Effect of Biogas Slurry on the Nutrient Cycling and Micro-organisms Community in Two Types of Soil

Pengcheng Li,[#,a,b,d] Dongqi Jiang,[#,a,b] Shuqiang Wang,[a,b,d] Xianying Zhang,[a,b] Yulan Zhang,[a,b,e,f,g,]* Yonghuan Wang,[c,]* Zhenhua Chen,[a,b] and Nan Jiang [a,b]

The biogas slurry (BS) generated through the anaerobic fermentation of biogas in pig farms is extensively employed as an organic fertilizer in Northeast China. BS is often used in large amounts because of fragmented farmland ownership resulting from previous local policies. In this work, 20 $m^3 \cdot 667m^{-2}$ of BS was applied to black soil twice and to aeolian sandy soil once to explore microbial-driven nutrient cycling. The results indicated that BS increased organic carbon, total nitrogen, and total phosphorus contents in black soil. The activities of C-cycling and P-cycling enzymes in black soil were enhanced, while the activities of P-cycling enzymes in aeolian sandy soil were reduced. The BS application increased the abundance ratio of fungi to bacteria in both soil types. Total carbon, total nitrogen, and total phosphorus primarily influenced the microbial community structure in black soil, while pH was the key factor in aeolian sandy soil. However, the excessive increase of heavy metals in black soil treated twice BS posed a potential risk to the environment. Utilizing BS as fertilizer is a viable strategy applicable for Northeastern China's agriculture, and application dosages must be adjusted according to experimental results.

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Contact information: a: CAS Key Laboratory of Forest Ecology and Silviculture, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China; b: Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 10016, China; c: Liaoning Agricultural Development Service Center, Shenyang 10016, China; d: University of Chinese Academy of Sciences, Beijing, 100049, China; e: Liaoning Key Laboratory of Modern Conservation Tillage and Ecological Agriculture, Shenyang Liaoning, Shenyang 10016, China; f: Shenyang National Field Scientific Observation and Research Station of Farmland Ecosystem, Liaoning Province, Shenyang Liaoning, Shenyang 10016, China; g: Key Laboratory of Terrestrial Ecosystem Carbon Neutrality, Liaoning Province, Shenyang 10016, China;
**Corresponding authors: ylzhang@iae.ac.cn (Y.Z.); lntfflk@163.com (Y.W.);*
 # These authors contributed equally to the work: lipengcheng22@mails.ucas.ac.cn (P.L.); dqjiangiae@126.com (D.J.).

INTRODUCTION

Efficient management and recycling of slurry from pig, cattle, and cow farms are deemed necessary and urgent (Hansen *et al*. 2006; Marques-dos-Santos *et al*. 2023). In recent years, anaerobic fermentation has been widely applied. China produces over 1.12 billion tons of biogas slurry (BS) annually through anaerobic fermentation of biodegradable organic wastes, including urine, feces, flushing water, and disinfectant. China produces more than half of the world's biogas slurry (BS) (Ma *et al*. 2018; Zou *et al*. 2020). Biogas slurry contains organic nitrogen (mainly amino acids), abundant mineral elements, and low-molecular-mass bioactive substances (*e.g.*, hormones, humic acids, vitamins, *etc*.) (Yu *et al*. 2010). Anaerobic fermentation is gaining increasing recognition from the public due to its numerous advantages, such as the effective reduction of odors, viruses, harmful substances, and so on (Demirer and Chen 2005; Holm-Nielsen *et al*. 2009). Anaerobic fermentation is characterized by reduced energy consumption, the production of consistent products, and improved preservation of nutrients (Khoshnevisan *et al*. 2021). China has emerged as the leading global producer of pigs (Dai *et al*. 2021; Zhao *et al*. 2022). The pig breeding sector in Northeastern China has undergone substantial expansion over the past 5 to 10 years (Wang *et al*. 2018). Manure management on pig farms in Northeast China typically involves segregating manure into liquid and solid fractions through intricate procedures. This practice is likely motivated by the convenience of disposal and transportation in the region's winter climate conditions. The nutrient-dense composition of BS has captured the attention of government agencies and researchers. One of the sustainable approaches employed for BS involves utilizing it as a valuable agricultural fertilizer or soil amendment to mitigate agricultural source waste pollution.

