Sustainable Management of Biogas Slurry Discharge in Biogas Engineering: As a Chemical Fertilizer Substitute for Garlic Cultivation

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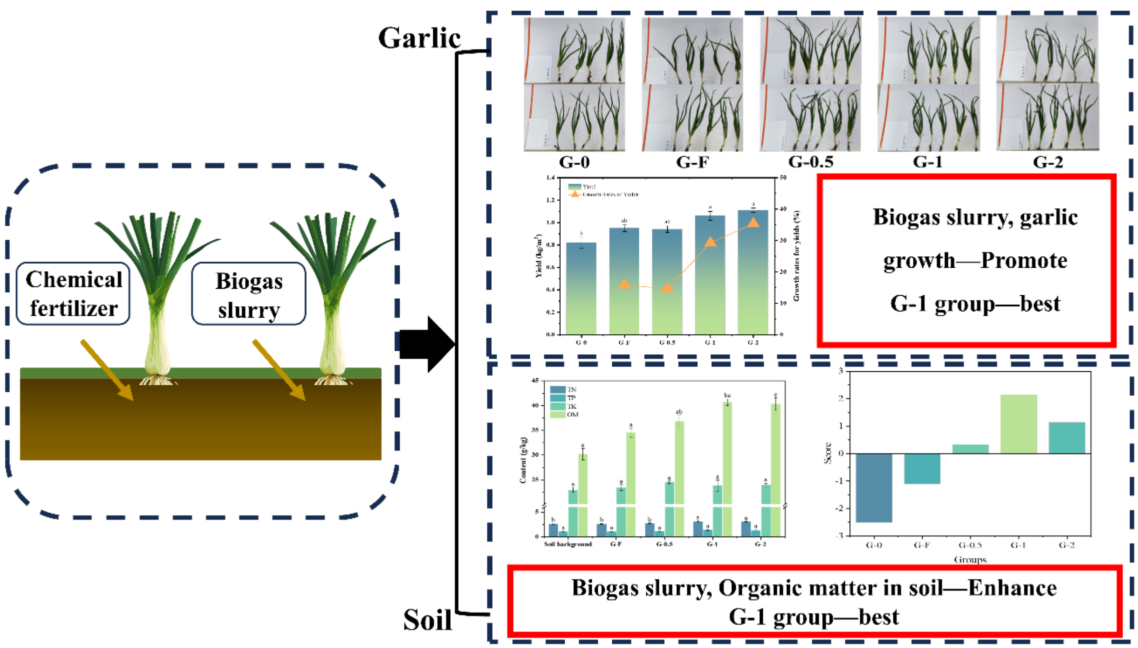
GRAPHICAL ABSTRACT



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To address the persistent challenge of managing livestock and poultry manure resulting from agricultural intensification and mechanization, the application of biogas engineering has steadily expanded. Biogas slurry, a valuable byproduct abundant in nitrogen and other nutrients, emerges as an attractive alternative to chemical fertilizers. This study investigated the effects of substituting chemical fertilizers with biogas slurry at varying application rates on garlic growth and soil properties. The results indicate that with increasing application of biogas slurry, the promotion of garlic growth and soil nutrients exhibited an initial increase followed by a decrease. Notably, application of biogas slurry with nitrogen content equivalent to chemical fertilizers (G-1 group) gave the most pronounced promotion effect. In the G-1 group, garlic yield and soil organic matter content reached 1.06 kg/m^3 and 40.6 g/kg, respectively, representing increases of 11.6% and 17.6%, respectively, compared to the chemical fertilizer group. Furthermore, after the application of biogas slurry, concentrations of both garlic and soil heavy metals remained within standard limits. Biogas slurry can be recommended as an effective substitute for chemical fertilizers, fostering garlic growth, boosting yields, enhancing soil organic matter content, and promoting biological carbon sequestration.

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Keywords: Biogas slurry; Garlic; Alternative fertilizer; Carbon sequestration

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INTRODUCTION

With the advancement of intensification and mechanization, the scale of livestock and poultry production worldwide is gradually expanding, resulting in an increasingly concentrated production of waste (Jia *et al*. 2018; Zhang *et al*. 2021). China stands as one of the largest producers and consumers of livestock and poultry products globally, with an annual production of livestock and poultry manure exceeding 4 billion tons nationwide. Over 40% of this manure remains not fully utilized, leading to serious environmental pollution. Therefore, the effective and comprehensive utilization of livestock and poultry manure is crucial (MARAC 2017; Zhou *et al*. 2019). Biogas engineering, which is primarily based on the anaerobic digestion of straw and livestock and poultry manure, represents a systematic approach for emissions reduction and carbon sequestration that integrates manure treatment, biogas production, and resource utilization. Biogas engineering not only manages livestock and poultry manure, increases the supply of renewable clean energy, and facilitates carbon peak and carbon neutrality, but it also promotes agricultural recycling through the application of biogas residue to fields, enhancing soil organic matter (Song *et al*. 2023; Chen *et al*. 2024). By the end of 2015, the government had subsidized 111,000 large-scale anaerobic digestion plants (NDRC and MOA 2017). Biogas engineering faces challenges in its practical promotion, such as difficulties in biogas slurry utilization and digestion (Gu *et al*. 2024; Zhou *et al*. 2024).

Biogas slurry (BS) is the liquid product of the anaerobic digestion of organic materials such as straw and livestock and poultry manure in biogas engineering (Mohanty *et al*. 2024). There are two main methods for treating BS: standard discharge and return to fields. Due to the high cost of BS treatment, its application to fields is the primary method of treatment. Nitrogen fertilizer is one of the indispensable important fertilizers in agricultural production, and it plays a vital role in the growth and development of crops (Tei *et al.* 2020; Zhang *at el.* 2022). Among them, nitrate (NO_3^-) and ammonium nitrogen (NH_4^+) are the main inorganic nitrogen sources of N (Ye *et al*. 2022). Nitrated nitrogen can be leached due to its high mobility, resulting in nitrogen loss. In addition, inorganic nitrogen is lost to the environment by participating in the volatilization of NH_2 and nitrification into forms that are not usable by plants, such as NO (Lyu *et al*. 2024). Chemical fertilizers are currently the most commonly used inorganic fertilizers due to their ease of use. However, the long-term application of inorganic nitrogen fertilizers accelerates soil compaction and acidification, affecting crop growth. Moreover, excessive use of chemical fertilizers leads to significant greenhouse gas emissions and water pollution issues, thus hindering the achievement of carbon peak and neutrality (Nishita *et al*. 2017; Iqbal *et al*. 2020; Ma *et al*. 2024). To develop sustainable low-carbon agriculture, using of nitrogen-efficient fertilizers is essential (Ma *et al*. 2024).

