Effects of novel bioorganic fertilizer application on soil enzymes and bacterial community in multi-site rice paddies in China

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Original article

Keywords: bioorganic fertilizer, rice paddy, weed management, bacterial community, soil enzyme

DOI: https://doi.org/10.21203/rs.3.rs-411933/v1

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Abstract

Application of the novel bioorganic fertilizer (BIO) is effectively used to inhibit weeds in rice paddies. To identify changes in soil bacterial community and enzymes in response to BIO treatments, field experiments were carried out in five major rice-growing areas in China. The dominant phylogenetic groups recorded included *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Acidobacteria*. *Anaeomyxobacter*, *Bacteroides*, *Bifidobacterium*, *Escherichia-Shigella*, *Geobacter* and *Haliangium* were significantly different and aided in general function (R), amino acid transport, metabolism (E) and transcription (K) clusters between BIO-treatment and untreated control. The soil chemical properties and enzyme activities were less affected by BIO at these study sites. RDA analysis showed that soil bacterial community had a significant positive correlations among northern latitude, eastern longitude, exchangeable K, total K, total P, soil pH, and total N, except for organic matter, hydrolytic N and extractable P. Overall, our work showed that application of BIO does not alter the main community structure and functional diversity of soil bacterial in rice paddies and should be encouraged for use as a sustainable weed management strategy.

Introduction

Soils provide the physical anchor for crops and therefore the management of its quality is key, in order to maintain agricultural productivity and ecosystems sustainability (Bardgett 2010). Soil bacterial community and enzyme activities are important components for soil functioning. Soil bacterial communities are essential part of the microbial community, and play roles in soil habitats in terms of their biodiversity and biomass (Bahram et al. 2018). Microbial communities play specific roles in biogeochemical cycling and soil functioning through traits such as their species richness, biotic interactions and decomposing activities (Kumar et al. 2018). Soil enzyme activities are good parameters to monitor both positive and negative effects on soil biological activity and crop productivity of agro-ecosystems (Christine et al. 2008). Soil enzyme data is the direct or indirect expression of the soil physio-chemical properties, microorganism, above-ground plants, disturbance and evolution (Zhu et al. 2019). Both changes in soil bacterial community and enzyme activities can affect ecosystem stability in relation to environmental conditions. Thus, the assessment of the influence of biotic and abiotic stressors on the soil microbial community and enzyme activities has become a hot research area for the sustainability of agricultural ecosystem.

To protect rice against weeds, the application of synthetic herbicides is by far the most common management strategy followed. Evaluating the negative effects of herbicides on soil functions is critical to maintaining sustainable utilization of soil and to avoid critical injury to agricultural ecosystems (Carvalho 2017). For instance, it was shown that there was no significant influence on the soil enzyme when it was treated with glyphosate at a rate of 360 g a.i. ha$^{-1}$ (Cheni et al. 2015). Nguyen et al. (2018) showed that effects on soil enzymes were lower in light textured Tenosol soil than Vertosol and Chromosol soils under glyphosate or Roundup CT application. Also, it was reported that the soil microbial structure of irrigated soils under herbicide (triasulfuron and prosulfocarb) treatment exhibited a higher proportion of *Actinobacteria* and lower relative amount of fungi than non-irrigated soils (Delgado et al. 2019). After bispyribac sodium (35 g ha$^{-1}$ and 70 g ha$^{-1}$) application, the soil microbial biomass carbon, dehydrogenase, alkaline phosphatase and urease activities were significantly decreased, and the heterotrophic bacteria, actinomycetes and fungal population were also declined (Kumar et al. 2020). Therefore, the soil type and herbicide formulation are important factors that influence soil functions (Borowik et al. 2017).

Bioorganic fertilizers are mostly organic fertilizer obtained by secondary fermentation involving particular microorganisms (Ling et al. 2012). Bioorganic fertilizers improve soil vitality and organic matter content, and strengthen the effectiveness of pest biocontrol agents (Barakat and Al-Masri 2009). Wu et al. (2014) suggested that a novel bioorganic fertilizer developed by them could inhibit bacterial wilt and suppress *Ralstonia solanacearum* growth. The early application of urea ammonium nitrate (UAN 28%-N) at 112 kg/ha has been shown to increase the emergence rate of certain weeds such as *Chenopodium album*, *Polygonum persicaria*, *Seteria faberi*, and *Abutilon theophrasti* (Pearson et al. 2008). The application of pig manure could reduce the effects of applying NPK fertilizers on rice paddy bacterial communities in heading and ripening stages, but the effects of straw returning was not obvious (Wang et al. 2019). Hence, bioorganic fertilizer is not only a fundamental soil quality resource, but also an effective carrier for the biocontrol of pests.

