



Effect of biogas slurry addition on soil properties, yields, and bacterial composition in the rice-rape rotation ecosystem over 3 years

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Abstract

Purpose Organic treatments may improve soil nutrient availability and ecological functions. This study aimed to determine the effect of biogas slurry (BS) on soil properties, yields, and bacterial community activity and to examine the main environmental factors impacting bacterial compositions in the ecosystem.

Materials and methods A 3-year field experiment was conducted in yellow soil under a rice-rape rotation to understand the effect of BS originating from anaerobically digested pig waste on soil chemical and bacterial compositions.

Results and discussion Compared with an inorganic nutrient treatment and a control, adding BS at a moderate dose (BS6, 165.1 t ha⁻¹) positively affected the rice and rape yields, soil fertility, and bacterial diversity. The BS was more suitable than chemical fertilizer for maintaining agricultural soil sustainability, especially the BS6 and BS7 treatments (165.1 and 182.1 t ha⁻¹, respectively), by improving nutrient content, increasing soil pH, and promoting soil crumb structure formation. The relative abundance of *Actinobacteria* in BS6 was decreased by 37.9% compared with the untreated soil and 27.7% compared with the fertilizer treatment, while the relative abundance of *Nitrospirae* in BS6 increased by 41.2% and 43.5% compared with these treatments, respectively. Redundancy analyses (RDA) showed that pH, rape yield, cation exchange capacity (CEC), and total nitrogen (TN) were significantly correlated with soil bacterial community composition and explained 56.0%, 46.4%, 43.2%, and 34.9% of the total community variability, respectively. The soil bacterial diversity depended mainly on soil pH, and crop rotation played an important role in changing the bacterial community.

Conclusions The soil bacterial composition was clearly altered after 3 years of BS treatments. These increases in bacterial diversity could be of ecological significance in maintaining soil fertility and functionality.

Keywords Bacterial composition · Biogas slurry · Pig waste · Rice-rape rotation

1 Introduction

Improving soil quality and increasing soil productivity are required to meet future food needs. Decreasing soil nutrient

availability or soil ecological function may cause soil degradation, resulting in these soils losing their ability to sustain production (Wang et al. 1999). Therefore, the application of organic amendments might prove to be a feasible option for the improvement of nutrient availability in the soil (Chen et al. 2016).

Biogas slurry (BS) originating from anaerobically digested animal waste is a good source of plant nutrients and is thus considered a nutrient-enriched organic fertilizer for crop production (Abubaker et al. 2013; Ozores-Hampton et al. 2011; Win et al. 2014). BS is used extensively to maintain sustainability in agricultural soils because (1) more nutrients are available during the period after its addition (Zirkler et al. 2014); (2) soil C and N are mineralized (Galvez et al. 2012); (3) greenhouse gas emissions are reduced (Terhoeven-Urselmans et al. 2009); and (4) nitrogen use efficiency

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(Sieling et al. 2013) and crop yield (Abubaker et al. 2012) are improved. Applying BS improves soil properties by increasing the nutrient content and bioactive substances, such as humic acids, consequently increasing plant productivity. Therefore, BS has attracted considerable attention for effectively improving soil fertility and ecological functions.

Soil microorganisms play key roles in promoting matter and energy flow conversion (Prosser et al. 2007; Zheng et al. 2016) and are sensitive to changes in soil conditions (Abubaker et al. 2013; Jiang et al. 2016; Zheng et al. 2016). Soil fertility and ecological functions are governed largely by the activity of soil microorganisms that regulate soil physicochemical processes (Elzobair et al. 2016; Wang et al. 2016). BS may affect soil microbial biomass when applied to soil (Odlare et al. 2008). Microbial community structures differ depending on various soil properties (Garbeva et al. 2004). Abubaker et al. (2013) showed that the effects of biogas residues and cattle slurry on bacterial community structures were greatest in sandy soil, indicating that bacterial communities in soils with lower biomass are more sensitive to organic inputs. Jiang et al. (2016) reported changes in microbial community structure in response to various crop rotations. Soil quality is governed primarily by the tillage practices used to meet the soil's contrasting physical and hydrological requirements of rice and rape crops, which in turn induce changes in the microbial community (Erenstein and Laxmi 2008). Most previous studies examining the effects of organic fertilizer on soil have focused on rice systems (Win et al. 2014), including rice-wheat rotation (Timsina and Connor 2001) and rice-legume-rice rotation (Jiang et al. 2016), while little research has considered the effects of organic fertilizer on microbial community shifts in a rice-rape system.

BS has advantages and disadvantages in its effects on soil functions. Field experiments and incubation tests have determined that BS positively affects plant growth, soil properties, and soil microbes. However, a previous study also showed that the lower C input from BS additions reduced soil microbial activity (Wentzel et al. 2015). Microbial biomass variability in rice-rape cropping ecosystems is complex and poorly understood. BS varies considerably in raw substrates containing varying hazardous elements (Abubaker et al. 2013). BS's effect on soil microorganisms depends on dose and aging time. Thus, the effects of adding BS originating from anaerobically digested pig waste to arable soils at different doses must be evaluated to ensure that soil functions are not adversely affected.

