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DECOMPOSING ACTIVITY OF BLACK SOLDIER FLY LARVAE *(Hermetia illucens)*

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Abstract

The increasing demand for sustainable waste management strategies has highlighted the potential of Black Soldier Fly Larvae (BSFL), Hermetia illucens, as biological agents for organic waste conversion. This study evaluated the effects of BSFL-mediated bioconversion on the culturable bacterial composition of sheep, pig, and chicken manure under ambient indoor rearing conditions. Seven treatments consisting of different manure combinations with and without BSFL were established and replicated four times. Before treatment, parasitological examination detected Heterakis spp. eggs in chicken manure at 100 eggs per gram (EPG), whereas no parasitic ova were detected in pig and sheep manure samples. Following bioconversion, bacterial isolates were identified using the VITEK® 2 Compact Microbial Identification System. Results revealed shifts in culturable bacterial communities among treatments. Untreated control treatments were dominated by Bacillus species, whereas BSFL-amended substrates contained additional bacterial genera, including Brevibacillus, Lysinibacillus, and Pediococcus. Treatments containing mixed manure substrates and BSFL exhibited greater bacterial richness, with Treatments 5 and 6 recording the highest number of detected bacterial taxa. Treatment 7 was distinguished by the presence of Pediococcus pentosaceus, a bacterium previously associated with compost stabilization and microbial antagonism. Several bacterial taxa reported in the literature to possess agronomic and biological control potential, including Bacillus subtilis, Bacillus amyloliquefaciens, Bacillus thuringiensis, and Lysinibacillus sphaericus, were also identified. The findings suggest that BSFL bioconversion influences the composition of culturable bacterial communities in livestock manure and may contribute to the development of microbiologically enriched organic amendments. Further studies employing molecular techniques and quantitative microbial analyses are recommended to verify microbial abundance, diversity, and functional activity.

Keywords: Black Soldier Fly Larvae, livestock manure, bioconversion, bacterial richness, organic fertilizer, sustainable agriculture, culturable bacteria.

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INTRODUCTION

Livestock production systems generate substantial quantities of manure that require proper management to minimize environmental and public health risks. The accumulation and improper disposal of livestock waste can contribute to nutrient leaching, greenhouse gas emissions, water contamination, and the proliferation of pathogenic microorganisms. Although livestock manure is widely recognized as a valuable source of organic matter and plant nutrients, untreated manure may contain undesirable microbial populations and parasite eggs that limit its direct agricultural application.

The Black Soldier Fly (*Hermetia illucens* L.) has emerged as an effective biological agent for converting organic waste into value-added products. Black Soldier Fly Larvae (BSFL) efficiently consume a wide range of organic substrates, including food waste, agricultural residues, and livestock manure. During feeding, BSFL reduce waste volume, accelerate organic matter decomposition, and produce nutrient-rich frass that can be used as organic fertilizer. Recent studies have demonstrated that BSFL bioconversion contributes to nutrient recycling and supports circular bioeconomy approaches in agricultural systems.

Beyond waste reduction, BSFL activity may influence the microbial ecology of decomposing substrates. Larval feeding alters substrate structure, moisture content, aeration, and nutrient availability, which may subsequently affect microbial succession and community composition. Previous studies have reported significant changes in microbial communities during BSFL-mediated waste conversion; however, information remains limited regarding the microbial composition of different livestock manure substrates following BSFL treatment under local production conditions.

Recent research has further expanded the understanding of BSFL-mediated waste conversion and its role in sustainable agricultural systems. BSFL bioconversion is increasingly recognized as an important component of circular bioeconomy strategies because it transforms organic wastes into value-added products while facilitating nutrient recovery and resource recycling. In addition to reducing waste volume, BSFL activity influences microbial succession and promotes the development of biologically active residues enriched with beneficial microorganisms. Recent studies have reported that BSFL-associated microbiomes contribute to organic matter degradation, nutrient mineralization, and pathogen suppression during waste processing (Abd Manan et al., 2024). Furthermore, the resulting frass has gained attention as a sustainable organic fertilizer due to its nutrient content, beneficial microbial populations, and potential to improve soil fertility, plant growth, and soil biological activity (Lomonaco et al., 2024). These findings support the growing interest in BSFL technology as an environmentally sustainable approach for livestock waste management and organic fertilizer production within circular agricultural systems (Ganesan et al., 2024).

Understanding microbial changes associated with BSFL bioconversion is important because microbial communities play critical roles in organic matter decomposition, nutrient mineralization, pathogen suppression, and compost stabilization. Therefore, the present study aimed to (1) determine the initial parasitological status of selected livestock manures, (2) identify bacterial taxa present following BSFL bioconversion, and (3) evaluate the potential functional significance of the detected microorganisms based on existing scientific literature.

Although previous studies have documented changes in microbial communities during BSFL-mediated waste conversion, information regarding culturable bacterial communities associated with different livestock manure substrates under local production conditions remains limited. Furthermore, the extent to which BSFL influences the composition of culturable bacterial populations in manure-derived substrates requires further investigation.

