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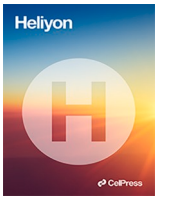

Research article

Flooding soil with biogas slurry suppresses root-knot nematodes and alters soil nematode communities

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ABSTRACT

Root-knot nematodes (RKNs) pose a serious threat to crop production. Flooding soil with biogas slurry, combined with soil heating before crop planting, has the potential for RKN disease suppression. However, the actual effect of this method has not been verified under field conditions. Here, we present the results of a two-year field experiment in a greenhouse demonstrating the control effect on RKN disease and plant growth using this method, as well as its influence on the soil nematode community. Four treatments were set: untreated control (CK), local control method for RKN (CC), soil flooded with 70 % biogas slurry (BS70), and soil flooded with undiluted biogas slurry (BS100). In the first year, all three RKN control treatments significantly reduced the root-knot index ($p < 0.05$). In the next year, only BS70 and BS100 still presented significantly suppressed effects ($p < 0.05$), and it was more obvious under BS70 with a relative control effect of 74.6 %. In the first year, BS70 and BS100 significantly inhibited the plant height of watermelon ($p < 0.05$). In the next year, however, all three RKN control treatments promoted the growth of watermelon, and their stem diameter was significantly greater than that of CK. The application of biogas slurry (BS70 and BS100) significantly increased nematode richness and the Shannon index in the second year ($p < 0.05$). However, the structure index showed no significant difference among treatments ($p > 0.05$), indicating that biogas slurry application did not increase the soil food web complex. Principal component analysis showed that the application of biogas slurry changed the nematode community, especially under BS70, which presented a more lasting influence. The high-level input of biogas slurry also caused soil NH_4^+-N and heavy-metal and arsenic accumulation in the first year, but these soil-pollution risks disappeared in the second year.

1. Introduction

Plant-parasitic nematodes have been reported to cause massive agricultural yield losses [1], and more than 50 % of these losses are attributable to root-knot nematodes (RKNs, *Meloidogyne* spp.) [2]. This nematode has a wide distribution area and host range [3] that can damage more than 5500 plants, including vegetable crops and weeds [4–6]. RKNs harm plant roots through the formation of giant cells and galls, which result in the disruption of plant water and nutrient uptake, thus leading to wilting and even death [7]. It was report that RKNs can reduce production by 10%–20 % and even up to 75 % in severe cases [8]. Nearly 157 billion USD per year in

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agricultural losses worldwide have been estimated as a result of RKNs [9].

RKN disease is particularly serious under closed greenhouse conditions, which have a relatively suitable soil temperature for nematodes and an imbalanced soil ecology caused by intensive management, including a large cropping index, continuous monoculture, and overfertilization [10,11]. Various approaches can be used to control RKNs, including nematicides, solar heating, soil flooding, rotation, organic soil amendment, biocontrol agents, and resistant cultivars [11–18]. Among these methods, the use of organic additives is a traditional approach for controlling plant-parasitic nematodes [19]. Some plant residues can serve as soil amendments, as they contain pre-existing nematocidal compounds to directly suppress nematodes [14,20]. Several green and animal manures release substances that are toxic to nematodes during the degradation process, such as ammonia and fatty acids [17,21,22]. Organic soil amendment also has indirect plant-parasitic nematode control effects through the regulation of the soil food web via a bottom up effect to boost the predators of plant-parasitic nematodes [23,24], or by helping nematodes with similar ecological niches to increase competition [14,25]. Moreover, the use of organic additives helps improve soil fertility and reduce chemical inputs [14], which is consistent with the ecological concepts of circular agriculture and sustainable development. This method is often used in greenhouse vegetable production, in combination with solar heating and soil flooding to enhance the control effect [26]. It is considered a practical, nonchemical approach to nematode disease management [27].

Biogas slurry, which is the byproduct of anaerobic digestion, is rich in nutrients, making it a viable liquid organic fertilizer [28]. The appropriate application of biogas slurry helps improve crop yield and quality as well as soil properties [29–31]. It is reported that biogas slurry can also be used to control various soilborne diseases [32–35]. Several studies have revealed that the application of biogas slurry at the vegetable growth stage can suppress plant-parasitic nematodes, including RKNs [33,36–38]. Compared with this conventional application method, our previous indoor pot study showed that flooding soil with a high amount of biogas slurry before planting could yield better control effect on RKNs [39]. In this experiment, the gall index decreased by 92.9 % compared with the untreated control, and control efficiency was as high as 97.1 % [39]. In addition, we also observed that high levels of biogas slurry input tended to inhibit crop growth [39]. It is widely known that pot conditions are very different from the field environment, and the obtained results might differ as well. It is necessary, therefore, to verify under real production conditions the control effect on RKNs and the state of crop growth using the method of flooding soil with biogas slurry.

Apart from plant-parasitic nematodes, there are other nematode trophic groups living in soil, including bacterivores, fungivores, and omnivore-predators [40]. Soil nematode assemblages occupy multiple trophic levels of the soil food web and play key roles in the functioning of belowground ecosystems [41,42]. Additionally, nematode communities are sensitive to soil disturbance, which can be used to reflect soil health [43–45]. Various nematode ecology indices, which are calculated based on the classification of nematodes into functional guilds, are efficient tools for assessing soil fertility, degradation pathways, and food web structure and function [46–49]. A few studies have demonstrated that nematode communities can reflect changes in the soil environment after biogas slurry application. Mahran et al. [50] found that the bacterial pathway was dominant as biogas slurry was added to soil. Li et al. [39] found that applying a high amount of biogas slurry led to a stressed soil food web condition. However, both experiments were carried out under pot conditions. Thus, field experiments with longer time scales are needed to draw better conclusions regarding the effect of biogas slurry application on soil nematode communities.

In this study, we conducted a two-year field experiment in a greenhouse to determine whether flooding soil with biogas slurry can effectively inhibit RKNs and to assess its effect on soil nematode communities. We expected that the crop-growth inhibition observed in previous pot experiments would be alleviated under this field condition. In addition, we hypothesized that more nematodes at high trophic levels would appear in the second year since the fertilizer efficiency of biogas slurry can stimulate omnivore-predators, as reported by Valocká et al. [38].

2. Materials and methods

2.1. Site description

Our field experiment was conducted in a commercial greenhouse owned and operated by the Beijing Haonongyou Agricultural Cooperative in Daxing District, Beijing, China (39°37′5″N, 116°14′9″E). The study site is in a warm temperate zone with a semihumid continental monsoon climate. Four seasons are distinct in this region, and the average multiannual temperature is 11.5 °C. The annual average frost-free period is 181 days, and the mean multiannual precipitation is 569.4 mm. The soil is classified as a Eutric Areni Fluvisol according to the FAO/WRB [51], and the soil texture is sandy clay loam (sand: 79.18 %; silt: 5.48 %; clay: 15.34 %). Watermelon is the main cultivated crop in this cooperative, while leafy vegetables are planted as rotation crops.