Compared to other organic fertilizers, BS contains abundant nitrogen, phosphorus, and other nutrients, providing sufficient nutrition for crop growth. BS also contains a rich array of nutrients, such as various trace elements (iron, zinc, manganese, *etc*.), amino acids, B vitamins, humic acid, plant growth hormones, and dozens of active substances that prevent crop pests and diseases, making it a high-quality organic fertilizer (Liang *et al*. 2023a; Nascimento *et al*. 2023). The application of BS has a good regulatory effect on the soil environment. Nutrient elements can improve soil fertility, while organic matter and biologically active substances can regulate soil physicochemical properties and physical structure, enhancing soil water and nutrient retention, thereby increasing crop yields (Zhang *et al*. 2018; Ibrahim *et al*. 2020). Soil total nitrogen includes nitrate nitrogen, ammonium nitrogen, and alkali-hydrolyzed nitrogen. The content of soil available nitrogen, namely nitrate nitrogen and ammonium nitrogen, reflects the nitrogen supply capacity of soil and nitrogen absorption by plants. In addition, alkali-hydrolyzed nitrogen is not easy to leach, and it is sensitive to soil nitrogen application, which is an important index to evaluate soil nutrients (Chen *et al*. 2016).

Garlic is an essential vegetable, seasoning, and medicinal material, with China ranking first globally in terms of garlic planting area, total production, and export volume. Allicin, a compound found in garlic, has been demonstrated to have negative effects on the growth of tumor cells (Brugnoli *et al*. 2021; Melguizo-Rodríguez *et al*. 2022). Liang *et al*. (2023b) showed that the combined application of biogas slurry and biochar improves soil health and increased soil carbon content. Li *et al*. (2023) found that a 3% addition of biogas slurry was most beneficial for the growth and disease resistance of cucumbers, without the risk of soil salinization. However, at present, the research on the effect of biogas slurry on garlic is still blank.

Biogas slurry is an organic alternative to chemical fertilizers, and its application could efficiently utilize resources and maintain soil health in future biogas engineering endeavors. Therefore, the objectives of this study are: (i) to elucidate the influence of biogas slurry application rates on garlic growth parameters and nutritional quality, (ii) to investigate the effects of different fertilization treatments on soil nutrients and heavy metal content, and (iii) to analyze the feasibility of substituting chemical fertilizers with biogas slurry for garlic cultivation to enhance crop yields while improving soil quality.

EXPERIMENTAL

Biogas Slurry

The original anaerobic digestion slurry used in the experiment was sourced from the discharge station of a biogas project in Ganzhou City, Jiangxi Province. Biogas slurry was used for this study after anaerobic fermentation (15 days) - photosynthetic bacteria purification (15 days) - struvite precipitation (15 days) - nitrifying bacteria treatment (15 days). The physicochemical properties of the treated biogas slurry are detailed in Table 1.

Table 1. Properties of Biogas Slurry

Parameters	Biogas Slurry
Total nitrogen (mg/L)	1070.58 ± 15.09
Water insoluble matter (g/L)	2.02 ± 0.12
Total salt (g/L)	2.45 ± 0.09
Electric conductivity (ms/cm)	15.77 ± 1.25
Odor concentration	577.76 ± 5.62
COD (mg/L)	1307.58 ± 6.99
Total phosphorus (mg/L)	15.97 ± 1.06
K (mg/L)	2078.50 ± 19.56
Mg (mg/L)	14.61 ± 1.36
Ca (mg/L)	140.32 ± 4.92
Cu (mg/L)	3.12 ± 0.09
Zn (mg/L)	11.43 ± 0.92
As (mg/L)	18.05 ± 0.85
Se (mg/L)	0.24 ± 0.02

Experimental Design

The garlic field experiment took place on a farm located in Ganzhou City, Jiangxi Province (24°90′N, 115°04′E), situated within a subtropical monsoon climate zone characterized by mild weather conditions. The area experiences an annual average temperature of 19.8°C and an average annual precipitation of 1320 millimeters.

The garlic field experiment involved five treatment groups: (a) G-0: Organic fertilizer was applied as a one-time base fertilizer during the embryonic stage, and no fertilizer was applied during the growing stage. (b) G-F: Organic fertilizer was applied as a one-time base fertilizer during the embryonic phase, and urea (about 46.6% nitrogen) was applied exclusively was the growth phase. (c) G-0.5: Organic fertilizer was applied as a disposable base fertilizer during the embryonic period, and anaerobic slurry (about 23.3% nitrogen) was applied alone during the growth period. (d) G-1: Organic fertilizer was applied as a disposable base fertilizer during the embryonic phase and anaerobic slurry (about 46.6% nitrogen) was applied alone during the growth phase. (e) G-2: Organic fertilizer was applied as a disposable base fertilizer during the embryonic phase and anaerobic slurry (nitrogen content of about 93.2%) was applied alone during the growth phase. Garlic received a total of three topdressings throughout the entire growth period. The first topdressing was applied during the embryonic stage (4 to 7 days after colonization). The second and third topdressings were applied during the bolting period, with a 15-day interval between each fertilization. The specific application rates for each treatment group are detailed in Table 2. Each treatment group comprised three replicates, totaling 15 plots. Garlic seeds were planted with a spacing of 8 cm between plants. The plots, measuring 4 m in length and 4 m in width, with an area of 16 m², were arranged in a north-south direction. A 0.5 m wide buffer zone was established between plots. Plot allocation followed a random principle, and on-site planting involved randomizing the layout through a drawing process. The fertilizer used for topdressing is sprayed as foliar fertilizer.

Table 2. Experimental Group Settings of Garlic (L/plot (16 m^2))

Treatments	Fertilizer	Embryonic stage 4-7 days after colonization	Seedling Stage	Bolting Period	
				Every 15 days	Every 15 days
G-0	Organic fertilizer	20.0	0	0	0
G-F	Urea	0	0.214	0.286	0.286
G-0.5	Biogas slurry	0	44.7	59.6	59.6
G-1	Biogas slurry	0	89.5	119	119
G-2	Biogas slurry	0	179	239	239

Garlic Analysis

After the initial fertilization and at harvest, garlic samples were randomly collected. Sampling was conducted within each experimental plot by selecting three random sample plots, each measuring 1 m × 1 m. Within each selected sample plot, the overall growth of garlic was observed, including plant height and leaf counts. Upon collection, the samples were transported to the laboratory, where the root soil was cleaned. Subsequently, the whole fresh weight and edible fresh weight of each garlic plant were measured. The relative chlorophyll content in garlic leaves was determined using a chlorophyll content meter (SPAD 502, Konica Minolta, Japan). Subsequently, a portion of the harvested garlic samples was ground. The vitamin C content was determined using high-performance liquid chromatography, following the method described by Wu *et al*. (2023), while the allicin content was determined using the mercury nitrate method. The remaining samples were dried at 60 °C, ground, and sieved for protein, soluble sugar, dietary fiber, and heavy metal content analysis. The protein content was analyzed using an automated Kjeldahl nitrogen analyzer (K9860, Hanon, China). The soluble sugar content was determined using the anthrone-sulfuric acid colorimetric method, and the dietary fiber content was determined as described by Li and Cardozo (1994).