MATERIALS AND METHODS

I. MASS REARING OF BLACK SOLDIER FLY

A. Larvae Rearing

Seven-day-old black soldier fly Larvae (BSFL) (hereafter called starter larvae) were reared on a mix of rice bran and water. The specimen was bought at Shamun BSF Insectary, Trinidad, Benguet. A group of starter larvae was separated to perform the test. At ages 9-16 days, 250 grams of larvae were collected randomly from the mass-rearing diet.

The BSFL were tested for microbial content, for species identification, and microbial analysis in the Cagayan Valley Integrated Agricultural Laboratory-Plant Health Clinic located at Carig Sur, Tuguegarao City, Cagayan. The researchers used 250 grams of Black Soldier Fly (Hermetia illucens) larvae per experimental unit to ensure uniformity and consistency across all treatments.

The researchers used 250 grams of Black Soldier Fly (Hermetia illucens) larvae per experimental unit to ensure uniformity and consistency across all treatments. The 250 g larval biomass corresponded to approximately 2,500 Black Soldier Fly Larvae (BSFL), based on an estimated average fresh weight of 0.10 g per larva at 9–16 days of age. Larval density varied according to the amount of substrate used in each treatment. Treatment 4 (2.5 kg sheep manure) received approximately 100 g BSFL kg^{-1} substrate, Treatments 5 and 6 (5.0 kg total substrate) received approximately 50 g BSFL kg^{-1} substrate, while Treatment 7 (7.5 kg total substrate) received approximately 33.3 g BSFL kg^{-1} substrate. The selected larval biomass was considered sufficient to support active feeding, efficient substrate conversion, and consistent evaluation of bioconversion performance across treatments.

B. Collection of Manure

Fifty kilograms each of sheep manure, pig manure, and chicken manure were collected to serve as the subjects for the different treatments in the study. A small portion from each collected manure type was taken for microbial analysis and fertilizer analysis testing.

C. Soil Analysis Test

Soil samples were collected from the field demo-establishment layout to determine their nutrient content and pH. Each soil sample weighed approximately 1kg and was taken from a 15 cm depth after removing the topsoil.

D. Nutrient Analysis Test

The prepared treatments were subjected to nutrient analysis, covering both micronutrients (Zinc (Zn), Boron (Bo), Iron (Fe), Manganese (Mn), Copper (Cu), Molybdenum (Mo), Silicon (Si), Nickel (Ni), Cobalt (Co), and Sodium (Na)) and macronutrients (Nitrogen (N), Phosphorus (P), and Potassium (K)). This analysis

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was conducted at the Department of Agriculture-Cagayan Valley Integrated Laboratory in Carig Sur, Tuguegarao City, Cagayan. The most suitable treatment, based on these analyses, was then selected for use in the vegetable demo-establishment.

Treatments were in a 1:1:1 ratio, replicated 4 times.

T1- Control (2.5 kg pure sheep manure)

T2- Control (2.5 kg pure chicken manure)

T3- Control (2.5 kg pure pig manure)

T4- 2.5 kg Sheep Manure + 250 grams BSF larvae

T5- 2.5kg Sheep Manure + 250 grams BSF larvae + 2.5 kg chicken manure

T6-2.5kg Sheep Manure + 250 grams BSF larvae + 2.5 kg pig manure

T7- 2.5kg Sheep Manure + 250 grams BSF larvae + 2.5 kg pig manure + 2.5 chicken manure

E. Experimental Setup and Bioconversion Conditions

The bioconversion experiment was conducted in an improvised indoor rearing area under ambient room-temperature conditions. The experimental units were maintained in a well-ventilated room protected from direct sunlight, rainfall, and extreme environmental disturbances. No artificial heating, cooling, or humidity-control systems were used during the study. Environmental conditions, therefore, reflected the natural ambient conditions of the locality throughout the experimental period.

The manure substrates were placed in plastic containers and inspected daily to ensure that they remained suitable for larval activity. Excess moisture accumulation and waterlogging were avoided by maintaining adequate ventilation and drainage. The containers were positioned to minimize variation in environmental exposure among treatments.

All treatments were maintained in identical plastic containers of the same dimensions and volume. The containers were placed within the same indoor rearing area to minimize environmental variation. Substrates were not mechanically turned during the experiment, allowing decomposition to proceed under uniform conditions. Moisture levels were monitored daily through visual inspection, and excessive water accumulation was prevented through drainage and ventilation. No additional water was added unless necessary to maintain conditions suitable for larval activity.

F. Determination of Bioconversion Completion

Bioconversion was considered complete when the manure substrate exhibited visible signs of stabilization and had been substantially processed by the Black Soldier Fly Larvae (BSFL). Completion was determined based on the following criteria: (1) marked reduction in substrate volume, (2) dark brown to black coloration, (3) fine and crumbly texture characteristic of BSFL frass, (4) substantial reduction in odor intensity, and (5) absence of recognizable fresh manure particles. These physical indicators were assessed through daily observations and were used to determine the endpoint of the bioconversion process for each treatment.

