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Original Article

Soil nutrient alterations and bacterial community shifts in media amended with black soldier fly larval frass: A metagenomic approach

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Abstract

Ameliorants improve soil's physical, chemical, and biological properties, boosting fertility and productivity. While many studies focus on their chemical effects, few have explored their impact on soil microbial changes. This study investigated changes in soil nutrients and bacterial communities in Red Yellow Podzolic soil amended with black soldier fly (BSF) frass, using metagenomic analysis. The soil, treated with BSF frass, lime, and arbuscular mycorrhizal fungi (AMF), was incubated for a period of three months. Samples included untreated soil (S), BSF frass (BF), and treated soil (TS). Nutrients were analysed using AAS and spectrophotometry, while bacterial communities were assessed via Illumina NGS. The nutrient profile of BSF frass (BF) differed significantly from that of the soil. Ameliorant application increased exchangeable K^+, Mg^{2+}, and Base Saturation. Metagenomic analysis revealed distinct bacterial compositions, with 753 ASVs in soil (S), 408 in BF, and 613 in treated soil (TS). The dominant genera in the soil included JG30-KF-AS9 (26%), *Acidothermus* (15%), and *Xanthobacteraceae* (5%). In treated soil (TS), the predominant genera were similar but differed in relative abundance, comprising JG30-KF-AS9 (29%), *Alicyclobacillus* (14%), *Acidothermus* (11%), and *1921-2* (6%). The abundance of *Alicyclobacillus* increased significantly, from 1% in the soil (S) to 14% in the treated soil (TS). The bacteriome community in BF was markedly differed, with predominant genera including *Galbibacter* (13%), *Brevibacterium* (8%), *Celvibrioraceae* (6%), and *Moheibacter* (5%). These bacteria are capable of decomposing organic matter; therefore, the use of BSF frass on acidic soil is expected to enhance soil fertility and health.

Keywords: Acid soil, Ameliorant, Bacterial, Black soldier fly (BSF) frass, Metagenomic

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Introduction

Ameliorants are substances applied for increasing soil fertility along with productivity, therefore improving its physical, chemical, and biological properties. Diverse soil complications like acidity, salinity, nutrient deficits, and insufficient soil architecture are frequently remedied via their efforts. A wide-ranging assortment of amendments exists which includes dolomite, gypsum, organic materials, biochar, zeolite, silica, mycorrhizae, and biostimulants. Biochar, as an example, improves cation exchange capacity (CEC) thereby enhances water and nutrient retention. Gypsum curtails salinity while fortifying the constitution of clay soils (Zhang, et al., 2023; Dariah et al., 2021; Mulatu and Bayata, 2024). Many investigations show that people employing enhancers refine edaphic states since those augment its concrete, synthetic, and organic attributes, hence easing prime flora development (Dariah et al., 2021; Mon et al., 2024; Herawati et al., 2021; Maswar et al., 2021).

Black Soldier Fly (BSF) frass is produced through the decomposition of organic matter by BSF larvae. It shows substantial potential as a bioameliorant—an amendment designed to improve the physical, chemical, and biological properties of soils, including fostering nitrogen-fixing and phosphate-solubilizing bacteria (Murtiningsih et al., 2023). BSF frass contains a diverse array of beneficial microorganisms that are essential for soil fertility, as these microbes help enhance nutrient use efficiency, bolster resistance to abiotic stresses, and support healthy plant growth (Poveda et al., 2019). The rich content of macronutrients (N, P, K, Ca, Mg) and micronutrients (Zn, Fe), along with the presence of these beneficial bacteria that decompose organic matter, makes BSF frass an excellent bioameliorant. According to Mulatu and Bayata (2024), bioameliorants can directly increase the soil's productivity by improving nutrient availability, and indirectly by enhancing its physical properties. Additionally, the relatively neutral pH of BSF frass can help mitigate soil acidity, making it an effective agent for improving soil health and fertility. It is also important to note that the composition of BSF frass varies depending on the type of feed it's derived from, particularly regarding phosphorus, potassium, and micronutrients (Lopes et al., 2022).

Many studies have researched the impacts of ameliorants on soil chemical properties and fertility (Ismail et al., 2025; Matfu'ah et al., 2023). However, very few studies have adequately studied the impacts of ameliorant application on the alteration of microbes within the soils themselves. This study investigated the impact of amelioration on microbiological properties of soils through NGS-based metagenomics. NGS-based metagenomics has been used to investigate nucleic acids in a variety of biological samples (Oulas et al., 2015; Tay et al., 2024). NGS sequencing allows the combined assessment of microbial communities, including bacteria, viruses, fungi, and other microorganisms.

In this study, the effect of BSF frass application on changes in soil nutrients and bacterial community dynamics was evaluated using a metagenomic approach. Study results were expected to provide additional evidence for the possibilities of BSF Frass as a sustainable soil amendment, and how it could contribute to the management of a soil microbiome that supports eco-friendly agriculture.

Material and Methods

This study utilised the Red Yellow Podzolic, an acid soil type native to Leuwiliang, Bogor, West Java. Physical and chemical analysis of the soil used in the study is presented in Table 1. The study incorporated three treatments: Black Soldier Fly frass (BF), Soil (S), and Treated Soil (TS). The Treated Soil had three different kinds of ameliorants added to them including, BSF frass, lime, and arbuscular mycorrhizal fungi (AMF). BSF frass was sourced from BSF farms located in Depok, West Java, Indonesia. The chemical characterisation of BSF frass was as follows: pH: 8.80; C-organic: 40.34%; total N: 2.10%; P_2O_5: 2.94%; and exchangeable K^{2+}: 105.99 cmol/kg. The lime used was commercial dolomitic lime with approximately CaO (29-32%) and MgO (18-22%). In this study the AMF consortium of *Glomus* sp., *Acaulospora* sp., and *Gigaspora* sp. was used.