In this study, field experiments were conducted for three consecutive years, each with two crops of rice and rape grown in sequence. This study aimed to assess the effect of BS originating from anaerobically digested pig waste on soil bacterial community activity and composition under field conditions with the following main objectives: (1) determine the effect of adding BS on the response of crop yields in yellow soil, (2)

estimate the changes in soil properties over 3 years, (3) investigate the responses of the bacterial communities and functions to the added BS after 3 years, and (4) evaluate the link between the bacterial community and environmental factors.

2 Methods and materials

2.1 Environmental materials

Field experiments were conducted from May 2009 to May 2012 on yellow soil in Heishi Village Qionglai City, Sichuan Province (30° 21' 6" N, 103° 32' 45" E), located in the southwest district of Sichuan in Southwest China. The local climate is a humid subtropical monsoon climate with an annual precipitation of 1117.3 mm and a mean annual temperature of 16.3 °C. The area receives sunlight 1107.9 h a year. Yellow soil contains large amounts of decaying organic material and can easily be obtained from cultivated farm land. The soil used here was silt loam composed of 37% sand, 36% silt, and 27% clay. The soil pH, total nitrogen (TN), available nitrogen (AN), organic matter (SOM), available phosphorus (AP), available potassium (AK), and cation exchange capacity (CEC) were 4.80, 2.11 g kg⁻¹, 173.4 mg kg⁻¹, 35.65 g kg⁻¹, 57.32 mg kg⁻¹, 31.48 mg kg⁻¹, and 13.26 cmol kg⁻¹, respectively.

BS originating from anaerobically digested pig waste was collected from a pig farm. The BS had a pH of 7.13, with 876.23 mg L⁻¹ TN, 634.7 mg L⁻¹ NH₃-N, 7.27 mg L⁻¹ NO₃-N, 83.19 mg L⁻¹ total phosphorus (TP), and 378.5 mg L⁻¹ total potassium (TK). The rice (*Oryza sativa* L., variety Yixiang-481) and the oilseed rape (*Brassica napus* L., variety Yiyou-15) were grown in sequence.

2.2 Experimental design and treatments

Field experiments were set up in a randomized block design (RBD) with 12 treatments each performed in triplicate and repeated for 3 years on the same plots. Treatments included different doses of BS (BS1–BS10, 59.9–264.4 t ha⁻¹), fertilizer (FT), and untreated soil (UT) (Table 1). BS was applied on a fresh weight basis. N, P, K, and B were applied via urea, calcium superphosphate, potassium chloride, and sodium borate. The chemical fertilizer doses for the rice were 0.06 t ha⁻¹ N, 0.10 t ha⁻¹ P₂O₅, and 0.07 t ha⁻¹ K₂O, and those for the rape were 0.25 t ha⁻¹ N, 0.27 t ha⁻¹ P₂O₅, 0.19 t ha⁻¹ K₂O, and 0.007 t ha⁻¹ B (boric fertilizer). A full dose of N, P, and K fertilizer was applied at the rice tillering stage, B was applied upon rape sowing, and the remaining fertilizer was applied at the tillering and panicle stages. Conventional farm management was consistently performed for the rice production system.

Table 1 Treatment application schedule for two cropping cycles of rice and rape (t ha^{-1})

Treatments	The total amount of biogas slurry	During the period of rice growth	During the period of rape growth
UT	0	0	0
FT	–	N 0.06, P ₂ O ₅ 0.10, K ₂ O: 0.07	N 0.25, P ₂ O ₅ 0.27, K ₂ O 0.19, B 0.007
BS1	59.9	9.8	50.2
BS2	86.5	17.5	69.0
BS3	113.1	25.3	87.9
BS4	132.3	33.0	99.3
BS5	147.8	40.8	107.0
BS6	165.1	48.5	116.6
BS7	182.1	56.3	125.9
BS8	208.7	64.0	144.7
BS9	236.5	73.0	163.5
BS10	264.4	82.0	182.4

UT untreated soil, FT fertilizer addition, BS1–BS10 different levels of biogas slurry additions, B boric fertilizer

The yield was recorded as kg ha^{-1} dry weight. Soil samples were collected after harvesting the rape in May 2012. After removing large debris and plant remnants, soil samples were collected from the top layer (0–20 cm) using a shovel sterilized with alcohol. Each soil sample was divided into two portions. The fresh part was stored at $-80\text{ }^{\circ}\text{C}$ for DNA isolation; the other was air-dried and pass through 0.15-mm and 2-mm mesh sieves to determine the chemical properties.