G. Feed Consumption and Observation

The manure consumption of BSF larvae was monitored daily. A video surveillance camera was installed in the plastic cage housing of the different treatments to observe the time taken for BSF larvae to consume the subjected treatments. Microbial content was also observed and later subjected to microbial analysis for proper identification of the microorganisms present.

H. Microbial Isolation and Identification

Following the bioconversion period, representative samples from each treatment were collected aseptically and submitted to the Cagayan Valley Integrated Agricultural Laboratory–Plant Health Clinic for microbial analysis. Approximately 10 g of each manure or frass sample was collected using sterile sampling procedures and placed in sterile containers for laboratory examination.

Each treatment consisted of four biological replicates. For microbial identification, representative samples from the four replicates of each treatment were composited to obtain a single sample for culture-dependent microbial analysis and bacterial identification. Consequently, the microbial profiles reported in Table 3 represent the bacterial taxa detected in the composite sample for each treatment rather than replicate-specific microbial communities.

Pure bacterial isolates were identified using the VITEK® 2 Compact Microbial Identification System (bioMérieux, France). Identification results were interpreted according to the probability scores generated by the instrument. Isolates exhibiting low-discrimination results were reported as presumptive identifications and interpreted with caution. Because the study relied on culture-dependent isolation techniques, only culturable bacteria present under the laboratory conditions employed were evaluated.

I. Statistical Analysis

Bioconversion period data were analyzed using one-way analysis of variance (ANOVA) to determine significant differences among treatments. When significant treatment effects were detected, mean comparisons were performed using the Least Significant Difference (LSD) test at the 5% level of significance. Coefficients of variation (CV) were calculated to assess experimental variability.

RESULTS (Style: Bold & UPERCASE, Font size: 10 pt)

Table 1. Mean Number of Days Required for Bioconversion of Livestock Manure Treatments

Treatment	Mean Bioconversion Period (Days)	LSD Grouping
T3 – Control (Pig Manure)	120.00	a
T1 – Control (Sheep Manure)	90.00	b
T2 – Control (Chicken Manure)	40.00	c
T7 – Sheep + Pig + Chicken + BSFL	33.25	d
T4 – Sheep + BSFL	27.50	e
T5 – Sheep + Chicken + BSFL	23.00	f

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T6 – Sheep + Pig + BSFL	22.50	f

LSD (5%) = 1.89 days

Means having the same letter are not significantly different at the 5% level based on the Least Significant Difference (LSD) test.

Table 1 presents an analysis of variance that revealed highly significant differences among treatments in terms of bioconversion period ($P < 0.01$). Mean separation using the Least Significant Difference (LSD) test showed that the BSFL-amended treatments decomposed significantly faster than the untreated controls. Treatment 6 recorded the shortest bioconversion period (22.50 days), followed by Treatment 5 (23.00 days), whereas Treatment 3 required the longest decomposition period (120.00 days). These findings demonstrate the effectiveness of BSFL in accelerating manure decomposition and organic matter conversion.

The untreated control treatments required considerably longer decomposition periods. Pig manure (T3) recorded the longest bioconversion period at 120 days, followed by sheep manure (T1) at 90 days and chicken manure (T2) at 40 days. The slower decomposition observed in the control treatments can be attributed to their dependence on naturally occurring microorganisms, which require more time to break down complex organic materials (Bernal et al., 2009).

In contrast, all BSFL-treated substrates decomposed much faster, requiring only 22.5 to 33.25 days. Among these treatments, T6 (2.5 kg Sheep Manure + 250 g BSFL + 2.5 kg Pig Manure) achieved the shortest bioconversion period of 22.5 days, followed closely by T5 (2.5 kg Sheep Manure + 250 g BSFL + 2.5 kg Chicken Manure) at 23 days. These findings demonstrate the ability of BSFL to accelerate organic matter degradation and nutrient transformation through active feeding and digestion (Diener et al., 2011).

The rapid decomposition observed in the BSFL-amended treatments may be attributed to the larvae's capacity to consume and fragment organic materials, thereby increasing the surface area available for microbial activity. In addition, larval movement improves aeration within the substrate, creating favorable conditions for microbial decomposition and nutrient mineralization (Gold et al., 2018; Surendra et al., 2016). The superior performance of T6 suggests that the combination of sheep and pig manure provided suitable nutrient and moisture conditions that enhanced larval activity and waste conversion efficiency (Lalander et al., 2019).

During the bioconversion process, noticeable changes occurred in the physical characteristics of the manure substrates. As the Black Soldier Fly Larvae (BSFL) actively fed on the manure, the substrates gradually became finer in texture, darker in color, and less odorous. The larvae continuously consumed organic matter and converted it into larval biomass and frass through digestion and nutrient assimilation. This feeding activity mechanically fragmented the manure and increased the surface area available for microbial decomposition. At the same time, digestive enzymes produced by the larvae facilitated the breakdown of complex organic compounds such as proteins, carbohydrates, and lipids into simpler substances.