The selected greenhouse was built in a typical format with a semiround arch, which is widely used in North China. It is 137 m in length along EW and 8 m in width along SN, with an area of 0.11 ha. Before the experiment was established, the RKN disease in the soil of this greenhouse was severe. The mean abundance of second-stage juveniles (J2s) of *Meloidogyne* was 3541 per 100 g of dry soil. The basic soil properties (0–20 cm) were also determined as follows: pH 8.03 and organic matter (SOM) 30.82 g kg^{-1}; total nitrogen (TN), available phosphorus (AP), and available potassium (AK) were 20.63, 406.1, and 1305.1 mg kg^{-1}, respectively.

2.2. Biogas slurry collection

The biogas slurry used in this study was produced from chicken manure with mesophilic anaerobic fermentation and a retention time of 15 d. The collection site was Liuminying Biogas Station, Daxing District, Beijing, China. About 22 t of biogas slurry was transported to the experiment site by a tank truck, and 400 ml of biogas slurry was saved in a bottle for basic property analysis. The

chemical characteristics of the biogas slurry were as follows: pH 8.3, and the content of total nitrogen, phosphorus, and potassium were 0.74 %, 0.25 %, and 0.59 %, respectively.

2.3. Experimental design

The experiment had a randomized block design with three replications that had four treatments: 1) CK: no control method for RKN was applied; 2) CC: local control method for RKN; 3) BS70: soil was flooded with 70 % biogas slurry; and 4) BS100: soil was flooded with 100 % biogas slurry. Each plot was 36 m^2 (4.5 m × 8 m). On August 20, 2018, we applied 70 % and 100 % biogas slurry to the BS70 and BS100 plots, respectively. The application rate in both treatments was 0.15 t m^{-2}, which was equivalent to an input of 770.0 kg total N ha^{-1}, 262.5 kg total P ha^{-1}, and 619.5 kg total K ha^{-1} for BS70 and 1100.0 kg total N ha^{-1}, 375.0 kg total P ha^{-1}, and 885.0 kg total K ha^{-1} for BS100. This high application rate was utilized to make the liquid level of biogas slurry 5–10 cm above the soils, which ensured that the soils could be completely submerged (100 % WHC) for several days [35]. Subsequently, mulch film was applied for two weeks to increase the soil temperature. After this solarization process, the mulch film was removed. When the fields could be plowed, the soils were sufficiently plowed and air dried for one week to reduce the possibility of burned seedlings. For the CC treatment, calcium cyanamide (1800 kg ha^{-1}) and sheep manure (150 t ha^{-1}) were applied according to local management practices. Then, the soils were plowed and covered with mulch film for solarization, which was synchronized with the solarization process of the two biogas slurry treatments. In the CK plots, only sheep manure was used, and the application rate was the same as for CC. After the above soil pretreatment process, no crop was planted in the experimental plots until February of the next year, when watermelon seedlings were transplanted to the plots with a line spacing of 150 cm and a row spacing of 30 cm. During the fruit expanding period, high potassium compound fertilizer (225 kg ha^{-1}) was applied to all of the treatments, which was equivalent to inputs of 42.8 kg total N ha^{-1}, 33.8 kg total P ha^{-1}, and 72.0 kg total K ha^{-1}. The experiment lasted two years, and each watermelon growing season was from February to July. Between the two growing seasons, no crop was planted in the plots, and the above soil pretreatment processes were not repeated. Only sheep manure was applied.

2.4. Soil and plant sampling and analysis

Soil and plant sampling were carried out at the harvest time of each watermelon growing season. Four plants were randomly selected from each plot. Plant height and diameter were measured to describe the growth of the watermelon, and the corresponding roots were collected to analyze the gall index. At each plant sampling site, one soil core of 0–20 cm was collected, and four soil cores from each plot were thoroughly mixed into one sample, which was used for soil nematode extraction and soil property analysis. Five other soil cores were collected from the 0–20, 20–40, and 40–60 cm soil layers in each plot in a zigzag pattern. These were used to assess the risk of soil pollution induced by the high-level input of biogas slurry, including soil ammonium nitrogen and nitrate nitrogen content, as well as soil heavy metal (Cu, Zn, Cd, Pb) and arsenic (As) content.

The pH of soil was determined using a handheld meter in a 1:2.5 soil:water solution (w/v). Electric conductivity (EC) was measured using a conductivity meter with a soil:H_2O ratio of 1:5 (w/v). SOM was measured using the method of potassium dichromate oxidation. TN was determined using Kjeldahl digestion. AP and AK were determined using the molybdate blue colorimetric method and the flame emission spectrometry method, respectively. Soil mineral nitrogen (NH_4^+ and NO_3^-) were extracted by 1 M NaCl (soil:solution ratio 1:2) and then analyzed using a Continuous Flow Analytical System AutoAnalyzer 3-Continuous-Flow Analyzer (Bran Luebbe, Norderstedt, Germany) [52]. The contents of Cu, Zn, Cd, Pb, and As were determined using inductively coupled plasma optical emission spectrometry (ICP-OES) [53].

2.5. Nematode analysis

The collected roots were washed with tap water; then, the degree of RKN disease was recorded. Following Chen et al. [54], the root gall indices were rated using a grading scale of 0–9, where 0: no gall; 1: <10 % roots with galls; 3: 11%–25 % roots with galls; 5: 26%–50 % roots with galls; 7: 51%–75 % roots with galls; and 9: >76 % roots with galls. The gall index and control efficacy were calculated using equations (1) and (2), respectively:

$$\text{gall index} = \frac{\sum \text{the number of diseased plants in each grade} \times \text{grade}}{\text{total number of plants investigated} \times \text{the highest grade}} \times 100\%, \quad (1)$$

$$\text{control efficacy } (\%) = \frac{\text{gall index in CK - gall index in the RKN control treatment}}{\text{gall index in CK}} \times 100. \quad (2)$$

Soil nematodes were extracted using a modified cotton-wool filter method [55]. In brief, 50 g fresh soil was spread over a paper tissue, which was placed on a screen. The screen was placed in an iron plate contained enough water to saturate the soil sample. After 48h, nematodes were collected from the water. Following extraction, the total number of nematodes were counted via a stereomicroscope, at least 100 specimens from each sample were identified to the genus or family level. If the total number of nematodes was less than 100, all nematodes were identified. The collected nematodes were divided into four trophic groups according to feeding habits: bacterivores (Ba), fungivores (Fu), plant parasites (PP), and omnivore-predators (OP) [40].