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 Heilongjiang, 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 et al. 2018). However, the BIO effects on soil bacterial community, functional capability and soil chemical properties in multisite soil rice paddy are not well known. In this study, we evaluated: 1) the influence of BIO on soil bacterial community and soil
enzyme and 2) the relationships among BIO, soil bacterial community and location (longitude and latitude). Results from this study might lay a theoretical foundation for BIO applications.

Materials And Methods

Bio-organic fertilizer (BIO) manufacturing

The organic substrates in the BIO 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 are provided in our previous study (Li 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 one day later. 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\textsubscript{2}O\textsubscript{5}, and 1.1% K\textsubscript{2}O.

Study site description

Our multi-site study was conducted in five rice paddies in China, which had already been in use for 30 years. These study sites described in Table 1 include the location, altitude, temperate climate, and growing season.

| Site       | Soil Type          | Latitude           | Altitude | Temperate Climate | Growing Season |
|------------|--------------------|--------------------|----------|-------------------|---------------|
| Hainan (SY)| Latosol            | N18°23'30" E109°11'33" | 9 m      | 22–28             | April-November |
| Henan (HN)| Yellow Brown Soil  | N 31°49'52" E114°04'79" | 87 m     | 25–34             | April-October  |
| Heilongjiang (HLJ) | Black Soil | N 45°97'82" E128°75'40" | 196 m    | 24–30             | May-November   |
| Jiangsu (JS)| Yellow Brown Soil | N 31°35'49" E 119°10'57" | 8 m      | 26–37             | April-October  |
| Guizhou (GZ)| Yellow Cinnamon Soil | N27°54'34" E106°74'78" | 1271 m   | 20–34             | May-November   |

Note: soil types were classified according to China soil classification system.

Field experiment and soil sample collection

For each site, field trials were arranged into six 30m×20m plots in April 25–27, 2018. Two or three of twenty days old rice seedlings were transplanted to each hill per plot with 15cm×15cm inter spacing seedlings in plot. The rice varieties selected were Chuanyou 6203 at Guizhou (GZ), Xiangeng 2369 at Henan (HN), Yuzhenxiang at Hainan sanya (SY), Longgeng 29 at Heilongjiang (HLJ) and Nangeng 9108 at Jianshu (JS). Three days after transplanting, BIO (3000 kg/ha) were spread over three plots as the treatment. Our choice of BIO dosage was drawn from our previous study which showed this dosage to be the most effective and economical for weed control. The other plots were not supplied with BIO fertilizer. The base fertilizer was applied uniformly in six BIO-treatments and untreated plots. All field management practices followed local and traditional practices, except for the irrigation during BIO application, as a 3-5cm water layer had to be maintained for 7 days. Rice plants were maintained as per site local agronomic practices. No top dressing or other weed control strategies were carried out in the experimental plots. Soil samples were collected from all plots on May 25–27, 2018, i.e. 1 month after BIO application. One hundred grams of surface soil (0–15 cm) was collected from 45 points and 15 samples were mixed together in 15 plastic bags from each plot. Soil samples were divided into two parts, one part was frozen and stored at -80°C, and the other part was air dried for 1 week and stored at 25°C.

Soil chemical properties measurements
Soil pH was measured in soil-water solution (W/V 1:5). Soil total N and K content were measured using an elemental analyzer (Carlo Erba, Milan, Italy) and total P content was assayed calorimetrically by the molybdate method (Willy et al. 2019). Other chemical properties which included hydrolytic N, extractable P, exchangeable K, and organic matter content were analyzed as described previously (Ballabio et al. 2019).

DNA extraction and library construction

Total genomic DNA was extracted using DNA extraction kit following the manufacturer's instructions. The quality and quantity of DNA was verified with NanoDrop and agarose gel. Extracted DNA was diluted to a concentration of 1 ng/μl and stored at -20°C until further processing. The diluted DNA was used as template for PCR amplification of bacterial 16S rRNA genes with the barcoded primers and Takara Ex Taq (Takara). For bacterial diversity analysis, V3V4 variable regions of 16S rRNA genes were amplified with the following primer pair: 343F-(5'-TACGGRAGGCAGCAG-3') and 798R-(5'-AGGGTATCTAATCCT-3').