The combined action of larval feeding and microbial activity accelerated nutrient mineralization, reduced moisture content, and promoted the stabilization of organic matter. These changes indicate that the manure was successfully transformed into a more mature and nutrient-rich organic fertilizer suitable for agricultural application (Diener et al., 2011; Surendra et al., 2016; Lalander et al., 2019).

Overall, the results demonstrate that BSFL significantly reduced the time required for manure decomposition compared with untreated controls. The findings support previous studies that identified BSFL as an effective biological agent for livestock waste management, nutrient recovery, and organic fertilizer production (Diener et al., 2011; Lalander et al., 2019).

Table 2. Laboratory testing before the experiment

Sample Description	Floatation Result	Eggs Present Per Gram of Feces (EPG)
Feces, Chicken	Heterakis spp.	100
Feces, Pig	No ova found	0
Feces, Sheep	No ova found	0

Environmental Condition: Temperature $22\underline{+}1^{\circ}$ C- Biological Laboratory

Relative Humidity: 45-50% RH

Table 2 presents the laboratory analysis of the livestock manures before BSFL application. The results showed that only chicken manure contained detectable parasitic contamination, with the presence of *Heterakis* spp. at 100 eggs per gram (EPG) of feces. In contrast, pig manure and sheep manure showed no detectable ova during laboratory examination. These findings indicate that poultry manure posed the highest initial biological risk among the manure sources evaluated.

The presence of *Heterakis* spp. in chicken manure is consistent with previous reports identifying poultry manure as a potential reservoir of parasitic organisms that may affect animal health and environmental sanitation if not properly managed (Permin & Hansen, 1998). Although the level detected in this study was relatively low, the occurrence of parasite eggs highlights the importance of appropriate manure treatment before agricultural utilization.

The absence of detectable ova in pig and sheep manure suggests lower parasitological contamination at the time of sampling. However, proper waste management practices remain essential to minimize potential microbial and parasitic hazards associated with livestock manure. These baseline results provide important information for evaluating the effect of BSFL treatment on the microbial quality and biological safety of the manure substrate.

Table 3. Laboratory Testing After the Experiment

TREATMENT	MANURE	RESULT	REMARKS
1 (Control 2.5 kg Sheep Manure)	Sheep Manure	*Bacillus licheniformis was found*	Vitek 2: 98% Probability; Excellent Identification
		Bacillus farraginis was found	Vitek 2: 88% Probability; Excellent Identification

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2 (Control 2.5 kg Chicken Manure)	Chicken Manure	*Bacillus subtilis, Bacillus amyloliquefaciens, and Bacillus atrophaeus*	Vitek 2: 98% Probability; Excellent Identification; Conduct Supplemental analysis tests to separate.
		Bacillus sporothermodurans	Vitek 2: 96% Probability; Excellent Identification
		Bacillus pumilus	Vitek 2: 95% Probability; Excellent Identification
3 (Control 2.5 kg Pig Manure)	Pig Manure	*Bacillus pumilus*	Vitek 2: 95% Probability; Excellent Identification
4 (2.5 kg Sheep Manure + 250g BSF Larvae)	Sheep Manure	*Staphylococcus sciuri*	Vitek 2: 96% Probability; Excellent Identification
		Bacillus subtilis, Bacillus amyloliquefaciens, and Bacillus atrophaeus	Vitek 2: 97% Probability; Excellent Identification; Conduct supplemental analysis tests to separate.
5 (2.5 kg Sheep Manure + 250 g BSF Larvae + 2.5 kg Chicken Manure)	Sheep Manure	*Bacillus cereus, Bacillus thuringiensis and Bacillus mycoides were found*	Vitek 2: 93% Probability; Excellent Identification; Conduct supplemental analysis tests to separate.
		Brevibacillus Brevibacillus choshinensis, Lysinibacillus sphaericus and Lysini	Vitek 2: Low Discrimination: 50% probability for each of the following taxon: **Brevibacillus** and **Lysinibacillus**; Conduct supplemental analysis tests to separate.
6 (2.5 kg Sheep Manure + 250 g BSF Larvae + 2.5 kg Pig Manure)	Sheep Manure	*Brevibacillus choshinensis,* *Lysinibacillus sphaericus, and Lysinibacillus* *fusiformis was found*	Vitek 2: Low Discrimination; 50% probability for each of the following taxon: Brevibacillus and Lysinibacillus; Conduct supplemental analysis tests to separate.
		Bacillus cereus, Bacillus thuringiensis, and *Bacillus mycoides was found*	Vitek 2: 93% Probability; Very Good Identification; Conduct supplemental analysis tests to separate.
7 (2.5 kg Sheep Manure + 250 g BSF Larvae + 2.5 kg Pig Manure + 2.5 kg Chicken Manure	Sheep Manure	*Bacillus cereus, Bacillus thuringiensis, and* *Bacillus mycoides was found*	Vitek 2: 93% Probability; Very Good Identification; Conduct supplemental analysis tests to separate.
		Pediococcus pentosaceus was found	Vitek 2: 85% Probability; Acceptable Identification

Table 3 presents the microbial profiles of the different manure treatments following bioconversion. The results showed that BSFL treatment substantially altered the microbial composition of livestock manures. Untreated control treatments were dominated primarily by Bacillus species, including Bacillus licheniformis, Bacillus farraginis, Bacillus subtilis, and Bacillus pumilus, which are commonly associated with the decomposition of natural organic matter and nutrient cycling in manure and soil environments.