Nematode diversity was described by taxa richness (S), the Shannon index (H′) (equation (3)), dominance (λ) (equation (4)), and trophic diversity (TD) (equation (5)). S = the number of the nematode taxon [56].

$$H' = \sum p_i(\ln p_i), \tag{3}$$

$$\lambda = \sum {p_i}^2, \tag{4}$$

where p_i is the proportion of the *i*th taxon in the total nematode assemblage.

$$TD = \frac{1}{\sum {p_i}^2}, \tag{5}$$

where p_i is the relative abundance of trophic group i [57]. Four other nematode functional indices were calculated to assess soil health under different treatments. The maturity index had nematode cp values of 2–5 (MI2-5), which was calculated using equation (6):

$$MI2\text{-}5 = \sum V_i \times f_i, \tag{6}$$

where v_i is the cp value of free-living nematode taxon i, and f_i is the frequency of the involved nematode taxa in a sample [58]. The enrichment index (EI) (equation (7)), structure index (SI) (equation (8)), and basal index (BI) (equation (9)) are useful indicators for assessing the condition of the soil food web and the disturbance of the soil environment [48].

$$EI = \frac{e}{e+b} \times 100, \tag{7}$$

$$SI = \frac{s}{s+b} \times 100, \tag{8}$$

$$BI = \frac{b}{e+s+b} \times 100, \tag{9}$$

in which e, b, and s are the weighted proportions of enriched, basal, and structured components in the soil food web, respectively [48].

2.6. Statistical analysis

The overall trend was first evaluated using multivariate analysis of variance (ANOVA). Two-way ANOVA was used for the plant growth indices, gall index, nematode data, and soil properties, with year and treatment as factors. For soil pollution characteristics, three-way ANOVA was used with year, soil depth, and treatment as factors. Then, one-way ANOVA was performed to analyze the treatment effect for each year or soil depth, and *t*-tests were used to evaluate the differences between years in each treatment. Least-significant difference (LSD) was used for multiple-comparison one-way ANOVA. Some data were transformed with ln(x) or the square root if they did not conform to normal distribution prior to statistical analysis. If the data still showed heteroscedasticity after transformation, the nonparametric Kruskal–Wallis test was performed. Subsequently, the Mann–Whitney test was conducted for multiple comparisons. SPSS (version 26.0, IBM Corp., Armonk, New York, USA) was used for all of the above statistical analyses, and differences were considered significant at $p < 0.05$. The differences in nematode assemblages among treatments were determined using principal component analysis (PCA). Nematode data were log (x + 1) transformed prior to analysis. PCA was carried out using Canoco software (version 5.0).

3. Results

3.1. Soil chemical characteristics

The contents of SOM, TN, AP, and AK, as well as the values of pH and EC, were significantly affected by sampling time and

Table 1
Soil chemical properties under different treatments.

	2019				2020				Year (Y)	Treatment (T)	Y*T
	CK	CC	BS70	BS100	CK	CC	BS70	BS100			
TN (g kg^{-1})	2.20a	2.16a	2.51a	2.61a	2.48b	2.43b	2.69 ab	2.93a	0.025	0.017	0.975
SOM (g kg^{-1})	32.83b	35.97b	42.90a	44.27a	32.47a	33.37a	32.63a	32.73a	<0.001	0.003	0.003
AP (mg kg^{-1})	424.2c	418.8c	557.2b	768.9a	420.6a	451.0a	430.1a	490.3a	0.002	<0.001	0.002
AK (mg kg^{-1})	1113.2d	1355.3c	1937.7b	2153.0a	1063.3c	1124.6bc	1418.1a	1247.6 ab	<0.001	<0.001	<0.001
pH	8.18d	8.36c	8.49b	8.73a	8.08a	8.15a	8.01a	7.97a	<0.001	<0.001	<0.001
EC (μs cm^{-1})	479.4d	607.1c	964.0b	1082.5a	469.6b	517.1 ab	534.7 ab	603.0a	<0.001	<0.001	<0.001

CK: no control method for RKN; CC: local control method for RKN; BS70: soil flooded with 70 % biogas slurry; BS100: soil flooded with 100 % biogas slurry. TN: total nitrogen; SOM: soil organic matter; AP: available phosphorus; AK: available potassium; EC: electrical conductivity. Different lowercase letters indicate significant differences between treatments for each year. The significance of the effect of year (Y) and treatment (T) is indicated. Values of $p < 0.05$ are considered significant.

treatments ($p < 0.05$) (Table 1). In 2019, all of the above soil properties yielded the highest value in BS100, followed by BS70. The application of biogas slurry (BS70 and BS100) significantly increased the soil properties compared with CC and CK ($p < 0.05$), except for TN, which did not exhibit a significant difference among all of the treatments ($p > 0.05$). In the second year, compared with CK and CC, BS100 was only significantly higher in TN, while BS70 was significantly higher in AK ($p < 0.05$). However, no significant difference was observed among treatments regarding SOM, AP, and pH ($p > 0.05$). Overall, the differences in various soil properties among the treatments were reduced in the second year, except for TN (Table 1).

3.2. Plant growth indices and RKN control effect

The comprehensive data for two years showed no significant difference in the stem diameter of watermelon among treatments ($p > 0.05$) (Fig. 1 A). However, the multiple-comparison results showed that stem diameter significantly increased under CC, BS70, and BS100 compared with CK in the second year ($p < 0.05$). The two biogas slurry treatments (BS70 and BS100) significantly inhibited plant height in the first year ($p < 0.05$) (Fig. 1 B). In the second year, all three RKN control treatments (CC, BS70, and BS100) showed a trend of increasing plant height, although no significant difference was found among treatments ($p > 0.05$). For year effects, plant height under CC, BS70, and BS100 increased significantly in the next year ($p < 0.05$).

The experimental treatment and sampling time had significant effects on the root-knot index of watermelon and the abundance of RKNs ($p < 0.05$) (Fig. 2 A C). In 2019, the overall incidence rate of nematode disease was relatively low, while in the next year, RKN disease broke out (the root-knot index reached 87.6 under CK, and the abundance of J2s of RKNs reached 5138 per 100 g of dry soil). In the first year, all three RKN control treatments significantly reduced the root-knot index ($p < 0.05$) (Fig. 2 A, Fig. S1), but in the second year, only two biogas slurry treatments presented significantly reduced effects ($p < 0.05$) (Fig. 2 A, Fig. S2), which were more obvious under BS70, with a relative control effect of 74.6 % (Fig. 2 B). The performance of the number of J2s was slightly different from the root-knot index. All three RKN control treatments reduced the number of nematodes in 2019, but only BS100 showed a significant difference compared with CK ($p < 0.05$) (Fig. 2C). This significant effect was found only under BS70 in 2020 ($p < 0.05$).

