

Article

Effects of Arbuscular Mycorrhizal Fungi and Biogas Slurry Application on Plant Growth, Soil Composition, and Microbial Communities of Hybrid *Pennisetum*

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Abstract: Biogas slurry with rich nutrients could be applied as fertilizer to improve nitrogen absorption and soil structure. Arbuscular mycorrhizal fungi (AMF) are beneficial soil microorganisms that establish mutualistic relationships with the plant roots. The purpose of this study was to study the effects of AMF and biogas slurry treatment on hybrid *Pennisetum* growth, soil chemical properties, and soil microorganisms. The results revealed that the biomass yield of hybrid *Pennisetum* was significantly increased after the application of biogas slurry, and it reached the peak values when the biogas slurry dosage was 900 t/hm², which were 13,216.67 kg/hm² and 13,733.33 kg/hm² in AMF– and AMF+ treatment groups, respectively. Moreover, biogas slurry treatment has a significant promoting effect on other agronomic traits related to biomass yield. As for soil chemical indicators, the contents of total nitrogen, nitrate nitrogen, ammonia nitrogen, and available phosphorus in the soil increased with the increase in biogas slurry application, while the soil organic matter was decreased. The addition of arbuscular mycorrhizal fungi significantly increased the species diversity of soil fungi with no biogas slurry application. Furthermore, when biogas slurry was applied, it had no significant effect on soil microbial diversity and composition, no matter the AMF+ or AMF– treatment. The research results can provide a reference for the long-term utilization of biogas slurry and it also can be used in the actual production of hybrid *Pennisetum*.

Keywords: biogas slurry; AMF; hybrid *Pennisetum*; agronomic characters; soil microorganism; soil



Citation: Cai, J.; Ran, Q.; Zhou, J.; Bi, M.; Liu, Y.; Yang, S.; Fan, Y.; Nie, G.; He, W. Effects of Arbuscular Mycorrhizal Fungi and Biogas Slurry Application on Plant Growth, Soil Composition, and Microbial Communities of Hybrid *Pennisetum*. *Sustainability* **2024**, *16*, 8416. <https://doi.org/10.3390/su16198416>

Academic Editor: Anna De Marco

Received: 22 July 2024

Revised: 17 September 2024

Accepted: 24 September 2024

Published: 27 September 2024



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1. Introduction

Biogas slurry, the primary by-product of the anaerobic fermentation of livestock and poultry manure, is rich in nutrients and can be utilized as a fertilizer in agricultural fields. Its application can enhance nitrogen uptake, improve soil structure, and reduce the overall cost of fertilizers required [1]. Nevertheless, improper treatment of biogas slurry can result in significant environmental pollution due to the high concentrations of nitrogen (N) and phosphorus (P). This can lead to eutrophication, air pollution, and soil degradation [2]. Additionally, biogas slurry application can increase plant height [3] and leaf area and leaf number [4], improve crop photosynthesis, promote plant tissue and organ development, accelerate the nutrient cycle [5], and change soil microbial community structure and microbial activity [6]. Meanwhile, although biogas slurry plays a positive role in improving the soil environment and promoting plant growth and development, excessive use of biogas slurry will still cause damage to the soil environment and eventually affect plant growth, increasing the risk of accumulation of harmful substances such as heavy metals [7] and antibiotics [8] in soil. Therefore, for sustainable development, it is

necessary to figure out a cost-effective way to dispose of these wastes and realize the safe utilization of biogas slurry.

Hybrid *Pennisetum* (*Pennisetum americanum* × *P. purpureum* Schumach L.) is a kind of fast-growing perennial C4 warm-season forage crop for ruminant cattle and sheep, which has the characteristics of high biomass production, strong adaptability to environmental stress, strong regeneration ability, and the ability to sequester carbon [9,10]. Studies have shown that hybrid *Pennisetum* can adapt well to irrigation by biogas slurry, which is used for wastewater treatment and can grow well by producing adventitious roots with internal air space [11]. Biogas slurry, as a better organic fertilizer, has a positive effect on plant biomass and quality. Compared with the control, the application of biogas slurry can significantly increase the biomass yield of *Pennisetum equisetum*, up to 119.0% and 174,754 kg/ha in the whole year [12]. The combination of biogas slurry and chemical synthetic fertilizer could significantly improve the growth, development, and yield of Italian ryegrass [13]. In addition, compared with the application of chemical fertilizer only, the application of biogas slurry increased the dry matter of Italian ryegrass by more than 9.00%, while the stem–leaf ratio decreased by more than 12%. However, the long-term application of biogas slurry may adversely affect the soil environment and soil microbial community [14–16].

Arbuscular mycorrhizal fungi (AMF) are common soil microorganisms, which establish mutually beneficial symbiosis with the roots of most plants, promoting the absorption of mineral nutrients in soil [17]. AMF could improve soil properties and the plant–water relationship through the AMF filamentous network, so that plants can find water and nutrients deeper and wider in soil profiles, thus reducing the need for chemical fertilizer and irrigation [18]. Studies have shown that the combined use of biogas slurry and AMF has a positive impact on most plants and the soil environment, especially plant growth, soil organic carbon, and nutrient content [18]. Compared with the corresponding separate applications, the combined application of biogas slurry and AMF was more obvious in promoting plant growth. The addition of biogas slurry improves the physical and chemical environment of the soil, increases mycorrhizal root colonization, and enhances symbiotic benefits to plant growth. Even in contaminated soil, AMF can significantly promote plant uptake of nutrients (especially P) [19–21], thereby helping plants grow and adapt to stress.

In this study, seven levels of biogas slurry amounts were applied to replace fertilizer for hybrid *Pennisetum* in a pot experiment, and arbuscular mycorrhizal fungi were added to soil as treatment. The agronomic characters, soil chemical properties, and soil microbial communities of hybrid *Pennisetum* were determined to evaluate the effects of arbuscular mycorrhizal fungi and biogas slurry on hybrid *Pennisetum*, providing a theoretical basis for solving the problem of environmental pollution caused by biogas slurry and promoting sustainable development. The research results can provide a reference for the long-term utilization of biogas slurry and be used in the actual production of hybrid *Pennisetum*. This study aims to provide a basis for the long-term use of biogas slurry in agriculture and the benign interaction between plants and microorganisms.

2. Material and Methods

2.1. Experiment Design

The experiment was conducted to explore the effect of arbuscular mycorrhizal fungi on biogas liquid absorption and utilization by hybrid *Pennisetum*. The experiment was carried out in a pot design. In the AMF group, a 200 g (containing 15–20 spores per 1 g) AMF (*Claroideoglomus etunicatum*, bought from National Microbial Resource Center) zeolite medium was added to the humus soil matrix in each pot (35 cm in diameter, 38 cm deep), and the same amount of the soil matrix was added to the CK group. The propagation of arbuscular mycorrhizal fungi propagules was carried out with maize (*Zea mays* L.) as material in the zeolite medium. In this study, hybrid *Pennisetum* (variety of Gui-Min Yin) was used as plant material, which was obtained from Sichuan Agricultural University, and propagated by stem node cuttage. After 3 months of cultivation, the plants with consistent growth were selected as test objects for transplantation, and 3 hybrid *Pennisetum* seedlings

were planted in each pot. The biogas slurry used in the experiment comes from Muyuan Food Co., Ltd. in Chengdu China, and the composition of biogas slurry was determined before the experiment (Table 1).

Table 1. Nutrient content of biogas slurry and nutritious soil used in the experiment.

Characteristics	Soil	Biogas Slurry
Organic matter (%)	18.62	0.35
Total nitrogen (%)	0.35	0.09
Ammoniacal nitrogen (mg/kg)	22.16	-
Nitrate nitrogen (mg/kg)	39.73	-
Total phosphorus (mg/L)	-	19.41
Available phosphorus (mg/kg)	6.20	-
Total potassium (mg/L)	-	833.6
Available potassium (mg/kg)	221.7	-

There were 7 different application levels of biogas slurry that were set in the AMF group and CK group (Table 2), respectively. Biogas slurry was applied three times during the treatment stage. Plant agronomic characters and soil chemical properties were determined at last.