Following BSFL treatment, bacterial richnessincreased, with the emergence of beneficial bacterial genera such as *Brevibacillus*, *Lysinibacillus*, and *Pediococcus*. These microorganisms have been reported in previous studies to be associated with organic matter degradation, nutrient mineralization, and compost stabilization of organic substrates. The observed shift in microbial communities suggests that BSFL bioconversion created conditions conducive to the establishment of a broader range of bacterial taxa, several of which have been reported in previous studies to have beneficial agronomic functions. Similar findings were reported by Gold et al. (2018) and Klammsteiner et al. (2020), who observed that BSFL activity modifies microbial communities and promotes the development of biologically stable organic residues.

Among the BSFL-amended treatments, Treatment 7 (2.5 kg Sheep Manure + 250 g BSFL + 2.5 kg Pig Manure + 2.5 kg Chicken Manure) was distinguished by the detection of *Pediococcus*

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pentosaceus, a bacterial species previously associated with compost stabilization and microbial antagonism. The presence of *Pediococcus pentosaceus*, a beneficial lactic acid bacterium, is particularly noteworthy because it is associated with pathogen suppression, production of antimicrobial compounds, and compost maturation (Papagianni & Anastasiadou, 2009). In addition, the detection of *Brevibacillus* and *Lysinibacillus* species suggests enhanced nutrient cycling and improved stabilization of organic matter. Although Treatment 6 recorded the shortest bioconversion period (22.5 days), Treatment 7 may possess characteristics associated with microbial stabilization due to the presence of Pediococcus pentosaceus. The microbial changes observed during bioconversion may be attributed to the feeding activity of BSFL, which continuously mixed the substrate, altered nutrient availability, and created favorable environmental conditions for beneficial microorganisms. As organic matter was degraded and transformed into frass, microbial communities became more diverse and adapted to the stabilized substrate. This process contributed not only to nutrient transformation but also to the development of a biologically active organic fertilizer with improved ecological quality (Diener et al., 2011; Lalander et al., 2019).

Overall, the results demonstrate that BSFL bioconversion promoted increased bacterial richness and nutrient stabilization while reducing the prevalence of undesirable microorganisms. The enhanced microbial community observed in Treatment 7 indicates its potential to produce a more mature, stable, and agronomically valuable organic fertilizer suitable for sustainable agricultural applications.

Table 4. Bacterial Species Richness Among Treatments Following BSFL Bioconversion

Treatment	Number of Bacterial Taxa (S)	Bacterial Species Richness
1. (Control 2.5 kg Sheep Manure)	2	0.693
2. (Control 2.5 kg Chicken Manure)	5	1.609
3. (Control 2.5 kg Pig Manure)	1	0.000
4. (2.5 kg Sheep Manure + 250g BSF Larvae)	4	1.386
5. (2.5 kg Sheep Manure + 250 g BSF Larvae + 2.5 kg Chicken Manure)	6	1.792
6. (2.5 kg Sheep Manure + 250 g BSF Larvae + 2.5 kg Pig Manure)	6	1.792
7. (2.5 kg Sheep Manure + 250 g BSF Larvae + 2.5 kg Pig Manure + 2.5 kg Chicken Manure	4	1.386

Table 4 presents the estimated bacterial richness among the different manure treatments following BSFL-mediated bioconversion. Treatments T5 and T6 exhibited the highest richness values, with six bacterial taxa identified in each treatment. These treatments contained bacterial groups associated with organic matter degradation, nutrient cycling, and biological control, suggesting the development of relatively complex microbial communities.

Treatment T7 contained four identified bacterial taxa and therefore exhibited lower richness than T5 and T6. However, T7 was distinguished by the presence of *Pediococcus pentosaceus*, a lactic acid bacterium commonly associated with fermentation processes, microbial antagonism, and compost stabilization. The occurrence of this bacterium may indicate a more biologically stable substrate despite the lower number of detected taxa.

Overall, the results suggest that BSFL treatment influenced bacterial community composition and increased bacterial richness relative to some untreated manure treatments. Because bacterial abundance data were not collected, the diversity estimates presented should be interpreted as measures of species richness rather than true Shannon diversity indices.

