



Soil microbial activity and community composition as influenced by application of pig biogas slurry in paddy field in southeast China

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Abstract

A 6-year field experiment was conducted to assess the effects of biogas slurry (BS) application on rice yield, soil nutrients, soil microbial activity (MicroRespTM) and community composition (16S rRNA gene sequencing) in paddy field in southeast China. The experiment included five treatments: (1) 270 kg N ha⁻¹ from urea (mineral fertilizers only, MF); (2) 135 kg N ha⁻¹ from urea and 135 kg N ha⁻¹ from BS (BS1); (3) 270 kg N ha⁻¹ from BS (BS2); (4) 405 kg N ha⁻¹ from BS (BS3) and (5) 540 kg N ha⁻¹ from BS (BS4). Results showed that no significant differences were found in average rice yield between the MF and BS treatments (BS1–BS4). Both soil available phosphorus (AP) and soil available potassium (AK) increased with an increasing application rate of BS. The average substrate-induced respiration was significantly higher in the treatment with lower rate of BS (BS1 and BS2) than in other treatments and then decreased with increasing application rate of BS. Soil microbial communities were affected by BS application. *Chloroflexi*, *Proteobacteria* and *Acidobacteria* were the dominant bacterial phyla across all soil samples. The BS application resulted in a relative abundance of *Nitrospirae* 2.6–3.7 times than that in MF. Our results also indicated that AP and AK were the two main factors affecting soil microbial activity and community. Overall, the results suggest that the replacement of chemical fertilizer with BS may be an alternative management practice for improving soil quality, soil fertility and nutrient balance in paddy field.

Keywords Biogas slurry · Rice yield · Soil fertility · Soil microbial activity · Soil microbial community

Introduction

The livestock and poultry breeding plants produce more than annual 400 million tons of manure and urea, which may result in ammonia emissions and thereby negatively affecting the environment in China (Jin and Chang 2011; Zhang et al. 2017a). Anaerobic digestion is not only an environmentally friendly approach of human and animal wastes, but also produces a renewable energy in the form of biogas (Lu et al. 2012; Wentzel et al. 2015). The 3800 anaerobic digestion plants in China produced more than 1 billion tons of digestion slurry in 2007 (Jin and Chang 2011; Cao et al.

2016). The increasing amount of biogas slurry (BS) creates a new environmental problem if not carefully managed (Lu et al. 2012; Bian et al. 2015; Zhang et al. 2017a). Therefore, a potential solution is urgent for the reasonable utilization of BS in China.

The biogas slurry has been widely used as a fertilizer for crops because it contains considerable concentrations of plant nutrients such as phosphorus (P), potassium (K) and especially N (Zhu et al. 2009; Lu et al. 2012; Zhang et al. 2017a). BS has the potential to replace mineral fertilizer (MF) and thereby reduce the application rate of MF in the agriculture (Abubaker et al. 2015). Terhoeven-Urselmans et al. (2009) reported that application of BS caused a higher aboveground biomass of barley than did a treatment with no slurry in a field experiment. In red soil, a combination of BS and MF significantly enhanced peanut grain yield compared to the application of only MF (Zheng et al. 2016). In the rice production in China, replacement of BS to chemical fertilizers can maintain or even increases rice grain yield compared to the use of only MF (Lu et al. 2012; Zhang et al. 2017a).

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However, excessive application of BS may result in a decline in crop yield and an increase in environmental risks (Chen et al. 2017b). Therefore, appropriate use of BS is crucial for improving crop yield and soil fertility, and reducing negative environmental impacts.

Rice is one of the most important cereal crops in the world and is the main staple food in China (Peng et al. 2010; Xu et al. 2016). The total grain yield of rice reached 207 million tons in 2016 in China, accounting for 34% of the total food production (NBSC 2017). Over 4 Tg of N fertilizer was applied annually for rice production in 2001–2010 in China (Zhang et al. 2017b). However, excessive N application may result in low N use efficiency and high N loss, which will lead to a series of negative environmental impacts, such as greenhouse gas emission, eutrophication of surface waters and soil acidification (Zhu and Chen 2002; Peng et al. 2010; Liu et al. 2016; Li et al. 2017). Rice paddy fields account for 33% of the total agricultural land in China (Peng et al. 2010; Yao et al. 2018). Paddy fields require a great amount of water to maintain floodwater at a 5-cm depth for a month after rice transplanting (Ji et al. 2013), and meanwhile, rice cultivation needs large quantities of mineral elements (Zhang et al. 2017a). Thus, they offer a good opportunity for assimilating large quantities of BS. Lu et al. (2012) estimated that paddy field could clear up about 1700-m^3 BS ha^{-1} in a single rice cropping system. This practice not only can reduce negative environmental impacts but also can improve soil fertility and crop production (Lu et al. 2012; Abubaker et al. 2013; Chen et al. 2013). Some farmers have already partially replaced MF with anaerobically digested slurry for rice production in China (Chen et al. 2013; Zhang et al. 2017a). Application of BS significantly increased rice grain yield by 12.4% compared to application of only MF (Chen et al. 2013). Lu et al. (2012) reported that BS application was beneficial to soil fertility and productivity in the paddy field. Irrigating BS to red soil has been shown to increase soil microbial biomass and soil enzyme activities (Zheng et al. 2016).