Preparation of arbuscular mycorrhizal fungi

The formulation of arbuscular mycorrhizal fungi (AMF) consortium used in the current study was created by researchers from the National Research and Innovation Agency and included mixed propagules of *Glomus* sp., *Acaulospora* sp., and *Gigaspora* sp., at an application rate of 2.5 tonnes per hectare. AMF multiplication occurred over three months using corn as the host plant and zeolite as the growth medium. Germinated corn seeds were sown in pots measuring 15 cm × 30 cm, filled with zeolite, and mycorrhizal inoculum was introduced in a 5 cm layer within the planting cavity. A low-phosphorus fertilizer was supplied in conjunction with routine irrigation. AMF harvesting was conducted at the peak of vegetative growth and during the early generative phase. The upper part of the plants was pruned, and the material was desiccated for approximately two weeks. After desiccation, the roots were separated from the stem base, sectioned into small pieces, and combined with the planting medium to generate AMF propagules.

Table-1. Physical and chemical analysis of the soil used in the study.

Characteristics	Value
Texture (%)	
- Sand	9
- Silt	37
- Clay	54
pH	4.6
C-org (%)	1.58
N Total (%)	0.14
C/N	11
P_2O_5 Bray1 (mg/kg)	7.7
P_2O_5 (mg/kg)	730
K_2O (mg/kg)	110
Exc. K^+ (cmol/kg)	11.43
Exc. Na^+ (cmol/kg)	2.87
Exc. Ca^{2+} (cmol/kg)	0.19
Exc. Mg^{2+} (cmol/kg)	0.18
Base Saturation (cmol/kg)	44
CEC (cmol/kg)	33.7
Al^{3+} (cmol/kg)	17.69
H^+ (cmol/kg)	3.59

Soil incubation

The incubation study was conducted in Bogor, West Java, using three treatment types: soil (S), BSF frass (BF), and treated soil (TS). For each treatment, 1,000 g of material were placed in plastic containers. The TS treatment consisted of a mixture of 987.5 g of soil, 5 g of BSF frass, 5 g of lime, and 2.5 g of arbuscular mycorrhizal fungi (AMF). All components were thoroughly homogenized and incubated at ambient room temperature for three months. After the incubation period, the samples were analysed for soil nutrient content and metagenomic profiles.

Soil analysis

The soil pH was measured using a glass electrode in a 1:5 soil-to-water solution ratio. Organic carbon content was determined using the Walkley and Black wet combustion method (Walkley and Black, 1934), while total nitrogen (N) was analyzed using the Kjeldahl method (Bremner and Mulvaney, 1982). Available phosphorus (P_2O_5) was extracted and quantified via the Bray 1 method (Bray and Kurtz, 1945). Exchangeable bases (Ca, Mg, K, Na) were extracted using 1 M ammonium acetate (NH_4OAc) at pH 7.0 and quantified using atomic absorption spectrometry (AAS) (Thomas, 1982). The cation exchange capacity (CEC) was assessed by saturating the soil with 1 M NH_4OAc at pH 7.0.

Metagenomic analysis

Metagenomic analysis was conducted to examine three sample types: soil (S), BSF frass (BF), and soil treated with BSF frass, lime, and arbuscular mycorrhizal fungi (TS). Genomic DNA was extracted using the Quick-DNA Magbead Plus Kit (Zymo Research, D4082). The initial quantity and purity of the extracted DNA were assessed using a Thermo Scientific Nanodrop 2000 spectrophotometer. PCR amplification was performed using the Phusion™ Plus PCR Master Mix (F631L), and the PCR products were visualized through agarose gel electrophoresis. The Qubit dsDNA HS Assay Kit (Thermo Scientific) was utilised for precise DNA quantification.

The DNA samples were sequenced employing the Illumina HiSeq 2 × 250 bp paired-end reads platform with 30k tags per sample. For bacterial species, the V3–V4 region of the 16S rRNA gene was amplified using the primers 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′), generating a 470 bp amplicon.

Library Preparation and Sequencing of gDNA samples were amplified with target-specific 16S V3-V4 primers. Library preparation was performed using the final PCR products. The Illumina platform sequenced the final library to generate paired-end raw reads.

PICRUSt2 was employed to predict the functional potential of microbial communities by inferring the presence of genes and enzymes. This prediction is based on the assumption that the functional potential of a microbial community can be inferred from its taxonomic composition. Functional profiling was further analysed through KEGG Orthology (KO) pathways, and a secondary PLS-DA was performed to evaluate the impact of treatments on microbial metabolic functions. Key functional enzymes and pathways were visualized using heatmap analysis across different treatment groups.

Statistical analysis

The analysis of the relationship between soil nutrient content data and the ten most abundant bacterial genera was carried out using the Partial Least Squares Discriminant Analysis (PLS-DA) method. The analysis process was carried out using the latest version of the MetaboAnalyst platform available online at https://www.metaboanalyst.ca/. The input data used had previously been normalized and log-transformed according to the standard protocol recommended by MetaboAnalyst to ensure the stability and reliability of the multivariate discriminant analysis results.

Adapter and PCR primer sequences from the paired-end reads were eliminated using Cutadapt (Bellemain et al., 2010). DADA2 was employed to rectify sequencing errors, eliminate low-quality sequences, and address chimeric errors (Martin, 2011). The resulting ASV data was used for taxonomic classification, utilising the SILVA (silva_nr99_v138.1) (16S) database. Downstream analysis and visualisations were performed using packages in RStudio (R version 4.2.3) (https://www.R-project.org/) and Krona Tools (https://github.com/marbl/Krona).

