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Effects of different application doses of black soldier fly frass *Hermetia illucens* (Diptera: Stratiomydae) on soybean plant performances and arthropod abundance

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Abstract. *Yudistira DH, Sandi YU, Wirabumi BA, Damayanti A, Wikandari P, Sato S. 2025. Effects of different application doses of black soldier fly frass* Hermetia illucens *(Diptera: Stratiomydae) on soybean plant performances and arthropod abundance. Asian J Agric 9: 40-51.* Food waste poses a global challenge, contributing approximately 3.49 billion tons of CO_2 to the atmosphere annually. Recycling this waste into valuable soil nutrients is a viable solution. This study focuses on black soldier fly *Hermetia illucens* L. (Diptera: Stratiomydae) larvae, which convert organic waste into protein-rich biomass and frass, a potential soil amendment. However, the effects of black soldier fly frass on crop performance and biodiversity remain underexplored. We investigated the impact of three different frass doses (0, 2.5, and 5 tons/ha) on the growth of the Tsuruoka soybean variety (*Glycine max* L.), *dadachamame*. Results showed no significant differences in plant height (p=0.07), but higher doses increased leaf count (p<0.01) and SPAD values significantly (p<0.001), with 2.5 tons/ha improving leaf count by 26% and SPAD values by 16%. Yield analysis revealed that 2.5 tons/ha increased the number of filled pods by 29% (p<0.05) and reduced empty pods by 52% (p<0.001), thereby enhancing pod quality. Arthropod analysis indicated that 5 tons/ha significantly increased overall arthropod abundance (p<0.001), particularly predators (p<0.001) and herbivores (p<0.001), while 2.5 tons/ha balanced predator abundance, thereby controlling herbivores and supporting biodiversity. These findings suggest that an application dose of 2.5 tons/ha is optimal for improving edamame yield and quality while maintaining a balanced arthropod ecosystem, demonstrating black soldier fly frass as a nutrient-rich, eco-friendly input that supports sustainable agriculture.

Keywords: Biodiversity, food waste recycling, organic farming, sustainable agriculture, trophic interaction

Abbreviations: BSF: Black Soldier Fly, WAT: Weeks after treatment

INTRODUCTION

Approximately one-third of the world's food production is wasted annually, accounting for roughly 40% of all waste (FAO 2011). Food waste is estimated to cost the global economy up to $936 billion and release around 3.49 billion tons of CO_2 into the environment (FAO 2011). In some countries, food waste has become a major problem. For example, food waste has long been an issue in Japan, where 17 million tons of food were wasted in 2017 alone, mostly from the retail, food, and wholesale industries (Oishi 2019).

Recycling food waste offers a cost-effective and environmentally friendly alternative by repurposing various nutrients, which aligns with the Sustainable Development Goals (SDGs) related to responsible consumption and production (SDG 12), climate action (SDG 13), and life on land (SDG 15). According to Noor et al. (2014), composting is an effective method for managing food waste, significantly reducing the volume of waste requiring landfill disposal. It also offers numerous benefits, including enhanced mineralization of plant nutrients, suppression of plant pests and diseases, and improved soil structure and stability (Martínez-Blanco et al. 2013; Ayilara et al. 2020). Advanced technologies have facilitated faster composting, such as through the use of microorganisms to decompose complex organic materials (Guanzon and Holmer 1993).

Recently, employing insects for organic material decomposition has emerged as a global trend. *Hermetia illucens* L. (Diptera: Stratiomydae), commonly known as the black soldier fly (BSF), is recognized for its beneficial role in reducing organic waste by converting it into biomass. The treatment of black soldier fly larvae (BSFL) is being explored as a waste reduction strategy in various countries (Fischer et al. 2021; Basri et al. 2022) due to the simplicity, speed, and minimal space requirements for BSFL rearing (Rummel et al. 2021). Beyond waste reduction, BSFL offers additional value by bioconverting low-value food waste into valuable fertilizer and protein-rich larvae (Basri et al. 2022). The residual material after harvesting the larvae, termed “frass”, consists of leftover BSFL feed, exuviae, feces, and microorganisms (Schmitt and de Vries 2020).

Organic farming was developed to reduce the impact of chemical use in conventional agriculture. Organic farming focuses on a comprehensive strategy that integrates high-quality output with environmentally friendly methods, thereby contributing to biodiversity, resource conservation, and animal welfare (Dinis et al. 2015). Organic yields are generally lower than conventional yields, but the main goal is sustainability (Seufert et al. 2012; Muller et al. 2017). Although yields may be lower, organic farming compensates for this by promoting higher biodiversity (Gong et al. 2022). Organic farming increases species diversity by approximately 30%, especially in areas with intensive land use (Tuck et al. 2014). This is due to organic farming avoids synthetic chemicals, making it safer for many species (Bengtsson et al. 2005). Organic fertilizer plays a key role in organic farming by enhancing microbial activity and improving soil physical properties and fertility (Chatzistathis et al. 2021). Among the various options, black soldier fly frass stands out as a promising component for organic fertilizers.

Black soldier fly frass (BSF frass) enhances soil quality, fertility, and nutrient content (Klammsteiner et al. 2020; Beesigamukama et al. 2021; Gärttling and Schulz 2022; Gebremikael et al. 2022; Beesigamukama et al. 2022). It is recommended to increase soil organic matter as well as nitrogen, phosphorus, and potassium levels (Anyega et al. 2021; Menino et al. 2021). According to Gärttling and Schulz (2022), BSF frass is notably rich in dry matter nutrients, containing nitrogen, ammonium nitrogen, phosphate, and potassium totaling over 1% of its dry weight. Several studies have shown that BSF frass positively affects plant growth and yield (Agustiyani et al. 2021; Anyega et al. 2021; Borkent and Hodge 2021; Fischer et al. 2021; Menino et al. 2021). Many studies have already investigated the effects of BSF frass on plant performance and soil nutrients. However, there is still limited information on the optimal application doses and its impact on organic farming agroecosystems, particularly concerning the structure of arthropod communities.