The integration of BS into agricultural land represents an effective approach for optimizing resource and improving soil quality. Farmers in intensive agricultural regions of China traditionally integrated significant amounts of BS into their fields (Holm-Nielsen *et al*. 2009; Khoshnevisan *et al*. 2021; Zhao *et al*. 2022). BS fertilization is an alternative to chemical fertilizers (Wang *et al*. 2018; Dai *et al*. 2021). Application of BS impacts crop yield, soil microbiological characteristics, and soil heavy metal pollution with BS incorporation (Massé *et al*. 2011; Kumar *et al*. 2022). BS has superior plant growth-promoting properties attributes in comparison to untreated slurries (Mukhtiar *et al*. 2023). Abubaker *et al*. (2013) examined the impact of applying cattle slurry (CS) at a level of 70 kg NH_4^+-N·ha^{-1} on the bacterial community structure and microbial activity in sandy, clay, and organic clay soils. Tang *et al*. (2022) highlighted that the long-term incorporation of BS improved soil microbial status and biodiversity, as well as stimulated enzyme activities, and influenced microbial community structure (Domingo-Olivé *et al*. 2016; Chen *et al*. 2020). Zheng *et al*. (2016) investigated the effects of dosage at 64, 128, and 192 t·ha^{-1} BS on dryland ecosystems. Xu *et al*. (2019) determined an optimal application rate of BS ranging from 59.9 to 264.4 t·ha^{-1} in rice cultivation to enhance agricultural soil sustainability. BS application resulted in neutralised soil pH (Zhu *et al*. 2023), increased nutrients levels, and influenced bacterial community compositions. BS incorporation generally effectively enhanced plant growth, crop yields, crop quality, soil nutrients content, soil aggregate structure, and reduced agricultural expenses (Abubaker *et al*. 2012; Huong *et al*. 2014). However, the composition of BS in China is intricate, characterized by elevated levels of ammonia nitrogen, suspended solids, and residual antibiotics, as well as heavy metals. Improper disposal of BS, including discharging directly into ditches, or storing openly, or high dosage application, adversely detrimental effected the quality of soil, water, and air, consequently impacting the flora, fauna, and microorganisms inhabiting these environments (Huong *et al*. 2014).

There are challenges in handling BS in Northeastern China, where farmers tended to apply high dosage BS in farmland (Shi 2020). The appropriate BS dosage for crops varies based on specific soil types, such as loam, sand, or clay (Abubaker *et al*. 2013). Excessive application of BS in arid regions led to soil structure degradation and an increase in heavy metal concentrations of groundwater and soil, posing a significant risk to global public health (Khodaverdiloo *et al*. 2020). The application of biogas slurry (BS) has negative impacts on the diversity and structure of microbial communities (Xu *et al*. 2019). Most previous studies examining the effects of pig slurry on soil have mainly focused on a specific soil type (Chantigny *et al*. 2004; Risberg *et al*. 2017; Shakoor *et al*. 2022). This narrowed focus complicates the ability to formulate overarching conclusions due to potential discrepancies in soil responses to pig farm BS. Therefore, it is crucial to investigate appropriate methods for utilizing pig farm BS in various soil environments. This study investigated the effects of pig farm BS on nutrients, enzyme activities, microbial communities, and heavy metal contents in black soil, at dosage exceeding 300 $t \cdot ha^{-1}$ twice a year in black soil, and once a year in aeolian sandy soil (considering that black soil has a stronger buffering capacity against environmental changes than aeolian sandy soil).

EXPERIMENTAL

Experimental Design

The field experiment was conducted in Kangping County, Liaoning Province, China (42°75′N, 123°35′E). The region has a temperate continental climate, with an average annual temperature of 6.9 °C and an average annual precipitation of 540 mm. The black soil in this experiment is classified as Chernozem. The aeolian sandy soil in the study area is classified as Sandic Entisols according to Chinese Soil Taxonomy (Revised Proposal). The crop grown in this area was maize.

Two 3000 m^2 areas were selected as test sites. At each site, two treatments were set up: pig farm biogas slurry and chemical fertilizer (as control), each with three replications (500 m^2). The raw material used for biogas slurry in this experiment was obtained from a large livestock company through a fully enclosed wastewater collection system in Kangping County, Liaoning Province. The slurry was stored for anaerobic fermentation in the black film facility, serving as storage ponds for 5 to 6 months. Pipe tapes were laid on the ground, and BS was applied by spraying using ground tank trucks. BS was applied to black soil plots at 300 $m^3 \cdot ha^{-1}$ dosage in autumn 2020 and spring 2021, respectively. BS was applied to aeolian sandy plots at 300 $m^3 \cdot ha^{-1}$ dosage in spring 2021. The control treatment during spring sowing followed the conventional fertilization in line with local farmers' practices: N 200 $kg \cdot ha^{-1}$ (urea), P_2O_5 90 $kg \cdot ha^{-1}$ ($(NH_4)_2HPO_4$), and K_2O 90 $kg \cdot ha^{-1}$ (KCl). Specific fertilizer application amounts are shown in Table 1.

Table 1. Nutrient Amount of Fertilizers in Each Treatment ($kg \cdot ha^{-1}$)

Nutrient	B (Chemical fertilizer)	BMS (BS 600 $m^3 \cdot ha^{-1}$)	S (Chemical fertilizer)	SMS (BS 300 $m^3 \cdot ha^{-1}$)
N	200	2712	200	1356
P_2O_5	90	3168	90	1584
K_2O	90	474	90	237

B = conventional fertilization in black soil, BMS = pig farm biogas slurry in black soil, S = conventional fertilization in aeolian sandy soil, SMS = pig farm biogas slurry in aeolian sandy soil.