Results

Soil nutrient changes

Partial Least Squares Discriminant Analysis (PLS-DA) revealed that the soil (S) and treated soil (TS) samples shared similar chemical properties, as indicated by their close proximity in the PLS-DA score plot (Figure 1). In contrast, the BSF frass (BF) sample displayed markedly different chemical characteristics. The combination of BF with lime and arbuscular mycorrhizal fungi (AMF) had a higher number of exchangeable potassium (K^+), exchangeable magnesium (Mg^{2+}), and base saturation (BS) than other samples (Table 2). Despite BF's elevated pH, it was insufficient to raise the overall soil pH. The high carbon-to-nitrogen (C/N) ratio in BF likely contributed to a slow nutrient release, promoting a prolonged fertilization effect. However, the three-month incubation period was inadequate to enhance immediate nutrient availability. This aligns with findings by Pei et al. (2019), who reported that nitrogen immobilization and release are positively correlated with the C/N ratio.

Table-2. Chemical components of BSF frass (BF), soil (S) and treated soil (TS) after 3-month incubation.

Characteristics	BSF Frass (BF)	Soil (S)	Treated Soil (TS)
Water Content (%)	29.08	-	-
pH	8.3	4.5	4.2
C-org (%)	40.34	1.58	1.53
N Total (%)	2.10	0.18	0.19
N-org (%)	1.02	-	-
C/N	19	-	-
P_2O_5 Bray1 (mg/kg)	-	9.96	11.91
P_2O_5 (mg/kg)	29.400	338	335
K_2O (mg/kg)	49.000	107.5	108
Exc. K^+ (cmol/kg)	195.99	0.43	3.32
Exc. Na^+ (cmol/kg)	116.60	0.18	0.26
Exc. Ca^{2+} (cmol/kg)	30.25	12.63	12.47
Exc. Mg^{2+} (cmol/kg)	6.18	2.93	4.74
Base Saturation (cmol/kg)	311.96	47.50	59.88
CEC (cmol/kg)	83.03	34.04	34.72

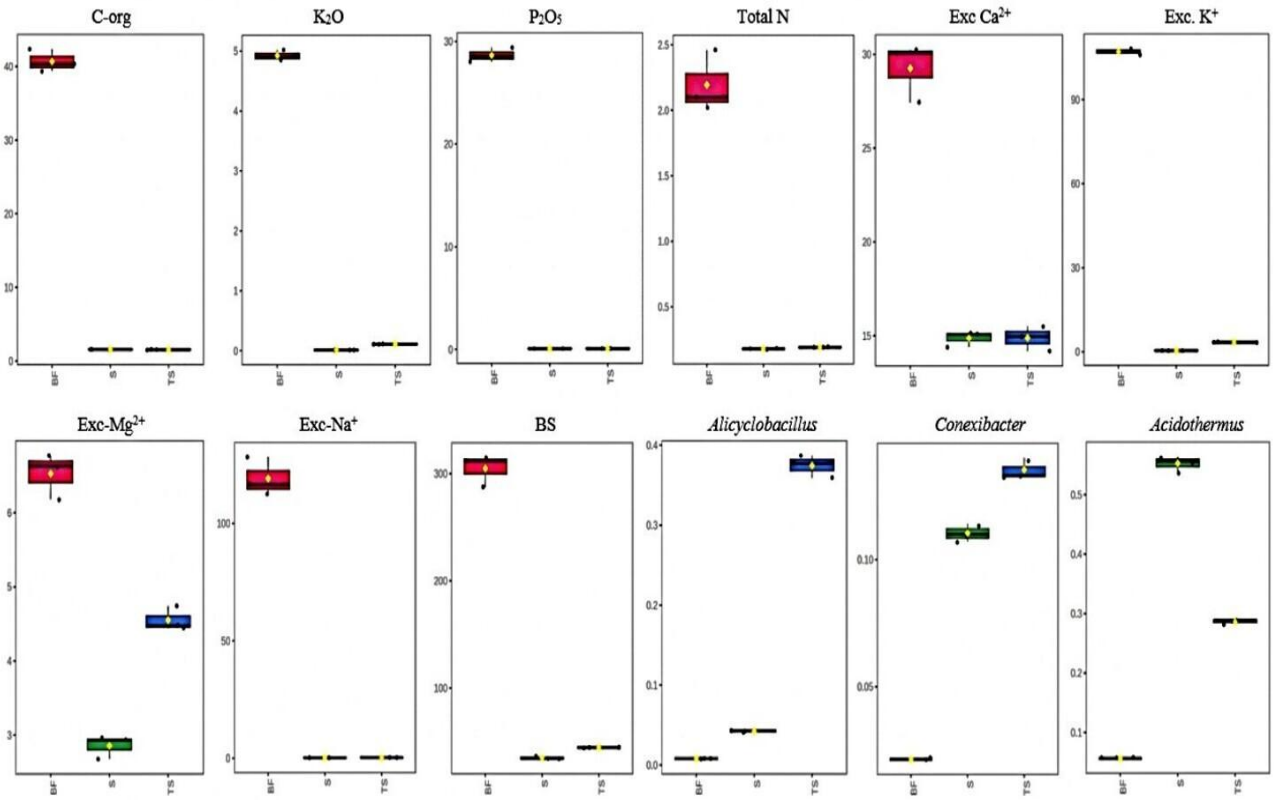

Figure-1. The PLS-DA score plot shows chemical component's and three dominant microbes distribution on BF, S, and TS samples.

