

*Full Length Research Paper*

# **Effects of biogas slurry and conventional fertilizer on the abundance, diversity, and function of the soil microbe community in continuously cropped Chinese chives**

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The effects of biogas slurry and conventional chemical fertilizer as topdressings on relative abundance and community diversity of soil microbes in fields under continuous cropping of Chinese chives were investigated. It was found that topdressing treatment altered soil bacteria community and diversity, where relative abundance and taxonomic diversity were greater in the biogas slurry treatment. Although average well color development was higher in the conventional fertilizer treatment than in the biogas slurry treatment and the control, indicating improved functional diversity of the carbon-using soil microorganism community, taxonomic diversity decreased (Shannon diversity and Chao 1 indexes). Of 24 bacterial genera that had a relative abundance greater than 1%, it was found that relative abundance of *Saccharibacteria\_genera\_incertae\_sedis* and Gp6 was correlated with soil organic matter content ( $r = 0.804$ ,  $P < 0.01$  and  $r = -0.85$ ,  $P < 0.01$ , respectively). The organic matter content in biogas slurry is richer than fertilizer. The result showed that biogas slurry as topdressings may be a useful biological fertilizer in sustainable continuous cropping systems.

**Key words:** Bacterial community, biogas slurry, Chinese chives, continuous cropping.

## **INTRODUCTION**

Chinese chives (*Allium tuberosum* Rottler ex Spreng.) is a vegetable traditionally favored by consumers in China due to its unique flavor and high nutritional value (Zhang et al., 2013). Continuous cultivation of Chinese chives has resulted in decreased yield and quality (Wang et al.,

2006) and while methods for their improvement have received little attention, it has been shown that biogas slurry applied as a topdressing increases chive quality through greater leaf uniformity and length, reduced number of dead leaf-tips, enhanced resistance to

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**Table 1.** Biogas slurry physicochemical properties.

Property	Contents (mg/L)	Property	Contents (mg/L)
Organic carbon	162	Total nitrogen	281
Ammonium nitrogen	269	Nitrate nitrogen	0.32
Total phosphorus	54	Total potassium	188

disease, and lower abundance of insect pests (Sun et al., 2012).

Biogas slurry, which is rich in soluble inorganic salts and biochemical products of anaerobic fermentation (Arthurson, 2009), has been shown to have positive effects on plant yield and soil chemical, physical and soil microbial biomass characteristics in artificial incubation (Sanger et al., 2011), greenhouse pot (Andruschkewitsch et al., 2013), and short-term field (Bachmann et al., 2011; Johansen et al., 2013) experiments.

Since effects on soil microbial community composition and diversity of biogas slurry applications to Chinese chives are unclear, the objectives of this study were to quantify microbial activity and soil organic carbon (C), total nitrogen (N), and soil pH from field plots of Chinese chives treated with either biogas or chemical fertilizer.

## MATERIALS AND METHODS

### Experimental design

The field experiment was carried out in Dongqiao, Hubei province, China (31°15'N, 113°41'E; 48 m asl). This region has a tropical monsoon climate, with an average annual precipitation of 1100 mm and an average temperature of 16°C and soils at the study site were a sandy loam.

Previously, the field had been cultivated as a paddy with moderate levels of fertility. We arranged three replicates of nine 5.0 × 10.0 m plots in a completely randomized block design. On April 28 2014, Chinese chives seedlings were planted along raised ridges (15 cm high and 160 cm wide) at 10 cm spacing in double rows that were separated by 25 cm. Blended fertilizer (750 kg·ha<sup>-1</sup>) was applied before seeding, with a ratio of 15:15:15 (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O and topdressing treatments were applied when the Chinese chives were harvested after two days. The treatments comprised applications of urea at 150 kg·ha<sup>-1</sup> with 46% the nitrogen content plus blended fertilizer at 150 kg ha<sup>-1</sup> (CP), biogas slurry (BS), and an unfertilized control (CK). CP and BS received the same amount of total N, while fertilizer supplemented P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O in the BS treatment. Pig dung and urine was the raw material for biogas slurry and was fermented for more than 3 months until it had become transparent with no obvious fecal odor. Slurry physicochemical properties are shown in Table 1; pH was found to be 7.67.