Soil Sampling

A total of 12 soil samples were collected at a depth of 0 to 20 cm in September 2021; 10 random cores were collected in each plot using a 10 cm diameter auger. Crop residues were removed from the soil surface before sampling. Ten soil samples were homogenized and made up as one mixed sample. Each sample was sieved (< 2 mm) and divided into four subsamples: one was stored at -8 °C for DNA extraction, another was stored at 4 °C for soil enzyme activity analysis within two weeks, the third was air-dried for soil physicochemical properties analysis, and the fourth was air-dried, ground, and sieved (< 0.15 mm) using a ball mill for determination of heavy metal content.

Determination of Soil Physiochemical Properties and Heavy Metals Content

Soil pH was determined with a ratio of 1: 2.5 (soil: water) by pH meter. Soil organic carbon (SOC) content was determined using the $K_2Cr_2O_7$ oxidation method (Nelson and Sommers 1982). Air-dried soil (＜0.149 mm, 0.1 g) was mixed with hydrofluoric acid, perchloric acid, and nitric acid. Digestion was performed, and then the product was evaluated using an inductively coupled plasma-optical emission spectrometer (Milestone ETHOS UP Microwave Dissolver, Nanjing, China). The total phosphorus content was determined using the molybdenum blue colorimetric method at 880 nm (Murphy and Riley 1962). The total potassium content was determined using a flame photometer. The soil's chemical properties were assessed using standard procedures. TC and TN levels were measured in 0.05 g of air-dried soil (< 0.149 mm) using an elemental analyzer (Vario MACRO cube, Elementar, Germany). NH_4^+-N and NO_3^--N concentrations were measured in the supernatant obtained by mixing 2.5 g of fresh soil (＜2 mm) with 25 mL of 2 mol L^{-1} KCl solution in 50 mL centrifuge tube. The mixture was shaken for 30 min in a shaking incubator (25°C, 180 rpm, SPH-2102C, China). Quantitative filter paper was used to collect the supernatant, and the concentrations of NH_4^+-N and NO_3^--N were determined using a continuous flow analyzer (Bran & Luebbe, Norderstedt, Germany) (Sparks *et al*., 2020). Soil samples were processed by adding 5 g of air-dried soil (< 2 mm) to a mixture of 50 mL of 1 mol L^{-1} ammonium acetate (CH_7NO_2). After shaking for 30 min, the filtrate was filtered, and the available potassium (AK) content in soil was determined using a flame photometer (Lehmann and Kleber 2015). The available phosphorus (AP) content of the soil was assessed using the molybdenum blue colorimetric method at 880 nm (Olsen 1954).

An inductively coupled plasma-mass spectrometer (ICP-MS) was used to analyze heavy metal content in soil (Moor *et al*. 2001). The soil samples were air-dried, ground, and sieved. Afterward, all samples underwent ablation using a mixture of acids, including nitric acid and hydrofluoric acid. After ablation, the samples underwent ionization using inductively coupled plasma to separate metal ions in a magnetic field and determine their charge-to-mass ratio. The mass spectrometer analyzed the ratio to determine the final concentration of heavy metal elements in the soil. A soil standard substance (SRM-2586) was used for quality control, and the results were compared with the soil environmental quality standard (Table S2).

Soil Enzyme Activity

Soil enzymes are commonly used as indicators of soil microbial nutrient status (Sinsabaugh *et al*. 2009). Representative soil enzymes involved in the carbon, nitrogen, and phosphorus cycling were selected: for C-cycling enzymes, ß-galactosidase; for N - cycling enzymes, urease (URE); and for P-cycling enzymes, acid phosphomonoesterase (ACP), alkaline phosphomonoesterase (AIP), and phosphodiesterase (PDE). Enzyme activities were assessed using Tabatabai's method (Tabatabai 1994). β - galactosidase (β - Gal) was treated with alkaline THAM buffer and color-developed to determine p-nitrophenol at 410 nm. For URE analysis, soil samples were treated with an equal amount of urea. Urease activity was determined by measuring the remaining urea after incubating soil samples with a urea solution at 37 °C for 5 h. Soil acid phosphatase (ACP) and alkaline phosphatase (AIP) were assayed using the same method with p-nitrophenyl phosphate as the substrate, modifying universal buffer at pH 11.0 and 6.5, respectively.

Phosphodiesterase (PDE) hydrolyzes dinitrophenyl phosphate, and its activity is measured by quantifying the released dinitrophenol content through a colorimetric method.