The heavy metal content in garlic was determined using the microwave digestion method (Yan *et al*. 2024). A 0.1 g sifted garlic sample was placed into a polytetrafluoroethylene digestion tubes, to which 5 mL of nitric acid and 1 mL of sulfuric acid were added. The digestion process was conducted in a microwave digestion system

(MDS-6G, Sineo, China), with three stages of temperature increase: the first stage at 150 °C for 10 min, the second stage at 180 °C for 5 min, and the third stage at 200 °C for 20 min. Subsequently, the solution was cooled to 60 °C and diluted with deionized water. After filtration through a 0.22 μm membrane, the heavy metal content was analyzed using an inductively coupled plasma mass spectrometer (OPTIMA 8000, PerkinElmer, USA). The plant heavy metal enrichment factor was calculated using Eq. 1,

$$AC = {C_{garlic}}/{C_{soil}} \quad (1)$$

where C_{galic} is the concentration of a specific heavy metal in plant leaves (mg/kg), and C_{soil} is the concentration of the same heavy metal in the soil (mg/kg).

Soil Analysis

After garlic harvesting, soil samples were collected from each plot area, with three random samples taken per plot. Upon mixing, approximately 1 kg of each sample was retained using the quadrat method. Stones, roots, and other debris were removed, and the samples were transported to the laboratory for air-drying. Subsequently, the samples were ground through a 50-mesh sieve and sealed in bags for nutrient and heavy metal analysis. Soil heavy metal content was determined as described previously. Total nitrogen content was determined using the Kjeldahl method, while total phosphorus content was measured using the molybdenum antimony anti-colorimetric method (Kowalenko and Babuin 2007). Total potassium content was determined using flame photometry. Available nitrogen was determined using the diffusion absorption method, and available phosphorus was determined using the sodium bicarbonate extraction-molybdenum antimony anti-spectrophotometric method. Available potassium content was determined by ammonium acetate-flame photometry. Organic matter was analyzed using a total organic carbon/total nitrogen analyzer (Multi N/C 3100+HT 1300, Analytik Jena, Germany).

Data Analysis

All data were statistically analyzed and calculated using Excel 2016 (Microsoft, Redmond, WA, USA). Figures and tables were generated using Origin 2021 software (OriginLab, Northampton, MA, USA). Data variability analysis and principal component analysis (PCA) were performed using IBM SPSS Statistics 22.0 software (SPSS, Inc, Chicago, IL, USA).

RESULTS AND DISCUSSION

Garlic Growth

The growth parameters of garlic following different fertilization treatments are shown in Fig. 1, both after the initial fertilization and at harvest. After the initial fertilization, compared to the chemical fertilizer (G-F group), the growth indexes (plant height, leaf number, SPAD value, fresh weight and edible fresh weight) of all treatment groups were higher than those of blank group (G-0), except the fresh weight and edible fresh weight of the G-F and G-2 group. Compared with the G-F group, G-0.5 and G-1 group improved the growth indexes of garlic to different degrees.

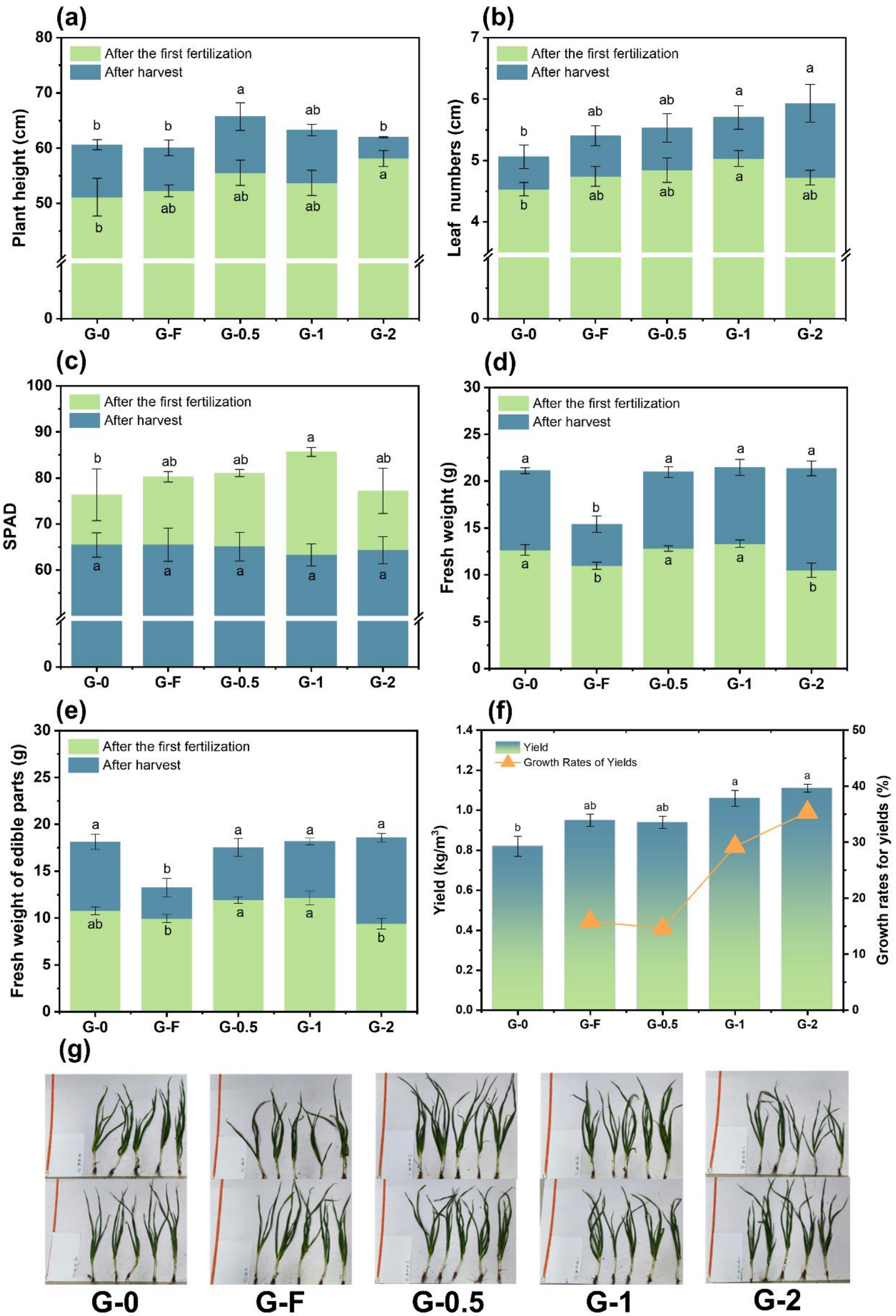

Fig. 1. Effects of different fertilization treatments on (a) plant height, (b) number of leaves, (c) SPAD value in leaves, (d) fresh weight, (e) fresh weight of edible parts, (f) yield and (g) growth photograph of garlic after the first fertilization and after harvest