Soybean (*Glycine max* L.) is an important crop, especially in Japan, where the immature seeds, known as edamame, are a popular product (Koshika et al. 2022). Tsuruoka, a city in Japan, is renowned for producing a local variety of edamame called ‘*dadachamame*.’ The high-quality cultivar *Shirayama-dadachamame* is particularly well-known for its umami and sweet flavor (Kamimura et al. 2016). This study aims to investigate the optimal application dose of BSF frass and its impact on agroecosystems, specifically focusing on the structure of arthropod communities. While previous research has highlighted the positive effects of BSF frass on soil quality, fertility, and plant growth, there remains a gap in our understanding of the appropriate amount of BSF frass to use in edamame crops and its potential effects on the broader ecosystem, particularly arthropod populations.

In this study, however, we did not include a chemical fertilizer as a positive control. Although chemical fertilizers are widely used in conventional farming and serve as a benchmark for evaluating the effectiveness of alternative fertilizers, this study focuses exclusively on organic frass applications. This choice aligns with our objective to prioritize organic methods; however, it limits the study’s ability to make broad claims about the efficacy of frass as a complete substitute for chemical fertilizers. By addressing this gap, we aim to provide valuable insights into the sustainable utilization of BSF frass in agriculture while minimizing any unintended ecological consequences.

MATERIALS AND METHODS

Land preparation and transplanting

The research was conducted on an experimental farm at the Faculty of Agriculture, Yamagata University, Tsuruoka, Japan, from May to June 2022. We utilized a local soybean variety, *dadachamame* cv. ‘Shirayama’. Seedlings were raised in a greenhouse using seed trays filled with commercial seedling soil Kumaya planter soil (Kumaya, Japan). The experimental field, measuring 13.0 × 15.0 m, was divided into 6.0 × 1.0 m plots (Figure 1). Soil preparation involved cultivation with a hand tractor two weeks before planting. Seedlings, approximately 14 days old, were transplanted into individual holes spaced 50 cm apart in both directions. The total number of plants in each plot was 15. The field maintenance was free from chemical input, including pesticides and chemical fertilizers, to represent organic field farming.

Frass preparation

Black soldier flies were obtained from the Yamadai Mizuabu Laboratory, a specialized research facility for black soldier fly studies within the Faculty of Agriculture at Yamagata University, Japan. The larvae were fed food waste collected from Yamagata University Co-op Vert and Tsuruoka Municipal Shonai Hospital, primarily composed of noodles, vegetables, bread, and rice, with a smaller portion of meat. This food waste was processed and homogenized before being provided to the larvae (Table 1). Over a 14-day period, approximately 7 kg of food waste and vegetables in a 5:2 ratio was given to the larvae in a 14-liter container. At the end of this feeding period, frass and prepupae were manually harvested using a 1 mm sieve. The frass contained insect skins, which are rich in chitin. The C/N ratio, total nitrogen (TN), and total carbon (TC) of the frass were analyzed using a total nitrogen analyzer (Sumigraph NC-220 F, Sumika Chemical Analysis Service, Ltd.). Total phosphorus (P) was measured by means of the vanadomolybdophosphoric acid colorimetric method (Kuo 1996) using UV-visible spectrometry, and total potassium (K) content was assessed through atomic absorption spectrometry (Mizuno and Minami 1980) (Table 2).