Chemical and microbial key variables

Figure 2 presents the results of Partial Least Squares Discriminant Analysis (PLS-DA), illustrating the distinctions among the three groups (S, TS, and BF) based on chemical and microbial parameters (Figure 2a). The analysis also highlights key variables, represented by loading vectors, that contribute significantly to this differentiation (Figure 2b). Soil chemistry variables, exchangeable Na^+, P_2O_5, C-Organic, N-Total, CEC, Base saturation trend to the left, strongly associated with BF. Exchangeable K^+ and K_2O (%) trend to the top left, also associated with BF, but slightly closer to the PC2 axis. This means that BF has very high macro and organic nutrient content. BF excrement is nutrient-dense, comprising N, P, K, organic matter, and chitin derived from larval moulting. Studies demonstrated that BF excrement contains much higher levels of N and K than other insects (Beesigamukama et al., 2022). Moreover, applying liquid organic fertiliser made from BSF frass increased exchangeable Na^+ significantly than the control (Sopha et al., 2025).

Figure 3b illustrates the Variable Importance in Projection (VIP scores) derived from the PLS-DA (Partial Least Squares Discriminant Analysis) analysis. It is utilised to identify the variables that most significantly differentiate the groups (in this case, BF, S, and TS). A VIP score > 1 indicates a substantial contribution to the separation of groups in the model. The higher the value, the stronger the role of the variable in influencing the separation between groups. Soil chemical variables (Exc. Na^+, P_2O_5, K_2O, Exc. K^+, C-Organic, etc.) are the main factors that distinguish the BF group from S and TS. Microbial genera such as *Alicyclobacillus* and *Conexibacter* are essential in distinguishing TS from other groups. This PLS-DA VIP plot provides complementary information that strengthens the previous PLS-DA, biplot, and heatmap results while confirming which variables are the most determinant in the data structure. Soil chemical factors dominate BF. Specific microbial communities dominate TS. S is transitioning, with certain typical microbes and low nutrient levels.

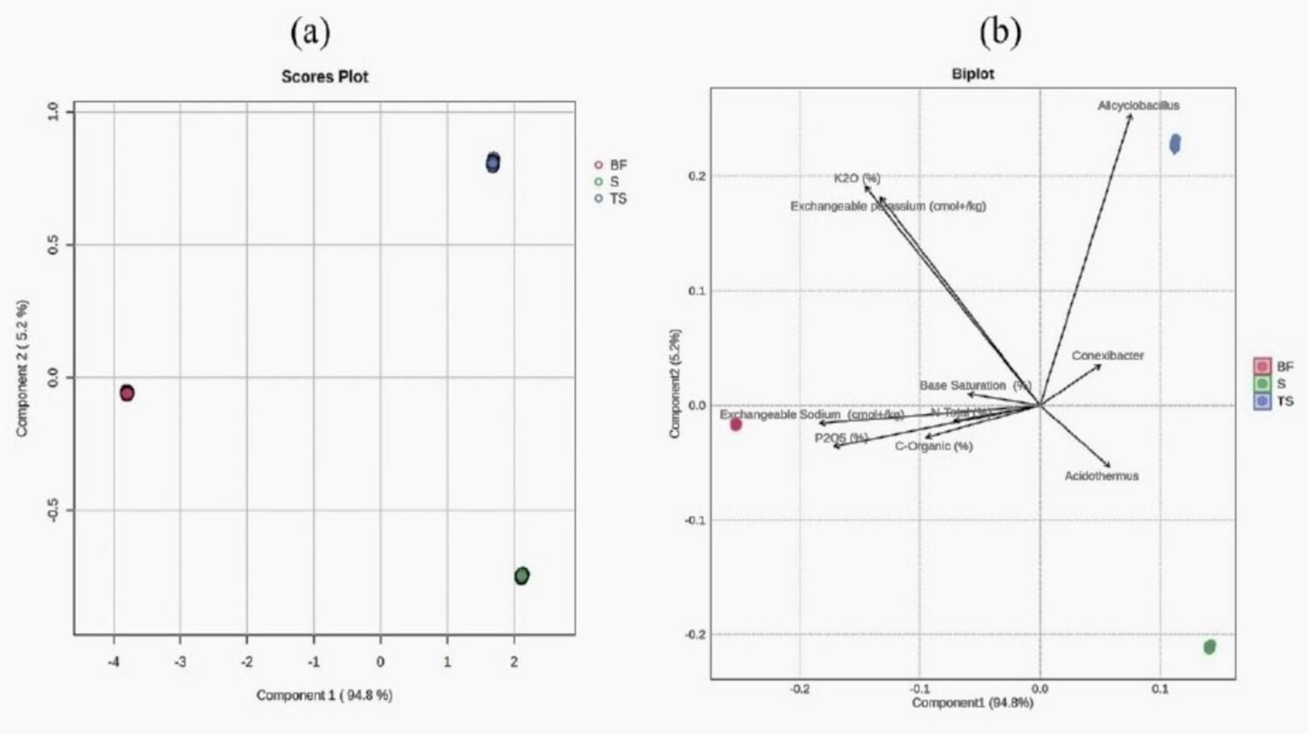

Figure-2. PLS-DA (Partial Least Squares Discriminant Analysis) of BF, S, and TS treatment: (a) differentiation based on physicochemical and microbiological parameters. (b) key variables with loading vectors contribute to the differentiation.