Among these, the growth rate of G-1 surpassed that of the G-0.5 group (excluding plant height), whereas the G-2 group exhibited only greater plant height compared to the G-F group, with other indicators being lower than the G-F group (Fig. 1). This suggests that, during garlic growth, the application of biogas slurry was more advantageous for promoting garlic growth compared to chemical fertilizer. The initial fertilization coincides with the vigorous growth stage of garlic, a critical period for its development. Biogas slurry, being richer in nutrients compared to chemical fertilizer, facilitates increased absorption of nitrogen, micronutrients, and other nutrients by garlic, thereby enhancing its growth (Cui *et al*. 2021).

At harvest, the plant heights of the G-0, G-0.5, and G-2 group were 60.57, 65.79, and 61.95 cm, and the average leaf numbers were 5.06, 5.52 and 5.98, respectively. The plant height and leaf number of garlic were significantly increased by nitrogen fertilizer application during growth period. As the slurry application increased, the garlic plant height decreased, while the leaf count gradually increased (Fig. 1a and Fig.1b). This study employed foliar spraying of biogas slurry, allowing for rapid nutrient absorption by the leaves to support garlic growth. Thus, foliar spraying can swiftly supplement the nutrients lacking in garlic. The nutrient content in slurry exceeded that in chemical fertilizers, and slurry application promoted garlic's nutrients absorption. The G-2 group exhibited the highest leaf numbers, as sufficient nitrogen serves as the basis for leaf formation and improved photosynthetic efficiency.

Varying concentrations of biogas slurry had no significant effect on increasing chlorophyll in mature garlic (Fig. 1c). After harvesting, the order of single plant fresh weight for each group was as follows: G-1 > G-2 > G-0 > G-0.5 > G-F (Fig. 1d), and the order of edible fresh weight for each group was as follows: G-2 > G-1 > G-0 > G-0.5 > G-F (Fig. 1e). The garlic yield of the G-1 group was 1.06 kg/m^3, representing increases of 29.3% and 11.6% compared to the G-0 and G-F groups, respectively. The highest garlic yield was observed in the G-2 group, reaching 1.12 kg/m^3, representing increases of 36.6% and 17.9% compared to the G-0 and G-F group, respectively (Fig. 1f).

Compared with the G-0 group, the yield of all treatment groups was increased to different degrees, which indirectly indicated the importance of nitrogen for garlic growth. Compared with chemical fertilizer, the growth advantage of the G-1 and G-2 groups of garlic was more obvious, and the yield increase was larger. Additionally, garlic yield increased with increasing slurry concentration. Apart from plant height, the other indicators of the G-1 group were the highest among the treatment groups. This indicates that the application of biogas slurry with the equivalent nitrogen content as chemical fertilizers was more suitable for garlic growth, with the highest nitrogen utilization in the slurry at this stage. The G-F group exhibited the lowest yield (0.95 kg/m^3), possibly due to the prolonged single application of chemical fertilizer in the soil, leading to soil degradation, severe compaction, inhibition of plant nutrient absorption, and antagonistic effects on soil micronutrients, resulting in incomplete expression of its immediate effect (Iqbal *et al*. 2020). Following biogas slurry application, the nutrients supply becomes more adequate and balanced, facilitating the adjustment of soil nutrient supply. Moreover, the antibiotics and bioactive substances in biogas slurry can inhibit the growth of pathogens and pests on leaf surfaces, aiding in disease and pest prevention and control, thereby further increasing garlic yield. However, it is worth noting that nitrogen is a major influencing factor in plant growth, and excessive field irrigation with biogas slurry, resulting in nitrate nitrogen exceeding the plant's absorption capacity, may adversely affect crop growth (Chen *et al*. 2013).

Garlic Nutrients

Figure 2 illustrates the impact of different fertilization methods on the nutritional components of garlic. Compared with the G-0 group, the nutritional composition of garlic in other treatment groups did not increase significantly ($p > 0.05$), on the contrary, the vitamin C content and dietary fiber content in the G-0.5 and G-2 group were significantly decreased ($p < 0.05$), which may be due to the influence of heavy metal content in biogas slurry. As the application rate of biogas slurry increased, the contents of garlic protein, vitamin C, dietary fiber, reducing sugars, and allicin exhibited a trend of initially increasing and then decreasing. This observation suggests that the accumulation of nutritional components within garlic plants is dependent on the concentration of biogas slurry. Compared to the G-0.5 and G-2 groups, the G-1 group exhibited the most favorable conditions for the accumulation of nutritional components within garlic plants, albeit less effective than the G-F group. The relatively low protein content in the G-0.5 group, measuring at only 5.81 ug/g, may be attributed to the reduced application of nitrogen, insufficient to support extensive protein synthesis in garlic.