Amplicon quality was visualized using gel electrophoresis, purified with AMPure XP beads (Agencourt), and amplified for another round of PCR. After a second round of purification with the AMPure XP beads, the final amplicon was quantified using Qubit dsDNA assay kit. Equal amounts of purified amplicon were pooled for subsequent sequencing.

Bioinformatics and statistical analysis

Raw sequencing data were in FASTQ format. Paired-end reads were then preprocessed using Trimmomatic software to detect and cut off ambiguous bases (N) (Tao JM et al. 2018). It also cut off low quality sequences with average quality score below 20 using sliding window trimming approach. After trimming, paired-end reads were assembled using FLASH software. Parameters of assembly were: 10bp of minimal overlapping, 200bp of maximum overlapping and 20% of maximum mismatch rate. Sequences were further performed with denoising as follows: reads with ambiguous, homologous sequences or below 200bp were abandoned. Reads with 75% of bases above Q20 were retained. Then, reads with chimera were detected and removed. These two steps were achieved using QIIME software (version 1.8.0).

Clean reads were subjected to primer sequences removal and clustering to generate operational taxonomic units (OTUs) using V search software with 97% similarity cutoff. The representative read of each OTU was selected using QIIME package. All representative reads were annotated and blasted against Silva database Version 123 (or Greengens) (16s/18s rDNA) using RDP classifier (confidence threshold was 70%). The alpha diversity indices (Chao1 and Shannon index) were calculated using QIIME (Version 1.7.0) in R software (Gomez-Sagasti et al. 2019). Principal component analysis (PCA) was performed using CANOCO 5.0. Analysis of variance (ANOVA) test was conducted with Genstat 13 (VSN International, Hemel Hempsstead, UK) to evaluate the effect of BIO treatment and control on the five sites. The relative abundance of OTU was inferred with FUNGuild (Wang et al. 2020). Significant differences in bacterial species were determined using the linear discriminant analysis (LDA) effect size (LEfSe) method with Kruskal-Wallis sumrank test. Differences were considered statistically significant at a level of $p < 0.05$. Phylogenetic analysis of communities by reconstruction of unobserved states (PICRUSt) analysis with the KEEG orthology database was used to predict and visualize bacterial function on the significant difference between bacteria. Redundancy analysis (RDA) was used to determine the correlation among relative abundance of bacterial community, soil chemical properties, and site location in R language.

Soil enzymatic activity determination

Five soil samples from treated and untreated sites were analyzed for three representative enzymes activities (Soil Urease (S-UE), Soil acid phosphatase (S-ACP) and Soil β-glucosidase (S-β-GC). Soil enzyme activities were analysed with the S-UE, S-ACP, and S-β-GC assay kit (Solarbio life Sciences, Beijing, China). S-UE was defined as 1 g of soil which produced 1µg NH₃-N (U/g) daily. S-ACP was considered to be 1 g of soil which liberated 1nmol phenol at 37°C (U/g) daily. S-β-GC was considered to be 1 g of soil which produced 1µmol p-nitrophenol (U/g) daily (Dick et al. 2013). All enzyme assays were conducted in duplicate.

Results

Effect of BIO on the soil chemical properties

The effects of BIO on the soil chemical properties at five sites are presented in Table 2. No significant differences were observed between BIO treatment and untreated plots for soil pH, the total N, total K and total P at all five rice paddy sites. The hydrolytic N and organic matter of the soil with BIO treatment were respectively lower than for untreated soil at JS and HN sites, and the other sites were not affected. The exchangeable K was higher in the BIO treatment (193 mg/kg and 243 mg/kg) than untreated plot (152 mg/kg and 142 mg/kg) at JS and GZ site rice paddy. The exchangeable K was not significantly different between treatments and control in HLJ and SY.
site. The exchangeable K of BIO soil was 23.66% lower than untreated soil. The exchangeable K was irregularly affected by BIO at five sites. The extractable P also showed irregularity in its influence by BIO in the five rice paddy sites.