3.3. Soil nematode community

A total of 38 nematode taxa were obtained during the two-year experiment (Table S1). Bacterivores were the richest trophic group, followed by omnivore-predators. Plant parasites were mainly RKNs. The relative abundance of fungivores was the lowest under all treatments (Fig. 3). The composition of nematode trophic groups varied greatly between the two years. In the first year, bacterivores were dominant in all treatments. In all treatments, both the proportion and abundance of plant parasites increased by different degrees in 2020 compared with 2019, and this trophic group was dominant under CK and CC. However, its abundance decreased significantly under BS70, compared with CK and CC (Fig. 4 B). The proportion of omnivore-predators under CK, CC, and BS100 was lower in 2020 than in 2019, while that under BS70 showed an opposite trend (Fig. 3). However, no year or treatment effect was found in the abundance of omnivore-predators (Fig. 4C).

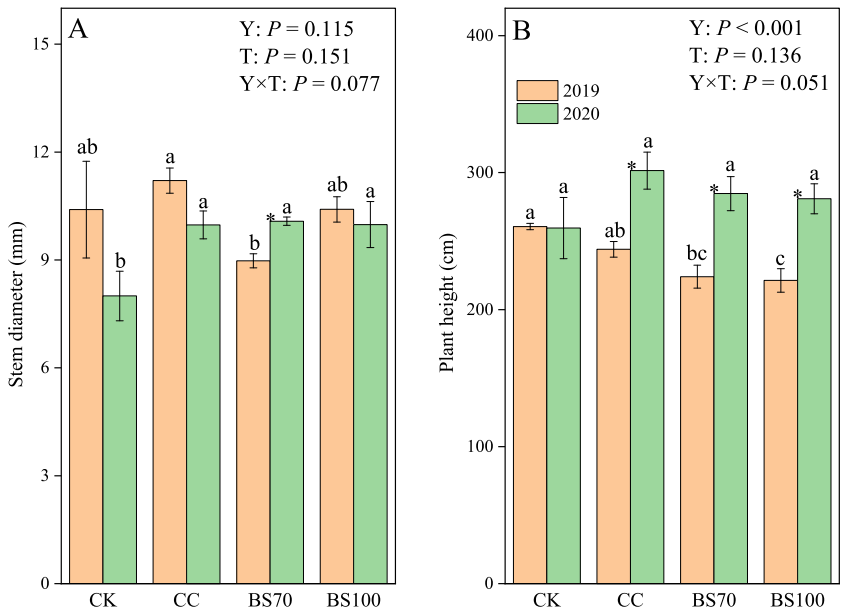

Fig. 1. Plant stem diameter (A) and height (B) under different treatments.

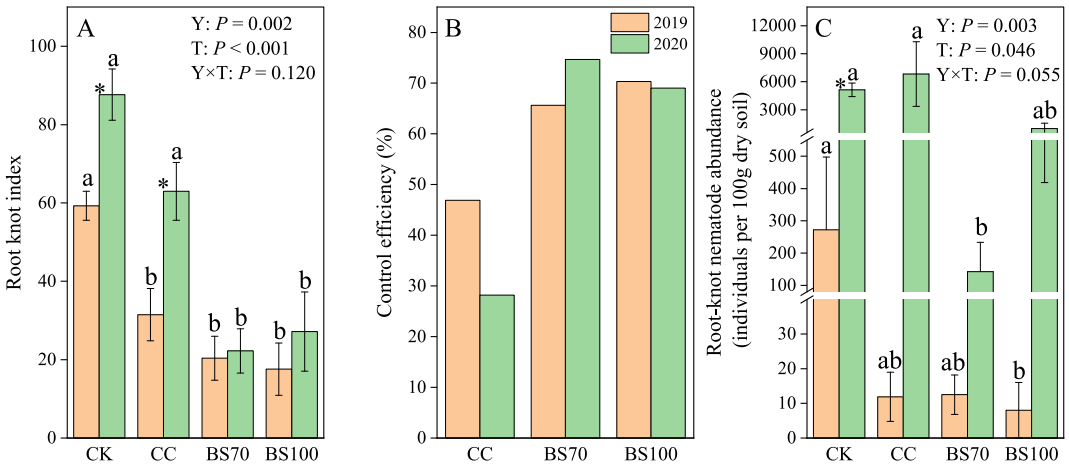

Fig. 2. Root knot index (A), control efficiency (B) and root-knot nematode abundance (C) under different treatments.

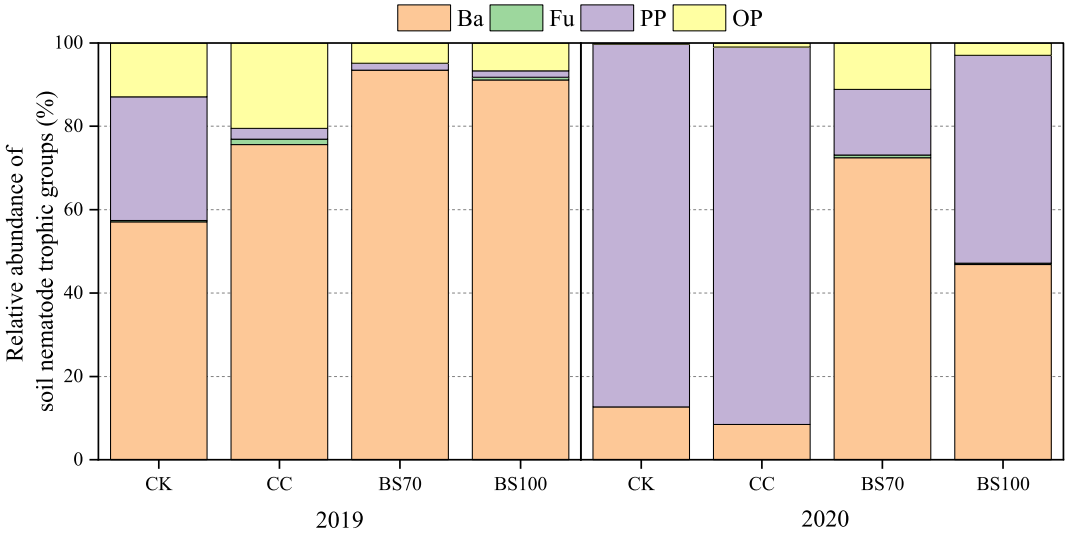

Fig. 3. Relative abundance of soil nematode trophic groups under different treatments.