Table 5. Taxonomic Classification, Morphological Description, and Benefits of Bacteria Identified in the Different Treatments

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Treatment	Bacteria Identified	Morphological Description	Potential Benefits	Number of Bacterial Species/Taxa Detected	Notable Functional Groups
T1 (Control: 2.5 kg Sheep Manure)	*Bacillus licheniformis*	Gram-positive, rod-shaped, endospore-forming, motile	Produces degradative enzymes, enhances organic matter decomposition, and improves nutrient mineralization.	2	Decomposers
	Bacillus farraginis	Gram-positive, rod-shaped, endospore-forming, motile	Assists in the decomposition of organic materials and nutrient recycling.		
T2	*Bacillus subtilis*	Gram-positive, rod-shaped,	Promotes plant growth, suppresses	5	Decomposers, Plant Growth-

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(Control: 2.5 kg Chicken Manure)		endospore-forming, motile	pathogens, and enhances compost maturation.		Promoting Bacteria (PGPB)
	Bacillus amyloliquefaciens	Gram-positive, rod-shaped, endospore-forming, motile	Produces enzymes and antimicrobial compounds; improves nutrient availability.		
	Bacillus atrophaeus	Gram-positive, rod-shaped, endospore-forming, motile	Contributes to organic matter decomposition and microbial antagonism.		
	Bacillus sporothermodurans	Gram-positive, rod-shaped, spore-forming	Participates in nutrient cycling and survives harsh environmental conditions.		
	Bacillus pumilus	Gram-positive, rod-shaped, endospore-forming, motile	Produces enzymes that degrade organic matter and promotes plant growth.		
T3 (Control: 2.5 kg Pig Manure)	*Bacillus pumilus*	Gram-positive, rod-shaped, endospore-forming, motile	Enhances decomposition, nutrient cycling, and compost quality.	1	Decomposer
T4 (2.5 kg Sheep Manure + 250 g BSF Larvae)	*Staphylococcus sciuri*	Gram-positive cocci arranged in clusters, non-spore-forming	Contributes to nutrient turnover and microbial community dynamics.	4	Decomposers, Biocontrol Agents
	Bacillus subtilis	Gram-positive, rod-shaped, endospore-forming, motile	Plant growth promotion and biological control.		
	Bacillus amyloliquefaciens	Gram-positive, rod-shaped, endospore-forming, motile	Produces extracellular enzymes and antimicrobial metabolites.		
	Bacillus atrophaeus	Gram-positive, rod-shaped, endospore-forming, motile	Assists decomposition and suppresses competing microbes.		
T5 (2.5 kg Sheep Manure + 250 g BSF Larvae + 2.5 kg Chicken Manure)	*Bacillus cereus*	Gram-positive, large rod-shaped, endospore-forming, motile	Produces enzymes involved in decomposition and nutrient release.	6	
	Bacillus thuringiensis	Gram-positive, rod-shaped, endospore-forming, motile	Produces insecticidal Cry proteins used in biological pest control.		

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	Bacillus mycoides	Gram-positive, rod-shaped, endospore-forming; rhizoid colonies	Contributes to decomposition and nutrient cycling.		
	Brevibacillus choshinensis	Gram-positive, rod-shaped, spore-forming	Produces extracellular proteins and aids organic matter degradation.		
	Lysinibacillus sphaericus	Gram-positive, rod-shaped, endospore-forming	Biological control of mosquito larvae and environmental bioremediation.		
	Lysinibacillus fusiformis	Gram-positive, spindle-shaped rod, endospore-forming	Participates in nutrient cycling and degradation of organic compounds.		
T6 (2.5 kg Sheep Manure + 250 g BSF Larvae + 2.5 kg Pig Manure)	*Brevibacillus choshinensis*	Gram-positive, rod-shaped, spore-forming	Protein production and decomposition activities.	6	Decomposers, Biocontrol Agents, Bioremediation Bacteria
	Lysinibacillus sphaericus	Gram-positive, rod-shaped, endospore-forming	Biological control and nutrient recycling.		
	Lysinibacillus fusiformis	Gram-positive, spindle-shaped rod, endospore-forming	Organic matter degradation and soil fertility enhancement.		
	Bacillus cereus	Gram-positive, rod-shaped, endospore-forming, motile	Organic matter degradation and nutrient mineralization.		
	Bacillus thuringiensis	Gram-positive, rod-shaped, endospore-forming, motile	Biological insect pest control.		
	Bacillus mycoides	Gram-positive, rod-shaped, endospore-forming	Decomposition and nutrient cycling.		
T7 (2.5 kg Sheep Manure + 250 g BSF Larvae + 2.5 kg Pig Manure + 2.5 kg Chicken Manure)	*Bacillus cereus*	Gram-positive, rod-shaped, endospore-forming, motile	Degrades proteins and carbohydrates, enhancing nutrient release.	4	Decomposers, Biocontrol Agents, Lactic Acid Bacteria
	Bacillus thuringiensis	Gram-positive, rod-shaped, endospore-forming, motile	Biological control agent against insect pests.		
	Bacillus mycoides	Gram-positive, rod-shaped, endospore-forming	Improves decomposition and nutrient cycling.		
	Pediococcus pentosaceus	Gram-positive cocci, non-spore-forming, facultative anaerobe; often occurs in pairs or	Produces lactic acid, inhibits pathogenic microorganisms, improves		

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		tetrads	fermentation processes, and contributes to compost stabilization.		