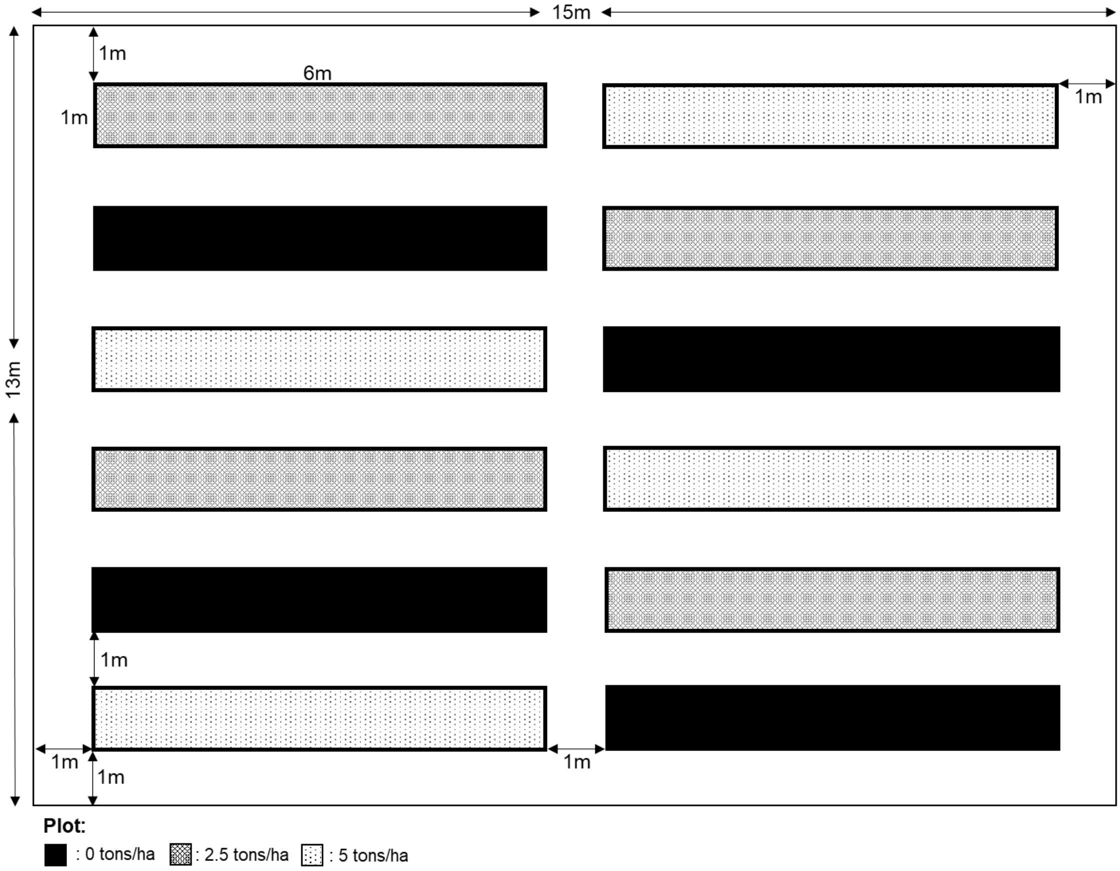

Figure 1. The experimental field plot design

Table 1. List of the BSF larvae feed and nutrient content

Product name	Source	Nutrient content (g)			
		Carbohydrate	Protein	Fat	Salt
Mini Tempura bowl (ミニ丼天丼)	University	75.2	9.4	7.8	2.4
Teriyaki chicken soba bowl (照秋チキンのそばる丼)	University	79	17.6	9.6	4.7
Fried rice and fried chicken (ミニチャハン＆唐揚げ)	University	63.4	11.9	13.6	1
Ramen (ラーメン)	University	45.8	15.4	8.7	5.4
Beef rib bowl (牛カルビ丼)	University	76.4	8.5	5.3	2.3
Chocolate marble snack (チョコマーブル)	University	41.3	5.2	10.5	0.8
Fried fish bento (のり弁当Ｂ（白身魚フライ）)	University	89.8	21.6	30	4
Sandwich（ゆでたまごとハムサンド)	University	28.1	10.5	12.7	2.2
Rice ball (おにぎり)	University	32.8	2.9	0.9	0.8
Carrot skin	Hospital	9.58	0.93	0.24	-
Raddish skin	Hospital	8.36	1.17	0.13	-
Cabbage	Hospital	5.58	1.44	0.12	-
Eggplant skin	Hospital	5.7	0.19	0.19	-

Note: The nutrient content of the product was provided by the company and is included on the product labels. The nutrient content of vegetable waste provided is the amount per 100 g of waste

Table 2. The chemical properties of BSF frass

Chemical properties	Concentration (%)
Total N	4.16±0.15
Total P	8.70±0.67
Total K	4.80±0.27
Total C	30.88±3.67

Frass application

The experiment involved applying BSF frass across different plots one week before transplanting the edamame plants. Three application dose treatments were applied: (i) 0 tons/ha, (ii) 2.5 tons/ha, and (iii) 5 tons/ha. Each treatment was replicated six times.

Plant performance

The characteristics of 20 edamame plants in each treatment were measured, including plant height (cm), number of leaves, and leaf color. The leaf color was measured on the shade of green it was, determined by the SPAD (soil plant analysis development) meter (SPAD-502, Konica). Other parameters were observed after the harvest time. The harvested edamame plants were assessed for their yield, total number of pods, number of empty pods, and the dry weight of the seeds. Drying was conducted in a natural convection oven (DS-64, Yamato) at a temperature of 70°C for three days.

Arthropod collection and identification

Soil arthropods were collected using pitfall traps made from 545 mL plastic cups (CP92-545, Daiso Industries Co., LTD). Each cup was filled with 200 mL of water and 1 mL of detergent to instantly kill the trapped arthropods. The traps were buried approximately 20 cm below the soil surface and placed in the middle of the experimental plots. Each plot contained three traps arranged in a straight line, spaced 15 cm apart. The traps were set for 24 hours and then collected and inspected. Pitfall traps were deployed during the 2nd, 3rd, and 4th weeks after transplanting (WAT). In this experiment, we used only macro-arthropods due to their measurable impacts on pest dynamics.

Foliar arthropods were collected using yellow sticky traps (Holiver, Arista Life Sciences). Each trap measured 10 × 10 cm and was mounted on poles 50 cm above the soil surface. These traps were also left for 24 hours before being collected and inspected. Yellow traps were set on July 12, 2022 and August 3, 2022. In each plot, two traps were placed between plants, with a distance of 50 cm between traps. Each treatment includes a total of 12 traps.

Collected arthropods were identified to the genus level in the laboratory at Yamagata University using a stereo microscope (SMZ800, Nikon). Arthropods were classified to morpho-genus level according to literature such as Higley and Boethel (1994) and Borror et al. (1996). The data on arthropods were then categorized according to their ecological functions.

Data analysis

All statistical analyses were performed using R version 4.2.1. Before conducting the analyses, the normality of the data was tested using the Shapiro-Wilk test ($p>0.05$), confirming that the data followed a normal distribution. Plant performance parameters and yield were analyzed using a one-way analysis of variance (ANOVA), followed by a post-hoc analysis using the Tukey test at a significance level of $p<0.05$. Abundances of soil and foliar arthropods were analyzed using a likelihood ratio (LR) test in the Generalized Linear Model (GLM) and implemented using Poisson error. After this, the deviance from the GLM was analyzed using the Chi-square test.

The community structure analyzed by the non-metric multidimensional scaling (NMDS) using the “vegan” package was performed to generate the visualization of assemblage composition on a matrix of Bray-Curtis dissimilarities of abundance data (square root transformed). To test the null hypothesis of species composition after the treatments, we applied analysis of similarities (ANOSIM) on the matrix of Bray-Curtis with 999 permutations. The Shannon diversity (H’) index were determined using the following formula (Ludwig and Reynold 1984):

$$H' = -\Sigma[(ni/N) \text{ In } (ni/N)]$$

The Richness (R) index was determined using the formula:

$$R = (S-1) / \text{In } N$$

The Evenness (E) index was determined using the formula:

$$E = H'/\text{In } S$$

The Simpson dominance (C) index was determined using the formula:

$$C = \Sigma \text{ } (ni/N)$$

Where:
Ni : Total individuals of species i
N : Total individuals found
S : Number of species found.

RESULTS AND DISCUSSION

Effect of different application doses of BSF frass on edamame plants

Plant performances

The study evaluated the impact of three different application doses (0, 2.5, and 5 tons/ha) on plant performance, by measuring plant height, number of leaves, and SPAD values (Table 3). No significant differences were found in plant height (df=2, χ^2=5.39, p=0.07), with a height average of 63.79±1.04 cm, 70.28±2.72 cm, and 71.18±3.10 cm for the application doses of 0, 2.5, and 5 tons/ha, respectively. However, plant height showed increases of 10 and 12% for the application doses of 2.5 and 5 tons/ha. The number of leaves was significantly affected (df=2, χ^2=6.87, p<0.01), with increases of 26% and 31% for the application doses of 2.5 and 5 tons/ha. SPAD values, which indicate chlorophyll content, significantly increased by 16 and 20% for the same treatments (df=2, χ^2=113.9, p<0.001).

Regarding the yield components, no significant differences were found in total pod count (df=2, χ^2=1.68, p=0.43) and pod dry weight (df=2, χ^2=1.18, p=0.55). However, the average number of empty pods showed significant differences across treatments (df=2, χ^2=16.63, p<0.001). The application dose of 2.5 tons/ha significantly increased the number of filled pods by 29% compared to the control (df=2, χ^2=5.88, p<0.05) and reduced the number of empty pods by 52%, indicating improvement of pod quality (Table 2).