Table 2. Biogas slurry treatment design, a total of 7 treatments; 3 times for biogas slurry application.

Amendment	Biogas Slurry Topdressing (L)			Total (t/hm ²)
	t1 (21 May 2022)	t2 (15 July 2022)	t3 (16 September 2022)	
T0	0	0	0	0
T1	0.5	0.5	0.5	150
T2	1	1	1	300
T3	1.5	1.5	1.5	450
T4	2	2	2	600
T5	2.5	2.5	2.5	750
T6	3	3	3	900

Note: The density of biogas slurry is close to that of water, which is calculated according to 1 g/mL.

2.2. Determination of Plant Agronomic Characters

A month after the last application of biogas slurry, the agronomic characters of hybrid *Pennisetum* were measured, including leaf length, leaf width, stem diameter, plant height, and total fresh biomass yield. The leaf length of the second stem node from the top of each plant was measured, and the leaf width was the width of the widest part of the leaf. The stem diameter was measured for the stem node close to the soil of hybrid *Pennisetum*. The plant height was the distance from the highest leaf of hybrid *Pennisetum* to the surface of soil, and then the fresh biomass of hybrid *Pennisetum* was measured.

2.3. Collection and Determination of Soil Chemicals

The 0–20 cm soil samples were taken from each pot and the samples were filtered by a 2 mm mesh sieve. After removing the plant residues, the samples were evenly divided into three parts. The first part of them were naturally air-dried for the determination of soil organic matter, soil total nitrogen, soil available phosphorus, and available potassium. The second part was stored in a refrigerator at 4 °C to determine soil moisture content, soil ammonium nitrogen, and soil nitrate nitrogen. The last part of the 0–20 cm soil samples were frozen in the refrigerator at −80 °C. The high-throughput sequencing technique was used to analyze the microbial and fungal community diversity in the soil rhizosphere.

Soil organic matter was determined by potassium dichromate volumetric method–external calorimetry, and soil total nitrogen content was determined by the method of Kjeldahl [22]. Soil nitrate nitrogen and ammonia nitrogen were determined by phenol disulfonic acid colorimetry and KCl extraction–indophenol blue colorimetry, respectively [23].

Soil available phosphorus and available potassium were determined by the $1/2\text{H}_2\text{SO}_4$ method and ammonium acetate extraction–flame spectrophotometry, respectively [22,24].

Calculation formula of soil organic matter:

$$\text{Soil organic matter} = \frac{(1000 * m1 * 1.724 * 1.32)}{m} * 100\% \quad (1)$$

$m1$ = The carbon content of soil is obtained from the standard curve.

m = Soil weight.

2.4. Determination of Soil Microbial Community

The community structure of soil fungi and bacteria was determined by high-throughput sequencing in the ITS region and 16 s region. The sample gDNA was purified by Zymo Kit (Cat# D4301) (Beijing, China). The concentration and integrity of gDNA were detected by Tecan F200 (Infinite ® 200 PRO, Mennedorf, Switzerland)(PicoGreen dye method) and 0.8% agarose electrophoresis [25]. The V4 region of bacterial DNA was amplified using gene primers 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), and the PCR reactions were performed as follows: a 50 μL reaction mixture including 5 μL 10 $\times$ PCR Buffer for KOD-Plus-Neo, 5 μL 2 mM dNTPS, 3 μL of 25 mM MgSO_4 , 1.5 μL of U515F, 1.5 μM of U806R, 1 μL of KOD-Plus-Neo (1 U/ μL), and 40 ng template DNA. The fungal DNA was amplified with primers ITS3 (5'-GATGAAGAACGYAGYRAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'), and the PCR programme was as follows: pre-denaturation at 94 $^\circ\text{C}$ for 1 min, denaturation at 94 $^\circ\text{C}$ for 20 s, annealing at 54 $^\circ\text{C}$ for 30 s and extension at 72 $^\circ\text{C}$ for 30 s for 25–30 cycles, 72 $^\circ\text{C}$ for 5 min for 1 cycle, and heat preservation at 4 $^\circ\text{C}$. Each sample was repeated by PCR technology for 3 times, and the linear phase PCR products were mixed in the same amount and used for the subsequent construction of the database. The PCR product was mixed with a 6-fold sample buffer, and then the target fragment was detected by 2% agarose gel electrophoresis. The samples that passed the test were collected and recycled using ZymocleanGelRecoveryKit (D4008) (Biomarker Technologies, Beijing, China) and we used Qubit® 2.0 Fluorometer (Biomarker Technologies, Beijing, China) (Thermo Scientific, Waltham, MA, USA) quantitatively, finally followed by equimolar mixing. The NEBNext Ultra II DNA Library Prep Kit for Illumina (NEB#E7645L) (NEW ENGLAND BioLabs, Beijing, China) was used to prepare the kit. Using the PE250 sequencing method, the sequencing kit uses Illumina NovaSeq 6000 SP Reagent Kit V1.5 (Illumina, Shanghai, China).

2.5. Statistical Analysis

The experiment was conducted with three biological replications. The data in the chart are represented as a mean value. IBM SPSS Statistics 25 (64-bit) software was used for a one-way analysis of variance (ANOVA), and the Duncan test was used. A principal component analysis (PCA) was performed through the OriginPro 2021 (64-bit) software; PCA is used to reduce the number of interrelated variables to smaller sets, which explains the overall variability.

In addition, the grey correlation degree analysis of the measured index was carried out, and the specific formula is as follows:

$$\text{Grey correlative} = \frac{\min_i \min_k |X_{0(k)} - X_{i(k)}| + 0.5 \max_i \max_k |X_{0(k)} - X_{i(k)}|}{\max_i |X_{0(k)} - X_{i(k)}| + 0.5 \max_i \max_k |X_{0(k)} - X_{i(k)}|} \quad (2)$$

Note: i stands for processing, and k for indicators.

3. Result

3.1. Effects of AMF and Biogas Slurry on Agronomic Characters

The changes in agronomic characters related to biomass yield of hybrid *Pennisetum* under arbuscular mycorrhizal fungi and different biogas slurry treatments are shown in Figure 1. The leaf width of hybrid *Pennisetum* increased with the increase in biogas slurry application. Specially, in the T3 treatment group, the leaf width of the arbuscular mycorrhizal fungi (AMF+) group was significantly higher than that of the treatment group without arbuscular mycorrhizal fungi (AMF−). The change in leaf length was similar to that of leaf width, and reached the maximum value at T5 treatment (100.72 cm), indicating that excess biogas slurry will inhibit the growth of the plant. There was no significant difference between AMF− and AMF+ treatment groups for leaf length.

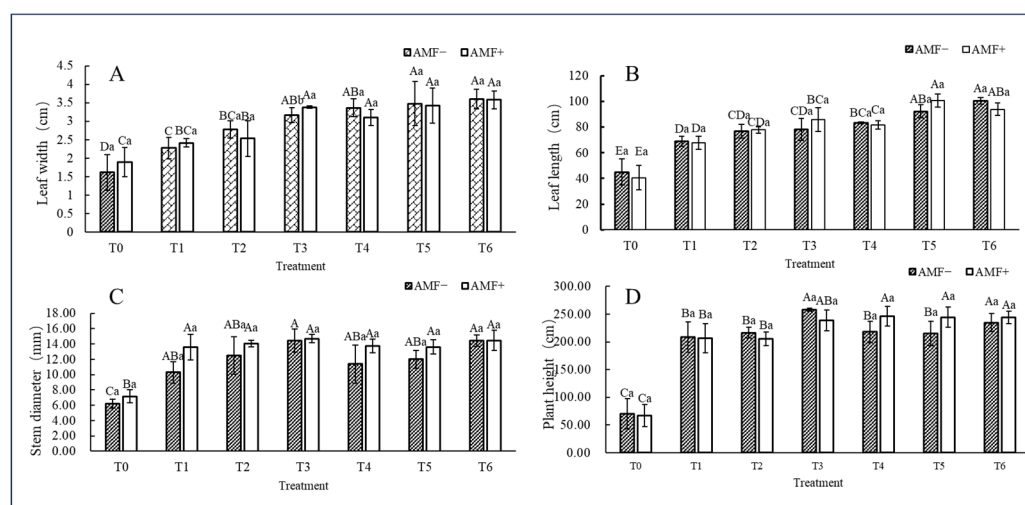


Figure 1. Effects of different biogas slurry treatments and AMF on different agronomic characters such as leaf width (A), leaf length (B), stem diameter (C), and plant height (D) of hybrid *Pennisetum*. Different capital letters in the figure indicate significant differences between different biogas slurry treatment conditions under the same fungus treatment, and different lowercase letters indicate significant differences between AMF+ and AMF− under the same biogas slurry treatment conditions ($p < 0.05$).