2.3 Soil chemical analysis

The soil samples were analyzed per the procedures described by Bao (2008). Soil pH was measured using a pH meter (1:5 w/v soil/water). AN was measured using the microdiffusion technique. AP was determined using NaHCO₃ extraction and the molybdenum blue colorimetric method. AK was extracted using 1 M CH₃COONH₄ and determined by flame photometry. TN contents were determined using a total N analyzer. SOM was determined by the oxidation technique. All chemical determinations were performed in triplicate.

2.4 Bacterial abundance analyses

Soil total DNA was extracted from soil samples (0.25 g wet weight) using a MoBio Power Soil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) per the manufacturer's instruction. The extracted DNA was stored at $-20\text{ }^{\circ}\text{C}$ until use. The V4 hypervariable region of the 16S rRNA gene was amplified using a set of two eubacterial primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGAC

TACHVGGGTWTCTAAT-3'). Three replicates of the DNA extracted from each sample were amplified by PCR. PCR was conducted using a Model C1000 PCR thermal cycler (Bio-Rad, Richmond, CA, USA). The total volume of the reaction mixture was 50 μL , consisting of 0.5 μL of each primer (50 pmol each), 5 μL of 2.5 mmol L⁻¹ dNTP mixture, 5 μL of 10 \times Ex Taq buffer (20 mmol L⁻¹ Mg²⁺; TaKaRa Inc., Dalian, China), 0.25 μL of Ex Taq DNA polymerase (TaKaRa), 1 μL of the environmental DNA template, and 37.75 μL of Milli-Q water. Cycle conditions for the PCR amplification were as follows: initial denaturation at 94 $^{\circ}\text{C}$ for 3 min, followed by 30 cycles of denaturation at 94 $^{\circ}\text{C}$ for 30 s, annealing at 50 $^{\circ}\text{C}$ for 30 s and extension at 72 $^{\circ}\text{C}$ for 30 s, extension at 72 $^{\circ}\text{C}$ for 30 s, and an extension step at 72 $^{\circ}\text{C}$ for 10 min after the cycling was complete. All samples were amplified in triplicate, and no-template controls were included in all steps. Next, 5 μL of each reaction mixture was analyzed on 2% agarose gel, and the PCR products were gel-purified using a QIAquick Gel Extraction Kit (Qiagen). The purified PCR amplicons were combined in equimolar ratios in a single tube after determining each amplicon's concentration using a Quant-iT PicoGreen dsDNA reagent kit (Life Technologies, Merelbeke, Belgium). The purified amplicons were then pooled in equimolar amounts, vacuum dried, and sequenced using an Illumina MiSeq platform at Macrogen, Inc. (<http://www.macrogen.com>, Seoul, South Korea). Sequence analysis was performed using Quantitative Insights Into Microbial Ecology (QIIME) (Fadrosh et al. 2014). Sequence reads belonging to the samples were extracted from the data obtained from the Illumina MiSeq platform. The primers were removed, and the sequences were trimmed to remove low-quality sequences. Singleton sequences were discarded. Operational taxonomic units (OTUs) were clustered based on 97% sequence similarity using the furthest neighbor algorithm at the phylum levels (Bodenhausen et al. 2013). Rarefaction curves and alpha diversity indices, which included richness indexes (Chao1 and abundance-based coverage estimator [ACE]) and a microbial community diversity index (the Shannon index), were calculated in Mothur based on the obtained sequencing (Yousuf et al. 2012).

2.5 Statistical analysis

The results were compared among samples using one-way analysis of variance (ANOVA), and paired comparisons of treatment means were achieved by Tukey's procedure at $P < 0.05$ using SPSS software (version 19.0). The beta diversity analysis was performed using UniFrac to compare the results of the principal component analysis (PCA) using the community ecology package, R-forge (Vegan 2.0 package). Correlations between environmental variables and community composition were analyzed using a redundancy analysis (RDA) by CANOCO (version 4.5). A manual forward

selection procedure was implemented in the RDA to determine the significance of environmental variables ($P < 0.05$) using a Monte Carlo test with 499 permutations.

3 Results

3.1 Effects of adding BS on soil properties and yields over 3 years

BS application significantly ($P < 0.05$) increased soil properties and crop yields compared with the UT (Tables 2 and 3). The pH increased after adding BS, especially in the BS10 treatment. The pH was slightly reduced in the FT compared with that in the control. The AN, AP, AK, SOM, and TN and CEC concentrations increased then decreased as the BS dose increased compared with those in the UT and FT. These changes in soil properties were greatest in the BS6 treatment. AP, AK, SOM, and TN concentrations did not significantly differ between the UT and FT. The BS6 treatment soil yielded more rice than did the other treatments, while the rape yield was the highest in the BS7 treatment (Table 3). However, the yield values did not significantly differ between the BS6 and BS7 treatments. Compared with the control and FT, the rape yields increased by 39.4–71.5% and 16.6–60.7%, while the rice yields increased by 39.9–69.8% and –16.7–41.4%, respectively, after adding BS (BS1–BS10). The trends for the rape yields in BS5 to BS10 were unremarkable.