The results indicate that although higher application doses did not impact overall yield or seed dry weight, they improved specific yield components, particularly with the application dose of 2.5 tons/ha. This application dose notably enhanced leaf number, chlorophyll content, and number of seeds, and reduced the number of empty pods. An application dose of 2.5 tons/ha is recommended to optimize edamame plant performance and pod quality.

Effects of different application doses of BSF frass on arthropods

Soil arthropod abundance

The study assessed the impacts of three different application doses of BSF frass on the abundance of soil arthropods in an edamame field, identifying 17 species across the treatments (Table 4). The results indicated a significant increase in the total number of arthropods with the highest dose (Table 4, df=2, χ^2=17.93, p<0.001). To be specific, the application of 5 tons/ha resulted in a substantial 313% higher number of arthropods per plot of about 19.40±8.50 individuals/plot compared to the control (0 tons/ha), which had the lowest number of arthropods of about 4.70±1.50 individuals/plot. Additionally, there was a 246% increase in the number of arthropods in the application of 2.5 tons/ha, which resulted in about 5.60±1.60 individuals/plot.

Table 3. Plant performances at three different application doses of BSF frass

Plant performances	Application doses		
	0 tons/ha	2.5 tons/ha	5 tons/ha
Plant growth characteristics			
Height (cm)	63.79±1.04^a	70.28±2.72^a	71.18±3.10^a
Number of leaves	20.26±1.8^a	25.58±2.08^b	26.59±1.57^b
SPAD	38.10±0.69^a	44.14±0.31^b	45.75±0.53^b
Yield attributes			
Yield (g/plant)	89.41±7.78^a	92.96±6.90^a	75.58±11.23^a
Number of filled pods	58.43±4.28^a	64.46±2.87^b	69.66±9.29^b
Number of empty pods	28.83±2.26^b	13.90±1.96^a	20.75±3.35^a
Seed weight (g)	86.60±06.53^a	111.83±04.51^a	77.400±16.17^a

Note: Average ± standard error. The same letters after means within the same column indicate there is no significant difference according to the Tukey test results, with a significance level of 5%

Table 4. The average number of individuals (± standard error) of soil arthropod species at three different application doses of BSF frass

Order	Species	Role	Application doses of BSF frass			Notation
			0 tons/ha	2.5 tons/ha	5 tons/ha	
Coleoptera (adult)	*Scarites terricola* Bonelli, 1813	PR	0.39±0.13	0.67±0.15	1.17±0.37	*
	Harpalus variipes Bates, 1883	PR	0.39±0.09	0.22±0.09	0.22±0.12	N.S.
	Tachys laetificus Bates, 1873	PR	0.00±0.00	0.72±0.30	1.56±0.42	***
	Bembidion spp.	PR	0.00±0.00	0.00±0.00	0.61±0.34	***
	Ectinoides insignitus Lewis, 1894	HV	0.00±0.00	0.11±0.11	0.00±0.00	N.S.
	Cardiophorus sp.	N	0.03±0.02	0.03±0.02	0.00±0.00	N.S.
	Harpalus sp.	PR	0.06±0.03	0.00±0.00	0.08±0.04	N.S.
	Holotrichia kiotonensis Brenske, 1894	HV	0.03±0.02	0.00±0.00	0.03±0.02	N.S.
	Anomala flavipennis Burmeister, 1844	HV	0.06±0.03	0.00±0.00	0.00±0.00	N.S.
	Popillia japonica Newman, 1838	HV	0.36±0.16	0.19±0.08	0.22±0.09	N.S.
Orthoptera	*Gryllus bimaculatus* De Geer, 1773	HV	0.22±0.09	0.17±0.07	0.50±0.23	N.S.
	Gryllotalpa orientalis Burmeister, 1838	HV	0.06±0.03	0.03±0.02	0.61±0.24	***
Isopoda	*Armadillidium vulgare* (Latreille, 1804)	N	0.00±0.00	0.08±0.04	0.00±0.00	*
	Armadillidium sp.	N	0.00±0.00	0.00±0.00	0.06±0.03	N.S.
Lithobiomorpha	*Lithobius* sp. (1)	PR	0.03±0.02	0.03±0.02	0.22±0.12	N.S.
	*Lithobius*sp. (2)	PR	0.22±0.07	0.14±0.08	0.19±0.06	N.S.
Trichoptera	Unknown	N	0.00±0.00	0.11±0.05	0.00±0.00	N.S.
Lepidoptera (larva)	*Spodoptera* sp.	HV	0.03±0.02	0.00±0.00	0.00±0.00	N.S.
	Spodoptera litura	HV	0.25±0.10	0.08±0.08	0.47±0.32	N.S.
Hymenoptera	*Tetramorium tsushimae* Emery, 1925	PR	0.03±0.02	0.11±0.05	5.39±1.97	***
Arachnida	*Pardosa astrigera* L.Koch, 1878	PR	0.78±0.14	0.69±0.15	1.00±0.21	N.S.
	Pardosa agraria Tanaka, 1985	PR	0.08±0.04	0.11±0.05	0.06±0.03	N.S.
	Pardosa spp.	PR	0.00±0.00	0.06±0.05	0.00±0.00	N.S.
Average			4.70±1.50^b	5.60±1.60^b	19.40±8.50^a	

Note: PR: Predator, N: Neutral, HV: Herbivore. Average ± standard error. Stars indicate statistical significance based on GLM analysis with Poisson distribution (*** p<0.001, ** p<0.01, * p<0.05, N.S. p<0.05). The same letters after means within the same column indicate there is no significant difference according to the Tukey test results, with a significance level of 5%