The maximum values of stem diameter under AMF− and AMF+ treatments appeared at T3 treatment with 14.45 and 14.71 mm. The maximum plant height appeared in T3 and T4 treatments under AMF− and AMF+ treatments, which were 258.44 cm and 246.50 cm, respectively. In total, plant growth had a positive correlation with the increase in biogas slurry application, while the difference between AMF− and AMF+ was not significant, indicating that the effect of arbuscular mycorrhizal fungi was mainly on soil properties and microbial diversity.

The change in forage yield of hybrid *Pennisetum* under arbuscular mycorrhizal fungi and biogas slurry treatment is shown in Figure 2. After the application of biogas slurry, the yield of hybrid *Pennisetum* was significantly higher than that of the control group, and the yield of hybrid *Pennisetum* in AMF− and AMF+ treatment was the highest under T6 treatment, which was 13,216.67 kg/hm² and 13,733.33 kg/hm², respectively. However, the effect of arbuscular mycorrhizal fungi on the yield of hybrid *Pennisetum* was not obvious, and there was no significant difference between AMF− and AMF+.

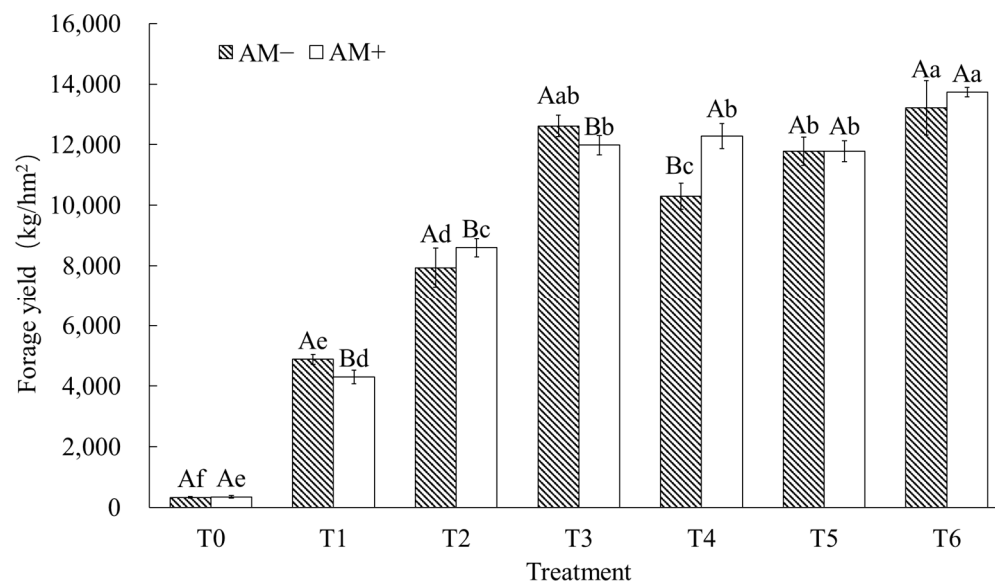


Figure 2. The effect of biogas slurry treatment on yield of hybrid *Pennisetum*. The lowercase letters above the histogram indicate the significance between different biogas slurry application rates under the same AMF, and the capital letters above the histogram indicate the significance of the difference between different AMF treatments with the same application amount ($p < 0.05$).

3.2. Effects of AMF and Biogas Slurry on Soil Chemical Properties

Soil total nitrogen is mainly determined by soil humus, including organic nitrogen and inorganic nitrogen, which is closely related to crop yield and used to guide fertilization in production. In this study, with the increase in biogas slurry application, the total of nitrogen in soil was also increased and reached the peak value at T6 treatment (5.76 g/kg and 4.05 g/kg, respectively), no matter at the AMF– or AMF+ level (Figure 3A). Additionally, the total nitrogen content of the AMF– treatment group was higher than that of the AMF+ treatment group, which may be due to the higher utilization efficiency of soil organic nitrogen by hybrid *Pennisetum* with the participation of arbuscular mycorrhizal fungi under biogas slurry treatment.

Soil inorganic nitrogen is the product of microbial activities mainly including nitrate nitrogen and ammonia nitrogen, which is easy to be absorbed by plants. In this study, the soil nitrate nitrogen content increased with the application amount of biogas slurry in AMF+ groups (Figure 3B), and reached the maximum value in the T6 treatment (153.49 mg/kg), and the content was higher than the AMF– group. The soil ammonia nitrogen content in each biogas slurry treatment group was significantly higher than that in the T0 group (Figure 3C), and the soil ammonia nitrogen content increased with the increase in the biogas slurry application amount.

Soil organic matter is an important substance, which is the basis of maintaining soil life and promoting plant growth. In this study, soil organic matter increased at first and then decreased with the increase in biogas slurry application, and the soil organic matter content reached the maximum under T2 treatment (Figure 4). The soil organic matter content of AMF– and AMF+ treatment under T2 treatment was 196.56 g/kg and 169.50 g/kg, respectively. The content of soil organic matter in AMF– treatment was higher than that in AMF+ treatment, which may be related to the participation of arbuscular mycorrhizal fungi in the utilization of soil organic matter by hybrid *Pennisetum*.

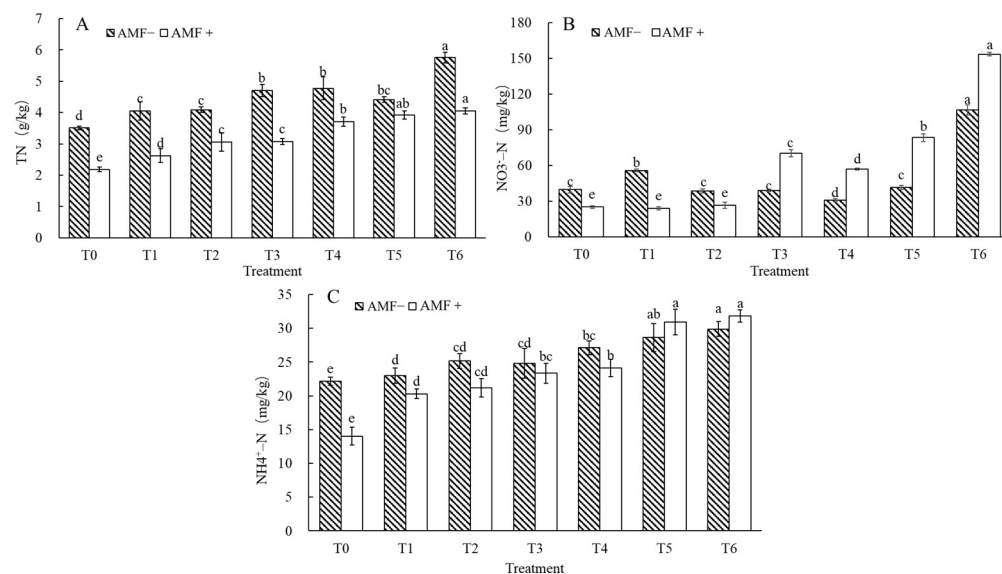


Figure 3. Effects of different treatments on total nitrogen (A), nitrate nitrogen (B), and ammonia nitrogen (C) in soil. The lowercase letters above the histogram indicate the significance between different biogas slurry application rates under the same AMF, and the capital letters above the histogram indicate the significance of the difference between different AMF treatments with the same application amount ($p < 0.05$).