Soil microorganisms play an important role in the function and structure of soil (Hupfauf et al. 2016). Soil microbial diversity is an important indicator of soil quality due to its rapid change in response to agricultural management (Abubaker et al. 2012; Wang et al. 2017). Bacteria are the most abundant soil microbes and are important for maintaining soil fertility in agricultural systems (Zhao et al. 2014b). Many studies have shown that organic fertilizer can stimulate soil microbial activity and change the abundance and composition of soil microorganisms (Ge et al. 2013; Zhao et al. 2016). Cao et al. (2016) reported that pig BS amendment increased soil microbial activity and diversity compared to no amendment. In a peanut field, application of BS not only increased the peanut grain yield but also enhanced the activities of urease and dehydrogenase (Zheng et al. 2016).

The single rice cropping system is an important practice of rice farming in Zhejiang Province, southeast China. Previous studies on this sites showed that short-term (2-year) application of BS resulted in improving rice yield and soil fertility (Lu et al. 2012; Chen et al. 2013). So far, numerous studies on BS have focused on the effect of BS application on the rice yield, soil fertility, ammonia loss and greenhouse gases emission in paddy field (Hou et al. 2007; Sasada et al. 2011; Lu et al. 2012; Chen et al., 2013; Win et al. 2013). However, there is a lack of detailed information on the effects of BS application on the microbial activity and bacterial community of paddy soil. Here, the main objectives of the present study were to investigate the effects of 6-year application of BS on rice yield, soil fertility, soil microbial activity and soil bacterial community composition in a single rice cropping system. The optimal application rate of BS was assessed for a paddy field in Zhejiang Province, southeast China.

Materials and methods

Site description

The field experiment began in 2007 in Jiaxing City, Zhejiang Province ($30^{\circ}49'N$, $120^{\circ}50'E$), southeast China, and continued for six seasons. This region has a typical subtropical monsoon climate, and the average annual rainfall and temperature are 1193 mm and $16.5^{\circ}C$, respectively. The soil at the experimental site is classified as a Mollic Endoaquept according to US soil taxonomy (USDA/NRCS 1999). The basal properties of soil (0–0.2 m) were as follows: pH of 6.6, 20.5 g kg^{-1} soil total organic C (TOC), 2.2 g kg^{-1} total nitrogen (TN), 203.9 mg kg^{-1} alkaline hydrolyzable N (AHN), 29.3 mg kg^{-1} available phosphorus (AP) and 165.0 mg kg^{-1} available potassium (AK).

The BS was collected from a pig farm near the experimental site. The BS was anaerobically digested for at least 30 days and then stored in a pond for at least 7 days before application. The mean values of chemical properties in the BS were total N: 571 mg N L^{-1} , $\text{NH}_4^{+}\text{-N}$: 405 mg N L^{-1} , total P: $72\text{ mg P}_2\text{O}_5\text{ L}^{-1}$, total K: $351\text{ mg K}_2\text{O L}^{-1}$ and COD: 1583 mg L^{-1} .

Experimental design

The field experiment was established in a single rice cropping system in 2007. The field trial had five treatments based on the N input from chemical fertilizer and/or ADS. The treatments consisted of (1) mineral fertilizers only, 270 kg N ha^{-1} from urea (MF); (2) 135 kg N ha^{-1} from urea and 135 kg N ha^{-1} from BS (BS1); (3) 270 kg N ha^{-1} from BS (BS2); (4) 405 kg N ha^{-1} from BS (BS3) and (5)

Table 1 Nutrient loadings from mineral fertilizers and biogas slurry

Treatment	Mineral fertilizers (kg ha ⁻¹)			Biogas slurry (kg ha ⁻¹)		
	N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O
MF	270	90	150	–	–	–
BS1	135	80.5	70.5	135	9.5	79.5
BS2	–	71	–	270	19	159
BS3	–	61	–	405	29	238
BS4	–	51	–	540	39	318

MF: 270 kg N ha⁻¹ from urea; BS1: 135 kg N ha⁻¹ from urea and 135 kg N ha⁻¹ from biogas slurry; and BS2, BS3 and BS4: 270, 405 and 540 kg N ha⁻¹ from biogas slurry, respectively

540 kg N ha⁻¹ from BS (BS4). Five treatments were laid out in a randomized complete block design with three replicates. There were 15 plots in total, and the area of each plot was 30 m² (5 m × 6 m). Each plot was separated by ridges (0.3 m wide × 0.4 m high), and each ridge was covered with a plastic film to prevent the movement of MF and BS. For the MF treatment, before the transplanting rice, basal fertilizers were broadcast by hand and incorporated into soil. N fertilizer was split-applied at transplanting, tillering and booting at the rate of 50%, 20% and 30%. For the BS1 treatment, N from urea and BS was equal at each application. MF was broadcast by hand and incorporated into soil, and BS was evenly irrigated into plot using flexible pipes. N fertilizer was also split-applied at transplanting, tillering and booting at the rate of 50%, 20% and 30%. For the remaining BS treatment (BS2–BS4), the BS was evenly irrigated into plot using flexible pipes and split-applied based on the N loading from BS at transplanting, tillering and booting at the rate of 50%, 20% and 30%. Following the BS application as basal fertilizer, the floodwater was moderately irrigated into plot after application of BS, and the BS was applied into paddy field at 3–5 days before rice transplanting according to the weather condition. All mineral P and K fertilizers were applied as basal fertilizers in each plot. All the treatments had the same application rate of P fertilizer. The mineral N, P and K fertilizer came from urea, superphosphate and potassium chloride, respectively. The application rates of mineral fertilizers and BS are detailed in Table 1. Rice (*Oryza sativa* L.) seedlings (30 days old) were transplanted annually in late June and harvested in early November during the 2007–2013 study period. The field management practices followed local farmers' practices.