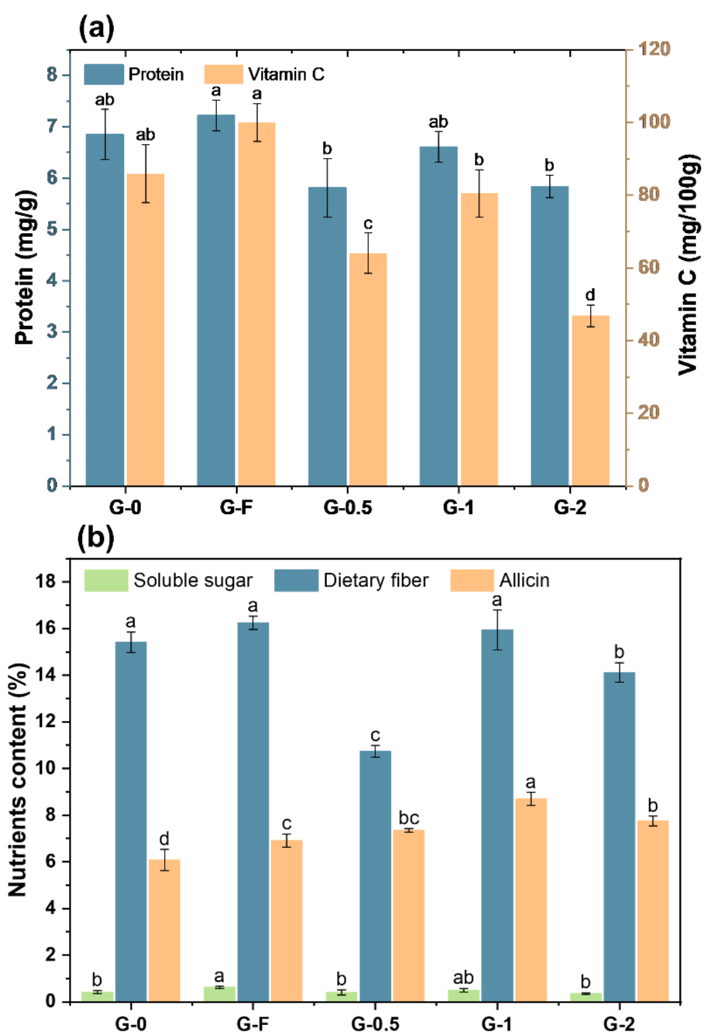

Fig. 2. Effects of different fertilization treatments on the content of nutrients in garlic: (a) protein and vitamin C; (b) soluble sugar, dietary fiber and allicin

Similarly, the protein content in the G-2 group, also registering at 5.84 ug/g, could be a consequence of excessive nitrogen application leading to the accumulation of nitrate ions in plant tissues, inhibiting the protein conversion process (Htwe and Ruangrak 2021). Additionally, copper (Cu) in biogas slurry is crucial for protein synthesis, and higher Cu content may exert toxic effects on garlic (Shin *et al*. 2012). The application of different concentrations of biogas slurry has no significant difference in the accumulation of soluble sugars in garlic plants ($p < 0.05$). Compared to the fertilizer group, the content of soluble sugars in garlic decreases after applying biogas slurry, and the reasons for this decrease require further investigation. While the application of an appropriate amount of biogas slurry to garlic may reduce dietary fiber content while enhancing its palatability, the resultant decrease is insufficient to impact its nutritional quality significantly. Compared with the G-0 group, application of nitrogen fertilizer significantly increased allicin content in garlic. Compared to the G-F group with the equivalent nitrogen content, the content of allicin in the G-1 group garlic increased by 26.0% (Fig. 2). Allicin, a sulfur-containing compound, is generated through the decomposition of alliin by alliinase in garlic. The synthesis of alliin necessitates amino acids and sulfur elements. Given the complex composition of biogas slurry, containing higher levels of sulfur elements and amino acids compared to chemical fertilizers, biogas slurry can promote the synthesis of alliin in garlic, with the application of biogas slurry at the equivalent nitrogen as chemical fertilizers being most conducive to the synthesis and accumulation of alliin in garlic.

The heavy metal content in garlic leaves serves as an indicator reflecting both plant physiology and soil conditions, playing a crucial role in assessing food safety and environmental risks. Table 3 shows the content of various metals in garlic leaves following different fertilization treatments. Compared with the G-0 group, the application of nitrogen fertilizer promoted the accumulation of Mg and Zn in garlic leaves, while the application of biogas slurry only promoted the accumulation of Zn. Compared with G-F group, the application of biogas slurry promoted the absorption and accumulation of Ca and K in garlic leaves but it was detrimental to the accumulation of Mg and Zn elements. With an increase in the application level of biogas slurry, the Mg, Ca, and Zn contents showed a trend of initially increasing and then decreasing, peaking at 546, 2450, and 18.8 mg/kg DM in the G-1 group, respectively, while the K content increased with the increasing application of biogas slurry. Biogas slurry had a significant impact on the absorption and accumulation of minerals in garlic leaves, and as the application of biogas slurry increased, the influence gradually diminished. This phenomenon may stem from the dilution effect of biogas slurry on minerals, leading to decreased absorption and accumulation by plants (Gupta *et al*. 2016). For heavy metals, compared to the G-F group, the application of biogas slurry reduced the accumulation of Cr and Pb in garlic, while significantly increasing the content of Cu, As, Se, and Cd in garlic leaves. Furthermore, with an increase in the application of biogas slurry, the content of Cu, Cr, and As in garlic leaves increases, reaching maximum values in the G-2 group of 4.95, 0.42, and 0.4 mg/kg DM, respectively (Table 3). The higher the dosage of biogas slurry, the higher the heavy metal content of garlic, which may be due to the high heavy metal content of biogas slurry itself. After applying biogas slurry, the content of Cr, Zn, As, Se, Cd, Hg, and Pb in garlic leaves does not exceed the limits specified in the national standard GB 2762-2022, indicating that the application of biogas slurry is safe for garlic cultivation. However, long-term application warrants further investigation into the accumulation of heavy metals.

Table 3. Effects of Different Fertilization Treatments on Metal Content in Garlic Leaves

Metal content (mg/kg DM)	G-0	G-F	G-0.5	G-1	G-2
Mg	614.74 ± 14.11[a]	615.66 ± 16.33[a]	458.01 ± 18.38[c]	546.12 ± 28.35[b]	525.33 ± 35.33[b]
K	5998.33 ± 262.93[a]	4521.55 ± 125.3[c]	5007.59 ± 344.07[bc]	5435.82 ± 148.25[ab]	5597.41 ± 214.81[ab]
Ca	4928.05 ± 169.66[a]	2028.63 ± 70.85[c]	1368.53 ± 70.43[d]	2449.48 ±1 47.08[b]	2128.08 ± 84.92[c]
Cr	0.57 ± 0.03[a]	0.51 ± 0.07[ab]	0.22 ± 0.03[d]	0.37 ± 0.04[c]	0.42 ± 0.06[bc]
Cu	5.38 ± 0.58[a]	3.76 ± 0.33[b]	2.58 ± 0.34[c]	4.8 ± 0.21[a]	4.95 ± 0.18[a]
Zn	12.39 ± 2.88[b]	20.9 ± 4.53[a]	12.5 ± 2.81[b]	18.8 ± 1.56[ab]	18.09 ± 1.56[ab]
As	0.46 ± 0.11[a]	0.10 ± 0.03[b]	0.12 ± 0.03[b]	0.31 ± 0.04[a]	0.40 ± 0.10[a]
Se	0.11 ± 0.06[bc]	0[c]	0.26 ± 0.06[a]	0.27 ± 0.03[a]	0.17 ± 0.06[ab]
Cd	0[c]	0[c]	0.19 ± 0.06[a]	0.09 ± 0.03[b]	0[c]
Hg	0	0	0	0	0
Pb	0.18 ± 0.04[a]	0.29 ± 0.07[a]	0.18 ± 0.07[a]	0.26 ± 0.04[a]	0.18 ± 0.06[a]

The average heavy metal enrichment coefficient of the G-1 group was 4.66, while the enrichment coefficient of G-F with the equivalent nitrogen content was 2.16 (Fig. 3).