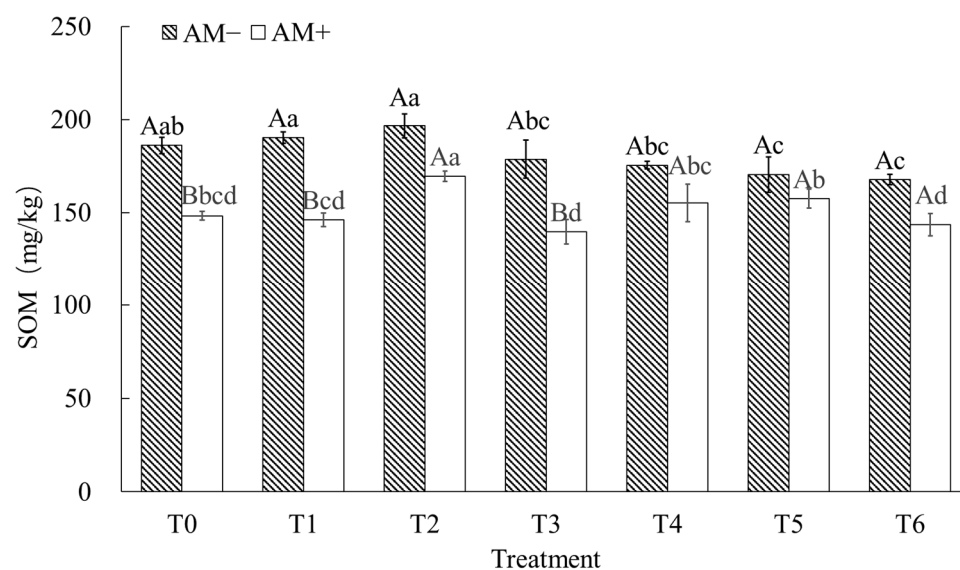


Figure 4. Effects of different treatments on soil organic matter in soil. The lowercase letters above the histogram indicate the significance between different biogas slurry application rates under the same AMF, and the capital letters above the histogram indicate the significance of the difference between different AMF treatments with the same application amount ($p < 0.05$).

Available phosphorus and available potassium are easily absorbed and utilized by plants in soil. The content of available phosphorus in soil increased with the increasing in biogas slurry application, and the maximum values in AMF- and AMF+ treatment groups were 253.06 mg/kg and 250.59 mg/kg, respectively (Figure 5). Under AMF- treatment, there was no significant difference in the application amount of high biogas slurry (T4~T6) among the treatment groups, while in the AMF+ treatment group, the application amount of high biogas slurry was significantly higher than that of low biogas slurry. In terms of soil available potassium, there was no obvious pattern among the amount of biogas slurry application.

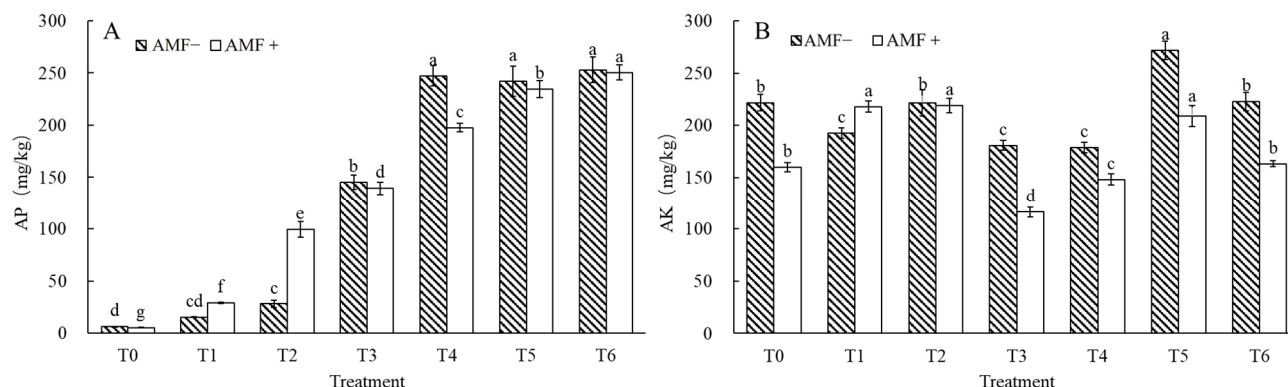


Figure 5. Effects of different treatments on available phosphorus (A) and available potassium (B) in soil. The lowercase letters above the histogram indicate the significance between different biogas slurry application rates under the same AMF, and the capital letters above the histogram indicate the significance of the difference between different AMF treatments with the same application amount ($p < 0.05$).

3.3. Clustering, Correlation, and PCA Analysis of Plant Growth and Soil Chemical Properties

In order to further determine the key index parameters for evaluating the agronomic and soil chemical properties of hybrid *Pennisetum*, 14 treatment indicators were selected to draw a heat map. As shown in Figure 6, 14 treatment groups were clustered into 3 groups: the type I group included T3–T6, which was a group with high biogas slurry application treatment; the type II treatment group included T1 and T2, which was a group with low biogas slurry application treatment, and type III was the T0 control group. Among the indicators, AK and SOM were negative indicators, and yield, LW, LL, SD, height, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, AP, and TN were positive indicators.

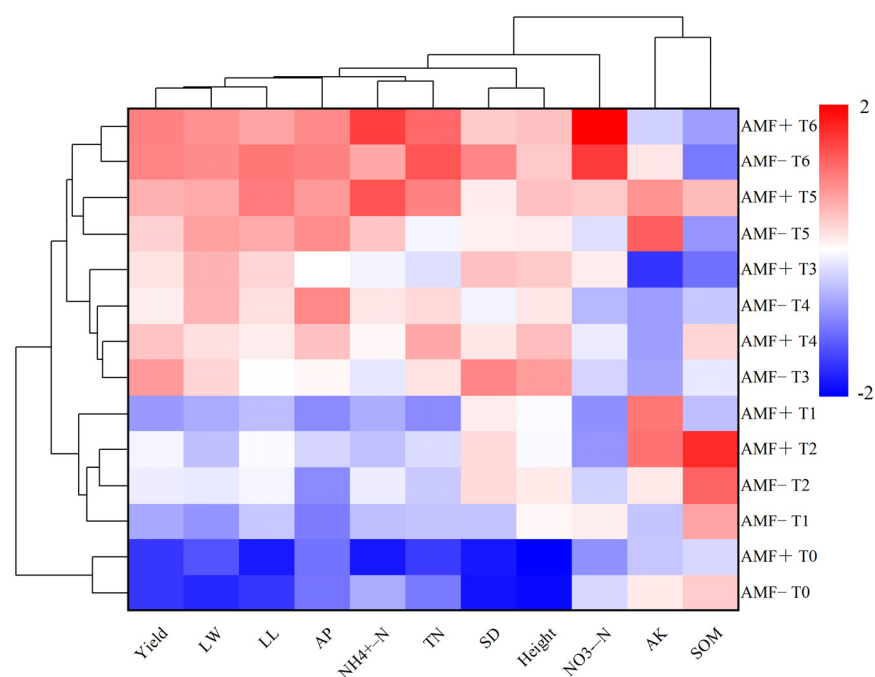


Figure 6. The cluster analysis of various indexes of hybrid *Pennisetum* under different treatments. In the picture, red is positive correlation and blue is negative correlation.

In order to determine whether there is a correlation between the agronomic characters and soil chemical indexes under 14 treatments, a correlation heat map was drawn for a total of 12 indexes. As shown in Figure 7, the correlation between available potassium (AK)

and the other indicators was lower, and the correlation between soil organic matter (SOM) and most other indicators was negative.