Table 5 presents the taxonomic classification, morphological characteristics, and potential benefits of bacterial species identified across the different manure treatments following BSFL bioconversion. The results showed that the microbial communities were predominantly composed of Gram-positive bacteria belonging to the genera *Bacillus*, *Brevibacillus*, *Lysinibacillus*, *Staphylococcus*, and *Pediococcus*. Most of these microorganisms have been reported in previous studies to be associated with organic matter decomposition, nutrient cycling, biological control, and soil fertility enhancement.

The untreated control treatments were dominated by *Bacillus* species, which are widely known for producing extracellular enzymes capable of degrading complex organic compounds such as proteins, starches, and cellulose. These bacteria contribute significantly to nutrient mineralization and the natural decomposition of organic materials. In particular, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Bacillus pumilus* have been reported to promote plant growth through the production of growth-promoting substances and antimicrobial metabolites that suppress soil-borne pathogens (Stein, 2005; Logan & De Vos, 2009).

Following BSFL treatment, bacterial richnessincreased considerably, as evidenced by the appearance of additional beneficial bacterial genera, including *Brevibacillus* and *Lysinibacillus*. These microorganisms are associated with enhanced decomposition, nutrient recycling, environmental bioremediation, and biological pest control. The emergence of *Lysinibacillus sphaericus*, known for its mosquito larvicidal activity, and *Brevibacillus choshinensis*, recognized for extracellular enzyme production, suggests that BSFL bioconversion created favorable conditions for the establishment of functionally diverse microbial communities. Such increased bacterial richnessis often associated with improved compost maturity and ecological stability (Berry, 2012; Klammsteiner et al., 2020).

Among the treatments, T5 and T6 exhibited the highest bacterial richness, with six bacterial taxa identified in each treatment. These treatments contained microorganisms involved in decomposition, nutrient mineralization, biological control, and environmental remediation. The presence of *Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus mycoides*, *Brevibacillus choshinensis*, *Lysinibacillus sphaericus*, and *Lysinibacillus fusiformis* indicates an active microbial ecosystem capable of accelerating organic matter degradation and nutrient transformation. The occurrence of these bacterial taxa may be associated with the decomposition and stabilization processes observed during bioconversion; however, their functional activity was not directly measured in the present study.

Treatment 7, although containing fewer bacterial taxa than Treatments 5 and 6, was distinguished by the presence of *Pediococcus pentosaceus*, a beneficial lactic acid bacterium. This microorganism produces lactic acid and antimicrobial compounds that inhibit pathogenic bacteria, improve fermentation processes, and enhance compost stabilization. The occurrence of *Pediococcus pentosaceus* may indicate characteristics consistent with compost stabilization based on previous reports; however, compost maturity was not directly assessed in this study. Together with the presence of *Bacillus* species involved in decomposition and nutrient cycling, this bacterium contributed to a balanced microbial community that supports the production of high-quality organic fertilizer (Papagianni & Anastasiadou, 2009).

Overall, the results indicate that BSFL bioconversion not only accelerated manure decomposition but also promoted the development of beneficial microbial communities with important agronomic functions. The predominance of decomposers, plant growth-promoting bacteria, biocontrol agents, and nutrient-cycling microorganisms highlights the potential of BSFL-treated manure as a biologically enriched organic fertilizer capable of supporting sustainable crop production and soil health improvement (Gold et al., 2018; Lalander et al., 2019).

Table 6. Biocontrol Agents Detected in the Treatments

Biocontrol Agent	Treatments Found	Mechanism of Biocontrol	Importance
Bacillus subtilis	T2, T4	Produces antibiotics, lipopeptides (surfactin, iturin, fengycin), and hydrolytic enzymes that inhibit fungal and bacterial pathogens.	Widely used as a biofertilizer and biopesticide for disease suppression.
Bacillus amyloliquefaciens	T2, T4	Produces antimicrobial metabolites and plant growth-promoting substances.	Controls soil-borne pathogens and enhances plant growth.
Bacillus pumilus	T2, T3	Produces antifungal and antibacterial compounds.	Protects plants from diseases and promotes healthy root development.
Bacillus thuringiensis	T5, T6, T7	Produces Cry and Cyt toxins that are toxic to insect larvae.	One of the most important biological insecticides worldwide.
Lysinibacillus sphaericus	T5, T6	Produces larvicidal toxins that kill mosquito larvae.	Used in mosquito control programs and environmental pest management.

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Pediococcus pentosaceus	T7	Produces lactic acid and bacteriocins that inhibit harmful bacteria.	Suppresses undesirable microorganisms and enhances microbial stability.

Table 6 presents the biocontrol agents identified in the different manure treatments following BSFL bioconversion. Several beneficial bacterial species known for their antagonistic activity against plant pathogens and insect pests were detected across the treatments. These microorganisms have been reported in previous studies to produce antimicrobial compounds, toxins, enzymes, and other biologically active metabolites that may contribute to biological control.

Among the identified biocontrol agents, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Bacillus pumilus* were detected in the control and BSFL-treated substrates. These species are widely recognized as plant growth-promoting rhizobacteria capable of producing antibiotics, lipopeptides, hydrolytic enzymes, and growth-promoting substances that suppress soil-borne pathogens and improve plant health (Stein, 2005). Their presence indicates the occurrence of bacterial taxa that have been reported to support crop growth and disease suppression under certain environmental conditions.

