800: 12000 kg/ha BIO-treated soil; HPS: herbicide-treated soil; CBF: common bio-fertilizer; CBFS: common bio-fertilizer-treated soil, and CK: untreated control soil ; Y: yield.

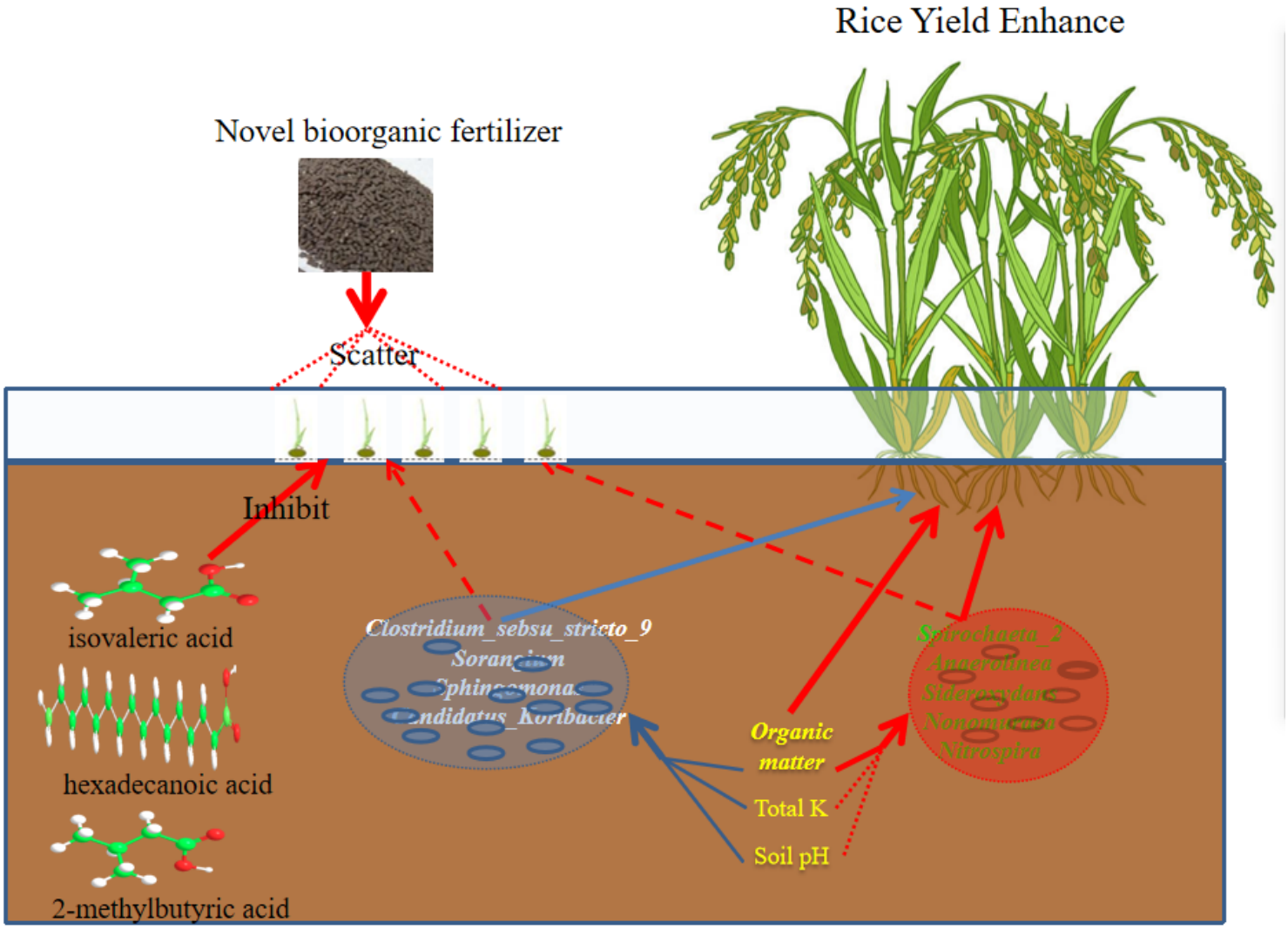

Figure 9

Scheme representation of the association between weed control effect and soil chemical properties and bacterial community. The blue and red arrows indicate negative and positive coefficients, respectively, while the solid and dashed lines show the significance levels.

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