On October 20 2017, 8 to 15 soil samples were randomly collected from each plot at a depth of 5 to 25 cm and combined to form single composite samples per plot, where loose soil was removed from the roots of the Chinese chives and any that remained strongly adhered to the roots was recovered as rhizosphere soil. Soil samples were divided into three subsamples that were stored at 4°C for determination of microbial abundance, -80°C for microbe DNA analysis or air-dried, ground, and passed

through 1- and 2-mm mesh sieves for physicochemical analysis.

The physicochemical properties of the rhizosphere soil are tested. The pH of soil was measured by preparing slurry of 1:2.5 fresh soils to water (v/v) and using a pH meter (OHAUS, Starter 3C). Soil organic matter (SOM) was determined using the standard Walkley-Black potassium dichromate oxidation method (Nelson and Sommers, 1982). Available N (AN) was measured using the alkali-hydrolysis and diffusion method (Cornfield, 1960), available P (AP) was extracted with 0.5 M NaHCO<sub>3</sub> using the Olsen method (Blakemore et al., 1972), and available K (AK) was extracted with 1 M NH<sub>4</sub>OAc (1:10 soil:solution ratio for 1 h) and analyzed using atomic absorption spectrophotometry (Lanyon and Heald, 1982).

### DNA extraction

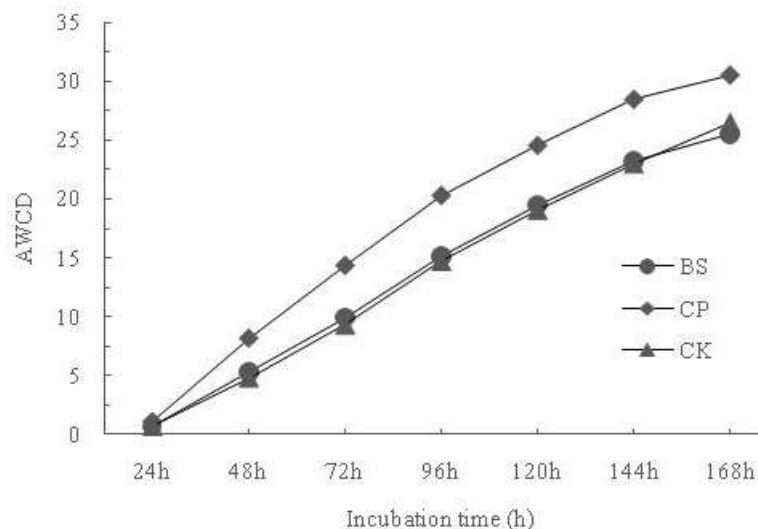
For each soil sample, three samples of total microbial genomic DNA were extracted from 0.5 g of the frozen soil using a PowerSoilDNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA), according to the manufacturer's instructions, and the three samples were pooled to reduce DNA extraction bias. Extracted DNA was evaluated on 1% agarose gel, where the quality and quantity of the extracts were determined using a NanoDrop ND-2000 spectrophotometer (ThermoScientific, DE, USA). All DNA samples were diluted to 10 ng μL<sup>-1</sup> and stored at -20°C until further use.