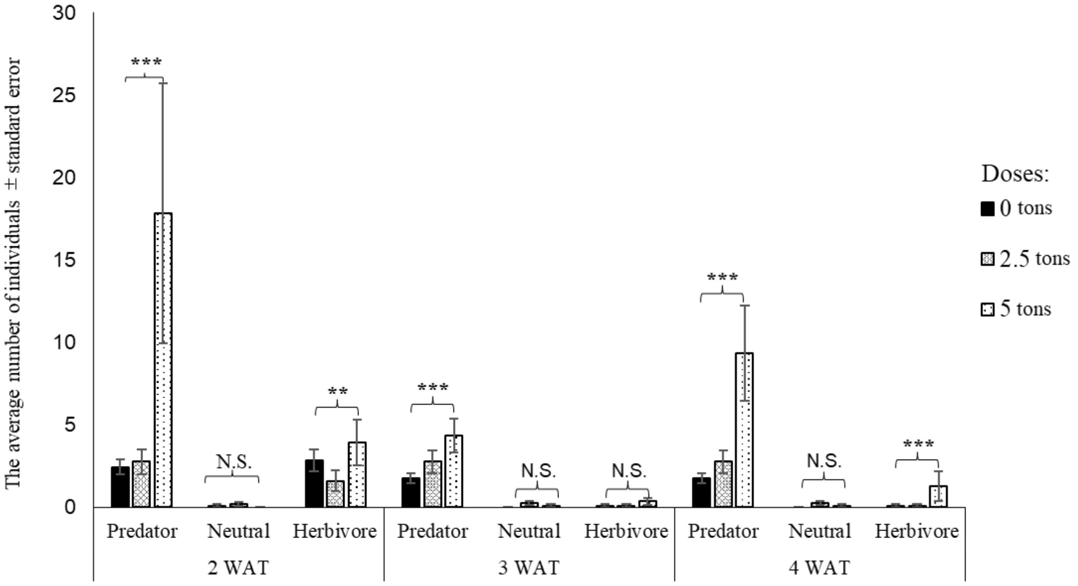

Figure 2. The average number of individuals of soil arthropod species according to their trophic groups at three different application doses of BSF frass at 2, 3, and 4 weeks after treatment (WAT). Significant differences were determined using multiple comparison tests (Tukey's test) (* p<0.05, ** p<0.01, *** p<0.001, N.S. p<0.05)

Predators were the dominant trophic group across all doses, with the 5 tons/ha treatment showing a significant 124% increase in predator abundance compared to the control (Figure 2, df=2, χ^2=18.12, p<0.001). The adult ground beetle was the dominant predator group, comprising seven species. Among these, the *Tetramorium tsushimae* ant was notably abundant in the highest dose treatment, with a statistically significant increase compared to other doses (Table 3, df=2, χ^2=63.65, p<0.05). Herbivores also showed increased abundance with the 5 tons/ha dose (Figure 2, df=2, χ^2=9.29, p<0.001), with *Gryllotalpa orientalis* as the most abundant herbivore, the number of which was significantly higher in the 5 tons/ha plots than that of the control (Table 3, df=2, χ^2=18.73, p<0.05). Detritivores, however, showed no significant difference across treatments (Figure 2, df=2, χ^2=4.21, p=0.12).

As seen in Figure 2, predator abundance at 5 tons/ha was approximately 433% higher than that of the control and 282% higher than that of the 2.5 tons/ha dose. Herbivores also reached peak abundance with the 5 tons/ha treatment, showing an 83% increase compared to the control and a 215% increase compared to the 2.5 tons/ha dose. In contrast, neutral arthropods did not show significant differences across doses (p<0.05) and displayed low average counts, suggesting negligible practical impact of BSF frass on neutral groups. Over time, no significant differences in trophic group abundances were found between weeks after application (predator: df=1, χ^2=1.35, p=0.244; detritivore: df=1, χ^2=3.92, p=0.4; herbivore: df=1, χ^2=0.0001, p=0.99), though specific weeks did show notable patterns. At 2 weeks after treatment (WAT), both predator and herbivore abundances were highest with the 5 tons/ha dose (predator: df=2, χ^2=217.92, p<0.001; herbivore: df=2, χ^2=12.43, p<0.001). At 3 WAT, predator abundance peaked again in the 5 tons/ha dose (df=2, χ^2=13.82, p<0.001). At 4 WAT, predator and herbivore numbers were significantly higher with the 5 tons/ha dose (predator: df=2, χ^2=83.14, p<0.001; herbivore: df=2, χ^2=22.62, p<0.001).

Considering the results above, the 5 tons/ha dose is recommended to increase predator and herbivore abundance, supporting overall arthropod diversity, while the 2.5 tons/ha dose has a minimal effect on neutral arthropods due to their low abundance across treatments.

Foliar arthropod abundance

A total of 2,634 foliar arthropods were collected across three different application doses of BSF frass (0, 2.5, and 5 tons/ha). The results indicated a significant effect of frass on the abundance of foliar arthropods (Table 5, df=2, χ^2=100.87, p<0.001). To be specific, the control treatment (0 tons/ha) had the highest number of arthropods at 105.00±17.80 individuals/plot. The application doses of 2.5 tons/ha resulted in a significantly lower number of arthropods at 52.00±4.50 individuals/plot, a 50.5% decrease compared to the control. The application doses of 5 tons/ha had a total number of arthropods at 62.30±17.20 individuals/plot, representing a 40.7% decrease compared to the control. These results suggest that although the control plots had the highest abundance of arthropods, the application doses of BSF frass at both 2.5 and 5 tons/ha reduced arthropod numbers significantly.

Table 5. The average number of individuals (± standard error) of foliar arthropod species at three different application doses of BSF frass