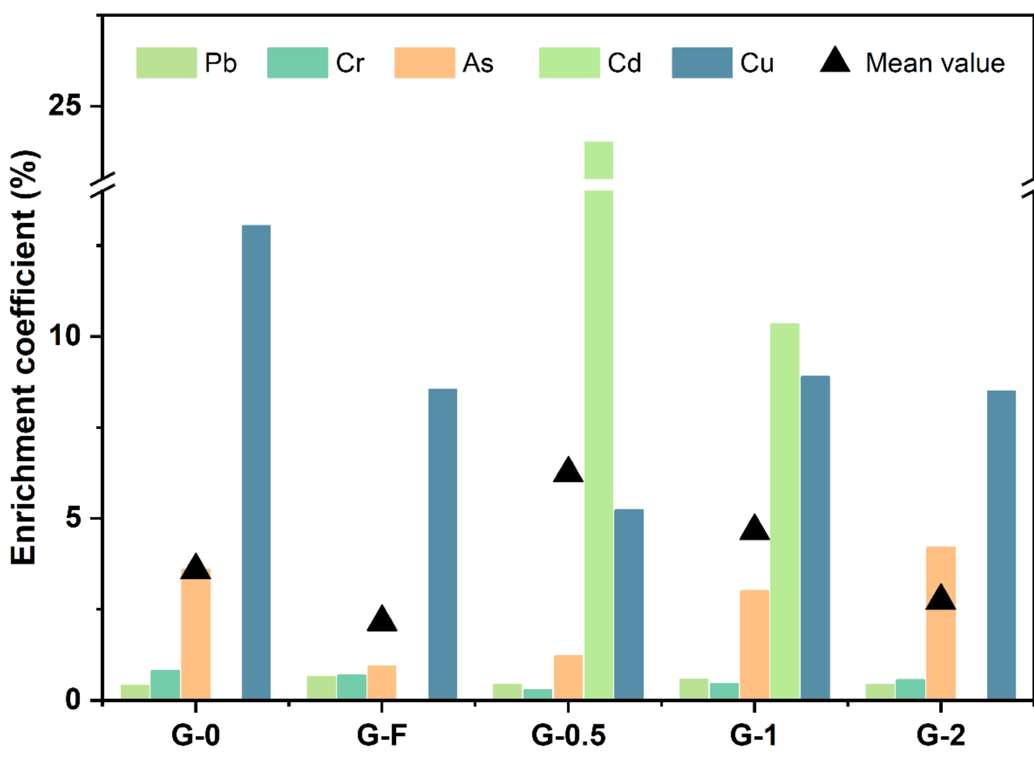

Fig. 3. Enrichment coefficient of heavy metals in garlic leaves under different fertilization treatment

The higher enrichment coefficient associated with biogas slurry application compared to the fertilizer group suggests that biogas slurry facilitated the absorption of heavy metals from the soil by garlic, thereby aiding the transfer of heavy metals from the soil to the edible part of garlic (*i.e.*, the leaves), particularly for Cd and As metals. The enrichment coefficients of Pd and Cr in different treatment groups were all below 1%, significantly lower than the other heavy metals, while the soil contained higher concentrations of Pb and Cr. This suggests that elevated levels of heavy metals in the soil can inhibit their migration from the soil to vegetables. Moreover, as the application rate of biogas slurry increased, the average enrichment coefficient of heavy metals decreased, possibly due to the high water content of biogas slurry, which dilutes the concentration of heavy metals. The enrichment coefficient of the G-F group was lower than that of the G-0 group, which may be due to a small amount of heavy metals contained in the fertilizer itself.

Heavy Metals and Nutrients in Soil

The experimental results from earlier sections indicate that compared to the application of chemical fertilizers, the application of biogas slurry exerted a more substantial promoting effect on the growth and development of garlic, culminating in a more pronounced increase in yield. However, there was a potential risk of heavy metal accumulation in the soil from biogas slurry application. To ensure the safety of biogas slurry application on soil, this study investigated the heavy metal content (Pb, Cd, Cr, Cu, Hg, and As) in the soil after different fertilization treatments, as shown in Table 4. Compared with the G-0 group, there was no significant change in soil heavy metal content in the G-F group ($p < 0.05$), yet there remained a risk of heavy metal accumulation with long-term application. Compared to the G-F group, the application of biogas slurry at the G-1 group significantly increased the As and Cu content by 210% and 27.7%, respectively. Se and Cd increased from "undetected" to 0.27 and 0.09 mg/kg DM, respectively, while the Cr content decreased by 27.4%. The differences in the content of other heavy metals were not significant ($p > 0.05$), and all heavy metal content remained within the limits stipulated by the national standard GB 15618-2018. Consequently, biogas slurry can effectively replace chemical fertilizers and applying appropriate amounts of anaerobic slurry can increase the yield of garlic and other vegetable crops.

Table 4. Content of Heavy Metals in Soil After Different Fertilization Treatments

Heavy metal content (mg/kg)	Soil background	G-F	G-0.5	G-1	G-2
Pb	43.22 ± 1.84^{ab}	44.55 ± 1.71^{ab}	40.55 ± 2.12^{b}	44.95 ± 1.33^{a}	41.13 ± 0.43^{ab}
Cd	0.25 ± 0.02^{a}	0.27 ± 0.02^{a}	0.25 ± 0.01^{a}	0.29 ± 0.03^{a}	0.29 ± 0.02^{a}
Cr	69.21 ± 2.46^{b}	73.56 ± 1.59^{b}	75.24 ± 3.26^{ab}	80.81 ± 2.68^{a}	73.98 ± 2.72^{ab}
Cu	41.26 ± 2.24^{c}	44.01 ± 1.36^{c}	49.27 ± 1.27^{b}	53.93 ± 1.78^{ab}	58.16 ± 2.69^{a}
Hg	0.12 ± 0.01^{b}	0.19 ± 0.03^{a}	0.12 ± 0.01^{b}	0.13 ± 0.02^{b}	0.15 ± 0.03^{ab}
As	12.78 ± 0.29^{a}	10.73 ± 0.33^{b}	9.83 ± 0.25^{bc}	10.30 ± 0.13^{bc}	9.51 ± 0.55^{c}

The nutrient content of soil after different fertilization treatments is shown in Fig. 4. Compared with the soil background, the content of soil organic matter in all treatments increased significantly after application of nitrogen fertilizer. The soil organic matter content in the G-1 group was the highest, reaching 40.6 g/kg, marking a 34.7% increase over the G-0 group and a 17.6% increase over the G-F group (Fig. 4a).