DNA Extraction and High-throughput Sequencing

Microbial DNA was extracted from each of the 12 composite fresh soil samples using DNA kits from Beijing Alwegene Technology Co., Ltd. The quality of the total DNA was assessed with a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, USA). The DNA was amplified with primers 338F (5' – ACTCCTACGGG AGGCAGCAG - 3') and 806R (5 '- GGACTACNNGGGTATCTAAT - 3'), targeting the V3–V4 16S rRNA region (Castrillo *et al*. 2017). The fungal internal transcribed spacer (ITS) region was amplified with universal primers ITS1F (5' CTTGGTCATTTAGAGGA AGTAA - 3') and ITS2 (5 '-TGCGTTCTTCATCGATGC - 3'), and each sample was replicated three times (Ji *et al*. 2022). The PCR reactions were prepared in triplicate for each sample using 16.5 µL of Power SYBR® Green PCR Master Mix (Applied Biosystems™, Thermo Fisher Scientific Inc., MA, USA), 0.8 µL of each primer, and 2 µL of DNA template. The PCR amplification conditions were: pre-denaturation at 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s. The final extension was carried out for 10 min at 72 °C. After completing the process, the amplification results were verified through 1% agarose gel electrophoresis, and the target fragments were extracted from the gel. Plasmid standards were diluted in a 10-fold gradient ranging from 10^1 to 10^5. 2 µL of each gradient was used to create a standard curve, and the copy numbers were determined using these curves. After the individual quantification step, amplicons were pooled in equal amounts, and pair-end 2×300bp sequencing was performed using the Illumina MiSeq platform. The sequencing data obtained from MiSeq is Pair-End (PE) double-ended sequence data. Trimmomatic (v0.36), Pear (v0.9.6), and Flash (v1.20) are utilized for quality filtering of merged sequences. Sequences that do not meet specific criteria are discarded: sequence length < 120 bp, ambiguous bases, and average quality score ≥ 20.

Statistical Analyses

One-way ANOVA was conducted with Tukey’s test, and LSD method was used for multiple comparisons to detect statistical differences among various treatments. A significance level of $P < 0.05$ indicates statistical significance. All statistical analyses were conducted using SPSS 16.0 software (SPSS, Chicago, IL, USA). Bacterial and fungal alpha diversity were compared using diversity indices like the PD-tree index, Shannon index, and OTU richness. To assess the impact of pig’s biogas slurry on microbial beta diversity, principal component analysis (PCA) was employed using the Bray-Curtis distance metric (Wang *et al*. 2012). The main factors driving changes in microbial community structure were identified through redundancy analysis (RDA).

RESULTS AND DISCUSSION

Effect of Biogas Slurry on Soil Physiochemical Properties

The pig farm biogas slurry enhanced soil nutrients significantly. Contents of soil nitrate - nitrogen (NO_3^--N), organic carbon (SOC), total carbon (TC), total nitrogen (TN) and total phosphorus (TP) were increased significantly ($P < 0.05$), which was consistent with the nutrients richness in BS (Table 2).

Table 2. Nutrient Content in the Collected Soil Samples

Treatments	NO_3^--N g·kg^{-1}	NH_4^+-N g·kg^{-1}	AP mg·kg^{-1}	AK mg·kg^{-1}	SOC g·kg^{-1}	TP g·kg^{-1}	TK g·kg^{-1}	TN g·kg^{-1}	TC g·kg^{-1}	pH
B	0.0100b	0.0124	20.900a	466.625a	14.87b	0.38b	20.35	1.87b	23.09b	8.47a
BMS	0.0858a	0.0171	19.663ab	240.287b	22.50a	0.61a	17.11	2.97a	42.31a	8.10b
S	0.0019d	0.0181	10.867b	149.753c	6.35c	0.22b	18.40	1.26c	11.92c	8.49a
SMS	0.0066c	0.0134	22.314a	166.214c	6.37c	0.34b	17.50	1.29c	11.88c	8.49a

B = conventional fertilization in black soil, BMS = pig farm biogas slurry in black soil, S = conventional fertilization in aeolian sandy soil, SMS = pig farm biogas slurry in aeolian sandy soil. NO_3^--N: Nitrate nitrogen; NH_4^+-N: Ammonium nitrogen; AP: Available phosphorus; AK: Available potassium; SOC: Organic carbon; TP: Total phosphorus content; TK: Total potassium content; TC: Total carbon content; pH: potential of hydrogen. Lowercase letters indicate significant differences between treatments ($P < 0.05$).