3.2 Bacterial diversity and correlation between bacterial diversity and soil properties

Table 4 shows the alterations in the bacterial richness indices of the bacterial communities in the soils treated with different

BS doses over 3 years. The Chao1 and ACE indices increased then decreased with increasing BS doses, while the Chao1 in BS5 and BS6 did not significantly differ. BS6 contained the highest bacterial richness, which was significantly higher than that in the control. The Shannon diversity index initially increased then decreased with increasing BS doses; however, no statistically significant changes were observed in the Shannon index in treatments BS4 to BS7. The BS6 treatment soil had a higher Shannon index value than that in the other treatments. The present study showed that adding BS at a relatively moderate dose (BS6, 165.1 t ha⁻¹) increased bacterial richness and diversity compared with those in the control, while adding BS at a high dose detrimentally affected bacterial growth.

The relationships between bacterial diversity and soil properties are shown in Table 5. The Chao1 and ACE values were significantly positively correlated with AN and SOM. The Shannon index was positively correlated with AN, AP, and SOM.

3.3 The abundance and composition of bacterial communities

The relative abundances of the soil bacterial phyla in all treatments are presented in Fig. 1. *Chloroflexi*, *Proteobacteria*, *Acidobacteria*, and *Actinobacteria* were the most dominant phyla, accounting for 72.92–78.82% of the total bacterial 16S rRNA gene sequences in all treatments over 3 years. *Chloroflexi* was the most represented phylum (27.42–36.62%) in the bacterial sequences in all treatments, except for the control. *Firmicutes*, *Nitrospirae*, TM7, *Gemmatimonadetes*, *Planctomycetes*, *Bacteroidetes*, *Verrucomicrobia*, AD3, and OD1 were identified in all soils but at relatively low abundances (< 5%).

Table 2 Soil properties and crop yields in soil under different fertilizer consecutive addition after 3 years

Treatments	pH	AN (mg kg ⁻¹)	AP (mg kg ⁻¹)	AK (mg kg ⁻¹)	SOM (g kg ⁻¹)	TN (g kg ⁻¹)	CEC (cmol kg ⁻¹)
UT	5.35 ± 0.24b	131.3 ± 11.3c	30.85 ± 1.32d	20.64 ± 1.06c	20.44 ± 0.16c	1.09 ± 0.04d	12.4 ± 1.01c
FT	5.26 ± 0.44b	126.0 ± 9.50d	31.46 ± 2.07d	21.42 ± 1.36c	21.54 ± 2.22bc	1.06 ± 0.13d	13.1 ± 1.22c
BS1	5.36 ± 0.14b	132.4 ± 11.2c	33.34 ± 2.14c	22.24 ± 2.14bc	23.39 ± 1.28bc	1.19 ± 0.05c	12.9 ± 0.91c
BS2	5.39 ± 0.33b	134.5 ± 10.1c	33.41 ± 1.18c	22.29 ± 1.06bc	23.43 ± 2.15bc	1.18 ± 0.12c	13.5 ± 1.23c
BS3	5.41 ± 0.23ab	137.3 ± 13.5c	34.28 ± 2.11c	22.56 ± 1.11bc	23.94 ± 2.05bc	1.19 ± 0.09c	14.6 ± 0.43b
BS4	5.44 ± 0.45ab	139.3 ± 8.56c	36.12 ± 3.12b	23.64 ± 2.13b	24.49 ± 2.18b	1.22 ± 0.12c	14.8 ± 0.63b
BS5	5.46 ± 0.12ab	143.8 ± 7.51b	37.85 ± 1.85b	24.74 ± 0.92b	25.05 ± 1.14b	1.35 ± 0.03ab	15.7 ± 1.41a
BS6	5.49 ± 0.01ab	155.8 ± 9.49a	39.59 ± 1.63a	26.02 ± 1.16a	27.03 ± 1.17a	1.48 ± 0.12a	15.9 ± 0.92a
BS7	5.50 ± 0.32ab	144.6 ± 13.5b	37.51 ± 3.25b	24.76 ± 0.81b	25.27 ± 3.02b	1.38 ± 0.07ab	16.3 ± 1.19a
BS8	5.53 ± 0.22ab	137.8 ± 11.4c	35.53 ± 2.11bc	24.35 ± 1.08b	22.97 ± 0.13bc	1.35 ± 0.09ab	15.6 ± 0.54a
BS9	5.57 ± 0.63ab	130.3 ± 16.6c	34.48 ± 1.18c	23.81 ± 1.14b	22.88 ± 2.19bc	1.33 ± 0.06ab	15.4 ± 1.32ab
BS10	5.61 ± 0.09a	127.6 ± 19.8d	33.99 ± 1.29c	23.52 ± 1.22b	21.68 ± 1.27bc	1.31 ± 0.14b	15.2 ± 0.75ab

UT untreated soil, FT fertilizer addition, and BS1–BS10 different levels of biogas slurry additions. Values are mean ± standard deviation ($N = 3$). Different letters in the same column indicate significant differences at $P < 0.05$ levels