Plant and soil sampling

All the rice from each plot was harvested by hand after reaching maturity and divided into grain and straw using a threshing machine. The harvested rice grains were dried at 70 °C to a constant weight for the determination of crop yield.

In 2013, five soil cores (0–20 cm depth, 5 cm diameter) were randomly collected after the rice was harvested in each plot, mixed well as a single soil sample and placed in ziplock bags. The fresh soil samples were ground and sieved through a 2-mm screen after the removal of plant material, roots and stones. The sieved samples were divided into three subsamples: One was stored at 4 °C to determine the pattern of soil substrate-induced respiration using the MicroResp™ system; one was stored at –20 °C to determine the soil microbial community; and the third was air-dried to determine the soil chemical properties.

Soil chemical properties

Soil pH was determined with an electrode using a soil-to-water ratio of 1:2.5. Soil TOC concentration was determined by oxidation with potassium dichromate and titration with ferrous sulfate (Lu 1999). TN concentration was measured using the Kjeldahl method (Lu 1999). Available P was measured by the Olsen method (Olsen and Sommers 1982), alkaline hydrolysable N was determined according to Roberts et al. (2011), and available K was extracted by 1 M ammonium acetate and determined by a flame photometer.

Soil respiration measurements by MicroResp™

The MicroResp™ system was used to determine the substrate-induced respiration (SIR) of soil with fifteen carbon sources according to a modified method (Campbell et al. 2003; Chapman et al. 2007). Before the soil was used, the moisture content of fresh soil samples was adjusted to 30%, and the soil was then incubated for 5 days at 25 °C (Lin et al. 2016). The fifteen carbon sources used in our study were 3,4-dihydroxybenzoic acid, α-ketoglutaric acid, D-galactose, D-fructose, D-trehalose, L-arabinose, L-alanine, L-arginine, L-cysteine HCl, L-lysine, L-malic acid, γ-amino butyric acid, oxalic acid, citric acid and D-glucose. The addition of only water was used as a control. L-alanine, L-arginine and 3,4-dihydroxybenzoic acid were added to a concentration of 7.5 mg g⁻¹ soil water, and the other carbon sources were added to a concentration of 30 mg g⁻¹ soil water. The

carbon sources were added to a deep-well plate with the soil samples, and the plate was then incubated at 25 °C for 6 h. The detection plate was measured at 570 nm at 0 and 6 h immediately after incubation using a microplate reader.

Soil DNA extraction and 16S rRNA high-throughput sequencing

Genomic DNA was extracted from 0.5 g of fresh soil using a Fast DNA spin kit for soil (MP Biomedicals, USA) according to the manufacturer's instructions. The compositions of bacterial community were measured using Illumina MiSeq high-throughput sequencing analysis of the 16S rRNA V4 region. The 16S rRNA was amplified using the universal primers 515F (5'-GTG CCA GCM GCC GCG GTA A-3')/907R (5'-CCG TCA ATT CMT TTR AGT TT-3'). PCR reactions of bacterial 16S rRNA contained 0.40 µl of each primer, 12.50 µl of 2× EasyTaq PCR SuperMix (TransGen Biotech, China) and 1.25 µl of DNA template. PCR reactions were denatured at 95 °C for 3 min and then amplified by 27 cycles at 95 °C for 30 s, 55 °C for 30 s and 72 °C for 45 s, followed by a final extension at 72 °C for 10 min. After amplification, the triplicate amplicons were pooled and purified using a PCR cleanup kit. Sequencing was conducted by Majorbio Bioinformatics Technology Co., Ltd. (Shanghai, China) with an Illumina MiSeq system.

Bioinformatic analysis

Raw sequences were processed using the Quantitative Insights into Microbial Ecology (QIIME) software package (Caporaso et al. 2010). The sequence reads were trimmed and assigned to each sample according to their barcodes. Low-quality sequences (quality < 20, length < 250 bp) were removed. All singletons were filtered out, and all chimeric reads were removed. Then, all the sequences were clustered

into operational taxonomic units (OTUs) at 97% similarity using USEARCH (Edgar 2013). Representative sequence from each OTU was classified by the Ribosomal Database Project (RDP) classifier (Wang et al. 2007).