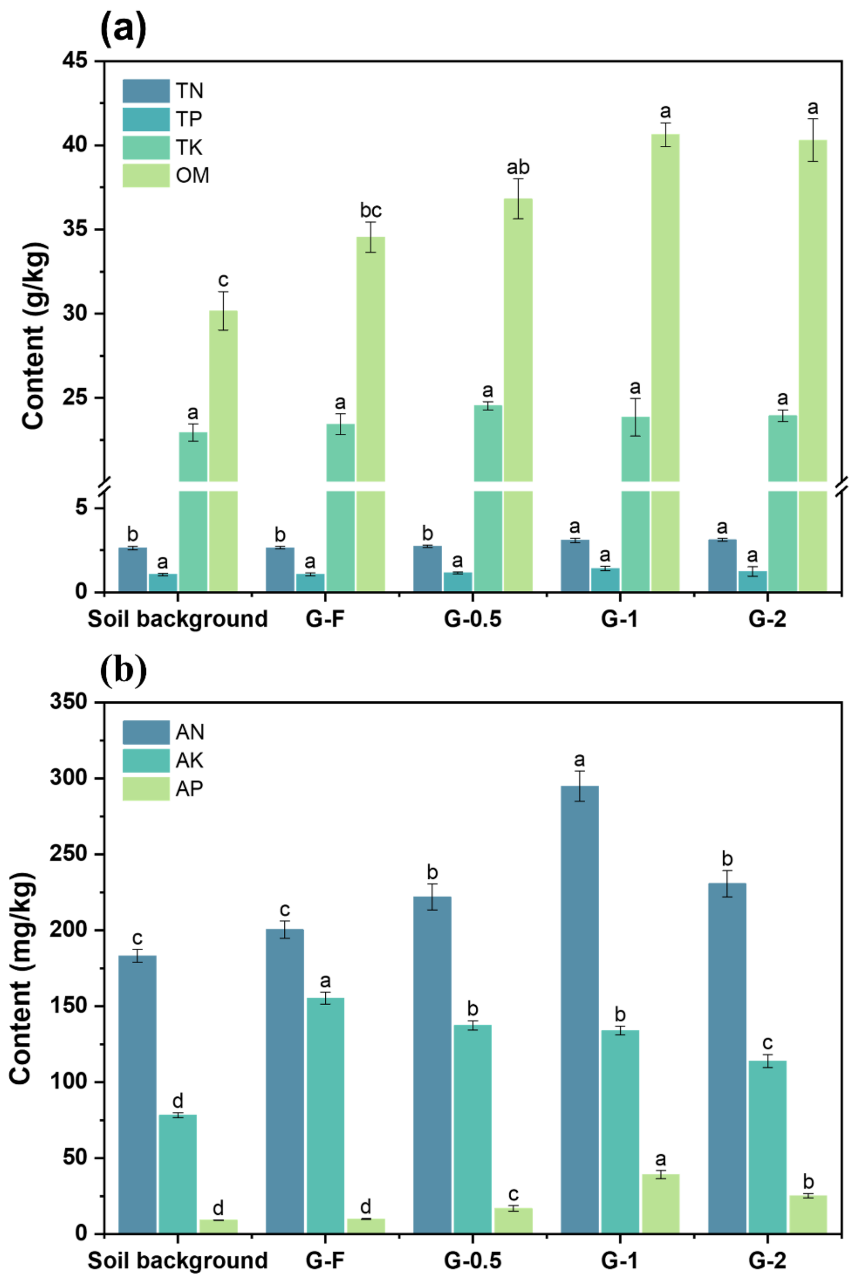

Fig. 4. Effects of different fertilization treatments on soil nutrients: (a) TN, TP, TK, OM; (b) AN, AK and AP. Note: TN: Total Nitrogen, TP: Total Phosphorus, TK: Total Potassium, OM: Organic Matter, AN: Available nitrogen, AP: Available Phosphorus, AK: Available Potassium

This indicates that biogas slurry had a more pronounced effect on enhancing soil nutrient organic matter compared to chemical fertilizers. An increase in soil organic matter content can accelerate the improvement of soil ecological environment and provide suitable carbon and energy sources for heterotrophic microorganisms, thereby augmenting the population and diversity of soil microorganisms. These microorganisms, in turn, accelerate the transformation of soil nutrients, particularly the release of mineral nutrients (Tang *et al*. 2021). Under the equivalent nitrogen level condition, the soil total nitrogen content in G-1 group (3.08 g/kg) was significantly higher than that in the G-F group (2.64 g/kg), with all treatment groups exhibiting higher total nitrogen content than the soil background. Specifically, the total nitrogen content in the G-1 and G-2 groups was significantly increased by 17.1% and 18.3%, respectively, compared to the soil background (Fig. 4a). This indicates that different fertilization treatments all increased the soil total nitrogen content, with the best effect observed in treatments with biogas slurry at doses of 1 time or higher. The difference in total nitrogen content between the G-1 and G-2 groups was only 0.03 g/kg, suggesting that the application of biogas slurry at twice the dosage had already reached the soil's capacity. There were no significant difference in the total phosphorus and potassium content of the soil between the background and the soils treated with chemical fertilizers or biogas slurry.

The application of biogas slurry resulted in a decrease in available potassium content in the soil (Fig. 4b), which was likely attributable to the relatively low total potassium content in the biogas slurry. After application in the field, most of the available potassium components were absorbed and utilized by plants, with little remaining in the soil. Additionally, the soil available nitrogen and available phosphorus content after applying biogas slurry exceeded those after chemical fertilizer application (Fig. 4b), indicating that biogas slurry application is more conducive to improving the quality of soil nutrients compared to direct chemical fertilizer application.

The readily available nutrients provided by chemical fertilizers lack the sustained and stable release of nutrients offered by biogas slurry, rendering them highly susceptible to loss. With the increase in biogas slurry dosage, the content of available nutrients in the soil exhibited an initial increase followed by a decrease. The availability of nutrients facilitates plant absorption and utilization, which is the fundamental reason for enhancing the stability of plant yield. The soil ecological environment and nutrient content are the foundation for plant absorption of nutrients, growth, and development. Therefore, the application of biogas slurry creates a superior rhizosphere environment and sufficient nutrition for plant root absorption, ultimately creating conditions to improve plant yield and quality. In soil application, biogas slurry can achieve the effect of “reducing fertilizer and fixing carbon”. Long-term application requires careful monitoring of changes in heavy metal concentrations in both vegetables and soil.