Table 3 Crop yields in soil under different fertilizer consecutive addition after 3 years

Treatments	Rice yield (kg ha ⁻¹)	Rape yield (kg ha ⁻¹)
UT	2496.0 ± 231.9e	920.7 ± 26.5d
FT	4837.5 ± 321.3d	1266.7 ± 175.2d
BS1	4146.4 ± 133.1d	1519.7 ± 138.5cd
BS2	4276.3 ± 432.0d	1840.0 ± 155.8cd
BS3	5109.8 ± 242.8c	2333.4 ± 120.5c
BS4	5943.4 ± 124.9c	2753.3 ± 180.3b
BS5	7279.6 ± 532.5b	2863.3 ± 111.5ab
BS6	8255.5 ± 533.6a	3050.0 ± 275.5ab
BS7	8167.6 ± 642.2a	3226.7 ± 175.7a
BS8	6818.8 ± 243.5b	3140.0 ± 150.8ab
BS9	5771.6 ± 642.3c	2966.7 ± 107.8ab
BS10	5093.1 ± 124.8c	2886.7 ± 41.3ab

UT untreated soil, FT fertilizer addition, and BS1~BS10 different levels of biogas slurry additions. Values are mean ± standard deviation ($N=3$). Different letters in the same column indicate significant differences at $P < 0.05$ levels

Compared with those in the control, the relative abundances of *Chloroflexi*, *Acidobacteria*, *Nitrospirae*, *Planctomycetes*, *Verrucomicrobia*, and AD3 increased after BS addition, whereas the relative abundances of *Proteobacteria*, *Actinobacteria*, *Firmicutes*, TM7, and *Bacteroidetes* decreased after BS addition.

The different BS doses changed the relative abundances of the main phyla (> 1%). The relative abundances of *Chloroflexi* and *Acidobacteria* increased with increasing BS dose, whereas that of *Firmicutes* decreased. With increasing BS addition, the abundances of *Actinobacteria*, *Proteobacteria*, TM7, and ODI decreased and then increased and were lowest in BS1

Table 4 Changes in the diversity index and richness indices of the soil bacterial communities in the different treatments

Treatments	ACE	Chao1	Shannon
UT	40,486~50370c	37,068~41756c	10.44~12.46c
FT	45,394~48340c	38,869~41143bc	10.97~12.05b
BS1	45,827~51887bc	39,552~44828b	10.80~12.22b
BS2	48,120~51586bc	42,056~42966b	10.67~12.44b
BS3	47,184~48902bc	39,223~41713bc	10.36~12.81b
BS4	43,459~57135b	41,782~43570b	9.68~13.77a
BS5	49,588~51756b	41,719~44601ab	10.36~13.24a
BS6	48,190~56834a	44,042~46708a	11.41~12.33a
BS7	42,408~47106c	38,358~39206c	11.17~12.23a
BS8	42,327~44169c	36,352~39,024cd	9.36~13.48c
BS9	40,868~42936d	35,005~38,011cd	10.22~12.32d
BS10	40,394~43296d	32,426~37882d	9.52~13.00d

UT untreated soil, FT fertilizer addition, and BS1~BS10 different levels of biogas slurry additions. Different letters in the same column indicate significant differences at $P < 0.05$ levels

Table 5 Correlation coefficients between soil properties and bacterial diversity and richness indices

Items	ACE	Chao1	Shannon
pH	-0.440	-0.438	-0.221
AN	0.585*	0.631*	0.840**
AP	0.371	0.400	0.687*
AK	0.150	0.179	0.451
SOM	0.599*	0.616*	0.813**
TN	0.007	0.046	0.319
CEC	0.067	0.070	0.295

**Significant at 0.01 level ($P < 0.01$), *Significant at 0.05 level ($P < 0.05$)

and highest in BS6. *Nitrospirae* abundance decreased and then increased and was lowest in BS10 and highest in BS3. The abundances of *Bacteroidetes*, *Gemmatimonadetes*, and AD3 initially increased and then decreased with increasing BS addition. *Planctomycetes* and *Verrucomicrobia* showed no response to the BS treatments.

3.4 Relationships between the bacterial community and environmental factors

Principal components analysis (PCA) showed that the bacterial community composition significantly differed between treatments, and 66.7% and 18.4% of the total bacterial variability were explained by the first two components, respectively (Fig. 2). The RDA showed that pH ($F = 12.7$, $P = 0.002$), rape yield ($F = 8.67$, $P = 0.02$), CEC ($F = 7.60$, $P = 0.002$), and TN ($F = 5.35$, $P = 0.01$) were significantly correlated with soil bacterial community composition and explained 56.0, 46.4, 43.2,