Statistical analysis

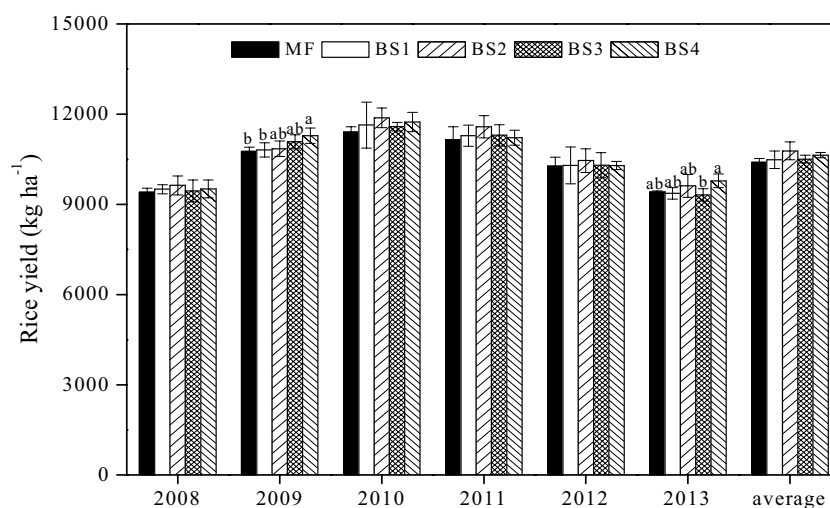
One-way analysis of variance (ANOVA) followed by Duncan's test was performed to compare the differences in rice grain yields, soil properties, soil microbial activities and bacterial abundances among the treatments using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Principal component analysis (PCA) was used to elucidate the differences in the patterns of C-source utilization, principal coordinate analysis (PCoA) was used to compare the beta-diversity of soil bacterial communities based on Bray–Curtis distances, and redundancy analysis (RDA) was carried out to assess the relationships among the microbial activities, community compositions and soil properties. The PCA and RDA were both conducted in R v. 3.4.2 (R Development Core Team) using the vegan package, and the PCoA was performed in R v. 3.4.2 (R Development Core Team) using the ape package.

Results

Crop yields

The grain yields of rice ranged from 9400 to 11,900 kg ha⁻¹ during 2008–2013 (Fig. 1). The rice yield was significantly higher in BS4 than in the other treatments in 2009 and 2013, but no significant differences were observed in the rice grain yields among the remaining treatments (Fig. 1). Among the five treatments, there were no significant differences in the rice yields in 2008, 2010, 2011 and 2012. The average rice

Fig. 1 Rice grain yield as affected by the different treatments. MF: 270 kg N ha⁻¹ from urea; BS1: 135 kg N ha⁻¹ from urea and 135 kg N ha⁻¹ from biogas slurry; and BS2, BS3 and BS4: 270, 405 and 540 kg N ha⁻¹ from biogas slurry, respectively. Vertical bars represent the stand error of the mean ($n=3$). Different letters above the bars represent significant differences at the level of $p<0.05$



yields over all years were similar among all treatments, ranging from 10,400 to 10,800 kg ha⁻¹.

Soil chemical properties

There were no significant differences in pH, TOC, TN, C/N and AHN among the treatments (Table 2). The highest soil AP (108.78 mg kg⁻¹) was found in the BS4 treatment; it was 41.8% and 50.3% higher than that in the BS1 (76.72 mg kg⁻¹) and MF treatments (72.36 mg kg⁻¹), respectively. Among the BS2, BS3 and BS4 treatments, the soil available P values were similar ($p > 0.05$),

ranging from 94.74 to 108.78 mg kg⁻¹. The BS4 treatment (238.30 mg kg⁻¹) had a significantly higher soil AK than did the MF treatment (178.98 mg kg⁻¹), while the soil AK in the MF treatment was similar to that in the BS1, BS2 and BS3 treatments ($p > 0.05$). Both soil AP and AK increased with an increasing application rate of BS.

Substrate-induced respiration pattern

The highest average substrate-induced respiration (SIR) was recorded in the BS2 treatment, being significantly higher than that in the MF, BS3 and BS4 treatments (Fig. 2).

Table 2 Soil chemical properties as affected by the different treatments

Treatment	pH	TOC (g kg ⁻¹)	TN (g kg ⁻¹)	C/N	AHN (mg kg ⁻¹)	AP (mg kg ⁻¹)	AK (mg kg ⁻¹)
MF	6.94 ± 0.08a	22.26 ± 0.41a	2.46 ± 0.06ab	9.07 ± 0.35a	202.45 ± 41.84a	72.36 ± 11.82b	178.98 ± 16.07b
BS1	6.93 ± 0.06a	21.95 ± 0.61a	2.47 ± 0.07ab	8.88 ± 0.48a	227.38 ± 76.16a	76.72 ± 16.58b	198.32 ± 56.89ab
BS2	6.92 ± 0.17a	22.68 ± 1.12a	2.54 ± 0.08a	8.92 ± 0.49a	210.02 ± 65.08a	94.74 ± 2.07a	206.98 ± 32.00ab
BS3	7.03 ± 0.29a	22.69 ± 0.31a	2.48 ± 0.04ab	9.14 ± 0.03a	217.45 ± 61.46a	97.55 ± 20.23a	219.98 ± 11.70ab
BS4	6.98 ± 0.11a	21.65 ± 0.86a	2.40 ± 0.07b	9.02 ± 0.19a	191.91 ± 10.98a	108.78 ± 17.47a	238.30 ± 15.83a

Values are presented as the mean ± standard deviation ($n = 3$)

TOC: total organic C; TN: total N; AHN: alkaline hydrolyzable N; AP: available P; AK: available K. MF: 270 kg N ha⁻¹ from urea; BS1: 135 kg N ha⁻¹ from urea and 135 kg N ha⁻¹ from biogas slurry; and BS2, BS3 and BS4: 270, 405 and 540 kg N ha⁻¹ from biogas slurry, respectively

Different letters in the same column represent significant differences at the level of $p < 0.05$