Table 2

| Variable source | pH   | Total K mg/kg | Total N mg/kg | Total P mg/kg | Exchangeable K mg/kg | Extractable P mg/kg | Hydrolytic N mg/kg | Organic matter mg/kg |
|-----------------|------|---------------|---------------|---------------|----------------------|---------------------|---------------------|---------------------|
| HN-CK           | 5.42 | 17.9a         | 2.33a         | 0.42a         | 131a                 | 0.26a               | 239a                | 34.1a               |
| HN-TRE          | 5.33 | 17.8a         | 1.55b         | 0.36a         | 100b                 | 0.26a               | 161b                | 26.1b               |
| JS-CK           | 6.13 | 13.4a         | 1.37a         | 0.46a         | 152b                 | 2.26a               | 174a                | 18.8a               |
| JS-TRE          | 6.60 | 14.7a         | 1.15a         | 0.50a         | 193a                 | 1.87b               | 120b                | 14.8b               |
| HLJ-CK          | 5.54 | 21.8a         | 1.70a         | 0.82a         | 131a                 | 1.40b               | 179a                | 34.0a               |
| HLJ-TRE         | 5.73 | 21.7a         | 1.73a         | 1.00a         | 128a                 | 8.91a               | 150a                | 32.1a               |
| GZ-CK           | 5.94 | 15.3a         | 2.16a         | 0.91a         | 142b                 | 4.25a               | 180a                | 34.3a               |
| GZ-TRE          | 6.47 | 16.7a         | 2.23a         | 1.06a         | 243a                 | 5.30a               | 182a                | 36.6a               |
| SY-CK           | 6.30 | 10.0b         | 1.03a         | 0.41a         | 121a                 | 2.17a               | 155a                | 15.5a               |
| SY-TRE          | 6.35 | 13.1a         | 1.12a         | 0.34a         | 128a                 | 2.01a               | 117a                | 16.1a               |

Values show mean (n = 3). Means with different letters represent significant differences at p < 0.05.

Soil bacterial community composition of the study sites

Sequencing the 16S rRNA genes revealed the bacterial diversity and community composition in the five sites of the rice paddies. The number of OTUs in all samples was 1660–4621 and the mean length of valid tags was 409.98-419.42 bp in all samples (Figure S1). Bacterial community structure at the phylum level in the BIO-treated soil and untreated soil samples is shown in Fig. 1a. The five most dominant phyla in HN soil samples were Proteobacteria, Bacteroidetes, Actinobacteria, Nitrospirae, and Acidobacteria. The five most dominant phyla in JS soil samples were Proteobacteria, Actinobacteria, Bacteroidetes, Acidobacteria, and Gemmatimonadetes. The five most dominant phyla were Proteobacteria, Bacteroidetes, Actinobacteria, Firmicutes, and Acidobacteria in HLJ, GZ, and SY soil samples. Bacterial community structure (at the genus level) in the BIO-treated and untreated soil samples is shown in Fig. 1b. The five most dominant genera in HN soil samples were Anaeromyxobacter, Haliangium, Geobacter, Ellin6067, and Thiobacillus. The five most dominant genera in JS soil samples were Anaeromyxobacter, Haliangium, Ellin6067, Geobacter, and Nocardoides. The five most dominant genus in HLJ soil samples were Anaeromyxobacter, Gouta6, Geobacter, Clostridium_sensu_stricto_1, and Ellin6067. The five most dominant phyla in GZ soil samples were Escherichia-Shigella, Bifidobacterium, Anaeromyxobacter, Bacteroides and Haliangium. The five most dominant genus in SY soil samples were Escherichia-Shigella, Bacteroides, Bifidobacterium, Anaeromyxobacter, and Nocardoides. Hence, the main phyla were Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, and Acidobacteria, and the main genera were Anaeromyxobacter, Escherichia-Shigella, Bacteroides, Haliangium, and Geobacter in the five rice paddies.

Soil bacterial community diversity from the study sites

The alpha diversity indices (Chao1 and Shannon) of the different soil treatments (BIO-treatment and untreated control) are shown in Fig. 2. The Chao1 indices indicated that any soil treatments of one site was not significantly separated (p < 0.05, Fig. 2a) and the Shannon indices also indicated that the overall bacterial species were not significantly separated (p < 0.05, Fig. 2b) among the BIO-treated and untreated soil samples at any one site. The indices were not found to be significantly different among the BIO-treated and untreated soil samples. The PCA of beta diversity indicated that all replicates of treated soils (BIO samples and untreated soil samples) clustered together (Fig. 3). The soil treatments of HN, HLJ, and JS site was not significantly separated (p < 0.05) and the GZ and SY also verified that the overall bacterial species were significantly separated (p < 0.05) among the BIO-treated and untreated soil samples at any one site. The PCA of beta diversity showed that the BIO treatment had minor influence on the beta diversity.