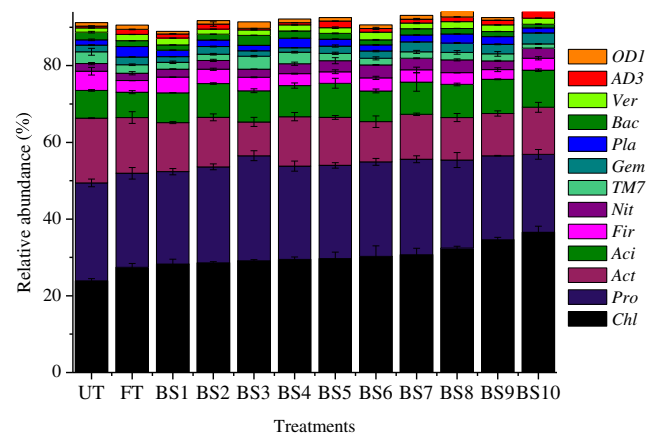


Fig. 1 Relative sequence abundance of different phyla in soils (relative abundance > 1%). Other represents all phyla with relative sequence abundances below 1%. *Chl* *Chloroflexi*, *Pro* *Proteobacteria*, *Act* *Actinobacteria*, *Acti* *Acidobacteria*, *Fir* *Firmicutes*, *Nit* *Nitrospirae*, *Gem* *Gemmatimonadetes*, *Pla* *Planctomycetes*, *Bac* *Bacteroidetes*, *Ver* *Verrucomicrobia*. UT represents untreated soil, FT represents fertilizer addition, and BS1–10 represent different levels of biogas slurry additions. Vertical bars represent the standard error ($n = 3$)

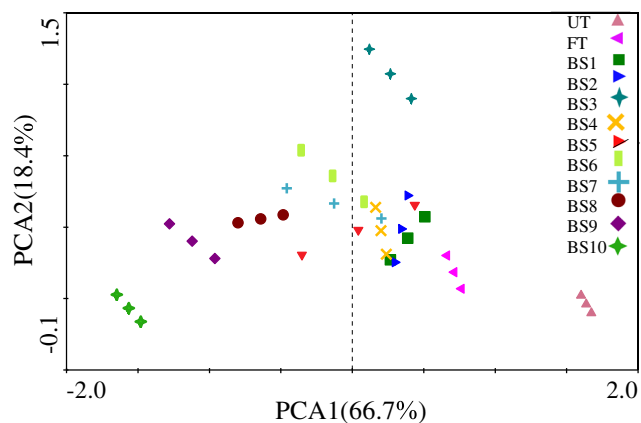


Fig. 2 Principal component analyses (PCA) of the bacterial community in soils from the different treatments. UT represents untreated soil, FT represents fertilizer addition, and BS1–10 represent different levels of biogas slurry additions

and 34.9% of the total community variability, respectively (Fig. 3). The first two RDA axes explained 72.9% and 16.3% of the total variation in the data, respectively.

4 Discussion

4.1 BS treatment increased soil nutrient availability and soil productivity

Several studies have shown significant agronomic benefits from adding BS (Banik and Nandi 2004; Bharde et al. 2003; Shankarappa et al. 2012; Wentzel et al. 2015). BS is produced after anaerobic digestion, and it has a high pH (pH = 7.13) and nutrient content (Wentzel et al. 2015). These characteristics significantly positively affect soil properties and productivity (Yan-li 2012). In this study, a similar result was observed. BS obtained from plant fermentation also has valuable agronomic properties.

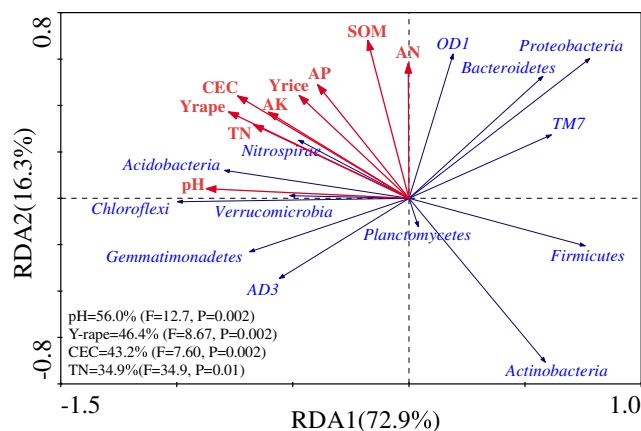


Fig. 3 Redundancy analyses (RDA) of the correlations between the environmental factors and bacterial community. AN available nitrogen, AP available phosphorus, AK available potassium, TN total nitrogen, CEC cation exchange capacity, SOM organic matter, Yrape rape yield, Yrice rice yield

Notably, BS was more suitable than FT for maintaining agricultural soil sustainability, especially in the BS6 and BS7 treatments. Adding BS may have affected the organic C composition differently than did the FT (Abubaker et al. 2013). BS treatment released nutrients more slowly than did the FT, and this benefit accrued over a relatively long time. This result indicated that slowly mineralized nutrients from BS could meet the crops’ nutritional requirements (Bharde et al. 2003). Additionally, the pH was slightly lower in the FT than in the BS treatments and the control. This trend demonstrated that FT inhibited soil ecological functions compared with the control and BS treatments due to acidification (Xu et al. 2014; Zhou et al. 2016). BS addition increased the soil pH, which is beneficial for reducing acidification.