Order	Species	Role	Application doses of BSF frass			Notation
			0 tons/ha	2.5 tons/ha	5 tons/ha	
Diptera (adult)	*Musca domestica* Linnaeus, 1758	N	4.50±0.81	0.67±0.14	1.42±0.42	***
	Sarcophaga peregrina (Robineau-Desvoidy, 1830)	N	0.00±0.00	0.25±0.25	4.42±0.16	***
	Bradysia sp. (Fungus gnat)	N	3.33±1.32	3.58±1.09	9.83±2.90	***
	Culiseta nipponica LaCasse & Yamaguti, 1950	N	8.00±2.32	0.83±0.30	2.25±0.79	***
	Dolichopus plumipes (Scopoli, 1763)	N	2.00±0.39	0.75±0.30	0.67±0.31	**
	Drosophila sp.	N	0.17±0.11	0.00±0.00	0.33±0.33	N.S.
	Cricotopus sp.	N	0.00±0.00	0.17±0.11	0.00±0.00	N.S.
	Agromyza wistariae Sasakawa, 1961	HV	0.00±0.00	7.33±0.92	0.42±0.42	***
	Cerodontha denticornis (Panzer, 1806)	HV	0.00±0.00	1.67±0.99	0.25±0.25	***
	Gnoriste mikado Okada, 1939	N	0.00±0.00	0.83±0.21	0.00±0.00	***
	Dysmachus sp.	PR	0.00±0.00	0.17±0.11	0.00±0.00	N.S.
	Eupeodes bucculatus Rondani, 1857	PO	0.17±0.11	0.83±0.51	0.00±0.00	**
Coleoptera (adult)	*Aulacophora* sp.	HV	0.08±0.08	0.33±0.22	0.33±0.14	N.S.
	Altica cyanea (Weber, 1801)	HV	0.00±0.00	0.00±0.00	0.17±0.11	N.S.
	Psylliodes sp.	HV	0.00±0.00	0.33±0.14	0.17±0.17	*
	Phyllotreta cruciferae (Goeze, 1777)	HV	0.00±0.00	0.33±0.22	0.00±0.99	N.S.
	Chaetocnema sp.	HV	0.33±0.22	0.00±0.00	0.17±0.11	N.S.
	Tachys sp.	PR	0.00±0.00	0.00±0.00	0.17±0.11	N.S.
	Propylea japonica (Thunberg, 1781)	PR	0.33±0.14	0.00±0.00	0.17±0.11	N.S.
	Coccinella septempunctata Linnaeus, 1758	PR	0.17±0.11	0.08±0.08	0.50±0.23	N.S.
	Ectinoides insignitus (Lewis, 1894)	PR	0.17±0.11	0.08±0.08	0.17±0.11	N.S.
Hymenoptera (adult)	*Pemphredon* spp.	PR	0.25±0.18	0.42±0.19	0.42±0.19	N.S.
	Xiphozele compressiventris Cameron, 1906	PR	0.00±0.00	0.17±0.11	0.00±0.00	***
	Cotesia plutellae (Kurdjumov, 1912)	PR	6.00±1.11	0.17±0.11	0.83±0.32	***
	Synopeas sp.	PR	0.50±0.23	12.08±1.53	3.67±1.79	***
	Pachyneuron formosum Walker, 1833	PR	0.00±0.00	0.00±0.00	0.17±0.17	N.S.
	Brachyponera chinensis (Emery, 1895)	PR	1.67±0.45	0.25±0.13	0.50±0.23	**
Hemiptera	*Acyrthosiphon pisum* (Harris, 1776)	HV	8.17±1.55	8.67±0.47	5.50±1.60	**
	Macrosteles quadrilineatus (Forbes, 1885)	HV	13.00±1.87	3.17±0.75	11.83±3.65	***
	Geocoris sp.	HV	0.33±0.14	0.25±0.13	0.83±0.44	N.S.
Lepidoptera (adult)	*Lycaena* sp.	HV	0.00±0.00	0.17±0.11	0.42±0.15	*
	Colias philodice Godart, 1819	HV	0.00±0.00	1.00±0.30	0.17±0.11	***
	Unknown	HV	0.08±0.08	0.17±0.11	0.00±0.00	N.S.
	Spodoptera sp.	HV	0.17±0.11	0.17±0.11	0.17±0.17	N.S.
Orthoptera	*Conocephalus longipennis* (Haan, 1843)	HV	0.75±0.43	0.08±0.08	0.83±0.37	**
	Gryllus bimaculatus De Geer, 1773	HV	0.67±0.43	0.08±0.08	0.67±0.36	*
	Chorthippus latipennis (Bolívar, 1898)	HV	0.00±0.00	0.08±0.08	0.00±0.00	N.S.
Thysanoptera	*Thrips* sp.	HV	54.33±14.60	6.83±1.22	14.92±9.55	***
Average			105±17.8^{a}	52±4.5^{b}	62.3±17.2^{b}	

Note: PR: Predator, HV: Herbivore, N: Neutral, PO: Pollinator. Average ± standard error. Stars indicate statistical significance based on GLM analysis with Poisson distribution (*** *p*<0.001, ** *p*<0.01, * *p*<0.05, N.S. *p*<0.05). The same letters after means within the same column indicate there is no significant difference according to the Tukey test results, with a significance level of 5%

The absence of BSF frass led to the highest overall arthropod abundance with herbivores particularly dominant. Thrips were significantly more abundant without BSF frass, constituting about 86% of their total population (Table 5, df=2, χ^2=588.47, *p*<0.001). The application doses of 2.5 tons/ha supported the highest abundance of predatory arthropods, which represented about 58% of the predator total, notably *Synopeas* sp. (Table 5, df=2, F=19.11, *p*<0.05). Neutral insects were most abundant in the application doses of 5 tons/ha, making up 38% of the total neutral population, but this difference was not statistically significant (df=2, χ^2=67.41, *p*=0.174). The Fungus gnat showed a significant increase at 5 tons/ha, comprising about 58% of their total (Table 5, df=2, χ^2=554.16, *p*<0.001). According to Figure 3, predators were significantly more abundant at 2.5 tons/ha, representing approximately 53% of the predator total (df=2, χ^2=29.48, *p*<0.001), and this dose also supported a diverse range of other beneficial insects.

Figure 3 showed there were no statistically significant differences in the abundance of all trophic groups between months (predator df=1, χ^2=1.24, *p*=0.26; neutral df=1, χ^2=3.03, *p*=0.08; herbivore df=1, χ^2=1.60, *p* =0.20) in all treatments (i.e., plots with 0, 2.5 and 5 tons/ha of BSF frass). However, predators were abundant in July (df=1, χ2=8.03, *p*<0.001) only, and herbivores were abundant in July and August (Figure 3: July df=1, χ^2=109.95, *p*<0.001; August df=1, χ^2=102.07, *p*<0.001). Given these findings, the application dose of 2.5 tons/ha of BSF frass is recommended as it optimally balances the promotion of beneficial predators while controlling the overall abundance of herbivores and neutral insects.