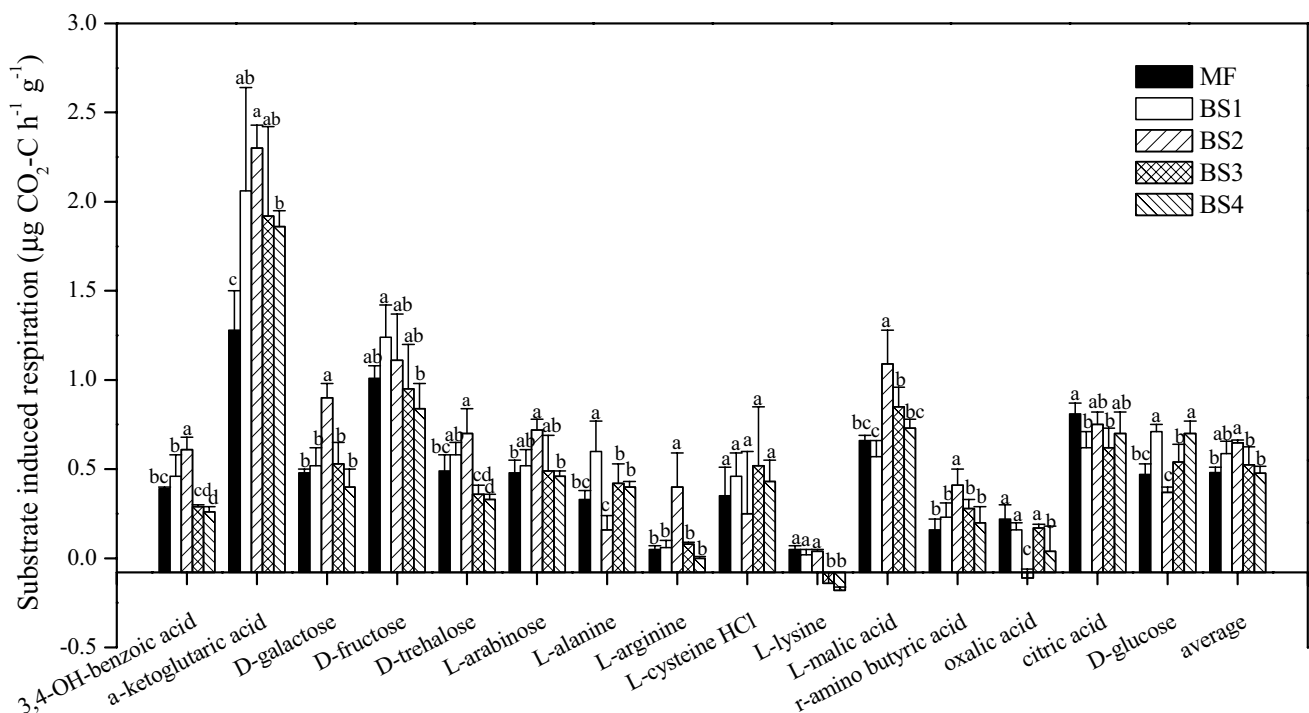


Fig. 2 Substrate-induced respiration as affected by the different treatments. Treatment is as given in Fig. 1. Vertical bars represent the stand error of the mean ($n = 3$). Different letters above the bars represent significant differences at the level of $p < 0.05$

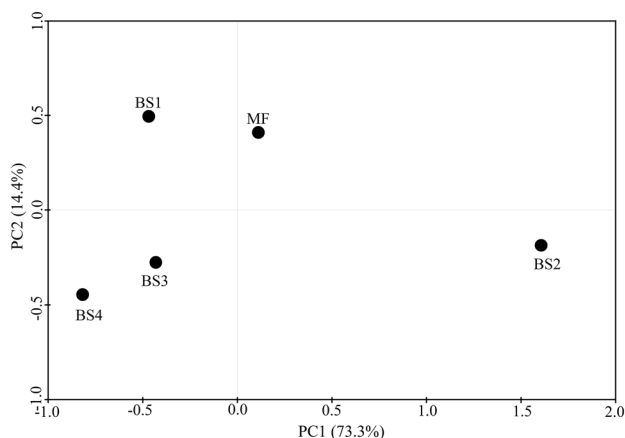


Fig. 3 Principal component analysis (PCA) of the MicroResp™ data for the different treatments. Treatments are as given in Fig. 1

However, no significant differences were found in the average SIR values among the MF, BS1, BS3 and BS4 treatments. The highest SIRs were observed for α -ketoglutaric acid and D-fructose, and the lowest was for L-lysine across all five soils. For 9 of the 15 C sources, the BS2 treatment had a higher SIR than did the MF treatment. However, for oxalic acid and citric acid, the SIR was significantly lower in the BS2 treatment than in the MF treatment. Soil microorganisms favored α -ketoglutaric acid, D-fructose, L-malic acid and citric acid across all five soils.

Principal component analysis (PCA) of the MicroResp™ data showed that the two principal components, PC1 and PC2, accounted for 73.3% and 14.4% of the overall variance, respectively (Fig. 3). The dominant substrates of PC1 were L-arabinose, L-arginine, L-lysine, γ -amino butyric acid, citric acid and D-trehalose, while the dominant substrates of PC2 were 3,4-dihydroxybenzoic acid, oxalic acid, L-malic acid and D-galactose. The C-source utilization profiles showed significant separation when comparing soil samples from MF and BS2 to those from BS1, BS3 and BS4 along PC1. A clear separation of MF and BS1 from BS2, BS3 and BS4 was observed when comparing the MicroResp™ data for soil samples along PC2.