The detection of *Bacillus thuringiensis* in Treatments 5, 6, and 7 further highlights the biological value of BSFL-processed manure. This bacterium is known for producing Cry and Cyt proteins that are toxic to various insect larvae and has long been utilized as a biological insecticide in sustainable pest management programs (Bravo et al., 2011). Likewise, *Lysinibacillus sphaericus*, identified in Treatments 5 and 6, produces larvicidal toxins effective against mosquito larvae and is commonly used in environmental pest control strategies (Berry, 2012).

One significant result was the detection of *Pediococcus pentosaceus* in Treatment 7. This beneficial lactic acid bacterium produces organic acids and bacteriocins that inhibit undesirable microorganisms and contribute to microbial stability during compost maturation. Its presence may indicate characteristics associated with microbial stabilization based on previous studies, and may enhance the safety and quality of the resulting organic fertilizer (Papagianni & Anastasiadou, 2009).

DISCUSSION

The results indicate that BSFL-mediated bioconversion was associated with changes in the culturable bacterial composition of livestock manure substrates. BSFL-treated treatments generally exhibited greater bacterial richness than untreated controls, with Treatments 5 and 6 recording the highest richness values. In addition, Treatment 7 was distinguished by the occurrence of Pediococcus pentosaceus, a bacterial species previously associated with compost stabilization and microbial antagonism. These findings suggest that BSFL activity may influence microbial succession and create environmental conditions favorable for a broader range of culturable bacterial taxa. Similar shifts in microbial communities have been reported during BSFL bioconversion of organic wastes, where larval feeding activity affects substrate aeration, nutrient availability, and microbial succession.

Several bacterial taxa identified in the present study, including members of the genera Bacillus, Brevibacillus, Lysinibacillus, and Pediococcus, have been reported in previous studies to participate in organic matter decomposition, nutrient cycling, microbial antagonism, and biological control. However, these ecological functions were not directly evaluated in the present investigation. Therefore, the functional significance of the detected microorganisms should be interpreted as potential roles inferred from published literature rather than confirmed activities within the experimental treatments.

Because bacterial identification was based on culture-dependent isolation and VITEK® 2 biochemical characterization, the microbial community described in this study represents only the culturable fraction of the manure microbiota. Numerous microorganisms may not have been detected because they were either non-culturable under the laboratory conditions employed or present at abundances below detection limits. Consequently, the results should be interpreted as describing shifts in culturable bacterial composition rather than the total microbial community.

Several limitations should be considered when interpreting the findings. Larval biomass relative to substrate mass differed among treatments because the same quantity of BSFL was applied to varying amounts of manure. Consequently, differences in larval density may have influenced substrate consumption rates, microbial composition, and overall bioconversion performance. In addition, several BSFL-treated treatments differed not only in the presence of larvae but also in manure composition. Because manure type can affect nutrient availability, moisture retention, and microbial succession, the independent contribution of BSFL activity could not be completely separated from substrate effects. Furthermore, bioconversion completion was determined using physical indicators such as color, texture, odor reduction, and substrate volume reduction. Although these criteria are commonly used in operational BSFL systems, objective measurements of compost maturity, nutrient stabilization, and organic matter reduction were not conducted.

Despite these limitations, the detection of bacterial taxa previously reported as decomposers, plant growth-promoting bacteria, and biological control agents suggests that BSFL-treated manure may possess potential agronomic value beyond waste reduction and nutrient recycling. Future studies employing molecular sequencing techniques, quantitative microbial analyses, and factorial experimental designs are recommended to better characterize microbial community dynamics, verify microbial functional activity, and distinguish the individual effects of BSFL activity and substrate composition during bioconversion.

CONCLUSION

The findings of this study indicate that Black Soldier Fly Larvae (BSFL) bioconversion was associated with changes in the culturable bacterial composition of livestock manure substrates and substantially reduced the time required for manure decomposition compared with untreated controls. BSFL-treated substrates contained a broader range of culturable bacterial taxa than several untreated manure treatments, including bacterial groups previously reported to be associated with organic matter decomposition, nutrient cycling, microbial antagonism, and biological control.

Among the BSFL-treated substrates, Treatment 7 was distinguished by the occurrence of *Pediococcus pentosaceus*, a bacterial species previously associated with compost stabilization

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and microbial antagonism. However, microbial functional activity, compost maturity, and fertilizer performance were not directly evaluated in the present study. Therefore, the ecological significance of the detected microorganisms should be interpreted cautiously.

The results demonstrate the potential of BSFL as a biological agent for livestock manure management and organic waste conversion. Nevertheless, because the study relied on culture-dependent microbial identification, the findings represent only the culturable fraction of the bacterial community. Future investigations employing molecular approaches such as 16S rRNA sequencing, metagenomics, and quantitative microbial analyses are recommended to provide a more comprehensive assessment of bacterial richness and functional activity during BSFL-mediated bioconversion.

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