Soil arthropod community structure

The study analyzed the diversity of soil arthropods in edamame fields treated with different doses of BSF frass using various diversity indices. In Table 6, the diversity index (H’) indicated a slightly higher diversity index at 2.5 tons/ha compared to 0 tons/ha, and lower diversity at 5 tons/ha, suggesting that moderate dose frass application promotes higher species diversity. The Richness (R’) index was highest at 2.5 tons/ha, indicating the presence of more species in this treatment. The Evenness (E’) index showed that species were more evenly distributed at lower doses, implying a balanced community structure. Simpson dominance (C’) index was higher at 5 tons/ha, suggesting that a few species dominated the community at this higher dose. Given the Bray-Curtis similarity coefficient, NMDS (Figure 4) was conducted on the community structure of soil arthropods. NMDS analysis showed a good fit (stress=0.08) and an overlap between treatments, indicating similar community structures across different frass applications. This was supported by ANOSIM results (R=0.03, *p*=0.0014), indicating statistically significant differences in arthropod communities among treatments, with an even distribution of high and low ranks within and between groups. Given these findings, an application dose of 2.5 tons/ha of BSF frass is recommended for edamame fields, as it optimally enhances species diversity and maintains a balanced arthropod community without the dominance issues observed at higher doses.

Table 6. Diversity, Richness, Evenness, and Simpson Dominance indices of soil and foliar arthropods at different application doses of BSF frass

Indices	Application doses		
	0 tons/ha	**2.5 tons/ha**	**5 tons/ha**
Soil arthropods			
H’	2.26	2.34	1.97
R’	5.41	5.64	5.41
E’	0.81	0.82	0.71
C’	0.13	0.13	0.22
Foliar arthropods			
H’	1.76	2.43	2.39
R’	7.01	8.94	8.52
E’	0.56	0.70	0.70
C’	0.30	0.13	0.13

Note: H’: Diversity indices, R’: Richness indices, E’: Evenness indices, and C’: Simpson dominance

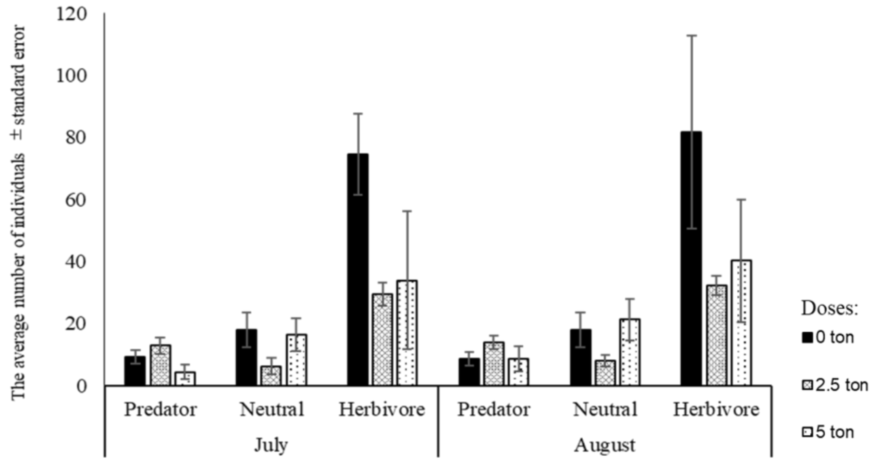

Figure 3. The average number of individuals of foliar arthropod species according to trophic groups at three different application doses of BSF frass at 2, 3, and 4 weeks after treatment (WAT). Significant differences were determined using multiple comparison tests (Tukey’s test) (* *p*<0.05, ** *p*<0.01, *** *p*<0.001, N.S. *p*<0.05)

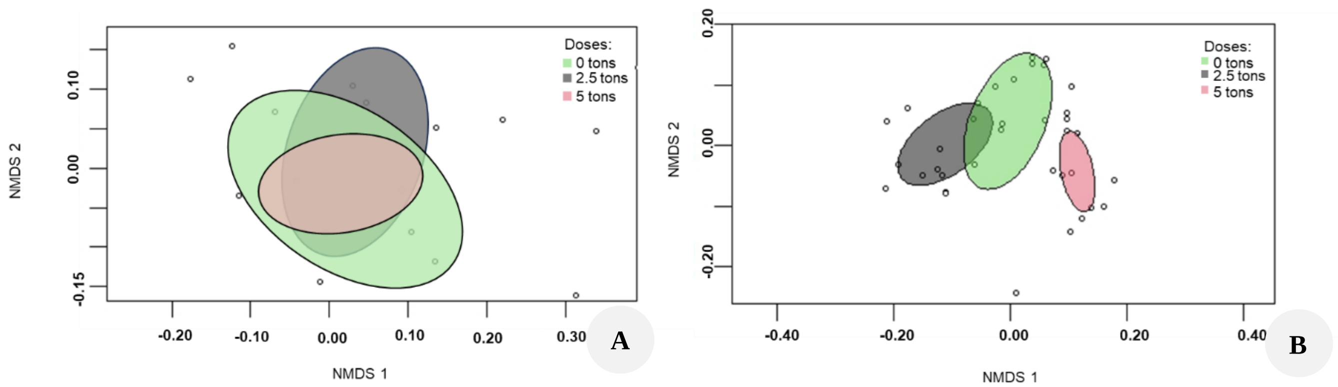

Figure 4. The non-metric multidimensional scaling (NMDS) ordination of A. Soil arthropods and B. Foliar arthropods community structure at three different application doses of BSF frass

Foliar arthropod community structure

The study analyzed the diversity of foliar arthropods in edamame fields treated with different doses of BSF frass using various diversity indices. In Table 6, the Shannon diversity (H') indices were higher at both 2.5 tons/ha and 5 tons/ha compared to the control (0 tons/ha), suggesting that frass application enhances species diversity. The Richness (R') index was highest at 2.5 tons/ha, indicating a greater number of species in this treatment. The Evenness (E') index showed that species were more evenly distributed at 2.5 tons/ha and 5 tons/ha, suggesting a balanced community structure at these doses. The Simpson dominance (C') index was highest at 0 tons/ha, indicating that a few species dominated the community without frass. The NMDS (Figure 4) analysis showed a good fit (stress=0.11) and an overlap existed between plots treated with 5 tons/ha and those with 0 tons/ha. However, this overlap was supported by ANOSIM results ($R=0.59$, $p<0.001$), indicating a significant difference in arthropod communities among treatments, with substantial differentiation between groups. Given these findings, an application dose of 2.5 tons/ha of BSF frass is recommended for edamame fields, as it optimally enhances species diversity and maintains a balanced arthropod community without the dominance issues observed at higher or zero doses.