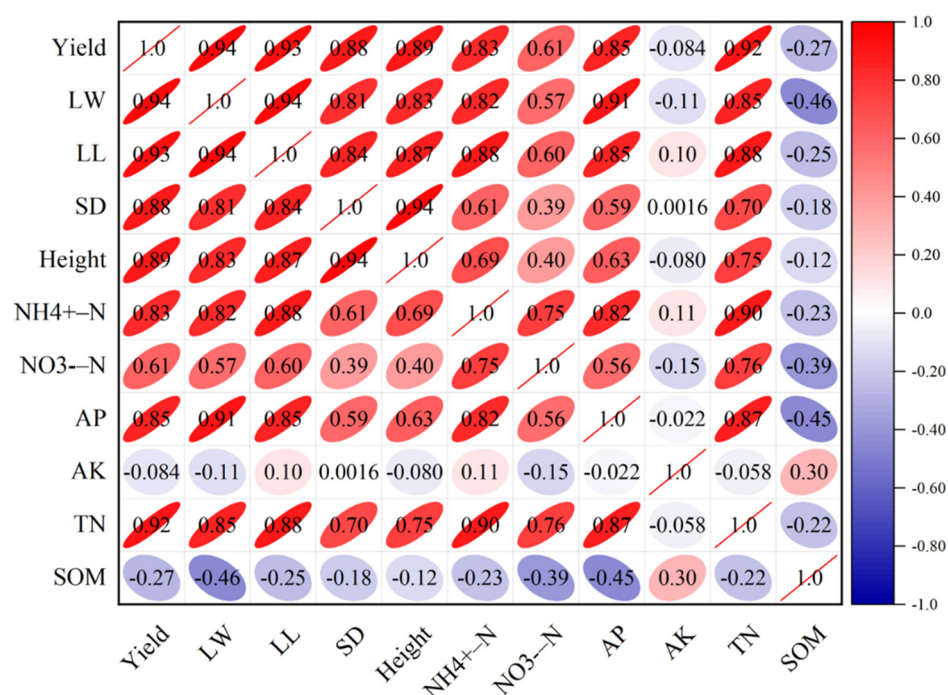


Figure 7. Correlation analysis of various indexes of hybrid *Pennisetum*. Red is positive correlation and blue is negative correlation.

Based on the PCA analysis, it could be seen that yield, LW, LL, SD, height, NH₄⁺-N, NO₃⁻-N, AP, and TN as positive indexes have an obviously promoting effect under treatment, while SOM was a negative index, which tends to decrease after treatment (Figure 8). Interestingly, available potassium (AK) seems to be not significantly affected after biogas slurry and arbuscular mycorrhizal fungi treatment.

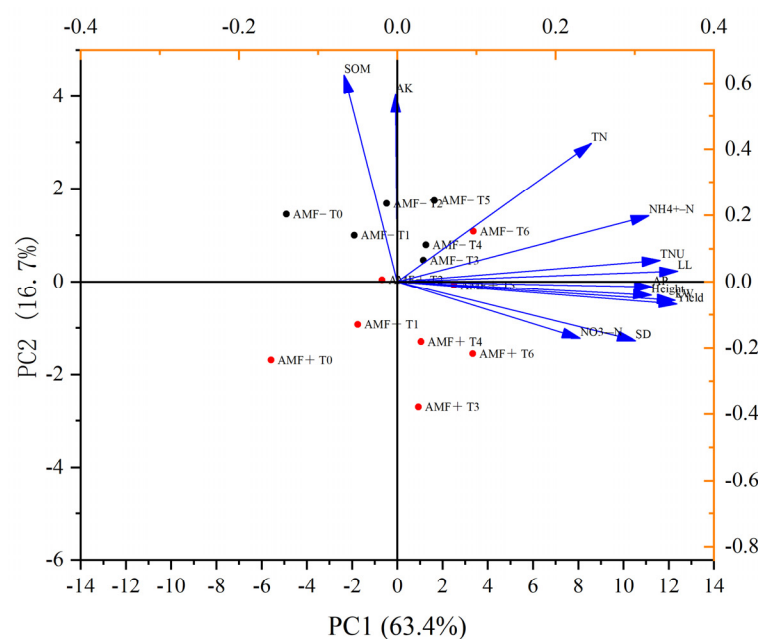


Figure 8. PCA analysis of different indexes of hybrid *Pennisetum* under different treatments.

In order to determine which treatment has the greatest positive influence on hybrid *Pennisetum* growth and soil chemical properties among 14 treatments, grey correlation degree analyses were carried out. The specific results are shown in Table 3. Among the 14 treatments, the grey correlative coefficient of AMF– T6 was the highest, reaching 0.879, followed by AMF+ T6, AMF+ T5, AMF– T5, AMF– T4, AMF– T3, AMF+ T4, AMF+ T3, AMF– T2, AMF+ T2, AMF– T1, AMF+ T1, AMF– T0, and AMF+ T0. The order of treatment based on weighted grey correlatives was consistent with previous analyses.

Table 3. Grey Relational degree Analysis of hybrid *Pennisetum* under different treatments.

Treatment	Grey Correlative	Order	Weighted Grey Correlative	Order
AMF– T6	0.879	1	0.823	1
AMF+ T6	0.861	2	0.798	2
AMF+ T5	0.799	3	0.753	3
AMF– T5	0.782	4	0.737	4
AMF– T4	0.740	5	0.698	5
AMF– T3	0.728	6	0.691	6
AMF+ T4	0.694	7	0.657	7
AMF+ T3	0.674	8	0.641	8
AMF– T2	0.659	9	0.628	9
AMF+ T2	0.635	10	0.604	10
AMF– T1	0.587	11	0.557	11
AMF+ T1	0.581	12	0.553	12
AMF– T0	0.506	13	0.477	13
AMF+ T0	0.450	14	0.425	14

3.4. Effects of AMF and Biogas Slurry on Soil Microbial Diversity and Composition

From the abundance diagram of soil microorganisms at the genus level, there was no significant difference in the species and abundance of soil bacteria among the treatment groups (Figure 9A). When biogas slurry was not applied, the addition of arbuscular mycorrhizal fungi increased the abundance of *Acidea* and reduced the abundance of *Plectosphaerella* compared with the AMF– treatment (Figure 9B). After the application of biogas slurry, there was no significant difference of the abundance levels of *Acidea* and *Plectosphaerella* between AMF+ and AMF–, regardless of whether arbuscular mycorrhizal fungi were added.

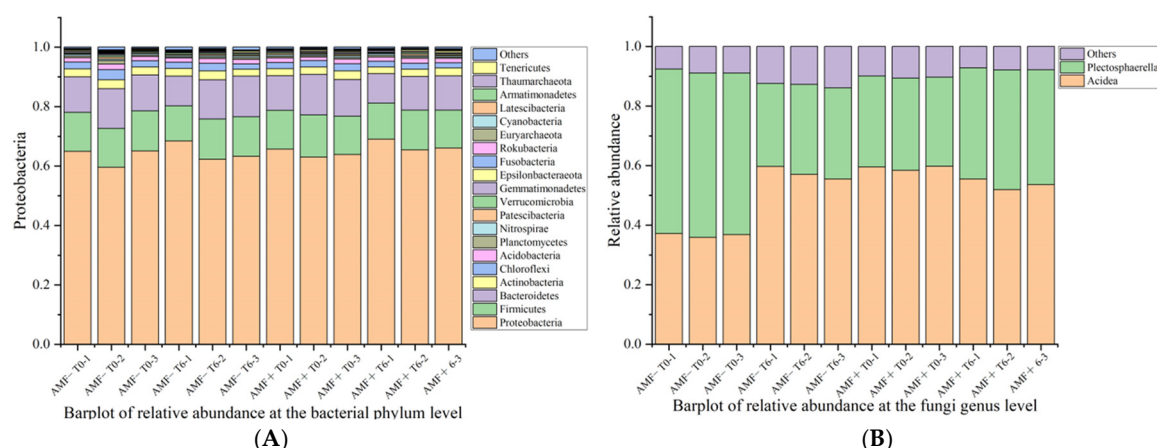


Figure 9. Abundance of soil bacteria (top 20 species) (A) and fungi (B). Different colours represent different genera.

The Alpha diversity of soil microorganisms showed that the Chao1 index and Shannon–Wiener index of soil bacteria had no significant difference among treatments (Figure 10), which indicated that biogas slurry and arbuscular mycorrhizal fungi had no significant effect on the species number and community diversity of bacteria. In terms of the Chao1

index and Shannon–Wiener index of soil fungi, the addition of arbuscular mycorrhizal fungi significantly increased the number and the species diversity of soil fungi with no biogas slurry application. The addition of biogas slurry decreased the Chao1 index and Shannon–Wiener index of fungi in the AMF+ group, which indicated the biogas slurry effect on soil microbial diversity and composition.