Changes on the soil bacterial community
To investigate the effects of BIO on the changes in soil bacterial community, ANOVA test was used to identify differential abundance between BIO-treatment and untreated control in five site soils (Supplementary Figure S1). At the phylum level, the top 10 significantly different abundant bacteria were Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Epsilonbacteraeota, Firmicutes, Gemmatimonadetes, Nitrospirae, Proteobacteria and Spirochaetes. At the genus level, the significantly different bacteria were Anaeromyxobacter, Bacteroides, Blifidobacterium, Escgerichia-Shigella, Geobacter, and Haliangium. LEfSe analysis identified 47 differentially abundant phyla and 963 differentially abundant genus with LDA scores > 2 (Fig. 4). There were 12 phyla in SY_CK and SY_TR with LDA scores > 5. The most significant contribution of BIO effect on soil community in the SY site was Gammaproteobacteria (at phylum level). In addition, Proteobacteria and Deltaproteobacteria were the major confounders of BIO effect on soil community in JS_CK and JS_TR. Of the 15 phyla in HN_CK and HN_TR with LDA scores > 4, the key contributor of BIO effect on soil community were Alphaproteobacteria and Nitrospirae. Nine phyla in HLJ and 7 phylum in GZ with LDA scores > 4, where Acidobacteria and Actinobacteria played key role of BIO effect on soil community, respectively.

Based on the KEEG data, 6 functional gene families were predominant and accounted for metabolism (50.69%), genetic information processing (16.29%) and environmental information processing (13.25%), at KEGG level 1 (Figure S2). At KEGG level 2, a total of 41 sub-functional gene families were identified to be involved in cell communication, sensory system and amino acid metabolism (Figure S3). In brief, only cell communication (P < 0.01) and sensory system (P < 0.01) were significantly reduced in all study sites. The other 39 sub-functional gene families were not concurrently affected at all the study sites. From baslt EggNOG data, a total of 25 functional clusters of COG were predicted in all sites (Fig. 5). In brief, the relative abundance of COG gene was enhanced by the BIO treatment in all sites. The top 3 functional clusters were general function prediction only (R), amino acid transport and metabolism (E) and transcription (K) in all study sites. The abundance of genes related to COG was similar between BIO treatments and controls at the multisites in China rice paddies.

Relationships of the bacterial community with soil chemical properties and site locations

The correlation among bacterial community, soil chemical properties, and site locations is shown in Fig. 6 and Table 3. We observed significant positive correlations among northern latitude, eastern longitude, exchangeable K, total K, total P, soil pH, and total N in our study, except for organic matter, hydrolytic N and extractable P. The northern latitude and eastern longitude of rice paddy sites were positively related to the abundance of Escherichia.Shigella (p = 0.022 and 0.001), Blifidobacterium (p = 0.007 and 0.001), Ellin6067 (p = 0.007 and 0.006), GOUTA6 (p = 0.006 and 0.046), Pesudolabrys (p = 0.039 and 0.010), Acidothermus (p = 0.030 and 0.036), Klebsiella (p = 0.069 and 0.069) and Fodinicola (p = 0.002 and 0.001). In addition, exchangeable K significantly correlated with Anaeromyxobacter (p = 0.001) Candidatus_Solibacter (p = 0.004), Knoellia (p = 0.021), Bradyrhizobium (p = 0.028) and Candidatus_Koribacter (p = 0.047).
dominant and most abundant bacteria including Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes and Acidobacteria) and the main genus (Anaeromyxobacter, Escherichia-Shigella, Bacteroides, Haliangium, and Geobacter) in five rice paddies in China. Proteobacteria was relatively the most abundant phylum in the soils of the five rice paddies located in Guizhou province (Southwestern China), and the other four phyla (Chloroflexi, Acidobacteria, Nitrospira, and Bacteroidetes) were the dominant species in all samples. This result is consistent with that of a previous study (Sun et al. 2015). Results from the taxonomic analysis indicated that the Cyanobacteria and Proteobacteria were dominant phyla in four soils, collected from Changchun, Jiangdu, Yingtian, and Yanting rice paddies (Wang et al. 2019). At Xiantang (Hunan Province, China), the dominant phyla were Proteobacteria (39.98%), Chloroflexi (17.10%) and Actinobacteria (12.70%) out of the total 41 phyla of bacterial community structure in the rice paddy (Guo et al. 2019). At Hengyang (Hunan Province, China), the Proteobacteria, Acidobacteria, Nitrospira, Gemmatimonadetes, and Verruco-microbia were the dominant phyla of bacterial community structure in three rice-based cropping paddies (Huang et al. 2020). From the above reports, we deduce that the dominant soil bacterial species were similar in all the major rice-growing areas in China. Hence, application of BIO did not change the soil bacterial structure within these rice paddies.