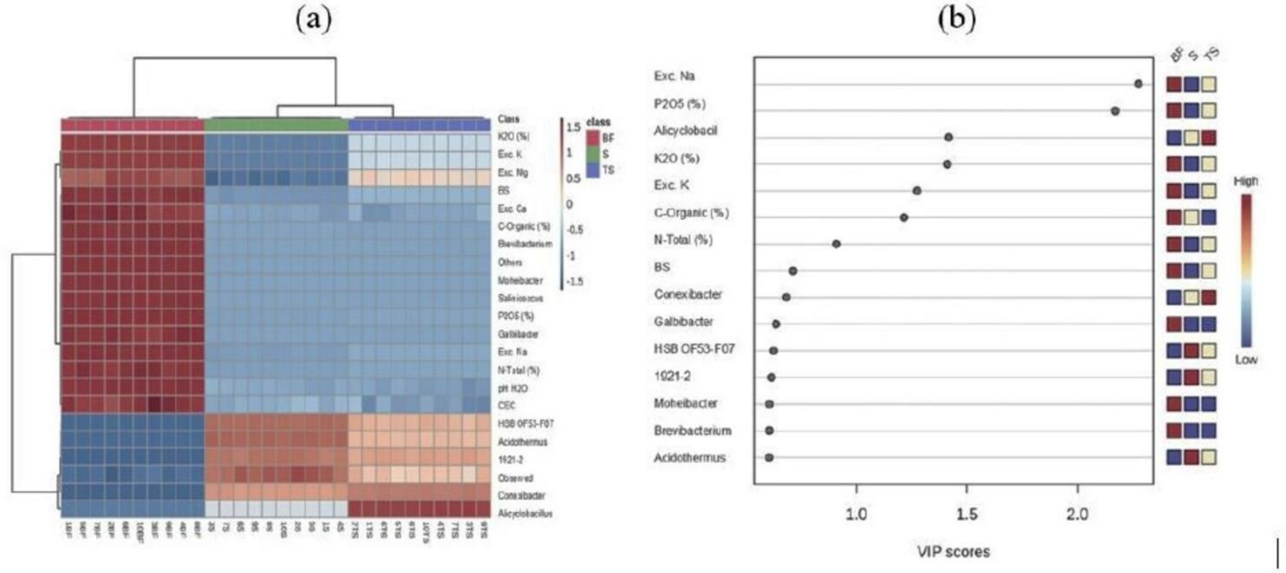

Figure-3. (a) Heatmap with hierarchical clustering and (b) Variable Importance in Projection (VIP scores) from the PLS-DA (Partial Least Squares Discriminant Analysis).

Bacterial diversity

Table 3 show the majority genera in each treatment. The predominant bacterial genera identified in the soil included JG30-KF-AS9 (26%), *Acidothermus* (15%), 1921-2 (5%), *Xanthobacteraceae* (5%), *Conexibacter* (3%), HSB OF53-F07 (3%), and *Ammoniphillus* (2%). In contrast, the ameliorant-treated soil exhibited dominant bacterial genera including JG30-KF-AS9 (29%), *Alicyclobacillus* (14%), *Acidothermus* (11%), 1921-2 (6%), *Xanthobacteraceae* (5%), *Conexibacter* (5%), HSB OF53-F07 (3%), and *Acidibacter* (2%). In BSF frass, the most abundant genera included *Galbibacter* (13%), *Brevibacterium* (8%), *Celvibrioraceae* (6%), *Moheibacter* (5%), *Ganicola* (3%), *Salinicoccus* (3%), *Lentibacillus* (3%), *Orrethobacterium* (3%), *Nocardiopsis* (2%), *Demecuina* (2%), *Pelagibacterium* (2%), *Paracoccus* (2%), *Marinobacter* (2%), *Cyclobacteriaceae* (2%), and *Membranicola* (2%). This variation underscores the impact of various treatments on microbial community composition.

Table-3. Taxa genus taxonomic classification of bacteria found in soil (S), BSF Frass (BF) and ameliorant-treated soil (TS).

No	S		TS		BF	
1	*Acidothermus*	0,430	*Alicyclobacillus*	0,304	*Galbibacter*	0,262
2	1921-2	0,152	*Acidothermus*	0,244	*Brevibacterium*	0,200
3	HSB OF53-F07	0,098	1921-2	0,127	*Salinicoccus*	0,105
4	*Conexibacter*	0,085	*Conexibacter*	0,113	*Moheibacter*	0,097
5	*Ammoniphilus*	0,059	HSB OF53-F07	0,058	*Nocardiopsis*	0,080
6	*Bacillus*	0,039	*Acidibacter*	0,051	*Garicola*	0,068
7	*Candidatus Solibacter*	0,036	FCPS473	0,030	*Ornithobacterium*	0,064
8	*Bryobacter*	0,035	1921-3	0,026	*Lentibacillus*	0,056
9	*Candidatus Koribacter*	0,033	*Sinomonas*	0,025	*Corynebacterium*	0,036
10	*Alicyclobacillus*	0,033	*Occallatibacter*	0,022	*Pelagibacterium*	0,033

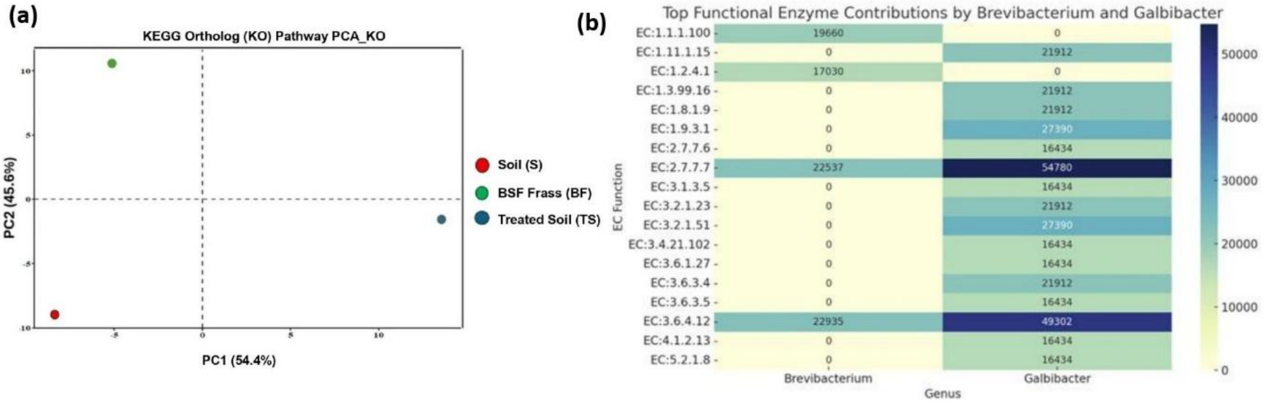

Figure-4. (a) Predictive functional analysis using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) and (b) Heatmap and stacked bar visualizations (X and Y) highlight functional specialization between the two genera.