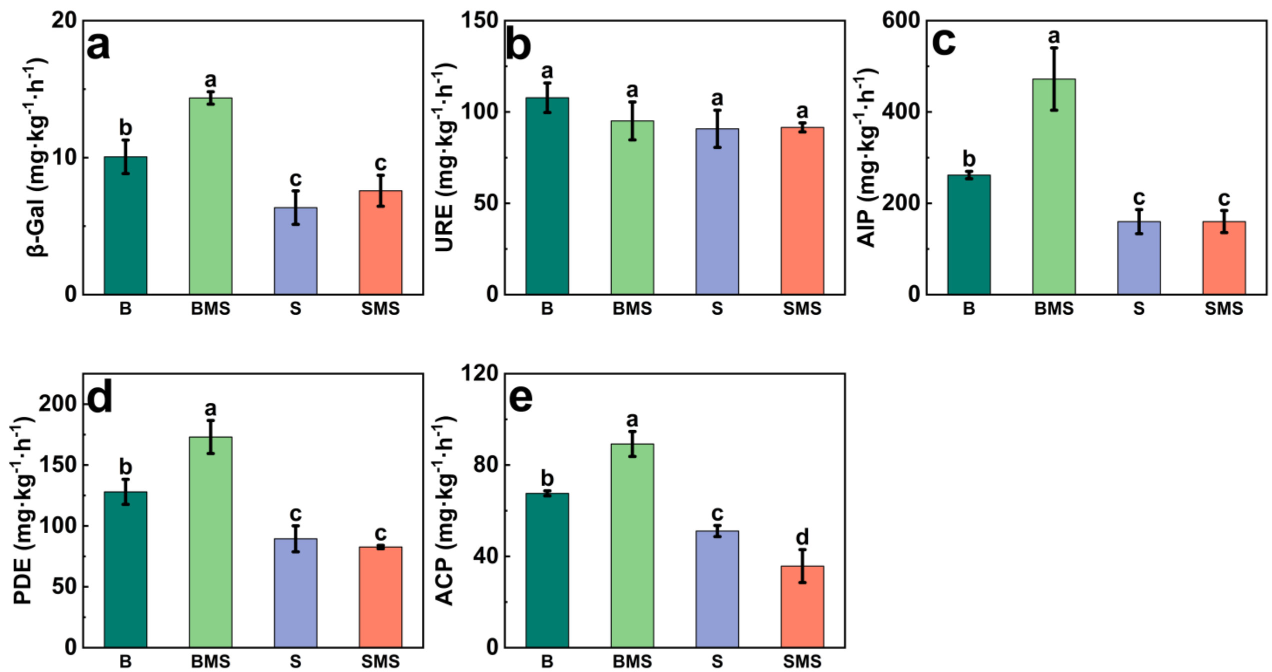

Fig. 1. The impact of pig's biogas slurry incorporation on the enzyme activities related to carbon, nitrogen, and phosphorus cycling in soil. This includes β-galactosidase (β-Gal), urease (URE), alkaline phosphatase (AlP), phosphodiesterase (PDE), and acid phosphatase (ACP). Lowercase letters indicate significant differences between treatments ($P<0.05$).

Nutrients improvement in black soil was significantly greater than that in aeolian sandy soil, showing that the impact of BS fertilizer on soil physicochemical properties varied greatly depending on soil type. Low nutrient content and poor aggregate structure in aeolian sandy soil limited its retention capacities of exogenous nutrients (Niu *et al*. 2023). The results showed that addition of BS reduced soil pH, which was consistent with many studies (Troy *et al*. 2013; Rahman *et al*. 2018). The contents of TK and AK in the aeolian sandy soil varied greatly, which may be related to the pH changes caused by the incorporation of BS (Table 2). Lower soil pH led to lower K contents or availability, and potassium tends to bind soil particles under low soil pH conditions (Han *et al*. 2023). The incorporation of pig farm biogas slurry as fertilizer plays an important role in soil nutrient cycling, thereby promoting the ecological function of agricultural ecosystems in black soil region.

Activity of Soil Enzyme, Abundances and Community Structures of Soil Microbe

BS incorporation stimulated activity of soil enzyme predominantly originating from microorganisms (Sinsabaugh *et al*. 2009; Jian *et al*. 2016; Tan *et al*. 2021). The increase of SOC, TN and TP contents in black soil enhanced the substrate availability, thereby promoting the proliferation of soil microbes subsequently (Craine *et al*. 2007). The response of soil enzyme activities in black soil was more pronounced than that in aeolian sandy soil (Fig. 1); this may be attributed to differences of their fertility. Higher soil P-cycling enzyme activities in black soil indicated P-cycling enhancement (Wang *et al*. 2024). The β - galactosidase enzyme activity processed by BMS was significantly higher than that of other treatments, which is related to the higher soil quality of the black soil itself. BS introduced a large quantity of soluble organic matter into soil, potentially serving as the primary factor influencing enzyme production (Möller 2015). However, BS inhibited the activity of soil acid phosphatase (ACP) in aeolian sandy soil, aligning with prior studies (Böhme and Böhme 2006; Mijangos *et al*. 2006). There was no significant difference in urease activity between the various treatments.