### Biolog ecoplate analyses

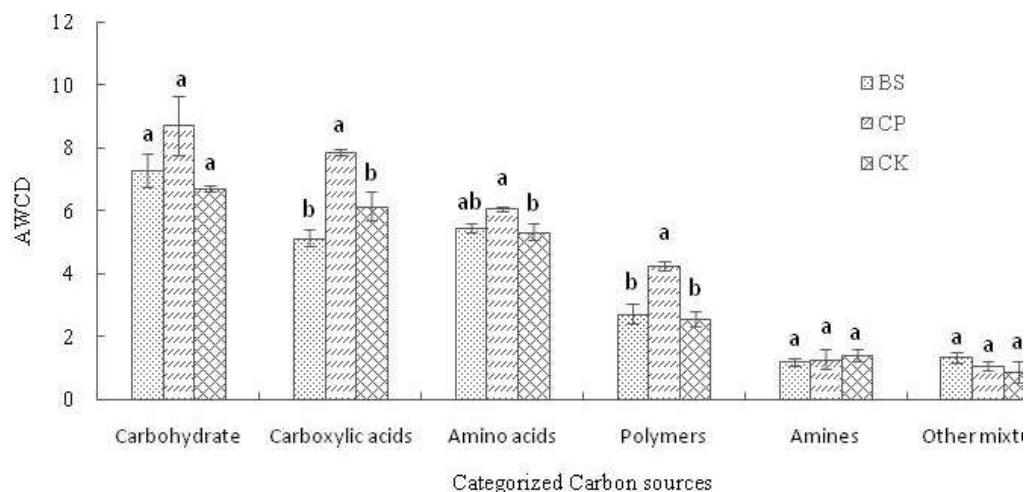
Fresh soils (5 g) were shaken for 20 min at 200 rpm with 100 ml 0.85% NaCl and then allowed to settle for 15 to 30 min. Ten-fold dilutions were performed until the desired (10<sup>-3</sup>) dilution was reached and then an aliquot (125 mL) of the diluted suspension was placed in each well of the Biolog Ecoplate using a multi-channel repetitive-dispensing pipette. The plates were incubated at 28°C, and absorbance at 590 and 750 nm was recorded at 24 h intervals for 7 days using a Biolog GEN III MicroStation™ (USA) to assess average well color development (AWCD). Three replicates per treatment and sampling time were performed. Each well of the Biolog Ecoplate was loaded with one of 31 single carbon sources that belonged to classes defined as carbohydrate (12), amino acid (6), carboxylic acid (5), amine (2), polymer (4) or phenolic acid (2). The well absorbance values were adjusted by subtracting the absorbance of the control well (water only) before data analysis, and substrates with an optical density (OD) < 0 were excluded from further analysis.

### Statistical analysis

Treatment differences (P < 0.05) between means were determined using t-test with LSDs in SPSS (v. 14.0 for Windows, Chicago, USA). The False Discovery Rate (FDR) of p-values was assessed using the BH method with the mt.rawp2adjp function in R. Redundancy analysis (RDA) was run in excel using the XLSTAT.



**Figure 1.** Treatment effects on changes in average well color development (AWCD) of 31 carbon sources.



**Figure 2.** Rhizosphere microorganism use of six major carbon sources.

add-on statistical software

**RESULTS**

**Carbon substrate metabolic profiles of soil microbial communities**

It was found that AWCD gradually increased with the cultivation, but there was no treatment effect on carbon utilization the first 24 h (Figure 1). Increase in soil microorganisms was logarithmic from 24 to 144 h, when AWCD of all soil samples increased to approximately 25, whereas rate of AWCD decreased after 144 h. The

AWCD was higher in CP than in BS and the control.

**Specific substrate utilization of soil microbial communities**

The relative absorbance of carbohydrates, carboxylic acids, amino acids and polymers was highest in CP and similar between the BS treatment and control (Figure 2).

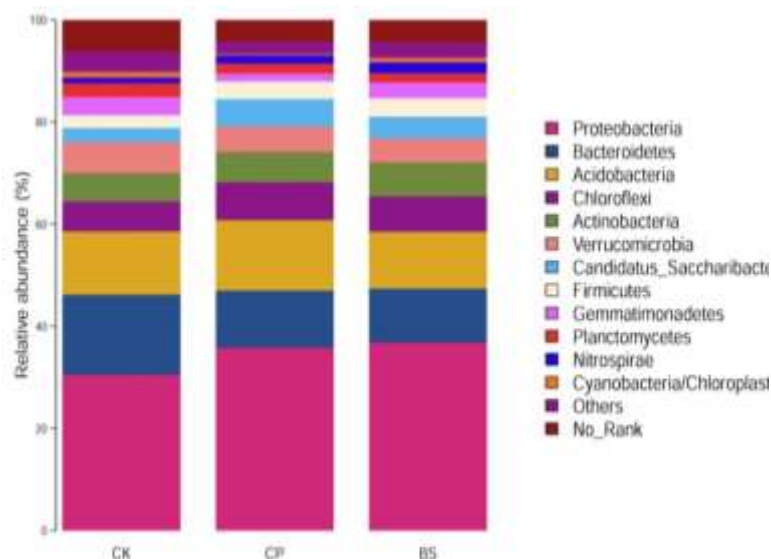
**Bacteria alpha-diversity**

There were treatment effects on the abundance and

**Table 2.** Treatment effects on diversity index of 16S rRNA.

Sample	Chao1	ACE	Shannon	Simpson
CK	3853.33±179.15 <sup>a</sup>	3802.54±170.99 <sup>a</sup>	6.5585±0.0959 <sup>a</sup>	0.0040±0.0004 <sup>a</sup>
CP	3302.10±485.99 <sup>a</sup>	3238.10±499.29 <sup>a</sup>	5.7643±0.5601 <sup>a</sup>	0.0082±0.0083 <sup>a</sup>
BS	3754.19±244.24 <sup>a</sup>	3714.24±227.08 <sup>a</sup>	6.3469±0.2276 <sup>a</sup>	0.0059±0.0021 <sup>a</sup>