Figure 4a presents a Principal Component Analysis (PCA) of KEGG Ortholog (KO) pathway profiles from three sample types, illustrating distinct microbial functional compositions among them. The application of BSF frass alters the soil's microbial KO pathway profile, indicating a shift in its functional potential, while Figure 4b presents a heatmap illustrating the contributions of the top functional enzymes by two bacterial genera: *Brevibacterium* and *Galbibacter*.

Figure 4b highlights the major KEGG functions contributed by *Brevibacterium* and *Galbibacter*. Based on taxonomy mapping and PICRUSt2 functional predictions, the bacterial genera *Brevibacterium* and *Galbibacter* made significant contributions to various metabolic pathways within the soil microbial community. *Galbibacter* is primarily predicted to contribute to the activities of nucleotidyltransferase (EC:2.7.7.7), ATP-dependent helicase (EC:3.6.4.12), glycoside hydrolase (EC:3.2.1.51), and oxidoreductase (EC:1.9.3.1), while *Brevibacterium* is predicted to play a more prominent role in oxidoreductase activity (Ec:1.1.1.100).

Amplicon sequence variant

Amplicon metagenomic analysis of the three samples is summarized in Table 4. The Shannon and Simpson indices for the soil (S) and ameliorant-treated soil (TS) samples were relatively similar, indicating comparable microbial diversity. The higher Shannon index observed in the S and TS samples suggests greater microbial diversity than the BSF frass (BF) sample, likely due to a larger number of species with more even abundance distributions.

Table-4. Amplicon metagenomic analysis of in soil (S), BSF Frass (BF) and ameliorant treated soil (TS).

Samples	Observed	Shannon	Simpson	InvSimpson
S	753	5.9443	0.9949	196.0080
BF	408	4.5958	0.9683	31.6416
TS	613	5.5556	0.9903	103.3334

Phylogenetic relationship

Figure 5 illustrates the phylogenetic relationships among bacterial ASVs in the three tested samples. The analysis reveals that the majority of ASVs from soil and ameliorant-treated soil exhibit clustering, indicating a close relationship. In contrast, ASVs derived from BSF frass, such as ASV1, ASV13, ASV39, ASV40, and ASV48, constitute a distinct group, suggesting a closer phylogenetic relationship among them. This diagram highlights the bacterial community present in BSF frass compared to untreated and ameliorant-treated soil.