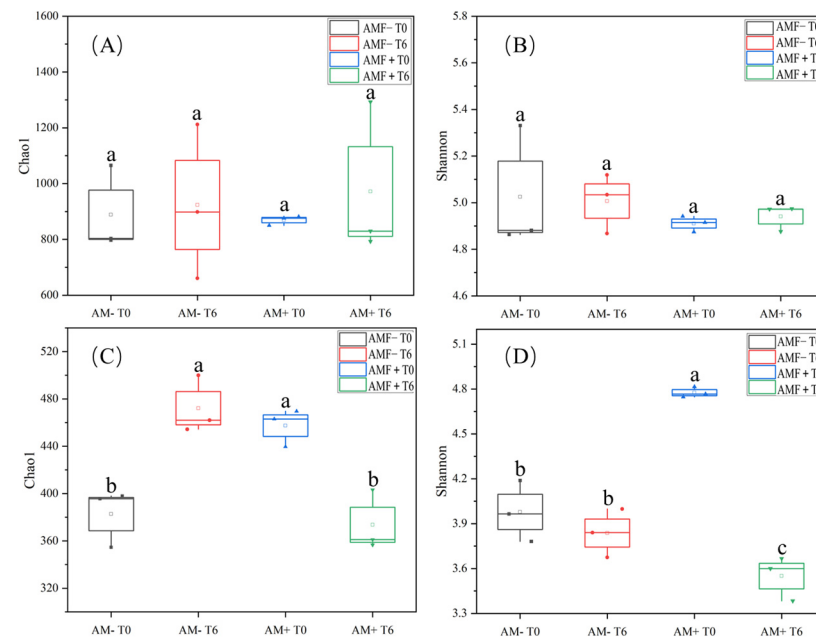


Figure 10. Test soil microbial bacteria (A) and fungi (C) Chao1 index and bacteria (B) and fungi (D) Shannon–Wiener index; different lowercase letters in figure indicate significant differences ($p < 0.05$).

It can be seen from the PCoA plot (Figure 11) that there was no significant difference in the soil bacterial community among the four treatments (Figure 11A), but the soil fungal community was obviously separated between different treatments (Figure 11B). In addition, there were significant differences in the fungal communities between the AMF– and AMF+ treatments when no biogas slurry was applied.

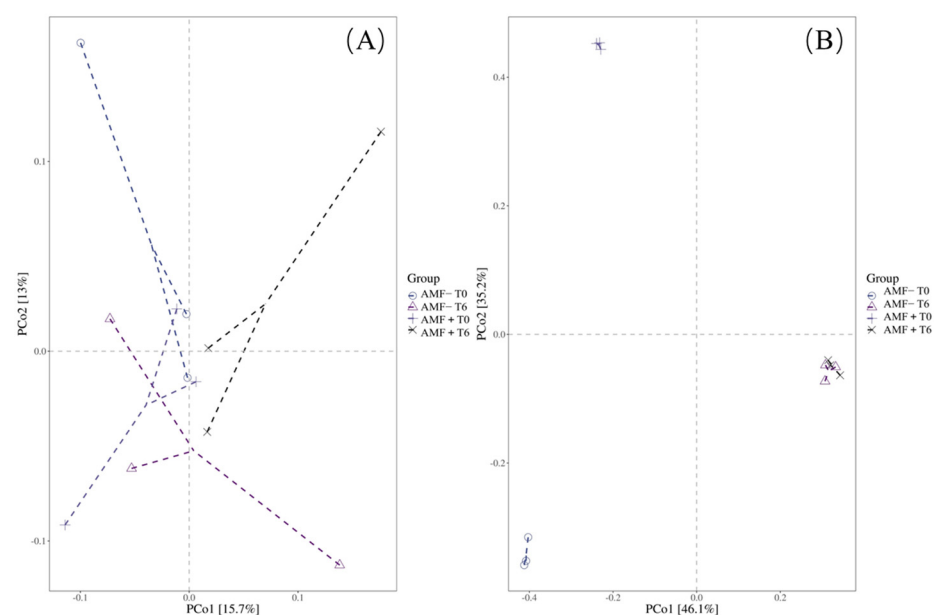


Figure 11. PcoA diversity of microbial bacteria (A) and fungi (B) in experimental soil.

4. Discussion

Biogas slurry has great fertilizer potential and is considered to be a popular source of nutrients, especially nitrogen, which play an essential role in sustainable organic agriculture development [26]. Biogas slurry has been widely used for crop productivity [27] and previous studies showed that using biogas slurry instead of chemical fertilizer in wheat (*Triticum aestivum* L.) and paddy fields could increase plant tiller and yield [28,29]. Additionally, crops such as peanuts (*Arachis hypogaea* L.) [30], tomatoes (*Solanum lycopersicum* L.) [31], and pepper (*Piper nigrum* L.) [32] can be harvested for a long time, suggesting that slow-release components in biogas slurry, such as organic matter and humic acid, provide an economic advantage by making fertilizers last longer. In this study, the biomass yield and related traits of hybrid *Pennisetum* treated with biogas slurry were significantly higher than those of the CK group, which was consistent with the previous studies, and the yield of hybrid *Pennisetum* increased with the increase in biogas slurry use. A combination of organic and synthetic fertilizers often increases crop yields and promotes optimal soil fertility. However, the cost of synthetic fertilizers is often beyond the reach of many small-scale farmers. Therefore, using biogas slurry instead of synthetic fertilizers in agricultural production is a proven way to reduce costs and increase crop yields [33,34].

Biogas slurry is a kind of organic fertilizer, which has many advantages to improve soil quality and plant growth, including increasing the availability of plant nutrients, enhancing water retention capacity, enhancing cation exchange capacity, improving soil texture, and promoting the growth of beneficial microorganisms in soil, such as nitrifying bacteria and phosphorus-solubilizing bacteria [35]. In addition, the addition of organic modifiers improves the physical properties of the soil, such as air permeability, water retention, stability, aggregates, and resistance to soil erosion, thus helping to prevent or mitigate the erosion process. Suitable biogas slurry provides a balanced proportion of nutrients and serves as a valuable source of slow-release minerals [36]. In this study, applying biogas slurry increased the content of total nitrogen, nitrate nitrogen, ammonia nitrogen, and available phosphorus in hybrid *Pennisetum* soil, while reducing the content of soil organic matter. The effect on the content of available potassium in the soil was not significant. This result was different from previous studies regarding that biogas slurry can be used as a viable alternative to chemical fertilizers and can effectively increase the content of soil organic carbon, especially when applied together with biochar, which can promote soil health [37].

As a habitat for plant and soil biodiversity, AMF can regulate soil composition and structure and its community plays an important role in maintaining the structure and diversity of a plant community and maintaining the stability and function of an ecosystem [38]. In this study, the exogenous addition of AMF could significantly improve the community richness and diversity of fungi in hybrid *Pennisetum* soil, but the effect of AMF reduced after the application of biogas slurry. AMF hyphae and GRSP outperform fine roots in controlling microbial diversity and soil multifunction [39]. AMF roots and AMF hyphae create specific habitats (that is, the rhizosphere and lower phosphorus) for a range of different microorganisms through secretions, thus forming specialized microbial communities [40]. For example, some phytate-degrading bacteria were isolated from the AMF mycelium chamber [41] and these bacteria may contribute to plant phosphorus nutrition along with AMF [42]. Research results showed that the effect of using biogas slurry instead of chemical fertilizer topdressing on the α -diversity of soil bacterial and fungal communities is not obvious, but different application ratios of biogas slurry and chemical fertilizers affect the separation of bacterial and fungal diversity indexes, among which the bacterial index value increases or decreases linearly with the increase in the ratio of biogas slurry to chemical fertilizer topdressing [43]. In the results of this study, the abundance of pathogenic fungi (*Plectosphaerella*) in the control group soil was higher than that in other treatment groups, while the abundance of saprophytic fungi (*Acidea*) was lower, indicating that the addition of biogas slurry and arbuscular mycorrhizal fungi would increase the abundance of saprophytic fungi in the soil and reduce the abundance of pathogenic fungi.