Data are means ±SD (n = 3). Different letters within a column indicate treatment differences at  $P < 0.05$ .



**Figure 3.** Treatment effects on the relative abundance of dominant phyla. Relative abundances are based on the proportional frequencies of those DNA sequences that could be classified.

**Table 3.** Treatment effects on phylum abundance.

Phylum	Comparison	Relative fold-change	P value
<i>Gemmatimonadetes</i>	CK/CP	2.31	0.030
<i>BRC1</i>	CK/BS	2.14	0.03*
<i>Gemmatimonadetes</i>	CP/BS	0.51	0.03*

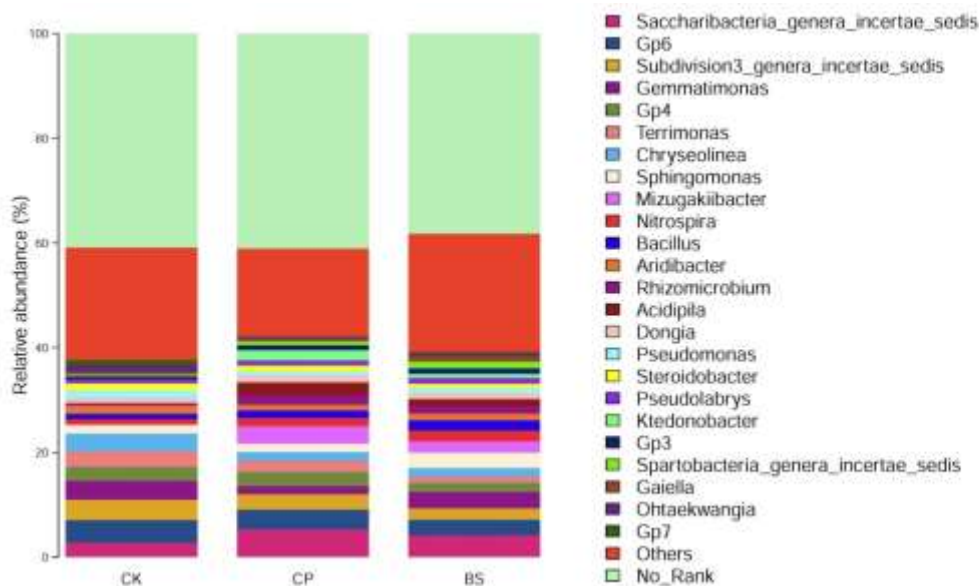
diversity of bacteria, where they are higher in the BS treatment and control than in the CP treatment (Chao1 and Shannon index; Table 2).

### Bacterial community composition in the soil

At the phylum level, a total of 30 phyla were shared across the three treatments, where the *Proteobacteria*, *Bacteroidetes*, *Acidobacteria*, *Chloroflexi*, *Actinobacteria*, *Verrucomicrobia*, *Candidatus\_Saccharibacteria*, and *Firmicutes* were dominant accounting for 80.9 to 94.6% of DNA sequences (Figure 3). The relative abundance of the *Gemmatimonadetes* and *BRC1* differed among the

treatments ( $P < 0.05$ ; Table 3). *Proteobacteria* was consistently the most dominant phylum, representing 29.1 to 41.1% of the phyla, where relative abundance was greater in the BS and CP treatments than in the control. *Bacteroidetes* was the second most dominant phylum (6.0-17.0%), where relative abundance was lower in the BS and CP treatments than in the control.

At the genus level, there are 24 genera with a relative abundance greater than 1%, including *Saccharibacteria\_genera\_incertae\_sedis*, *Gp6*, *Gemmatimonas*, *Gp4*, *Subdivision3\_genera\_incertae\_sedis*, *Terrimonas*, *Chryseolinea*, *Sphingomonas*, etc. (Figure 4). The relative abundance of some genera was significantly affected by the different topdressing (Table S1).