Redundancy analysis (RDA) of the MicroResp™ data showed that the coordinates of the first and second ordination axes explained 80.6% and 9.4% of the total variance, respectively (Fig. 4). Microbial respiration was distinctly separated based on the application of BS. Soil AP and AK had the greatest effect on soil microbial respiration.

Soil bacterial community composition

In the present study, a total of 41 phyla were identified across all soil samples. The two dominant bacterial phyla were *Chloroflexi* and *Proteobacteria* in all treatments,

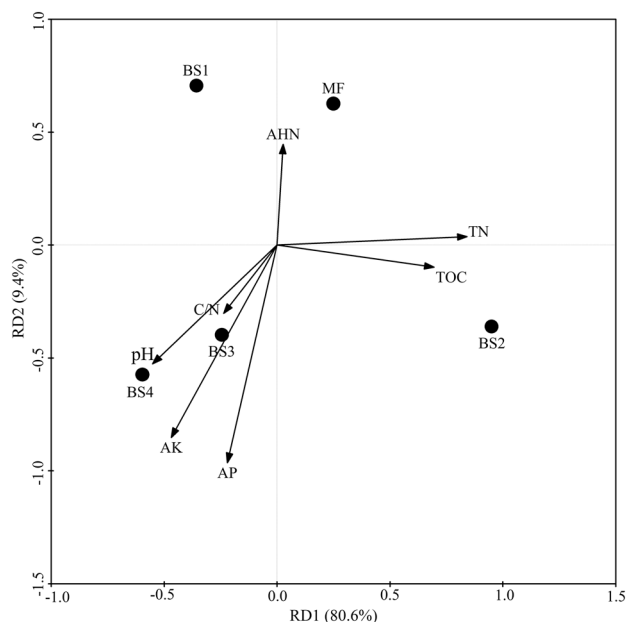


Fig. 4 Redundancy analysis (RDA) of the relationships between the MicroResp™ data and soil properties for the different treatments. TOC total organic C, TN total N, AHN alkaline hydrolyzable N, AP available P, AK available K. Treatments are as given in Fig. 1

with proportions ranging from 24.0 to 33.5% and 21.6 to 26.8%, respectively (Fig. 4). *Acidobacteria* was the third most common bacterial phylum in the five treatments, accounting for 17.0–21.6% of total bacterial abundance. Furthermore, the relative abundance of seven phyla (*Nitrospirae*, *Planctomycetes*, *Bacteroidetes*, *Chlorobi*, *Actinobacteria*, *Gemmatimonadetes* and *Verrucomicrobia*) was less than 10% but greater than 1% (Fig. 5). The BS applications resulted in a relative abundance of *Nitrospirae* 2.6–3.7 times that in the MF treatment. As shown by the principal coordinate analysis (PCoA) using Bray–Curtis distances, the bacterial community compositions were clearly separated by the BS application (BS2 and BS3) along the first axis (Fig. 6). The two axes of the PCoA explained 64.7% of the total variance in the composition of the bacterial communities. (The first axis explained 42.8%, and the second 21.9%.)

The RDA ordination plot showed the relationships between soil bacterial community composition and soil properties (Fig. 7). The first two RDA components could explain 90.0% of the total variation. (The first axis explained 59.2%, and the second 30.8%.) MF, BS1 and BS3 had higher scores than did BS2 and BS4 along RD1. Along the second axis, the score of MF was higher than that of the biogas slurry treatments (BS1–BS4). Soil AP and AK were the two main factors affecting soil bacterial community compositions along the axis 2.

Fig. 5 Relative abundance of dominant phyla as affected by the different treatments. Treatments are as given in Fig. 1

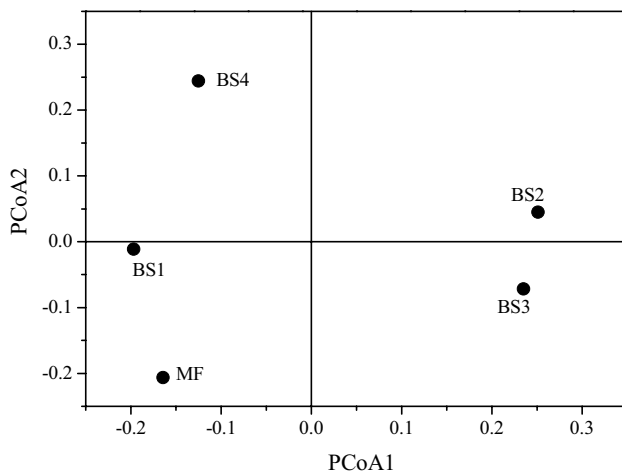
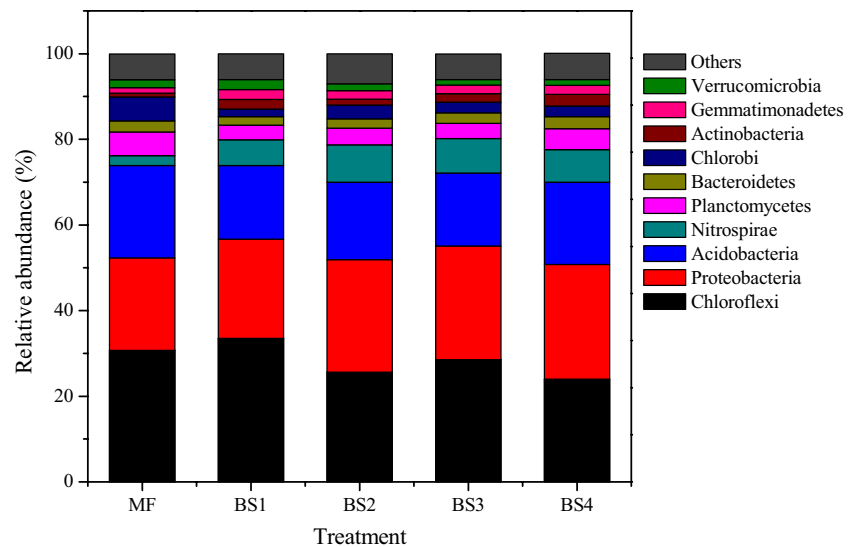