3.4. Soil nematode ecology indices

Sampling time and treatment had significant effects on the S, H′, and λ of nematodes ($p < 0.05$) (Table 2) but had no significant effect on TD ($p > 0.05$). In the first year, there was no significant difference in S, H′, and λ among the treatments, but in the second year, S and H′ under the two biogas slurry treatments were significantly higher than those under CK and CC ($p < 0.05$). In the first year, TD under CK and CC was significantly higher than that under the two biogas slurry treatments ($p < 0.05$), and it showed the opposite trend in the next year.

EI was significantly higher under the two biogas slurry treatments than under CK and CC in 2019 ($p < 0.05$) (Table 2). In the next year, EI significantly decreased under BS100 ($p < 0.05$), and only BS70 still showed a significantly higher value compared with CK and CC ($p < 0.05$). BI presented an opposite trend to EI across treatments and sampling dates. No significant difference was found among treatments in SI and MI2-5 in 2019, as well as that in 2020 for the SI index. Under CK and BS100, MI2-5 significantly decreased in the next year ($p < 0.05$) and was significantly lower under CK than BS70 ($p < 0.05$).

3.5. PCA based on nematode community

In 2019, the soil nematode communities were similar under BS70 and BS100, and these two treatments exerted greater effects than the CC base on the CK nematode community (Fig. 5 A). In the next year, the nematode communities under CC and BS100 shifted to CK, and the communities under CC were similar to those under CK, and BS100 was between CK and BS70 (Fig. 5 B). For 2019, the first and second PCA axes explained 40.30 % and 19.17 % of composition variation, respectively; the corresponding values for the next year

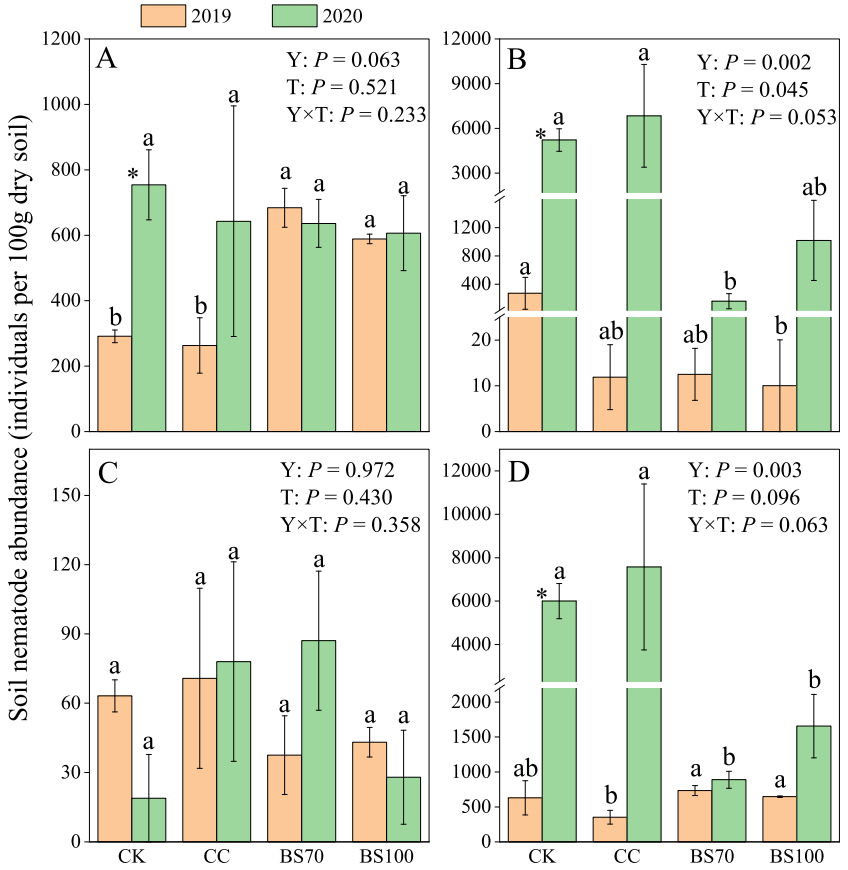

Fig. 4. The abundance of bacterivores (A), plant parasites (B), omnivore-predators (C) and total nematode (D) under different treatments.

Table 2
Soil nematode ecological indices under different treatments.

	2019				2020				Year (Y)	Treatment (T)	Y × T
	CK	CC	BS70	BS100	CK	CC	BS70	BS100			
S	13.00a	13.00a*	13.33a	14.33a	7.33b	7.33b	16.33a	12.67a	0.024	0.008	0.022
H′	1.91a*	1.97a*	1.94a	2.14a	0.65b	0.49b	2.21a	1.48a	<0.001	0.002	0.003
λ	0.24a*	0.20a*	0.21a	0.17a	0.74 ab	0.81a	0.15c	0.40bc	<0.001	<0.001	0.002
TD	1.87a*	1.60a	1.14b*	1.19b	1.30bc	1.21c	1.70a	1.63 ab	0.904	0.487	<0.001
EI	38.69b	61.28b	93.72a	93.58a*	45.23b	36.67b	85.38a	51.30b	0.029	<0.001	0.135
SI	30.42a	13.13a	44.65a	24.16a	10.26a	14.62a	6.54a	5.87a	0.035	0.728	0.422
BI	48.44a	36.36a	6.05b	6.20b*	53.98a	55.72a	14.40b	46.62 ab	0.020	0.003	0.331
MI2-5	2.40a*	2.54a	2.73a	2.52a*	2.06b	2.33 ab	2.49a	2.09 ab	0.033	0.221	0.924

S: taxa richness; H′: Shannon index; λ: dominance; TD: trophic diversity; EI: enrichment index; SI: structure index; BI: basal index; MI2-5: maturity index with nematode cp values from 2 to 5.

were 59.20 % and 11.39 %, respectively.

3.6. Soil environmental indicators

Flooding soil with biogas slurry significantly increased the content of soil NH_4^+-N in the first year ($p < 0.05$), especially for the top two soil depths. The values under BS70 plus BS100 were 42.6 and 47.4 times those under CK plus CC for soil depths of 0–20 cm and 20–40 cm, respectively (Fig. 6 A). In the second year, the content of soil NH_4^+-N sharply decreased under the two biogas treatments but significantly increased under CC and CK ($p < 0.05$) (Fig. 6 B), largely reducing the significant gaps between the biogas treatments and the two other treatments. The differences in soil NO_3^--N among treatments were relatively small compared with NH_4^+-N (Fig. 6C D), especially in 2020. Compared with 2019, in the second year, the content of soil NO_3^--N significantly decreased at the 0–20-cm soil depth ($p < 0.05$) but significantly increased at the 40–60-cm soil depth ($p < 0.05$) across all treatments.