Discussion

This study demonstrated the impact of different application doses of BSF frass on the performance and yield of edamame plants and on the abundance of soil and foliar arthropods. The results revealed varied effects, providing insights into optimal dose recommendations for maximizing plant growth, yield, and insect diversity. Plant performance data indicated that an application dose of 2.5 tons/ha was optimal. These findings are consistent with a previous study on lettuce, which revealed that higher concentrations of BSF frass led to stunted plant growth (Chiam et al. 2021). A previous study by Beesigamukama et al. (2021) also indicated that 2.5 tons/ha is sufficient for plant performance and yield. Similarly, Kawasaki et al. (2020) noted that a high dose of BSF frass could cause damage. However, these findings differ from other studies, which observed increased plant height with high applications of BSF frass on mustard, kale, swiss chard, and maize (Abiya et al. 2022; Tanga et al. 2022). This suggests that each plant has different requirements for BSF frass dose, with edamame needing only 2.5 tons/ha.

The edamame yield study further illustrated the specific effects of different application doses of BSF frass. It increased plant nitrogen (N) uptake by up to 23% and had higher nitrogen recovery efficiencies than those of other organic fertilizers (Beesigamukama et al. 2020). The quality of the frass also plays a crucial role in influencing soil nitrogen availability and subsequent plant growth (Kagata and Ohgushi 2012). The enhanced nitrogen levels in the soil are particularly beneficial for soybean pod filling, given soybeans' high nitrogen requirement (Serafin-Andrzejewska et al. 2024). These findings highlight the efficacy of using 2.5 tons/ha of BSF frass to optimize edamame yield through improved nitrogen management and pod filling, thereby enhancing overall plant productivity.

Furthermore, the study explored the community structure and abundance of soil and foliar arthropods in response to different application doses of BSF frass. Given the study's findings, an application dose of 2.5 tons/ha of BSF frass is recommended for edamame fields. This dose optimally enhances soil and foliar arthropod diversity, maintains a balanced arthropod community, and supports the highest abundance of beneficial predatory arthropods while controlling herbivore populations. The mechanism behind these changes likely involves multiple factors, one of which is the nutrient enrichment in the soil resulting from treatment with BSF frass (Hasibuan et al. 2022; Shahbuddin et al. 2023). Increased plant biomass offers more foliage for herbivores to feed on, supporting larger herbivore populations (Bonser and Reader 1995; Katz 2016). Predators, benefiting from the larger prey base, also increase in number, contributing to a more balanced and dynamic ecosystem (Snyder and Evans 2006).

The results showed that higher doses of organic fertilizer can increase the population of soil arthropods (Lin et al. 2013; Wang et al. 2015). In this research, BSF frass increased organic matter and nutrients in the soil, which can create a more favorable environment for soil arthropods. This is also consistent with studies that found that the high content of organic matter enhances arthropod abundance (Gunadi et al. 2002; Kautz et al. 2006; Salah et al. 2018; Viketoft et al. 2021). BSF frass contains nitrogen, phosphorus, potassium, micronutrients, and beneficial microbes that enhance soil health (Watson et al. 2021; Boudabbous et al. 2023). Research by Peng et al. (2023) found that insect frass improves soil structure by forming aggregates, enhancing aeration and root penetration. This improved structure also retains moisture longer, benefiting plants and soil organisms (Beesigamukama et al. 2021). The microbes in BSF frass increase microbial activity, which is crucial for nutrient cycling (Houben et al. 2020; Poveda 2021; Gebremikael et al. 2022), and can work synergistically with plant growth-promoting rhizobacteria (PGPR) (Fuertes-Mendizábal et al. 2023). Additionally, nutrients such as calcium and magnesium in BSF frass play key roles in plant physiology and soil pH regulation, promoting a balanced soil environment (Gärttling and Schulz 2022; Lopes et al. 2022). The results showed that ants dominate in the highest doses. Ants thrive in soil with high nutrient content (Farji-Brener and Werenkraut 2017; Vaidya and Vandermeer 2021), which is supported by Vaidya and Vandermeer (2021), who found that ants prefer plants with higher fertilizer levels. These findings highlight the positive impact of high organic matter and nutrient content on predator populations, particularly ground beetles and ants, in agricultural fields. The organic material might change the environmental condition of the soil. In the soil that was treated with organic fertilizer, the number of microorganisms was higher by 7% compared to the soil without any application of fertilization (Bebber and

Richards 2022). The soil microorganisms are highly likely to become the source of food for microfauna such as detritivores and indirectly increase the predator presence. In other words, organic fertilization enhances the complexity of ecological relationships between microbes and microfauna, impacting food web dynamics and predator-prey interactions (Suleiman et al. 2019). This combination supports soil-dwelling arthropods, contributing to pest control and nutrient cycling, which enhances overall soil health and plant productivity (Kuťáková et al. 2018).

The application of high doses of BSF frass decreased the number of herbivores. This condition is in contrast with the application of other types of fertilizer that result in herbivores having the highest abundance in the higher doses (Chen et al. 2008; Trisnawati et al. 2015; Rashid et al. 2017). The nutrient enrichment of the soil from fertilizer enhances plant growth, providing more food and habitat resources for arthropods (Hasibuan et al. 2022; Shahbuddin et al. 2023). However, BSF frass potentially decreases the herbivore population by increasing the plant defense mechanism (Lopes et al. 2022). Plants grown with organic fertilizer often have higher concentrations of plant defense chemical compounds than they do if grown with synthetic fertilizers (Staley et al. 2011). In addition to enhancing plant resilience to herbivores, organic fertilizers can support biocontrol activities. Through bottom-up impacts, herbivore abundance is decreased and parasitism rates are increased due to better soil nutrient content and plant metabolism (Gu et al. 2022).

In conclusion, the study provides valuable insights into the multifaceted effects of different application doses of BSF frass on edamame plant performance, yield, and arthropod communities. Given these findings, an application dose of 2.5 tons/ha of BSF frass is optimal for edamame fields, resulting in enhanced plant performance, yield, and arthropod diversity. The findings align with previous studies indicating that moderate dose frass applications improve plant growth and soil health, driven by nutrient enrichment and improved soil structure. However, further research is warranted to elucidate the long-term effects of BSF frass on plant-soil-arthropod interactions and its broader implications for sustainable agriculture.

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