Fig. 6 Principal coordinate analysis (PCoA) based on Bray–Curtis distances of the bacterial communities for the different treatments. Treatments are as given in Fig. 1

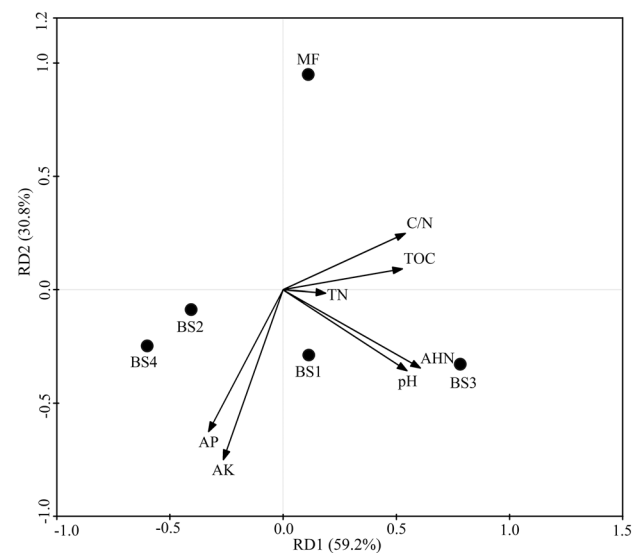


Fig. 7 Redundancy analysis (RDA) of the relationships between bacterial phyla and soil properties for the different treatments. *TOC* total organic C, *TN* total N, *AHN* alkaline hydrolyzable N, *AP* available P, *AK* available K. Treatments are as given in Fig. 1

Discussion

It is widely recognized that BS application to paddy soil is a highly valuable solution to the organic waste management problem (Lu et al. 2012; Moreno-García et al. 2017; Zhang et al. 2017b). Further, application of BS could improve soil fertility and crop yield in flooded rice field (Hou et al. 2007; Lu et al. 2012). In the present study, there were no significant differences in the average grain yields of rice between the MF and BS treatments (BS1–BS4, Fig. 1), indicating that the N from BS can meet the N demand of rice during the rice-growing season. Many studies have shown that BS could replace chemical N fertilizer in rice production (Lu et al. 2012; Chen et al.

2013; Win et al. 2013). Similarly, Lu et al. (2012) reported that application of BS could maintain or even increase the rice grain yield compared to the use of only MF. Ammonium-N accounts for 80% of total N in BS and is the main form absorbed and utilized by rice (Chen et al. 2013). Lu et al. (2012) estimated that BS application to paddy soil at 540 kg N ha⁻¹ was beneficial to rice yield with no environmental risks in Zhejiang Province. However, high level of BS application may result in rice lodging and thus decrease the rice grain yield (Win et al. 2013). Combination of pig BS and MF was a recommended method for rice production in east China (Zhang et al. 2017b). In this

study, the rice yield was slightly lower in high level of BS application than that in the BS2 treatment, but there was no significant difference (Fig. 1). We suggested that the application of pig BS at 270 kg ha⁻¹ might be the effective practice for the rice production. The substitution of BS for chemical N fertilizer at moderate rate is an alternative management practice in a single rice cropping systems in southeast China.

Soil nutrients, such as organic C, total N, available P and available K, are good indicators of soil fertility and quality (Zheng et al. 2016). Previous studies have demonstrated that a combination of BS and MF improved soil available N and P relative to the application of only MF in a red soil (Zheng et al. 2016). However, the results from the present study showed that application of BS does not significantly affect the soil TOC, TN and AHN (Table 2). The reason for this result may be that the duration of the field experiment was relatively short, and the initial soil TOC and TN were relatively high in the paddy field. However, the soil available P and available K increased with increasing application rates of BS (Table 2). Similarly, Lu et al. (2012) found that application of BS increased soil fertility (TOC, AN, AP and AK) compared to application of only MF. Bachmann et al. (2014) reported that 3-year application of cattle slurry increased soil water-soluble P relative to application of MF only. The rate of K fertilizer was higher in the high level application of BS than that in the MF treatment due to considered K₂O concentration in the BS (Table 1), which resulted in an increase in soil AK in paddy field.