It is generally accepted that farming practices cause changes in the soil microbial community (Zwetsloot et al. 2020). Likewise, the dominant and most abundant bacteria including Gammaproteobacteria, Proteobacteria, Deltaproteobacteria, Alphaproteobacteria, 

### Soil enzymatic activity

The activities of three soil enzymes in the BIO-treated and untreated soil at five sites were not significantly different (Fig. 7). Soil urease and acid phosphatase activity in BIO treated soil decreased when compared to its activities in untreated soil at GZ, SY, HN site of rice paddies. On the contrary, these enzymes exhibited opposite activity in the other sites (JS and HLJ). However, the BIO treated soil experienced decreased β-glucosidase activity when compared to untreated soil at GZ, JS, HLJ, and HN sites except for SY sites. In majority of the sites, the three enzyme activities under BIO-treatment were equivalent to untreated.

### Discussion

In this study, we identified the main phyla (Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes and Acidobacteria) and the main genus (Anaeromyxobacter, Escherichia-Shigella, Bacteroides, Haliangium, and Geobacter) in five rice paddies in China. Proteobacteria was relatively the most abundant phylum in the soils of the five rice paddies located in Guizhou province (Southwestern China), and the other four phyla (Chloroflexi, Acidobacteria, Nitrospira, and Bacteroidetes) were the dominant species in all samples. This result is consistent with that of a previous study (Sun et al. 2015). Results from the taxonomic analysis indicated that the Cyanobacteria and Proteobacteria were dominant phyla in four soils, collected from Changchun, Jiangdu, Yingtian, and Yanting rice paddies (Wang et al. 2019). At Xiantang (Hunan Province, China), the dominant phyla were Proteobacteria (39.98%), Chloroflexi (17.10%) and Actinobacteria (12.70%) out of the total 41 phyla of bacterial community structure in the rice paddy (Guo et al. 2019). At Hengyang (Hunan Province, China), the Proteobacteria, Acidobacteria, Nitrospira, Gemmatimonadetes, and Verruco-microbia were the dominant phyla of bacterial community structure in three rice-based cropping paddies (Huang et al. 2020). From the above reports, we deduce that the dominant soil bacterial species were similar in all the major rice-growing areas in China. Hence, application of BIO did not change the soil bacterial structure within these rice paddies.