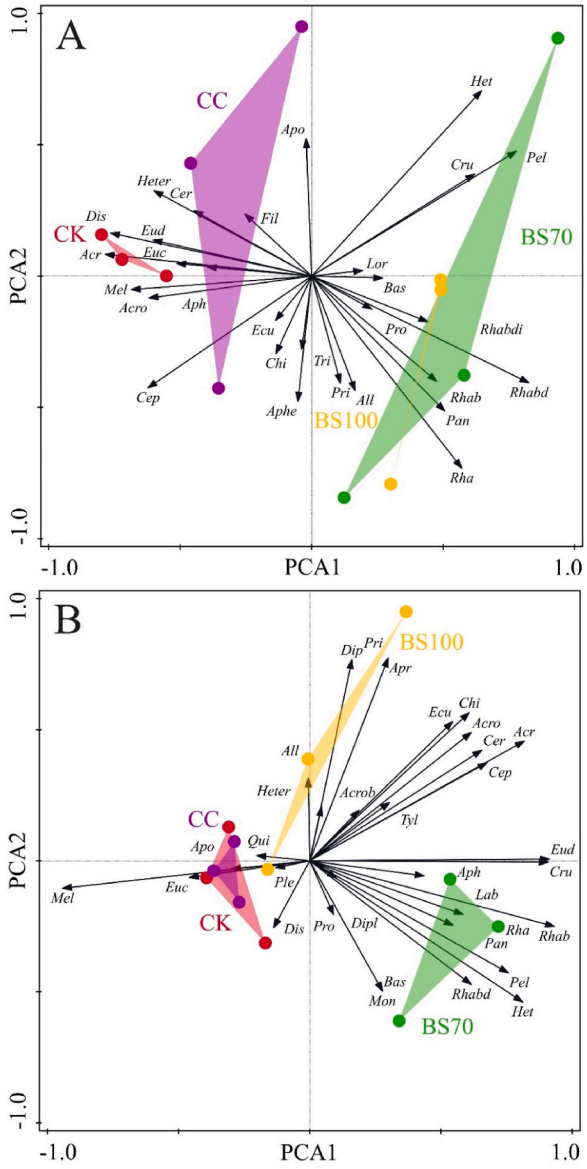

Fig. 5. Biplot representation of principal component analysis based on soil nematode communities for 2019 (A) and 2020 (B).

In the first year, compared with CK, the two biogas slurry treatments significantly increased the content of soil Cu, Zn, As, Cd, and Pb across all soil depths ($p < 0.05$) (Fig. 7 A C E G H). In the second year, all of the above indicators were reduced under the two biogas slurry treatments (Fig. 7 B D F H J), and the differences were significant in Cu, Zn, and As ($p < 0.05$). According to the national standard of the People's Republic of China (GB15618-2018), the content of Cd under BS100 and the content of As under BS70 and BS100 exceeded the threshold for trace metal concentrations in soil in 2019, but in the second year, the contents of all metals and As were below the threshold.

4. Discussion

4.1. Effects of flooding soil with biogas slurry on RKN control

This study was a continuation of a previous pot experiment [39]. It aimed to verify, under field conditions, the real control effect on RKNs of applying biogas slurry to submerged soil before planting. The results showed that this method (BS70 and BS100) achieved an average relative control effect of more than 65 % over two years. Although this value was lower than that in the pot experiment, in which a control effect of more than 90 % was obtained under similar treatment [39], it was superior to the conventional method of calcium cyanamide application.

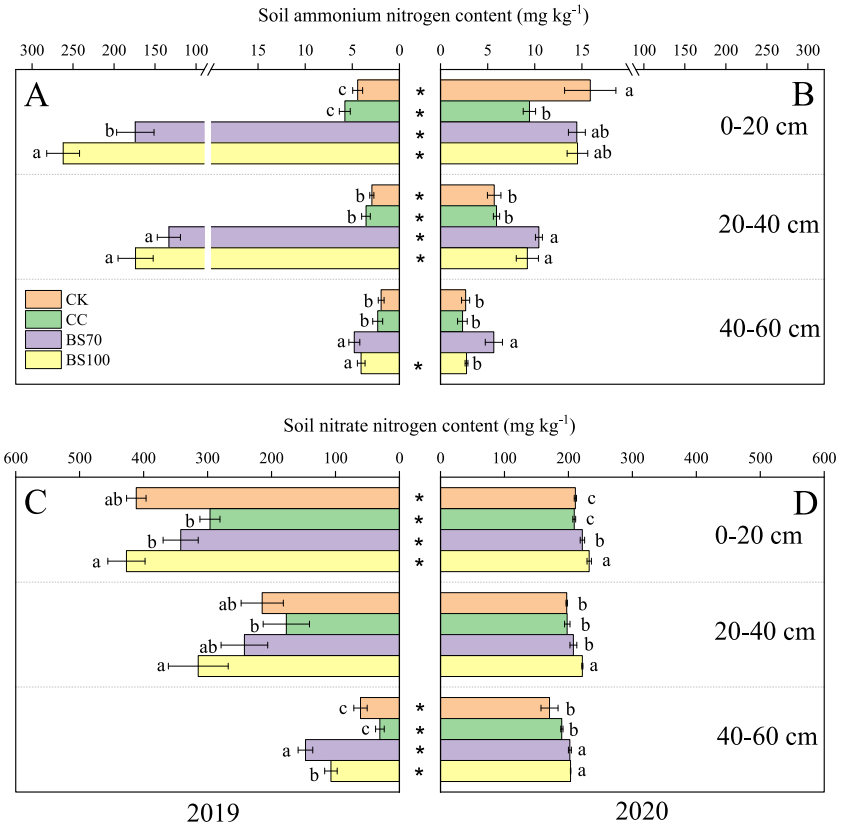

Fig. 6. Contents of soil ammonium nitrogen (A and B) and nitrate nitrogen (C and D) under different treatments at soil depths of 0–20, 20–40, and 40–60 cm.