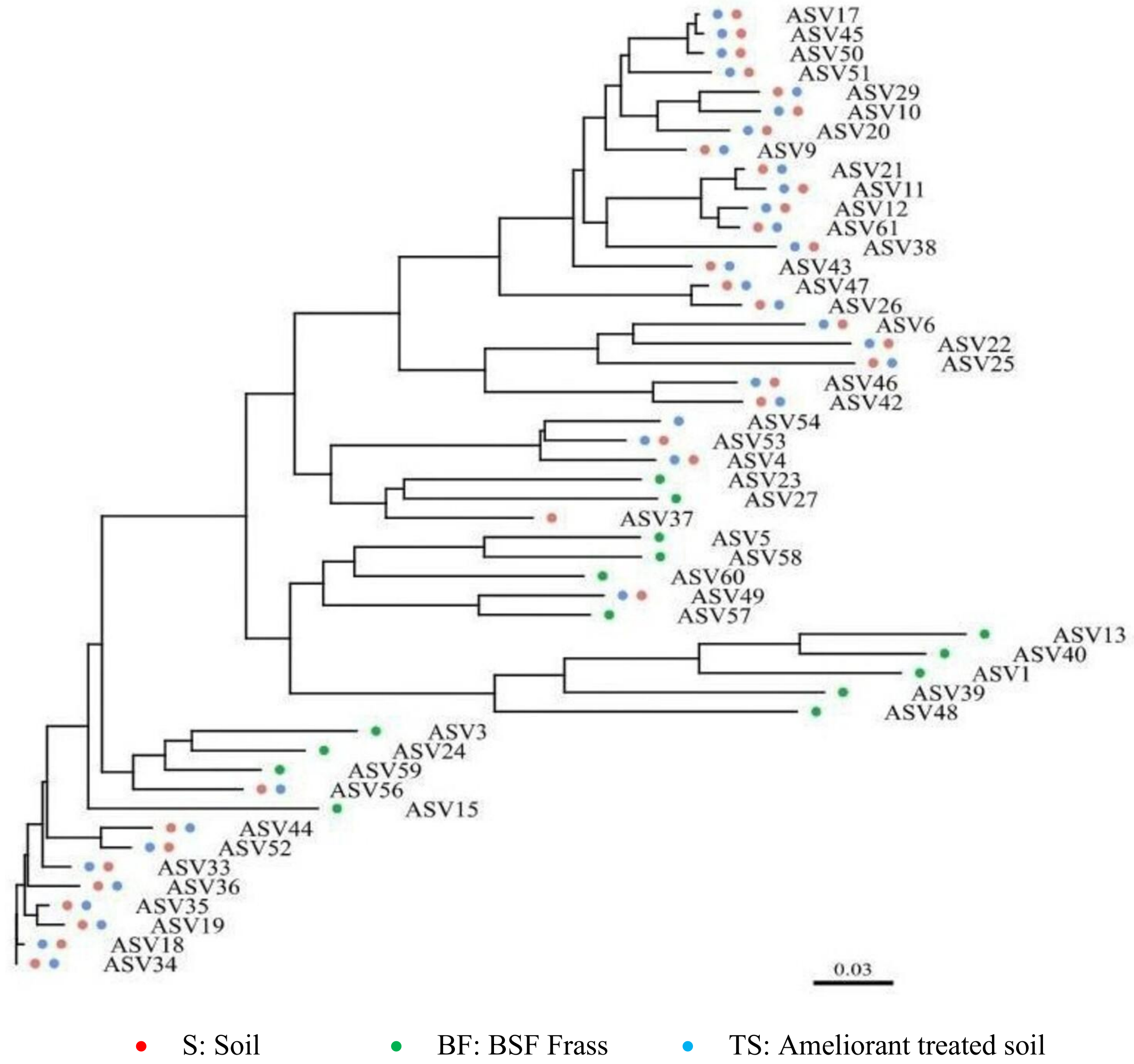

Figure-5. Phylogenetic trees of bacteria in soil (S), BSF frass (BF), and ameliorant treated soil (TS).

Discussion

Figure 1 further illustrates that the BF sample had distinct chemical characteristics compared to both S and TS samples. While S and TS samples showed minimal variation, particularly in exchangeable K^+, Mg^{2+}, and BS, the BF sample exhibited significantly higher exchangeable sodium (Na^+) levels—approximately 110–120 cmol/kg—compared to near-zero levels in the S and TS groups. Exchangeable Na^+ emerged as a key variable distinguishing the BF sample. Moreover, boxplot analyses reinforced the multivariate findings, particularly highlighting the elevated phosphorus content in BF as a defining characteristic.

K_2O is one of the dominant parameters that is a sharp differentiator between the BF treatment and the other two treatments. The potassium content difference can significantly influence microbial responses, especially in sensitive samples or dependent on the availability of macronutrients. In the PLS-DA loadings plot shown in Figure 1, K_2O is also one of the variables located far from the canter, indicating that it has a significant weight in forming the main components. This indicates that K_2O plays a strong role in defining the structure of differences between treatment groups.

The BF sample shows very high exchangeable K^+ levels, with values above 100 cmol/kg. S and TS are much lower, where S is close to zero (seemingly very small), and TS has a slightly higher value than S. BF was chemically very different from S and TS regarding available potassium. This finding reinforces the understanding that BF is a group with a nutrient advantage, which is likely to also impact the microbial community structure. The addition of BSF to the soil can increase K exchange so that it is beneficial for plants, where K becomes available to plants and can be easily exchanged with other ions in the soil cation exchange complex. According to Ragel et al. (2019), K plays an important role in regulating cell osmotic pressure, which controls the opening and closing of stomata. This mechanism affects the efficiency of water use and photosynthesis, especially under water stress conditions.

Conversely, the BF sample exhibited clear separation, indicating divergent chemical characteristics. This differentiation is ascribed to the distinctive composition of BF, the residual by-product produced after BSF larvae digest organic substances. BF is nutrient-dense and comprises larval excreta, undigested feed remnants, moulted exoskeletons, and microbial biomass. The soil utilised in this investigation was a red-yellow podzolic variety, distinguished by elevated levels of iron (Fe) and aluminium (Al), low pH, little organic matter content, and reduced cation exchange capacity (CEC), collectively resulting in its restricted potential to retain and provide nutrients.

The results of this study display distinct differences in the chemical characteristics of BSF frass and soil. These differences are primarily due to their differing origins: frass is derived from the decomposition of organic waste (Siddiqui, et al., 2022), while soil is formed through the weathering of minerals and organic matter (Adhikari et al., 2024). The composition and nutrient content also differ, with frass being richer in nutrients and active organic matter (Bohm et al., 2023). Additionally, the biological processes involved are distinct—frass results from larval activity (Siddiqui et al., 2022), whereas soil forms through long-term natural decomposition (Adhikari et al., 2024).

The results revealed that the application of BSF frass during a 3-month incubation period did not substantially alter soil chemical parameters. This is likely due to the slow decomposition of the organic matter in the frass. The low soil pH may have limited the effectiveness of the frass. The highly acidic soil, combined with its high buffering capacity, likely prevented any substantial pH change. Additionally, the frass dosage may have been insufficient to neutralize soil acidity, and microbial activity was likely suppressed under these acidic conditions (Li et al., 2023), resulting in suboptimal decomposition and nutrient release.

Three dominant microbes, *Alicyclobacillus, Conexibacter*, and *Acidothermus*, are microbial indicators significantly differentiating TS from BF and S groups (Figure 1). Their presence is dominant in TS, intermediate in S, and very low in BF. This pattern supports the role of this genus as a determinant of microbial community structure due to different planting media treatments or soil conditions. It can be used as a biological marker in interpreting metagenomic or soil ecology results. *Alicyclobacillus* is very prominent in the upper right and is closely associated with the TS group, supporting the previous boxplot results. *Conexibacter* is also skewed towards TS, but more towards the centre may favour TS. *Acidothermus* is closer to S, suggesting this genus may be more prevalent in the S group than in TS and BF.

Figure 3a, a heatmap with hierarchical clustering, showing the distribution pattern of variable values (soil chemistry and microbial genera) across samples from three treatment groups: BF (red), S (green), and TS (blue). BF group shows intense red colour in almost all chemical elements such as K_2O, exchangeable K^+, exchangeable Mg^{2+}, C-Organic, P_2O_5, and exchangeable Na^+. This group has consistently high nutrient content. In contrast, the colour is blue for most microbial genera (especially *Alicyclobacillus*, *Conexibacter*, etc.), indicating low presence. TS group, red colour is seen in microbial genera such as *Alicyclobacillus*, *Conexibacter*, HSB OF53-F07, *Acidothermus*, etc. Nutrient values tend to be low (blue colour). This indicates that the microbial community in TS is very different, possibly due to soil conditions that are not too rich in nutrients but support specific microbes. In the S group, in terms of pattern, nutrient values are low, like those of TS, but the microbial composition is in the middle between BF and TS. Some genera, such as *Moheibacter* or *Salinicoccus*, tend to be higher in S.