BS increased the ratio of fungal abundance to bacterial abundance, indicating enhancement in soil resilience (Fig. 2A). A higher biomass ratio of soil fungi to bacteria was considered to indicate a more sustainable agro-ecosystem more resilient to severe environmental conditions (Bahram *et al*. 2018).

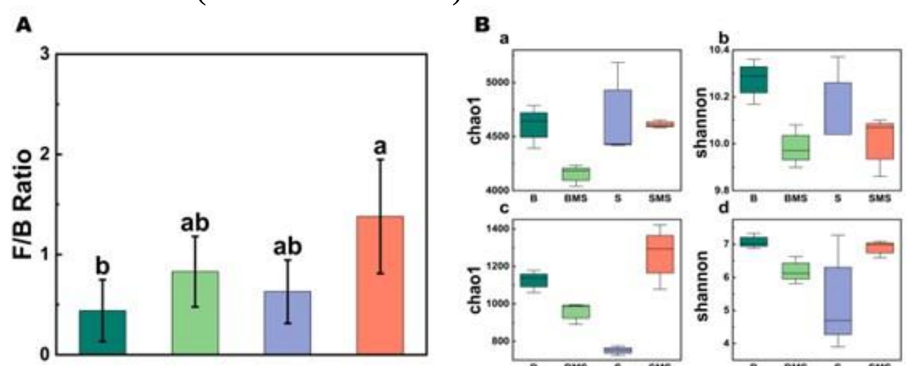

Fig. 2. A. Ratio of fungal to bacterial abundance. B. Effects of pig's biogas slurry incorporation on the chao1 and Shannon indices of two soil bacteria (a,b) and fungal (c,d). Lowercase letters indicate significant differences between treatments (P<0.05).

The importance of the ratio between fungi and bacteria lay in their different lifestyles and different roles in nutrient cycling (Wang *et al*. 2021). Communities dominated by bacteria show faster cycling rate due to bacteria's characteristic, whereas fungi generally have a longer life cycle (Kirchman 2012).

The diversity and richness of soil bacteria in black soil and aeolian sandy soil decreased in a short period of time, which may be due to the sensitivity of soil bacterial communities to external environmental changes (Yin *et al*. 2010). The diversity and richness of fungi in aeolian sandy soil increased, while those in black soil decreased. BS incorporation introduces a significant amount of nutrients into typically poor Aeolian sandy soil, positively impacting the short-term growth and reproduction of fungi (He *et al*. 2020). Soil fungi rely more on external nutrients for growth and reproduction, which may explain the above phenomenon (Dix 2012).

The main three phyla of bacterial communities in both types soil were Proteobacteria, Actinobacteria, and Firmicutes, and the main three genera were genera *RB41*, *Sphingomonas* and *MND1* (Fig. 3a; 3b). In terms of soil fungal communities, the main two phyla were Ascomycota and Basidiomycota. The relative abundance of Ascomycota and Basidiomycota in black soil were increased, while the abundance of Basidiomycota in aeolian sandy soil were decreased by BS incorporation significantly (Fig. 4). The main four genera were *Tausonia*, *Metarhizium*, *Mortierella*, and *Botryotrichum* (Fig. 4a, 4b).

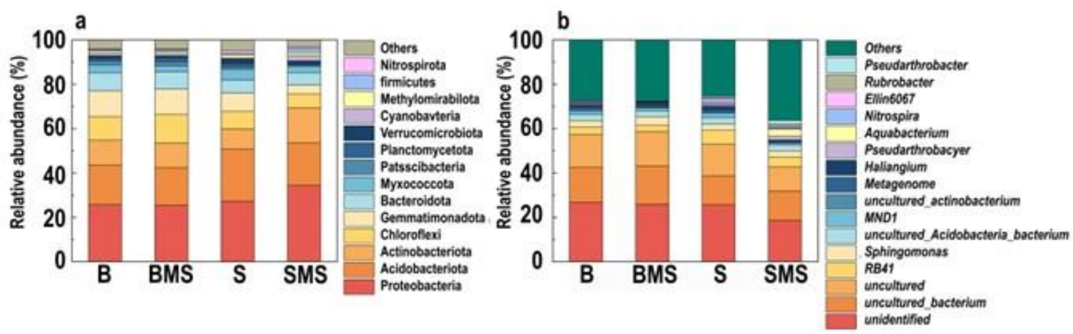

Fig. 3. Effect of pig's biogas slurry incorporation on the structure of soil bacterial at phylum levels (a) and genus levels(b)