**Figure 4.** Treatment effects on relative abundance of genera. Relative abundances are based on the proportional frequencies of those DNA sequences that could be classified.

**Table 4.** Treatment effects on key soil physicochemical properties.

Treatment	pH	Organic matter (g·kg <sup>-1</sup> )	Available N (mg·kg <sup>-1</sup> )	Available P (mg·kg <sup>-1</sup> )	Available K (mg·kg <sup>-1</sup> )
BS	6.21±0.00 <sup>c</sup>	2.73±0.16 <sup>a</sup>	77.93±3.22 <sup>a</sup>	245.04±13.22 <sup>b</sup>	272.67±4.18 <sup>b</sup>
CP	5.70±0.05 <sup>b</sup>	2.51±0.86 <sup>a</sup>	76.30±1.65 <sup>a</sup>	199.36±4.02 <sup>b</sup>	176.00±0.00 <sup>a</sup>
CK	4.94±0.02 <sup>a</sup>	1.76±0.15 <sup>a</sup>	87.27±12.45 <sup>a</sup>	94.20±2.98 <sup>a</sup>	173.00±3.51 <sup>a</sup>

Different letters within a column indicate treatment differences at  $P < 0.05$ .

*Cystobacter*, *Sphingobium* and *Anaerosalibacter* dramatically differed ( $p < 0.01$ ) between CK and BS. *Desulfosporosinus*, *Pontibacter* and *Solibacillus* dramatically differed ( $p < 0.01$ ) between CK and CP. *Arthrobacter*, *Gp2* and *Niastella* dramatically differed ( $p < 0.01$ ) between CP and BS.

#### Relationship between bacterial community and environmental factors

There were treatment effects on soil physicochemical properties (Table 4), where pH was the highest in the BS treatment and lowest in the control; AP was higher in the BS and CP treatments than in the control; and AK was the highest in the BS treatment. There were no treatment effects on SOM content. Redundancy analysis (RDA) of the 17 most abundant genera and their associations with SOM, pH, and AN, AK, and AP content showed that the decreasing rank order of influence on genus abundance was SOM, AK, AN, pH, and AP (Figure 5). Genus abundance in the BS treatment was positively associated with AK, SOM, AP, and AN, and negatively related to pH,

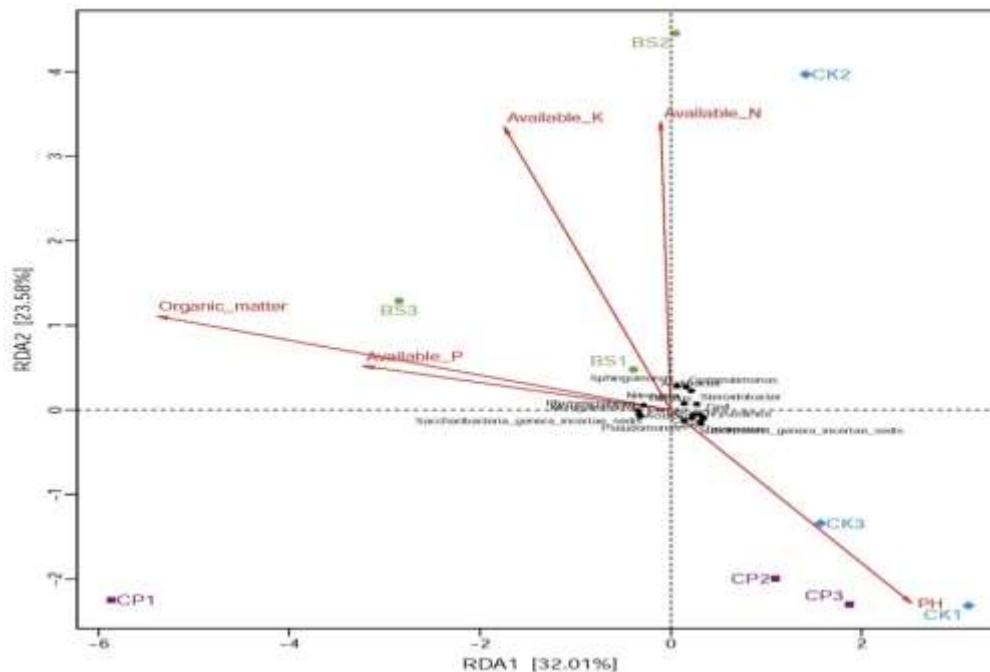
while in the CP treatment and control, genus abundance was negatively associated with AK, OM, AP, and AN, and positively associated with pH.