MicroResp™ has been widely used to determine the substrate-induced respiration (SIR) in the soil and is sensitive to the environmental changes, such as soil fertility and pollution (Bérard et al. 2014; Lin et al. 2016; Long et al. 2016). However, the turnover of soil microbial community based on molecular technique needs some time to occur, because DNA still exists in dead microbes (Wang et al. 2014; Pan et al. 2016). Studies have reported that availability of C sources played a vital role in the activity and growth of soil microorganisms (Lagomarsino et al. 2012; Abujab-hah et al. 2016). In the present study, soil microbes favored α -ketoglutaric acid, D-fructose, L-malic acid and citric acid (Fig. 2). A study of soil microbial activities in a paddy soil showed that citric acid, oxalic acid, α -ketoglutaric acid and L-malic acid were easily utilized by soil microorganisms (Pan et al. 2016). Long et al. (2016) also found that microbes from landfill cover soils favored C sources of L-malic acid, α -ketoglutaric acid and citric acid. Compared to MF treatment, appropriate rate of BS application enhanced soil microbial activities in our study (Fig. 2). An incubation was conducted by Abubaker et al. (2013), who indicated that clay soil treated with cattle slurry had a higher SIR than soil without slurry. A short-term field experiment showed that manure amendment significantly increased the average

SIR of soil compared to application of MF only (Lin et al. 2016). Pan et al. (2016) reported that application of rice straw caused higher microbial activity than no application of straw. Our results showed that high levels of BS application (BS3 and BS4) significantly reduced the SIR rates for eight C sources compared to the effects of low application levels (BS1 and BS2) (Fig. 2). In a paddy soil, excessive use of manure may decrease the SIR rates due to accumulation of heavy metals (Lin et al. 2016). Bérard et al. (2014) found that soil SIR decreased with increasing soil Cd concentration. In the Taihu Lake Basin, Bian et al. (2015) reported that the concentrations of lead (Pb) and cadmium (Cd) in soil irrigated with BS exceeded the permissible limits set by Chinese government. Thus, the risk assessment of heavy metals in the soils applied with BS needs to be conducted in the future. According to the PCA, both of BS3 and BS4 were distinctly separated with MF and BS2 on the PC1 and with BS1 on the PC2, which indicated that high level of BS application significantly influenced the soil microbial functional diversity (Fig. 3). In the present study, soil AP and AK were significantly increased by high level of BS application, which would affect the soil SIR in the paddy field. The results of RDA analysis showed that soil AP and AK were the two main factors affecting soil microbial activity (Fig. 4). Manzoni et al. (2012) reported that the availability of soil P and N could confine the microbial C use efficiency.

In the present study, the bacterial community compositions in all the treatments were dominated by three phyla, including *Chloroflexi*, *Proteobacteria* and *Acidobacteria* (Fig. 5). This is in agreement with Guo et al. (2017), who found that the relative abundances of *Proteobacteria*, *Acidobacteria* and *Chloroflexi* were 37.3%, 19.5% and 12.7% in an agricultural soil, respectively. The phylum *Chloroflexi* is the most abundant bacterial phylum, and its average relative abundance is 28.5% in our study. Similarly, Chen et al. (2016) reported that the relative abundance of *Chloroflexi* was 30% in an arable soil in southeast China. During the rice season, the anaerobic environment associated with flooding is beneficial to anaerobic bacteria (Zhao et al. 2014a). Most of phyla *Chloroflexi* and *Proteobacteria* are facultative anaerobic bacteria (Chen et al. 2017a). The phylum *Acidobacteria* is an oligotrophic bacteria and favors the conditions with lower soil organic C (Fierer et al. 2007; Wang et al. 2017). Application of pig BS may increase the nitrification of paddy soil by increasing the relative abundance of *Nitrospirae*. The results from this study showed that relative abundance of *Nitrospirae* was 2.6–3.7 times higher in the soils receiving slurry than in that in the MF treatment (Fig. 5). Ammonium-N is the main form of N in pig BS and makes up 60–80% of TN in the BS, which is the substrate for nitrification. The results of PCoA showed that MF, BS1 and BS4 were clearly separated with BS2 and BS3 along the axis 1 (Fig. 6), indicating that moderate application of

BS could influence the compositions of soil microbial community. High level application of BS could suppress the soil microbial communities due to the accumulation of heavy metals (Bian et al. 2015; Caracciolo et al. 2015). Many studies have showed that soil pH was an important factor affecting soil microbial community compositions (Fierer and Jackson 2006; Xiong et al. 2012; An et al. 2019). However, in the present study, soil pH does not affect soil bacterial community compositions according to the RDA analysis (Fig. 6). The reason may be that soil pH was similar among all treatments. This is consistent with results of Zhao et al. (2014a) and Chen et al. (2016), who reported that soil pH was not correlated with soil bacterial communities due to no changes in soil pH among treatments. As shown in RDA analysis, soil AP and AK were the two main factors affecting soil bacterial community structure (Fig. 7). The reason may be that BS application changed the soil AP and AK, but had no effect on other soil properties (Table 2). Zhao et al. (2014b) found that soil organic matter (SOM), AK, TP and TN were significantly correlated with soil bacterial phyla taxa in a paddy soil in southeast China.

Conclusions

The results from the present study demonstrated that replacement of mineral fertilizer with BS could maintain the grain yields of rice in a single rice cropping system. The rice yield was not significantly affected by the application rate of BS. Application of BS increased soil available P and K concentrations compared to the application of only MF. Pig BS application changed the soil microbial activities and microbial community composition. The BS2 treatment had the highest average SIR. The BS application might increase soil nitrification potential through stimulating the growth of *Nitrospirae*. Our results also indicated that soil AP and AK were the main factors affecting soil microbial community. These results are helpful in establishing an alternative management practice with BS in paddy fields, which can reduce the use of chemical fertilizers and alleviate negative environmental impacts. This study revealed that the application of pig BS at 270 kg ha⁻¹ may be the effective practice for the rice production. To improve soil productivity while reducing environmental risks, a long-term field experiment is needed to verify the present findings.

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