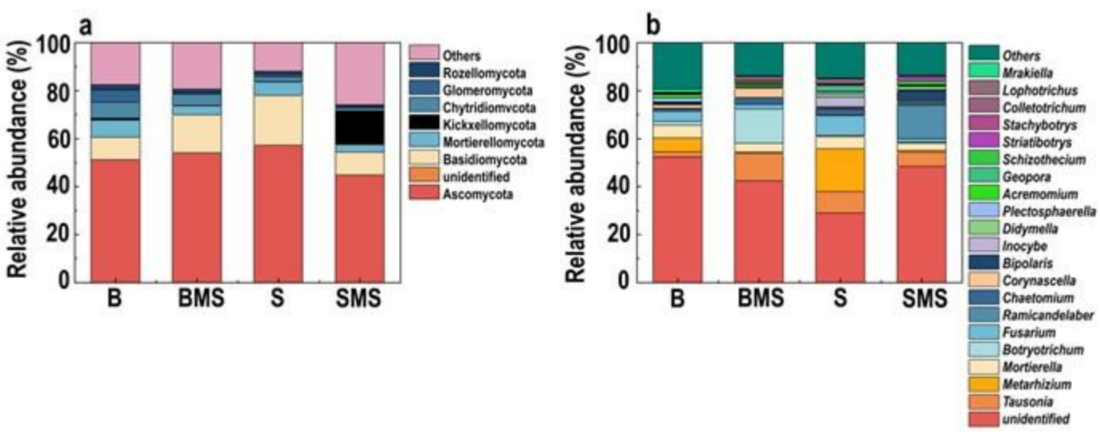

Fig. 4. Effect of pig's biogas slurry incorporation on the structure of soil fungal at phylum levels (a) and genus levels (b)

Soil microbial community similarity was analyzed using PCA (Fig. 5), and relationships with environmental factors were assessed through Redundancy Analysis

(RDA) (Fig. 6). The BS incorporation significantly affected bacterial community composition of aeolian sandy soil, but less affected soil bacteria of black soil (Fig. 6a). Fungal communities in both soils were changed significantly with BS incorporation (Fig. 6b). With pig farm BS treatments, TC, TN, and TP were the main factors affecting the microbial communities' structure in black soil, while pH was the main factor affecting the microbial communities' structure in aeolian windy soil (Fig. 6).

Significant differences in bacterial species distribution were observed between two types of soil under various treatments (Fig. 6a). This finding is consistent with previous research indicating that the use of organic fertilizers alters bacterial community structure (Francioli *et al*. 2016; Wang *et al*. 2016). At the fungal level, there is minimal difference in the distribution of fungal species between the traditional fertilized area and the pig's biogas slurry area in aeolian sandy soil (Fig. 6b). BS incorporation increased fungal biomass without altering fungal community composition, which was consistent with previous research (Yuan *et al*. 2013). In summary, BS incorporation with different dosage had diverse effects on soil microorganism abundance and community structure, and the effects depended on the soil types.