Of the 24 genera with a relative abundance greater than 1%, the abundance of nine was associated with SOM; there were no associations between the other physicochemical properties and abundance of genera. Abundance of *Saccharibacteria\_genera\_incertae\_sedis* was positively correlated ( $r = 0.804$ ,  $P < 0.01$ ) and *Gp6* was negatively correlated ( $r = -0.850$ ,  $P < 0.01$ ) with the SOM content (Table 5).

## DISCUSSION

### Effects on bacterial community composition

The depletion of soil organic matter is a major factor in the degradation of ecosystem services and ecosystem resilience to perturbations (Feller et al., 2012) and studies have suggested that amendment of organic soil may be an approach to improve the economics of viable crop production whilst minimizing the impacts of environmental



**Figure 5.** RDA correlation analysis of the soil bacteria communities and physicochemical properties in the treatments.

**Table 5.** Association between genus relative abundance and physicochemical properties (n=20).

Genus	Pearson's correlation coefficient				
	Available K	Available N	Available P	Organic matter	pH
<i>Acidipila</i>	0.008	0.003	0.315	0.767*	-0.148
<i>Gp6</i>	-0.279	-0.308	-0.294	-0.850**	0.28
<i>Steroidobacter</i>	-0.301	0.4	-0.332	-0.741*	0.291
<i>Nitrospira</i>	0.456	-0.333	0.49	-0.299	-0.553
<i>Saccharibacteria_genera_incertae_sedis</i>	0.019	0.073	0.197	0.804**	-0.08
<i>Dongia</i>	-0.034	-0.219	0.165	-0.785*	-0.085
<i>Aridibacter</i>	-0.072	0.648	-0.269	-0.489	0.12
<i>Pseudomonas</i>	-0.095	-0.309	-0.165	-0.472	0.17
<i>Terrimonas</i>	-0.473	-0.226	-0.499	-0.784*	0.51
<i>Bacillus</i>	0.414	0.089	0.472	0.635	-0.444
<i>Chryseolinea</i>	-0.317	0.01	-0.507	-0.641	0.448
<i>Sphingomonas</i>	0.615	0.272	0.413	-0.097	-0.599
<i>Mizugakiibacter</i>	0.078	0.003	0.405	0.709*	-0.235
<i>Gp4</i>	-0.264	-0.316	-0.266	-0.632	0.236
<i>Gemmatimonas</i>	0.198	0.516	-0.401	-0.274	0.097
<i>Rhizomicrobium</i>	0.114	0.118	0.343	0.850**	-0.226
<i>Subdivision3_genera_incertae_sedis</i>	-0.481	-0.268	-0.553	-0.835**	0.56

pollution. Biogas slurry, which is rich in organic matter, has high levels of bioactivity and associated nutrient utilization efficiency and is known to reduce disease incidence and improve stress tolerance that lead to increased production and product quality (Gao et al.,

2011). Soil microorganisms play an important role in nutrient cycling and decomposition (Kennedy and Smith, 1995) and are affected by the application of fertilizer (Ge et al., 2015); understanding the soil microbe community and its response to various agricultural management

practices will allow the selection of a suitable management strategy for more stable and sustainable agroecosystems (Li et al., 2012; Zhao et al., 2014). Ai et al. (2018) reported that microbe functional diversity was high in paddy soils subjected to long-term, high levels of fertilizer application. Here, AWCD was found to be higher in the CP treatment than in the BS treatment and the control, indicating that fertilizer topdressing improved the functional diversity of the carbon source-using soil microorganism community, as reflected by increases in utilization of the carboxylic acids, amino acids, and polymers (Figure 2).