The SOM, AN, AP, AK, and TN concentrations in the soil increased then decreased with increasing BS doses. This pattern also suggested that the extent of the agricultural benefit from the BS was dose-dependent. A higher BS dose was not always better due to the reduction in soil nutrients, which was consistent with previous studies (Yan-li 2012; Yi-qi et al. 2016). Organic additives play important roles in improving SOM concentration and soil structure (Ladha et al. 2004). In our study, the added BS increased the SOM content, which was consistent with previous study result (Ozores-Hampton et al. 2011). However, Wentzel et al. (2015) and Abubaker et al. (2013) showed that BS treatment did not strongly impact SOM content because the impact of the BS on SOM content varied with the original soil C. Win et al. (2014) reported higher CH₄ emissions with higher BS doses, showing a higher stimulatory effect on soil CH₄ emissions with relatively low soil C content (28 g kg⁻¹). In our study, the SOM contents were relatively low (20.64–27.03 g kg⁻¹); thus, additional C input via adding BS to the soil at high doses may more strongly affect the CH₄. This may explain the lower SOM content in the BS treatments at higher doses. Furthermore, the reduced nutrients in the BS treatments at higher doses may have been caused by the low CEC in the soil with high BS doses. Higher BS doses over longer periods had insignificant effects on soil crumb structure because the soil function was destroyed. This in turn affects major processes, including biogeochemical nutrient cycling and soil quality (Chodak et al. 2013; Gu et al. 2017). Accordingly, nutrient loss due to low nutrient retention is a potential risk of enhanced BS treatment (Niyungeko et al. 2018). These results demonstrated that adding BS effectively improved soil properties and crop yields, and soil nutrients in the BS6 and BS7 treatments best improved the plant and soil fertility.

4.2 Effects of BS treatment on bacterial diversity

Soil microbial diversity is important for agricultural ecosystems to properly function. Soil bacteria are sensitive to soil property changes induced by applying fertilizer. The shifts in

bacterial community compositions depend on fertilizer properties such as type and dose (Marschner et al. 2003).

In this study, applying BS induced changes in the bacterial communities. Adding BS generally enhanced the number of nutrient sources for the microorganisms, which could explain the BS's benefit to the bacterial communities (Win et al. 2014). BS addition may have affected the bacterial communities differently than did the FT and the control due to the increased C content and altered C composition, thus altering the bacterial community (Abubaker et al. 2013). Organic C enhances soil ecological functions. BS produced from anaerobic digestion and with a higher C content more efficiently increased the C content and stability.

The extent of the increased bacterial growth varied with the BS dose. High doses of BS at 236.5–264.4 t ha⁻¹ (BS9–BS10) poorly affected bacterial growth, while a relatively moderate dose of 165.1 t ha⁻¹ (BS6) benefitted the bacteria. This result was consistent with that of a previous study (Zheng et al. 2015) and most likely resulted from reduced nutrients and CEC in the BS9–BS10 treatments after 3 years (Tables 2 and 4).

The positive correlation between the diversity index (Shannon) and richness index (i.e., Chao1 and ACE) values indicated that bacterial diversity was lower in the soil with low nutrient contents. Bacteria may also act as nutrient sources in soils and are involved in maintaining soil structure (Chodak et al. 2013). These interactions in turn affect soil quality and functions. Additionally, low CEC negatively affects bacteria due to low absorption, which does not affect bacterial retention in runoff. The results showed that moderate BS (165.1 t ha⁻¹) significantly increased the bacterial diversity and richness compared with those of the FT and the control in yellow soil.

4.3 Effect of BS treatment on bacterial communities

The results showed that adding BS significantly affected the bacterial communities. BS addition induced alterations in bacterial community distributions, which may have resulted from the additional nutrients that the BS provided, which could enhance specific bacterial communities (Bending et al. 2002).

The relative abundance of *Chloroflexi* increased significantly in all BS treatments compared with that in the control. *Chloroflexi* was the phylum most sensitive to BS, with relative abundances increasing by 34.6% and 25.0% compared with those in the UT and FT, respectively, which was consistent with the results of a previous study (Chodak et al. 2013). The higher *Chloroflexi* diversity in the BS-treated soil was mainly due to the higher pH. Chodak et al. (2013) reported decreased soil bacterial community diversity in acidic soils compared with less acidic soils. Higher soil pH favored *Chloroflexi*, which was more abundant in neutral soil (Xu et al. 2014).

The relative abundances of *Proteobacteria* initially increased then decreased as the BS dose increased and were

highest in the BS3 treatment. *Proteobacterial species* colonize nutrient-rich environments (Xu et al. 2016); thus, the added BS favored *Proteobacteria* (Kuffner et al. 2012). BS treatment positively affected *Proteobacteria*, and BS3 (113.1 t ha⁻¹) was more suitable for this phylum than were the other treatments.