Among them, saprophytic fungi play an important role in the growth of hybrid *Pennisetum*. In addition, there could be interaction between intracellular bacterial microbiomes and AMF to produce characterized functions, which require further proof in the future [44].

5. Conclusions

In this study, the agronomic traits, soil physicochemical properties, and rhizosphere soil microbial communities of hybrid *Pennisetum* were analyzed by applying biogas slurry and adding exogenous AMF; the results will provide key information for the balance between hybrid *Pennisetum* production and biogas slurry digestibility, and make recommendations for managing soils to promote soil health and productivity, benefiting the sustainable development of the livestock sector. The results showed that the application of biogas slurry could promote the growth of hybrid *Pennisetum*, and the yield and related agronomic traits of hybrid *Pennisetum* increased with the increase in biogas slurry application. With the increase in biogas slurry application, the contents of total nitrogen, nitrate nitrogen, ammonia nitrogen, and available phosphorus in the soil also increased, while the content of soil organic matter decreased. The effect of adding AMF on the growth of hybrid *Pennisetum* and soil nutrients was not obvious, but from the results of soil microorganisms, the addition of AMF could increase the richness and diversity of fungal communities in the soil when biogas slurry was not applied, and the addition of biogas slurry and AMF could reduce the abundance of *Plectosphaerella*. Under the condition of applying biogas slurry, it had no significant effect on soil microbial diversity and composition, no matter for AMF+ or AMF− treatment, and the reasons for this need further study.

Author Contributions: Data curation, G.N., J.C., M.B., Y.L. and J.Z.; Funding acquisition, Q.R., G.N., W.H. and Y.F.; Investigation, J.C., S.Y. and Y.L.; Methodology, G.N. and W.H.; Project administration, Q.R., W.H. and Y.F.; Resources, J.C.; Software, M.B.; Validation, J.Z. and S.Y.; Writing—original draft, G.N. and J.C.; Writing—review and editing, G.N. and J.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Chongqing performance incentive guide special project (21528) and Innovation Training Program for College Students (S202410626020).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Bachmann, S.; Gropp, M.; Eichler-Loebermann, B. Phosphorus availability and soil microbial activity in a 3 year field experiment amended with digested dairy slurry. *Biomass Bioenergy* **2014**, *70*, 429–439. [\[CrossRef\]](#)
2. de Godos, I.; Vargas, V.A.; Blanco, S.; González, M.C.G.; Soto, R.; García-Encina, P.A.; Becares, E.; Muñoz, R. A comparative evaluation of microalgae for the degradation of piggery wastewater under photosynthetic oxygenation. *Bioresour. Technol.* **2010**, *101*, 5150–5158. [\[CrossRef\]](#)
3. Xu, Z.-M.; Wang, Z.; Gao, Q.; Wang, L.-L.; Chen, L.-L.; Li, Q.-G.; Jiang, J.-J.; Ye, H.-J.; Wang, D.-S.; Yang, P. Influence of irrigation with microalgae-treated biogas slurry on agronomic trait, nutritional quality, oxidation resistance, and nitrate and heavy metal residues in Chinese cabbage. *J. Environ. Manag.* **2019**, *244*, 453–461. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Ghimire, K.; Gautam, D.; Mishra, K.; Pande, K.; Gc, A. Influence of biogas slurry and urea on yield and quality of okra (*Abelmoschus esculentus* L.) fruits. *J. Agric. Environ.* **2015**, *16*, 161–169. [\[CrossRef\]](#)
5. Xu, C.; Tian, Y.; Sun, Y.; Dong, L. Effects of Biogas Slurry Irrigation on Growth, Photosynthesis, and Nutrient Status of *Perilla frutescens* Seedlings. *Commun. Soil Sci. Plant Anal.* **2013**, *44*, 3381–3390. [\[CrossRef\]](#)
6. Alejandro Iocoli, G.A.; Zabaloy, M.C.; Pasdevicelli, G.; Gómez, M.A. Use of biogas digestates obtained by anaerobic digestion and co-digestion as fertilizers: Characterization, soil biological activity and growth dynamic of *Lactuca sativa* L. *Sci. Total Environ.* **2019**, *647*, 11–19. [\[CrossRef\]](#)
7. Bian, B.; Zhou, L.J.; Li, L.; Lv, L.; Fan, Y.M. Risk assessment of heavy metals in air, water, vegetables, grains, and related soils irrigated with biogas slurry in Taihu Basin, China. *Environ. Sci. Pollut. Res.* **2015**, *22*, 7794–7807. [\[CrossRef\]](#)