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### Table 3

| Bacterial genus        | Organic matter N | Hydrolytic K | Exchangeable K | pH | Total N | Total P | Total K | N  | E  | Extractable P |
|------------------------|------------------|--------------|----------------|----|---------|---------|---------|----|----|--------------|
| Anaeromyxobacter       | 0.865            | 0.838        | 0.001          | 0.331| 0.560   | 0.044   | 0.657   | 0.216| 0.407| 0.401        |
| Escherichia_Shigella   | 0.527            | 0.418        | 0.116          | 0.037| 0.243   | 0.360   | 0.200   | 0.022| 0.001| 0.231        |
| Geobacter              | 0.178            | 0.204        | 0.828          | 0.096| 0.024   | 0.367   | 0.003   | 0.026| 0.260| 0.602        |
| Bifidobacterium        | 0.178            | 0.204        | 0.894          | 0.682| 0.537   | 0.946   | 0.313   | 0.007| 0.001| 0.498        |
| Ellin6067              | 0.892            | 0.707        | 0.206          | 0.811| 0.682   | 0.279   | 0.279   | 0.007| 0.006| 0.881        |
| Gemmatimonas           | 0.313            | 0.349        | 0.166          | 0.331| 0.448   | 0.035   | 0.973   | 0.702| 0.367| 0.121        |
| Candidatus_Solibacter  | 0.427            | 0.470        | 0.004          | 0.296| 0.279   | 0.014   | 0.407   | 0.298| 0.634| 0.383        |
| Knoellia               | 1                | 0.838        | 0.021          | 0.407| 0.759   | 0.073   | 0.919   | 0.335| 0.449| 0.336        |
| Bradyrhizobium         | 0.892            | 1            | 0.028          | 0.707| 0.492   | 0.039   | 0.178   | 0.016| 0.046| 0.455        |
| GOUTA6                 | 0.178            | 0.263        | 0.947          | 0.014| 0.066   | 0.232   | 0.001   | 0.006| 0.046| 0.841        |
| Bryobacter             | 0.492            | 0.537        | 0.089          | 1    | 0.218   | 0.049   | 0.049   | 0.009| 0.058| 0.894        |
| Candidatus_Koribacter  | 0.733            | 0.514        | 0.047          | 0.060| 0.838   | 0.073   | 0.919   | 0.576| 0.492| 0.132        |
| Pesudolabrys           | 0.583            | 0.865        | 0.828          | 0.514| 0.973   | 0.584   | 0.232   | 0.039| 0.010| 0.777        |
| Acidothermus           | 0.919            | 0.811        | 0.185          | 0.010| 0.838   | 0.838   | 0.080   | 0.030| 0.036| 0.614        |
| Klebsiella             | 0.891            | 0.759        | 0.424          | 0.448| 0.707   | 0.367   | 0.232   | 0.069| 0.069| 0.700        |
| Fodinicola             | 0.492            | 0.560        | 0.815          | 0.514| 0.946   | 0.448   | 0.154   | 0.002| 0.001| 0.960        |

N: Northern latitude; E: East longitude; Boldface: significance; Italic boldface: top effect on soil chemical properties and soil sites.
Nitrospirae, Acidobacteria, and Actinobacteria were present in the five rice paddies. Meanwhile, the top three functional clusters were only of general function prediction, amino acid transport and metabolism and transcription in all study sites. Land-use changes of desert soils resulted into a significant decrease in Alphaproteobacteria, Actinodbacteria, Bacteroidetes and Firmicutes and sharply increased Acidobacteria, Chloroflexi, Nitrospira and Gammaproteobacteria (Wang et al. 2012). The phylum Bacteroidetes and Acidobacteria were increased, while Actinobacteria and Firmicutes decreased under combined antibiotics (sulfadiazine, sulfamethoxazole, trimethoprim, florfenicol, and clarithromycin) treatment in rice system (Uddin et al. 2019). Amino acid transport and metabolism was significantly different among the soils in the paddies under four common fertilizer treatment and control without fertilizer treatment (Wang et al. 2019). Similar to these studies, soil bacterial community and function were slightly different between application of BIO and untreated plots in the five rice paddies.

Farming practices commonly cause changes in soil chemical properties and enzyme activity (Kumar et al. 2020). However, there were minor modifications after application BIO in the five rice paddies (Table 2, Fig. 7). This result is in agreement with other studies. For instance, soil chemical properties and enzyme activity differed significantly between the organic site and conventional site, while no changes were stimulated in response to lupin (Lupinus angustifolius) amendment at 4 and 8 t level during short-term incubation (Strak et al. 2008). The soil properties were not different between optimum reduced fertilization (OPT) treatments and unfertilized control (CK) and the soil pH, total phosphorus, and total potassium showed no significant difference among all treatments under long-term fertilization field experiment at rice-rice rotation producing area in China (Zhu et al. 2019). The activities of urease and acid phosphatase in the mesotrione-treated and control soil were not different from 2nd to 20th day after application (Du et al. 2018). AgNPs had minor influence on the soil physico-chemical properties and enzyme activities (Oca-Vasquez et al. 2020). Similarly, the application of BIO in our study did not change the main soil chemical properties and enzyme activity in China rice paddies.

The present study illustrates how BIO induces influence on bacterial community, soil enzyme and soil chemical properties. Application of BIO did not change the main soil bacterial phyla and genus, but resulted to a slightly different bacterial community and minor modification of soil enzyme and chemical properties in the rice paddies. These finding suggests that BIO application may be a sustainable weed management strategy in rice system. Future research under long-term field studies at multiple sites on rice paddies will shed more light on our findings.

**Abbreviations**

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

**Declarations**

*Ethics approval and consent to participate*

This article does not contain any studies with human participants or animals performed by any of the authors.