The mechanisms of controlling RKNs by applying biogas slurry to submerged soil could include the following: 1) The high-level input of biogas slurry put a large amount NH_4^+-N into the soil, and NH_4^+-N is considered a key factor for suppressing RKNs [36,37,59]. Ammonium nitrogen itself has no effect on nematodes [59]; however, it can be transformed into NH_3, especially in alkaline soil environments [35]. NH_3 can pass through the cell membrane and change the pH of cytoplasm [60] to damage nematodes. In addition, the input of biogas slurry can induce various organic acids [61], which have been reported to have inhibitory effects on soil nematodes [26]. 2) Flooding soil with biogas slurry can form an anaerobic environment, which is conducive to maintaining high NH_4^+-N content and releasing organic acids [35,62], thus indirectly inhibiting RKNs. Moreover, a high-level concentration of biogas slurry is viscous [63], which helps maintain a soil-saturated state to enhance the inhibition effect. 3) The solarization process could increase soil temperatures and therefore kill soil nematodes [27,64]. Previous reports showed that soil heating induced by greenhouse closure and mulch film cover can raise topsoil temperatures to over 50 °C [26,65], which is sufficient for eradicating nematodes, as well as weed seeds and fungal pathogens [66–68]. Although we did not monitor soil temperatures in this experiment, the role of soil heating cannot be ignored. 4) We applied biogas slurry before watermelon planting, which is different from previous studies that applied it during planting [33,36–38]. Under this condition, when the soil was submerged by biogas slurry, RKNs in the soil were mainly J2s, which were greatly reduced before they entered the host root [39].

It is noteworthy that the proportion of *Meloidogyne* (J2s) in the soil under BS70 was less than 2 % in the first year, and this value increased to 14.09 % in the second year (Table S1). However, the relative control effect did not decrease but was improved. This seemingly contradictory phenomenon, which also occurred under BS100, was mainly related to the difference in overall RKN disease severity between the two years. It was obvious that the root-knot index was relatively low in 2019, while in the next year, nematodiasis broke out under the CK treatment. We can infer, therefore, that when disease severity was low, the control effect measures were not reflected, while when disease was serious, biogas slurry flooding could present a better control effect compared with the conventional method under field conditions.

4.2. *Effects of flooding soil with biogas slurry on watermelon growth*

In our previous pot experiment, the high input of biogas slurry inhibited crop growth [39]. We expected that this negative effect would be alleviated owing to stronger soil runoff, and a leaching effect would appear under the field condition compared with the pot condition [36]. In the current field experiment, however, the inhibitory effect still occurred in the first year, mainly reflected in the

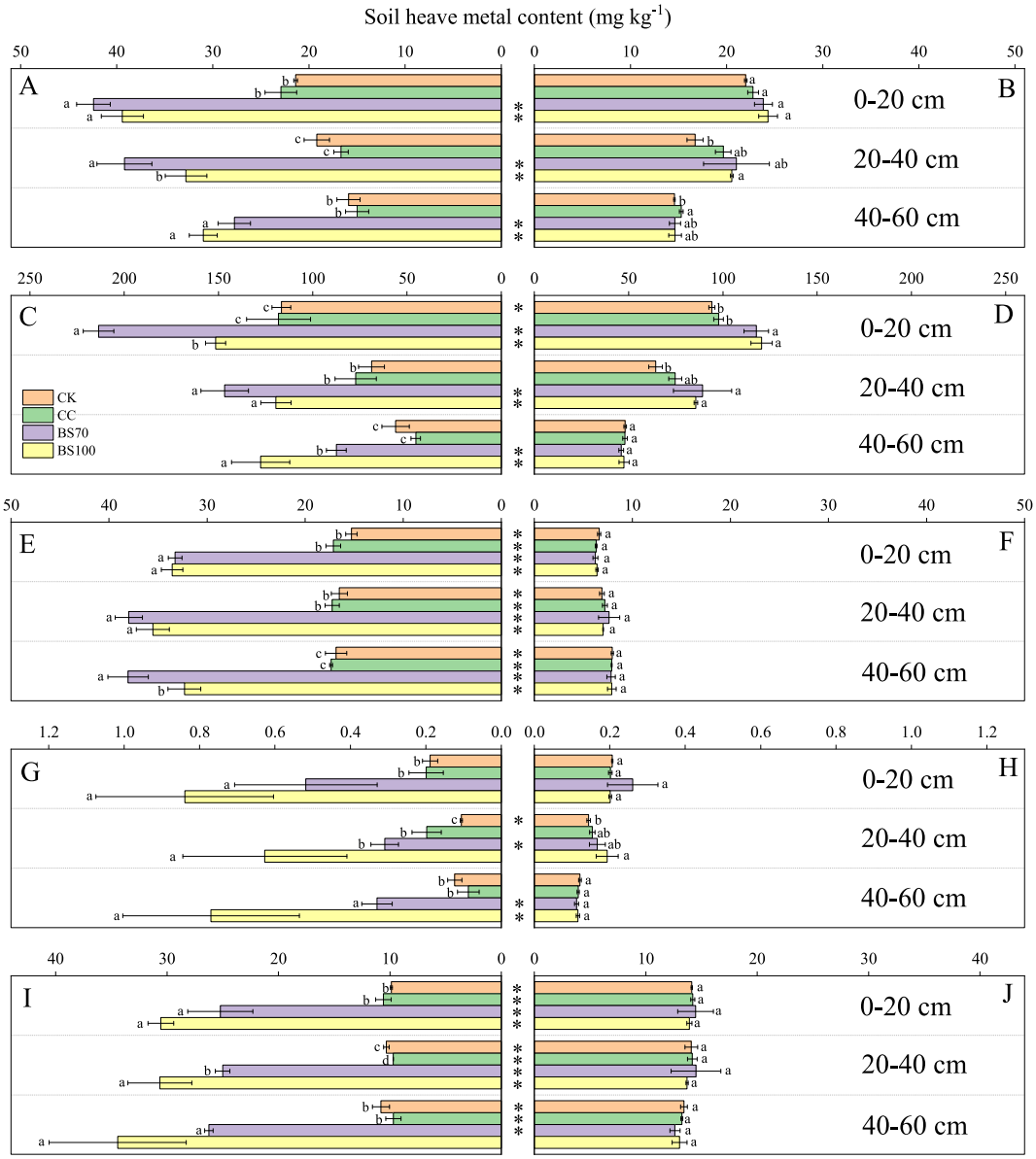

Fig. 7. Contents of Cu (A and B), Zn (C and D), As (E and F), Cd (G and H) and Pb (I and J) in soil under different treatments at soil depths of 0–20, 20–40, and 40–60 cm.

inhibition of plant height under BS70 and BS100. This phenomenon was probably related to the existence of a great deal of ammonium nitrogen and organic acid in the soil induced by biogas slurry application. Although these two substances can suppress nematodes as discussed above, excessive ammonium nitrogen and organic acid can also inhibit plant growth [59,69]. In the next year, as we expected, there was an opposite trend in plant height, although no significant difference was found among treatments. In addition, significantly larger stem diameters were obtained under CC, BS70, and BS100 compared with CK. The improved growth of watermelon under the two biogas treatments in the second year can be explained by the outbreak of RKNs under CK, while biogas slurry suppressed RKNs. Another reason for this phenomenon could be the fertilizer efficiency of biogas slurry [28,29,31], such that the high input in the first year could still maintain a continuous supply of nutrients to crops in the second year.