Principal Component Analysis

Principal component analysis (PCA) was employed to elucidate the relationship between garlic growth indicators (post-harvest plant height, leaf numbers, SPAD value, fresh weight, fresh weight of edible parts, and yield), garlic nutritional components (protein, vitamin C, soluble sugars, dietary fiber, and allicin), and basic soil parameters (total nitrogen, total phosphorus, total potassium, organic matter, available nitrogen, available phosphorus, and available potassium) with fertilizer types and quantities. The correlation loadings of PCA impact factors are shown in Fig. 5a. PCA1 and PCA2 can explain 79.2% of the data variability. PCA1 is mainly influenced by organic matter, total nitrogen, allicin, available phosphorus, total phosphorus, leaf numbers, and available nitrogen, which constitute fundamental soil indicators.

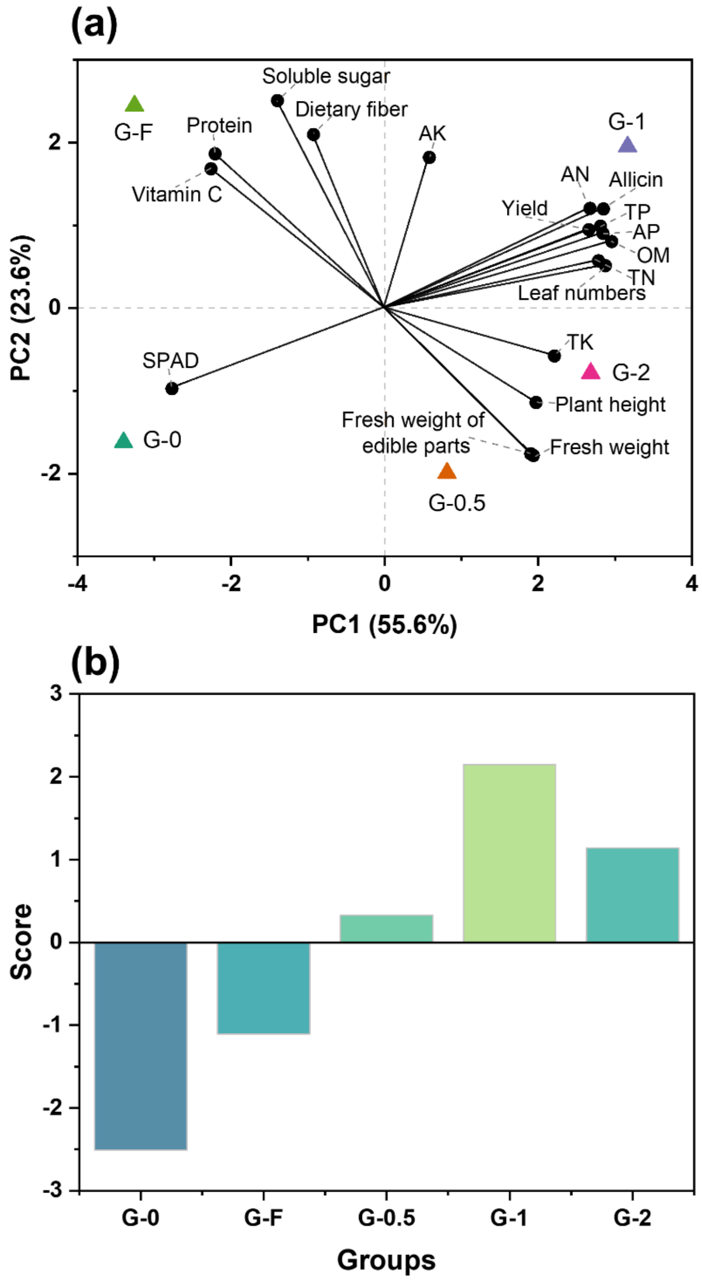

Fig. 5. Correlation loadings (a) and score plots (b) of garlic growth index, nutrient component and soil basic index in relation to fertilizer type and quantity. Note: TN: Total Nitrogen, TP: Total Phosphorus, TK: Total Potassium, OM: Organic Matter, AN: Available nitrogen, AP: Available Phosphorus, AK: Available Potassium.

PCA2 shows higher correlation with soluble sugars, dietary fiber, and available potassium, related to the nutritional composition of garlic. The G-0 and G-F groups are situated on the left side of the multivariate space, showing distinct separation along PCA1 from the G-0.5, G-1, and G-2 groups on the right side, with the G-1, G-2, and G-0.5 groups arranged from right to left within the right-side groups. Compared to chemical fertilizers, the application of biogas slurry can regulate soil physicochemical properties, improving soil health, with the application of biogas slurry containing the equivalent nitrogen content as chemical fertilizers yielding the best results. The G-1 and G-F groups are positioned on the upper side of the multivariate space, exhibiting noticeable separation on PCA2 compared to the G-0, G-0.5, and G-2 groups on the lower side. The G-1 group favors the synthesis and accumulation of garlic nutritional components, whereas applying either less or more nitrogen is detrimental to garlic quality.

The score plots of PCA impact factors are shown in Fig. 5b. According to Luo *et al*. (2019), the numerical values of influencing factors such as garlic growth index, garlic nutrient composition and soil basic parameters were first standardized, and then the index weights were assigned to different factors through principal component analysis, and the comprehensive score coefficient was calculated. The comprehensive score of different fertilization treatments was in the following order: G-1 (2.15) > G-1 (1.14) > G-0.5 (0.33) > G-F (-1.1) > G-0 (-2.51) (Fig. 5b). It is evident that biogas slurry positively influences garlic growth and soil health in way that chemical fertilizers cannot achieve. Biogas slurry contains abundant nutritional substances such as amino acids and trace elements, providing the nutrients necessary for garlic growth. Additionally, the high moisture content of biogas slurry enables it to alleviate soil compaction to a certain extent, facilitating nutrient absorption. Therefore, in soil application, biogas slurry not only can substitute chemical fertilizers, but it also enhances soil organic matter, fosters biological carbon sequestration, and contributes to carbon peak and carbon neutrality, thereby achieving the effect of "educing fertilizer and fixing carbon".

CONCLUSIONS

1. Biogas slurry, a byproduct of biogas engineering, is an effective substitute for chemical fertilizer. The garlic yield and soil organic matter content of biogas slurry with nitrogen content equivalent to chemical fertilizer (G-1 group) reached 1.06 kg/m^3 and 40.6 g/kg, respectively, representing increases of 11.6% and 17.6%, respectively, compared to the chemical fertilizer group. The impact of different biogas slurry doses varied for both garlic and soil, with optimal results observed when biogas slurry is applied with nitrogen content equivalent to chemical fertilizers.
2. The application of biogas slurry stimulates garlic growth and enhances allicin accumulation, while also increasing soil fertility, particularly organic matter content, compared to chemical fertilizers.
3. After applying biogas slurry, the heavy metal content of garlic leaves and soil were kept within the prescribed range of national standards, which especially reduced the accumulation of Cr and Pb in garlic.

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