*Consent for publication*

Not applicable

*Availability of data and materials*

The datasets generated during and/or analysed during the current study are available in the NCBI, https://submit.ncbi.nlm.nih.gov/subs/sra/SUB8184095/overview.

*Competing interests*

Zuren Li declares that he has no conflicts of interest. Jingcai Han declares that he has no conflicts of interest. Haodong Bai declares that he has no conflicts of interest. Di Peng declares that he has no conflicts of interest. Lifeng Wang declares that he has no conflicts of interest. Lianyang Bai declares that he has no conflicts of interest.

*Funding*

This study was funded by the National Key Research and Development Programme (2016YFD020 0809), China Agriculture Research System (CARS-16-E19), Scientific-Innovative of Hunan Agricultural Sciences and Technology (2019LS05, 2019LS06, 2019TD03, 2020CX58 and 2020CX60), and Major Science and Technology Programs of Changsha (No. kq1804011).
Authors’ contributions

LY Bai and ZR Li conceived and designed the experiments. Jingcai Han, HD Bai, and LF Wang performed experiments. ZR Li and Di Peng analyzed the data. ZR Li wrote the article. All authors commented on the manuscript.

Acknowledgements

Not applicable

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**Figures**
Figure 1
Relative abundances (%) of bacterial composition (phyla and genera level) in BIO-treated and untreated soil samples at five rice paddy sites. (a) Relative abundance of the top 15 phyla among the BIO-treated soil samples. (b) Relative abundance of the top 15 genera among the BIO-treated soil samples. The HN_CK, HN_TRE, JS_CK, JS_TRE, HLJ_CK, HLJ_TRE, GZ_CK, GZ_TRE, SY_CK and SY_TRE represent BIO treatment and untreated control at the five rice paddy sites in China.

Figure 2
Alpha-diversity indices, (a) Chao1 and (b) Shannon indices, of the bacterial community structure in BIO-treated and untreated soil samples at five rice paddy sites. The HN_CK, HN_TRE, JS_CK, JS_TRE, HLJ_CK, HLJ_TRE, GZ_CK, GZ_TRE, SY_CK and SY_TRE represent BIO treatment and untreated control at the five rice paddy sites in China.
Figure 3

Beta-diversity indices (PCA plot) of bacterial community structure in BIO-treated and untreated soil samples at five rice paddy sites. The HN_CK, HN_TRE, JS_CK, JS_TRE, HLJ_CK, HLJ_TRE, GZ_CK, GZ_TRE, SY_CK and SY_TRE represent BIO treatment and untreated control at the five rice paddy sites in China.
Linear discriminant analysis (LDA) effect size (LEfSe) analysis on the different biomarkers between BIO-treated and untreated soils at five rice paddy sites. Biomarkers that are significantly associated with each treatment, with LDA scores larger than 2 are shown. Significantly discriminant taxon nodes are colored. Each circle's diameter is proportional to the taxon's abundance. Labels are shown of the phylum, class and order levels. The LDA scores of each identified biomarker from the phylum to genus levels are shown in Supplementary Figure S2. The HN_CK, HN_TRE, JS_CK, JS_TRE, HLJ_CK, HLJ_TRE, GZ_CK, GZ_TRE, SY_CK and SY_TRE represent BIO treated and untreated soil samples at the five rice paddy sites in China.
Figure 5

Clusters of Orthologous groups of proteins (COG) functional prediction of the significantly different abundant bacteria between BIO-treated and untreated soil samples at five rice paddy sites. The HN_CK, HN_TRE, JS_CK, JS_TRE, HLJ_CK, HLJ_TRE, GZ_CK, GZ_TRE, SY_CK and SY_TRE represent BIO treated and untreated soil samples at the five rice paddy sites in China.
Figure 6

Redundancy analysis (RDA) of microbial community and soil chemical properties (p value=0.02) between BIO-treated and untreated soil samples at five rice paddy sites. E: East longitude, N: Northern latitude, ExchnK: Exchangeable K, HydrIN: Hydrolytic N, ExtracP: Extractable P.

Figure 7

Soil enzyme activity in the BIO-treated and untreated soil at five site soils. a: Soil urease, b: Soil acid phosphatase, c: Soilβ-glucosidase. Vertical bars indicate standard deviation of the mean (n=3). For each parameter, different letters indicate significant differences between means at P < 0.05. The HN, JS, HLJ, GZ and SY represent BIO treated and untreated soil samples at the five rice paddy sites in China.

Supplementary Files

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