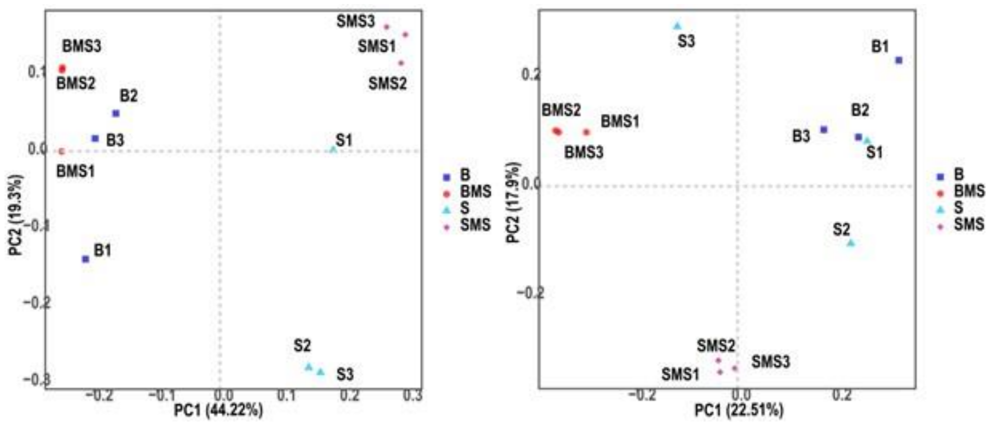

Fig. 5. Bacterial level (a) and fungal level (b) OTU level based PCA analyses

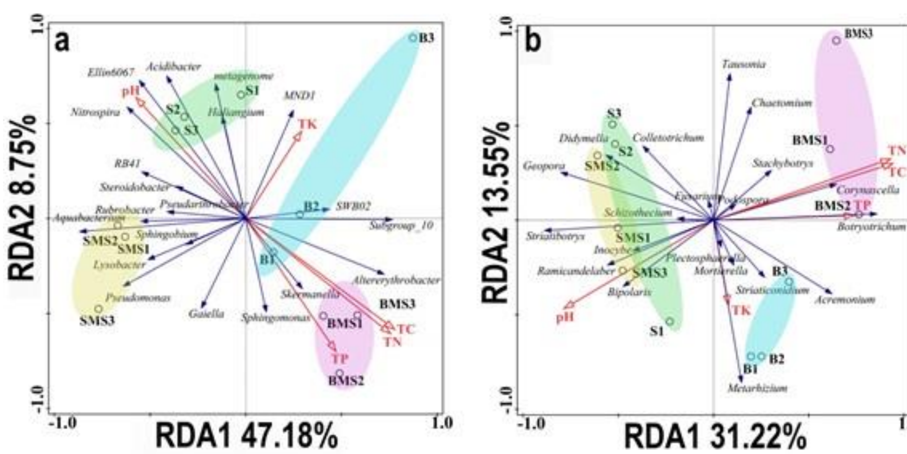

Fig. 6. RDA analysis of soil bacteria (a) and fungi (b) at the genus level, with environmental factors

Effect of Biogas Slurry on Soil Heavy Metal Elements Level

The concentrations of various heavy metal elements of black soil were notably higher than those in aeolian sandy soil (Fig. 7). BS incorporation at 600 t ·ha^{-1} dosage elevated tested heavy metal elements level of black soil significantly (*P<0.05*). BS incorporation at 300 t ·ha^{-1}·year^{-1} in aeolian sandy soil impacted tested heavy metals contents slightly, although only mercury (Hg) content was decreased significantly (*P<0.05*).

The increasing pattern of soil heavy metal elements level affected with BS incorporation also was consistent with previous studies (Guo *et al*. 2018; Kang *et al*. 2020). According to the "Chinese Soil Environmental Quality Standards (1995)", the concentrations of heavy metals with BS incorporation fall within the safe range as indicated in Table S1 and Figure S1. This study suggested that BS incorporation did not pose a threat to soil health. However, excessive increase of heavy metal content in black soil after twice high doses posed a potential risk to the environment, which meant BS application at high levels is not suitable. When farmers utilize pig farm BS fertilizer as an agronomic strategy applicable in practical agricultural production of Northeastern China, adjusting application dosage of BS must be carried based on the experimental results.

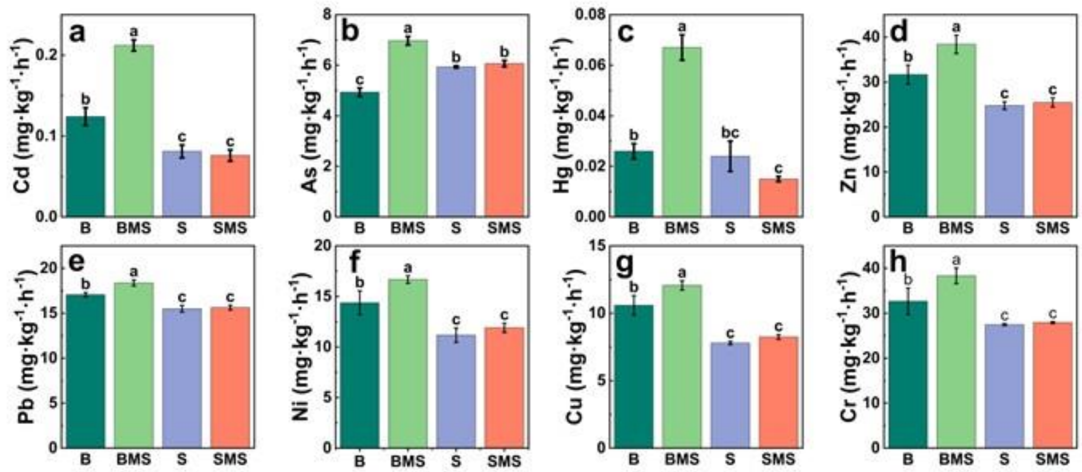

Fig. 7. The impact of pig's biogas slurry incorporation on soil heavy metal content. Lowercase letters indicate significant differences between treatments (P<0.05)

CONCLUSIONS

1. Incorporating pig farm biogas slurry at high dosages showed greater potential for improvement in black soil areas compared to aeolian sandy soil. Pig farm biogas slurry significantly enhances the physicochemical properties and enriches soil nutrient elements in black soil.
2. Soil microbial communities' adjustments were observed, and mechanisms for the soil microbial driven nutrient cycling varied lay on soil types. Incorporating pig farm biogas slurry significantly increased P-cycling enzyme activities in black soil, accelerating the enzymatic turnover process. After the addition of biogas slurry, there was no significant change in the C cycling enzyme in aeolian sandy soil. However, the activity of the P cycling enzyme decreased, along with reductions in total potassium and available potassium content.
3. High doses of BS should not be applied to aeolian sandy soil for multiple years due to its poor quality. Heavy metal levels in the black soil area have risen significantly.

Although still within safe limits, they pose a potential environmental threat. In conclusion, adding biogas slurry positively impacts soil nutrient cycling driven by microorganisms, but excessive application should be avoided. Reduce the dosage to an appropriate level.

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