4.3. Effects of flooding soil with biogas slurry on the soil nematode community

Bacterivores were dominant in all treatments in the first year, which accords with previous reports showing that bacterivores are more abundant under greenhouse conditions [42,57]. By contrast, fungivores accounted for the lowest proportion of trophic groups in our study. The bacterial pathway indicated by the above microbivores is closely related to the long-term organic input and strong

disturbance of such an intensive management system [42]. Omnivore-predators are considered indicators of a better soil environment and complex food web [46,70]. They are also predators of herbivores and can therefore help regulate the population of pest species [23,24]. We expected that more omnivore-predators would appear in the two biogas slurry treatments in the second year since organic input might boost soil organisms at high trophic levels through the bottom-up effect [24,71]. Among the treatments, however, no significant difference was obtained in SI, which was mainly based on the proportion of omnivore-predators and the abundance of omnivore-predators in 2020. These indicate that a high level of biogas slurry input did not form a more complex soil food web compared with other treatments as the plantation time expanded. Thus, although a higher relative abundance of omnivore-predators was found under BS70 compared with other treatments in the second year (Fig. 3), the inhibition of RKNs could not be attributed to the top–down effect within the soil food web but to the toxic effect of biogas slurry itself.

Calcium cyanamide, which can serve as a fumigant, has been widely used to control various soil-borne diseases in greenhouse systems [72–74]. It is metabolized to nitrogen and calcium in soil and has little toxicity to belowground ecosystems [75]. Shi et al. [73] found that calcium cyanamide application only had a short-term impact on soil microbial community. This is similar to our nematode results, which showed that the CC treatment exerted a relatively small influence on the nematode communities, and they almost recovered to the level of that in CK in the following year (Fig. 5 B). In comparison, the application of biogas slurry had greater impacts on nematode community, especially under BS70, presenting a more lasting influence. The difference in nematode communities among treatments in the second year may have been closely related to the proportion of RKNs, which accounted for more than 85 % in CK and CC, but only 14.1 % in BS70 (Table S1).

In our previous pot experiment, the high-level input of biogas slurry significantly increased the nematode Shannon index compared with untreated sick soil [39]; this only accorded with the results for 2020 in the present study. It was obvious that the relatively low nematode diversity occurring in diseased RKN soil was mainly attributable to the high proportion of RKN. Thus, in 2019, RKN disease was not serious in our experimental greenhouse, and in turn, the nematode Shannon index was not significantly lower under CK than in other treatments. Even without considering the factor of nematode proportion, more nematode taxa were obtained under the two biogas treatments than under CC and CK in 2020. Cao et al. [35] also found that, based on the Biolog method, biogas slurry soil flooding could increase microbial diversity. This implies that, for diseased soil, flooding soil with biogas slurry is beneficial for soil biodiversity.

4.4. Limitations of current study and future experimental presumption

In the current experiment, as mentioned in the previous discussion, the application rate of biogas slurry was extremely high, being more than 46 times that of conventional methods in arable crop systems [76,77]. It was also much higher than that in another experiment using a similar method of biogas slurry application [35]. Thus, from the perspective of nutrient management, this excessive fertilization is not appropriate, and it would not only harm crop growth, but could also cause soil environmental risks [78,79]. The most concerning risk is heavy-metal pollution. Previous reports revealed that the application of biogas slurry with a common dose could induce heavy-metal accumulation in soil [28,80,81], and a high application dose poses a greater risk. In our experiment, the high level of biogas slurry application indeed caused various heavy-metal accumulations in the soil, even leading to standard-exceeding levels of As and Cd in the first year. Although this light level of contamination was not sufficient to have a negative impact on crop growth [82], it might have affected the nutrient absorption of the crop though altering or negatively impacting the soil microbial structure and diversity [83,84]. In addition, the heavy metals in the soil could be transferred to the fruit though bioconcentration and translocation effects mediated by soil microbes [53,85], in turn posing health risks to humans [53]. Therefore, although these heavy-metal risks disappeared in the second year, they still warrant attention and should be monitored continuously, not only for soil but also for roots, plants, and fruit.

Considering the limitations of flooding soil with biogas slurry for RKN control in our study, further research is needed to optimize this method. Since the RKN control effect not only depended on the biogas slurry itself, as it was also closely related to the anaerobic environment formed [11], and because diluted biogas slurry was found to have a better control effect than undiluted biogas slurry, future studies should explore the suppression of RKNs using lower-concentration biogas slurry to reduce the negative effects on crops and the environment. It has been found that heavy-metal pollution differs in different livestock and poultry manure [86,87], as well as in the corresponding biogas slurries. Cao et al. [35] found that flooding soil with pig manure biogas slurry did not cause any heavy metal pollution, and there was no significant difference in the heavy-metal contents of the treatments. Thus, future studies can explore the use of other types of biogas slurry to control RKN disease.

5. Conclusions

The results of this study confirm that flooding soil with biogas slurry before watermelon planting could suppress RKNs under field conditions. It was found that the application of diluted biogas slurry had a better controlling effect than undiluted biogas slurry. Both of these biogas slurry treatments exhibited higher relative control effects than that of the local control method of calcium cyanamide application. In addition, using large amounts of biogas slurry was conducive to improving nematode diversity in diseased soil. The two biogas slurry treatments induced changes in the nematode community, especially BS70, which showed a more lasting influence on nematode communities. High-level biogas slurry application before planting also produced plant-growth inhibition and soil-environment risk, but these negative effects disappeared in the second year. Further study is needed to improve the new biogas slurry application method proposed in this study, which aimed to give consideration to both the guarantee of RKN control effects and the alleviation of negative effects on crops and the environment.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Yufei Li: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Conceptualization. **Bensheng Liu:** Resources, Methodology, Investigation. **Jijin Li:** Resources, Investigation. **Guoyuan Zou:** Supervision, Project administration, Data curation. **Junxiang Xu:** Investigation. **Lianfeng Du:** Supervision, Funding acquisition. **Qianqian Lang:** Writing – review & editing, Investigation. **Xiang Zhao:** Investigation. **Qinping Sun:** Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30226.

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