### Effects on bacterial diversity

Soil health is one of the most vital requirements for crop production in agricultural systems, where soil microorganisms play a major role in its development and maintenance. Yu et al. (2017) found long-term application of inorganic nitrogen fertilizer reduced the diversity of soil bacteria and here, it was found that biogas slurry increased soil microbe taxonomic diversity (Table 2). Soil organic matter is essential in the maintenance of soil structural stability and improvement of physical, chemical, and biological properties (Oo et al., 2015) and the addition of organic matter in soil remediation is considered essential for sustainable land use and crop productivity. In the present study, the dominant phyla Proteobacteria, Bacteroidetes and Acidobacteria accounted for more than 50% of bacteria abundance, which was consistent with Lauber et al. (2009) and Chu et al. (2010) (Figure 3). Nine genera had significant differences with SOM, but none of them had significant differences with the other physicochemical properties (Table 5). Thus, the application of biogas slurry to vegetable, fruit tree, flower and field crops may provide multiple benefits, including increases in SOM content and microbial diversity, as a biological fertilizer.

### Conclusions

It was found that biogas slurry as topdressing is conducive to increase relative abundance and taxonomic diversity of the soil bacteria community in continuous cropping. Of the 24 genera with a relative abundance greater than 1%, the abundance of nine was positively associated with SOM content. Relative abundance of *Saccharibacteria\_genera\_incertae\_sedis* and *Gp6* was positively and negatively correlated with SOM, respectively, and RDA indicated that SOM was a key driver of the composition of the soil bacteria community and physicochemical properties. Biogas slurry contains the essential nutrients for plant growth (NPK), improves SOM content, and enhanced the soil microbe community, so we suggest its application in sustainable vegetable production.

### CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

### ACKNOWLEDGEMENTS

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**Table S1.** Treatment effects on relative abundance of bacteria genera.

Genus	Relative fold-change	
	CK/CP	p value (*p < 0.05, **p < 0.01)
Desulfosporosinus	0.27	0.003**
Pontibacter	33	0.005**
Solibacillus	0.29	0.008**
Chlorophyta	2.35	0.01*
Gp2	5.09	0.013*
Geminicoccus	3.96	0.016*
Ignavibacterium	4.26	0.018*
Geothermomicrobium	0.54	0.021*
Albidovulum	7.5	0.023*
Nannocystis	3	0.026*
Blastococcus	3.1	0.029*
Paenispodosarcina	2.92	0.031*
Taibaiella	0.17	0.034*
Curvibacter	2.75	0.036*
Microvirga	2.85	0.039*
Clostridium_XIVa	0.42	0.042*
Gemmatimonas	2.31	0.044*
Solirubrobacter	3.74	0.047*
Desulfobacca	0.32	0.049*
	<b>CK/BS</b>	<b>p value (*p&lt;0.05, **p&lt;0.01)</b>
Cystobacter	3.98	0.003**
Sphingobium	0.08	0.005**
Anaerosalibacter	0.08	0.008**
Singulisphaera	0.29	0.010*
Albidovulum	6.00	0.013*
Cupriavidus	0.31	0.015*
Thiobacillus	0.00	0.018*
Chlorophyta	2.27	0.020*
Blastococcus	3.56	0.023*
Gaiella	0.52	0.026*
Skermanella	3.37	0.028*
Arthrobacter	0.29	0.031*
Sporacetigenium	1.50	0.033*
Microvirga	2.22	0.036*
BRC1_genera_incertae_sedis	2.14	0.038*
Oryzihumus	3.33	0.041*
Azoarcus	0.04	0.043*
Pirellula	1.78	0.046*
Brevibacterium	0.18	0.049*
	<b>CP/BS</b>	<b>p value (*p &lt; 0.05, **p &lt; 0.01)</b>
Arthrobacter	0.11	0.003**
Gp2	0.14	0.005**
Niastella	0.08	0.008**
Bdellovibrio	0.34	0.010*
Armatimonadetes_gp4	0.19	0.013*
Turicibacter	0.12	0.016*
Gaiella	0.47	0.018*
Methanocella	2.52	0.021*

**Table S1.** Contd.

Thermobifida	0.08	0.023*
Fervidicella	3.00	0.026*
Azoarcus	0.04	0.029*
Armatimonadetes_gp5	0.18	0.031*
Aggregicoccus	0.24	0.034*
Solibacillus	2.38	0.036*
Schlesneria	0.24	0.039*
Gp13	0.11	0.042*
Amycolatopsis	0.07	0.044*
Gemmatimonas	0.51	0.047*
Stenotrophomonas	0.21	0.049*