8. Pu, C.; Liu, H.; Ding, G.; Sun, Y.; Yu, X.; Chen, J.; Ren, J.; Gong, X. Impact of direct application of biogas slurry and residue in fields: In situ analysis of antibiotic resistance genes from pig manure to fields. *J. Hazard. Mater.* **2017**, *344*, 441–449. [\[CrossRef\]](#)
9. Premaratne, S.; Premalal, G.G.C. Hybrid Napier (*Pennisetum purpureum* × *Pennisetum americanum*) VAR. CO-3: A resourceful fodder grass for dairy development in Sri Lanka. *J. Agric. Sci. Sri Lanka* **2006**, *2*, 22–33. [\[CrossRef\]](#)
10. Li, X.; Geng, X.; Xie, R.; Fu, L.; Jiang, J.; Gao, L.; Sun, J. The endophytic bacteria isolated from elephant grass (*Pennisetum purpureum* Schumacher) promote plant growth and enhance salt tolerance of Hybrid *Pennisetum*. *Biotechnol. Biofuels* **2016**, *9*, 190. [\[CrossRef\]](#)
11. Jampeetong, A.; Muenrew, J. Interactive effects of NH_4^+ concentration and O_2 availability on growth, morphology, and mineral allocation of hybrid Napier grass (*Pennisetum purpureum* × *P. americanum* cv. Pakchong1). *Ecol. Eng.* **2016**, *91*, 409–418. [\[CrossRef\]](#)
12. Yang, Q.; Zhong, Z.M.; Weng, B.Q.; Huang, X.S.; You, X.F. Effects of Biogas Slurry on Yield of *Pennisetum* and Inorganic Nitrogen and Enzymatic Activities in Soil. *Fujian J. Agric. Sci.* **2016**, *31*, 1353. [\[CrossRef\]](#)
13. Xu, W.; Zhu, Y.; Wang, X.; Ji, L.; Wang, H.; Yao, L.; Lin, C. The effect of biogas slurry application on biomass production and forage quality of *Lolium multiflorum*. *Sustainability* **2021**, *13*, 3605. [\[CrossRef\]](#)
14. Huang, J.C.; Peng, Z.P.; Xu, P.Z.; Tu, Y.T.; Yang, L.X.; Wu, X.N.; Lin, Z.J. Effects of paddy field disposal of biogas slurry on rice production, soil fertility and environmental safety. *Guangdong Agric. Sci.* **2016**, *43*, 8. [\[CrossRef\]](#)
15. Chen, Z.; Ma, J.; Ma, J.; Ye, J.; Yu, Q.; Zou, P.; Sun, W.; Lin, H.; Wang, F.; Zhao, X.; et al. Long-term biogas slurry application increases microbial necromass but not plant lignin contribution to soil organic carbon in paddy soils as regulated by fungal community. *Waste Manag.* **2024**, *175*, 254–264. [\[CrossRef\]](#)
16. Sun, R.; Ding, J.; Li, H.; Wang, X.; Li, W.; Li, K.; Ye, X.; Sun, S. Mitigating nitrate leaching in cropland by enhancing microbial nitrate transformation through the addition of liquid biogas slurry. *Agric. Ecosyst. Environ.* **2023**, *345*, 108324. [\[CrossRef\]](#)
17. Brundrett, M.C. Mycorrhizal associations and other means of nutrition of vascular plants: Understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant Soil* **2009**, *320*, 37–77. [\[CrossRef\]](#)
18. Zhao, Y.; Naeth, M.A. Soil amendment with a humic substance and arbuscular mycorrhizal Fungi enhance coal mine reclamation. *Sci. Total Environ.* **2022**, *823*, 153696. [\[CrossRef\]](#)
19. Ahammed, G.J.; Shamsy, R.; Liu, A.; Chen, S. Arbuscular mycorrhizal fungi-induced tolerance to chromium stress in plants. *Environ. Pollut.* **2023**, *327*, 121597. [\[CrossRef\]](#)
20. Ma, X.; Li, X.; Ludewig, U. Arbuscular mycorrhizal colonization outcompetes root hairs in maize under low phosphorus availability. *Ann. Bot.* **2021**, *127*, 155–166. [\[CrossRef\]](#)
21. Lourdes, G.-C.M.; Stéphane, D.; Maryline, C.-S. Impact of increasing chromium (VI) concentrations on growth, phosphorus and chromium uptake of maize plants associated to the mycorrhizal fungus. *Heliyon* **2021**, *7*, e05891. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Faithfull, N. Methods in Agricultural Chemical Analysis: A Practical Handbook. *Grass Forage Sci.* **2003**, *58*, 98. [\[CrossRef\]](#)
23. Hata, T.; Kono, Y.; Banno, Y. Determination of Nitrogen Oxides of Low Concentration in Exhaust Gas by Improved Phenol Disulfonic Acid Method. *Tetsu-to-Hagane* **2010**, *64*, 297–302. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Gota, H.G.; Madalcho, A.B.; Kerse, B.L.; Szwagrzyk, J.; Solomon, T. The impact of native trees, *Cordia africana* and *Ficus sur*, and the economically valuable *Manihot esculenta* on soil chemical properties in an agroforestry system. *Trees For. People* **2024**, *15*, 100471. [\[CrossRef\]](#)
25. Lavaee, P.; Taghdisi, S.M.; Abnous, K.; Danesh, N.M.; Khayyat, L.H.; Jalalian, S.H. Fluorescent sensor for detection of miR-141 based on target-induced fluorescence enhancement and PicoGreen. *Talanta* **2019**, *202*, 349–353. [\[CrossRef\]](#)
26. Guo, G.; Guan, J.; Sun, S.; Liu, J.; Zhao, Y. Nutrient and heavy metal removal from piggery wastewater and CH_4 enrichment in biogas based on microalgae cultivation technology under different initial inoculum concentration. *Water Environ. Res.* **2020**, *92*, 922–933. [\[CrossRef\]](#)
27. Tang, J.; Davy, A.J.; Wang, W.; Zhang, X.; Wu, D.; Hu, L.; Yin, J. Effects of biogas slurry on crop yield, physicochemical properties and aggregation characteristics of lime concretion soil in wheat–maize rotation in the North China Plain. *J. Soil Sci. Plant Nutr.* **2022**, *22*, 2406–2417. [\[CrossRef\]](#)
28. Kalkhajeh, Y.K.; He, Z.; Yang, X.; Lu, Y.; Zhou, J.; Gao, H.; Ma, C. Co-application of nitrogen and straw-decomposing microbial inoculant enhanced wheat straw decomposition and rice yield in a paddy soil. *J. Agric. Food Res.* **2021**, *4*, 100134. [\[CrossRef\]](#)
29. Rahaman, A.; Zhan, X.; Zhang, Q.; Li, S.; Lv, S.; Long, Y.; Zeng, H. Ammonia Volatilization Reduced by Combined Application of Biogas Slurry and Chemical Fertilizer in Maize–Wheat Rotation System in North China Plain. *Sustainability* **2020**, *12*, 4400. [\[CrossRef\]](#)
30. Du, Z.; Xiao, Y.; Qi, X.; Liu, Y.; Fan, X.; Li, Z. Peanut-Shell Biochar and Biogas Slurry Improve Soil Properties in the North China Plain: A Four-Year Field Study. *Sci. Rep.* **2018**, *8*, 13724. [\[CrossRef\]](#)
31. Zheng, J.; Qi, X.; Shi, C.; Yang, S.; Wu, Y. Tomato comprehensive quality evaluation and irrigation mode optimization with biogas slurry based on the combined evaluation model. *Agronomy* **2022**, *12*, 1391. [\[CrossRef\]](#)
32. Wang, Y.X.; Fu, J.R.; Wang, Q.; Wang, J.M.; Ma, J.W.; Jiang, L.N. Effect of biogas slurry farmland disposal and utilization on pepper's yield, quality and soil fertility. *Acta Agric. Zhejiangensis* **2010**, *22*, 859–863. [\[CrossRef\]](#)
33. Choudhary, V.; Machavaram, R. Need of automation in paddy nurseries for raising paddy seedlings in India: A Review. *J. Biosyst. Eng.* **2022**, *47*, 209–222. [\[CrossRef\]](#)

34. Mukhtiar, A.; Mahmood, A.; Zia, M.A.; Ameen, M.; Dong, R.; Shoujun, Y.; Javaid, M.M.; Khan, B.A.; Nadeem, M.A. Role of biogas slurry to reclaim soil properties providing an eco-friendly approach for crop productivity. *Bioresour. Technol. Rep.* **2023**, *25*, 101716. [[CrossRef](#)]
35. Cao, W.; Wang, X.; Sun, S.; Hu, C.; Zhao, Y. Simultaneously upgrading biogas and purifying biogas slurry using cocultivation of *Chlorella vulgaris* and three different fungi under various mixed light wavelength and photoperiods. *Bioresour. Technol.* **2017**, *241*, 701. [[CrossRef](#)]
36. Kominko, H.; Gorazda, K.; Wzorek, Z. The Possibility of Organo-Mineral Fertilizer Production from Sewage Sludge. *Waste Biomass Valorization* **2017**, *8*, 1781–1791. [[CrossRef](#)]
37. Liang, X.; Wang, C.; Wang, H.; Qiu, X.; Ji, H.; Ju, H.; Wang, J. Synergistic effect on soil health from combined application of biogas slurry and biochar. *Chemosphere* **2023**, *343*, 140228. [[CrossRef](#)]
38. Koziol, L.; Bever, J.D. The missing link in grassland restoration: Arbuscular mycorrhizal fungi inoculation increases plant diversity and accelerates succession. *J. Appl. Ecol.* **2017**, *54*, 1301–1309. [[CrossRef](#)]
39. Qin, M.; Zhang, Q.; Pan, J.; Jiang, S.; Liu, Y.; Bahadur, A.; Peng, Z.; Yang, Y.; Feng, H. Effect of arbuscular mycorrhizal fungi on soil enzyme activity is coupled with increased plant biomass. *Eur. J. Soil Sci.* **2020**, *71*, 84–92. [[CrossRef](#)]
40. Faghihinia, M.; Jansa, J.; Halverson, L.J.; Staddon, P.L. Hyphosphere microbiome of arbuscular mycorrhizal fungi: A realm of unknowns. *Biol. Fertil. Soils* **2023**, *59*, 17–34. [[CrossRef](#)]
41. Shintaro, H.; Masanori, S. Isolation of Inositol Hexaphosphate (IHP)-Degrading Bacteria from Arbuscular Mycorrhizal Fungal Hyphal Compartments Using a Modified Baiting Method Involving Alginate Beads Containing IHP. *Microbes Environ.* **2016**, *31*, 234–243. [[CrossRef](#)]
42. Desirò, A.; Salvioli, A.; Ngonkeu, E.L.; Mondo, S.J.; Epis, S.; Faccio, A.; Kaech, A.; Pawlowska, T.E.; Bonfante, P. Detection of a novel intracellular microbiome hosted in arbuscular mycorrhizal fungi. *ISME J.* **2014**, *8*, 257–270. [[CrossRef](#)] [[PubMed](#)]
43. Liang, X.; Wang, H.; Wang, C.; Yao, Z.; Qiu, X.; Ju, H.; Wang, J. Disentangling the impact of biogas slurry topdressing as a replacement for chemical fertilizers on soil bacterial and fungal community composition, functional characteristics, and co-occurrence networks. *Environ. Res.* **2023**, *238*, 117256. [[CrossRef](#)] [[PubMed](#)]
44. Torres-Cortés, G.; Ghignone, S.; Bonfante, P.; Schüßler, A. Mosaic genome of endobacteria in arbuscular mycorrhizal fungi: Transkingdom gene transfer in an ancient mycoplasma-fungus association. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 7785–7790. [[CrossRef](#)]

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