The relative abundance of *Acidobacteria* increased, possibly because of the higher C content in the BS treatments. Previous studies showed that *Acidobacteria* may be able to adapt to environments with large varieties of C sources, such as those in BS treatments (Xu et al. 2016). Additionally, a previous study showed that the increased pH or soil organic matter may increase the *Acidobacterial* abundance (Navarrete et al. 2013). These direct effects require further study because different *Acidobacterial* subgroups respond differently to abiotic soil factors.

The relative abundances of *Actinobacteria* in BS6 decreased by 37.9% and 27.7% compared with those in the UT and FT, respectively. The relative abundance of *Actinobacteria* was reduced in the BS-treated soil. The soil in BS6 had lower relative abundances of *Actinobacteria* than those in the other treatments. This trend showed that BS reduced the activities of microbes involved in C cycling (Har-Peled et al. 2015), especially in BS6, suggesting a lower SOM degradation rate and, subsequently, slower organic C turnover (Har-Peled et al. 2015; Zheng et al. 2016).

Firmicutes and *Bacteroidetes* play significant roles in degrading organic materials (Nozomi et al. 2009). *Bacteroidetes* are reported to be enriched in soils with high C content (Zheng et al. 2016). The relative abundances of *Firmicutes* and *Bacteroidetes* decreased after adding BS. As the BS dose increased, the relative abundance of *Firmicutes* decreased, while that of *Bacteroidetes* increased then decreased and was highest in the BS3 treatment. The increase in *Bacteroidetes* in BS3 indicated that the BS increased the C resources. The reduction in *Firmicutes* indicated that the C availability decreased after incorporating BS. These bacterial community changes demonstrated that the BS3 treatment-induced C resources become more recalcitrant.

Compared with those in the UT and FT, the relative abundances of *Nitrospirae* in BS6 increased by 41.2% and 43.5%, respectively. The increase in *Nitrospirae* sequences in the BS-treated soil indicated that BS might increase N emissions (Xu et al. 2016) although not all *Nitrospirae* members are intricately linked to N cycling. Adding BS increased rape growth, thus shifting the soil bacterial community and, therefore, likely increased N cycling. This may be attributed to crop growth due to improved soil N availability (Xu et al. 2014).

4.4 Relationships between the bacterial community and environmental factors

Due to their sensitivity to changes in soil conditions, soil bacterial community abundances have been used to indicate

changes in soil properties (Shen et al. 2010; Zhao et al. 2014). Adding BS to soil significantly affects soil microorganisms via changes in soil nutrients (Wang et al. 2015).

Previous studies showed that soil pH was a major factor determining soil bacterial communities (Griffiths et al. 2011; Chodak et al. 2013; Geisseler and Scow 2014; Stroobants et al. 2014; Xu et al. 2014). In this study, variations in soil pH induced by adding BS were also central factors in shifting the soil bacterial community, explaining 56.0% of the total variation. Rape growth also affected the soil bacterial community, which was consistent with the findings of previous studies (Xu et al. 2014; Jiang et al. 2016; Zhou et al. 2016). BS addition increased rape growth by improving nutrient availability, resulting in a shift in soil bacterial communities (Xu et al. 2014). This result demonstrated that plant growth contributes less than do the chemical properties of BS to variations in soil bacterial communities; however, this should not be generalized, as plant root systems vary greatly among species. In this study, the CEC may have affected bacterial dynamics by adjusting the soil's absorption capacity. These results are consistent with those discussed in Section 4.2. The RDA showed that soil bacterial biomass and community composition were also correlated with TN, which was similar to observations in previous studies (Wang et al. 2015; Jiang et al. 2016). Changes in TN induced a shift in the soil bacterial community by increasing crop yields and N availability. The RDA results indicated that BS could shift the soil bacterial community by changing soil chemical properties, which might modulate N cycling.

5 Conclusions

BS was found to be more suitable than chemical fertilizer for maintaining the sustainability of agricultural soils, especially in the BS6 and BS7 treatments (soils treated with BS doses of 165.1 and 182.1 t ha⁻¹, respectively), by improving nutrient content, increasing soil pH, and promoting the formation of soil crumb structure. BS application at a relatively moderate dose (BS6, 165.1 t ha⁻¹) increased bacterial diversity and richness compared with those the control in yellow soil. Compared with that in the untreated soil and that in the fertilizer treatment, the relative abundance of *Actinobacteria* in BS6 decreased by 37.9% and 27.7%, respectively, whereas that of *Nitrospirae* in BS6 increased by 41.2% and 43.5%, respectively. The results indicated that BS addition probably reduced C cycling and increased N emissions in a rice-rape cropping system. RDA showed that pH and crop rotation play central roles in changing bacterial community. Our results showed clear changes in both soil fertility and bacterial community structure after 3 years in BS amendments. The observed increases in soil nutrient and bacterial diversity could

be of ecological significance in maintaining the stability and functionality of soil.

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