The row (variable) dendrogram shows two main clusters. The soil chemical variables tend to cluster together (at the top), and the microbial variables cluster alone at the bottom. This confirms that the different treatments affect soil chemistry and change the microbial community's overall structure. The column (sample) dendrogram groups samples by treatment type, showing that each group's responses are very consistent and significantly different between groups. BF has a very high soil chemistry profile for all elements, but with low or different microbial diversity. TS shows a dominance of specific microbes such as *Alicyclobacillus* and *Conexibacter*, but is low in nutrients. S is in the middle, with moderate nutrient levels and microbial composition, not extreme.

The predominant bacterial genera identified in the soil (S) and ameliorant treated soil (TS) *Acidothermus*, *Conexibacter* and *Alicyclobacillus*. *Acidothermus* has been identified as the most abundant genus in both the rhizosphere and endosphere compartments (Berrios et al., 2023), and is closely associated with the biosynthesis and accumulation of bioactive compounds in certain plants (Li et al., 2023). *Acidothermus* is generally classified as a thermophilic and acidophilic bacterium (likes hot and acidic environments) that can degrade cellulose (Wang et al., 2020). The cellulose enzyme produced can decompose plant biomass, so that it can increase soil nutrient content and support plant growth (Lin et al., 2022). These bacteria have the potential to be used in biofertilizers or soil decomposers.

Conexibacter represents a key genus within the bacterial network of primary forests (Pedrinho et al., 2020). *Conexibacter* plays an important role in carbon and nitrogen cycles, particularly in nitrification; however, the abundance of *Conexibacter* is affected by changes in ecosystem (Zhang et al., 2020). The genus *Alicyclobacillus* includes thermo-acidophilic, strictly aerobic, heterotrophic, endospore-forming bacteria (Stackebrandt, 2014), which frequently inhabit orchard soil and places with extreme acidity in the fruit processing industry (Ciuffreda et al., 2015). The ability to thrive in thermal and acidic environments can be attributed to the combination of fatty acids that exist on the cell membrane (Sarma et al., 2023).

Despite the composition of bacteria in S and TS, the bacteria present in BF plays an important role in organic matter degradation. *Galbibacter* typically exists in marine environments and is able to decompose complex organic matter and therefore contributes to nutrient cycling (Wei et al., 2023). *Brevibacterium* occurs in a variety of environments, including soil where it facilitates the decomposition of organic matter. Some strains of *Brevibacterium* produced antimicrobial components and can be considered for applications in bioremediation (Kalsoom et al., 2020). Some species of *Moheibacter* can be found in both soil and waterbodies where it plays a role in organic matter turnover and nutrient cycling (Liu et al., 2024; Zhao et al., 2024). *Salinicoccus* is a genus capable to grow in very high pH (Kiledal et al., 2021).

Figure 4b indicates that *Galbibacter* is mostly predicted to contribute to the activity of enzymes involved in the decomposition of organic matter, such as glycoside hydrolases (EC:3.2.1.51), and oxidoreductase (EC:1.9.3.1), while *Brevibacterium* is predicted to have a greater involvement in oxidoreductase activity (Ec:1.1.1.100). Jin et al. (2020) reported that *Brevibacterium* produces carbohydrate-active enzymes (CAZymes), a group of enzymes involved in the breakdown of complex carbohydrates (Cantarel et al., 2008).

Glycoside hydrolase is an enzyme that catalyses the hydrolysis of glycosidic bonds between two sugar units (carbohydrates) or between a sugar and a non-carbohydrate compound (aglycone). It plays a crucial role in the decomposition of organic matter, particularly by breaking down complex polysaccharides into simpler sugars (Davies and Henrissat, 1995; Withers, 2001). Oxidoreductase enzymes play a vital role in the decomposition of organic matter, particularly by oxidizing complex and recalcitrant compounds such as lignin (Kirk and Farrell, 1987; Bugg et al., 2011). In the absence of these enzymes, microbial decomposition would proceed significantly more slowly and with reduced efficiency

The microbial community was more diverse than the original acid soil whilst still having some traits from BSF frass. The acid tolerant microbes were predominantly in the acid soil, while the BSF frass samples showed a more equally distributed microbial community with added microbes that break down all of the organic matter rich in protein. The ameliorant treated soil had a more complex microbial community, having more plant beneficial microbes compared to the acid soil. Acidified soil will increase the abundance of bacteria that thrive in low pH (Shi et al., 2021; Guan and Liu, 2020), while the high organic matter in BSF frass will support the abundance of organic matter decomposers (Raza et al., 2023; Xu et al., 2024; Séneca et al., 2021).

The addition of ameliorants (BSF frass, lime, AMF) changed the dominant bacterial genera in the soil. In untreated soil, the dominant genera were JG30-KF-

AS9 (26%), *Acidothermus* (15%), 1921-2 (5%), *Xanthobacteraceae* (5%), *Conexibacter* (3%), HSB OF53-F07 (3%) and *Ammoniphilus* (2%). After the ameliorant was added, the dominant genera were JG30-KF-AS9 (29%), *Alicyclobacillus* (14%), *Acidothermus* (11%), 1921-2 (6%), *Xanthobacteraceae* (5%), *Conexibacter* (5%), HSB OF53-F07 (3%), and *Acidibacter* (2%). The major difference we noted was the greater dominance of the genus *Alicyclobacillus* after the ameliorant treatment. The genus *Alicyclobacillus* has the potential to survive acidic conditions and high temperatures (Bevilacqua et al., 2025; Tyfa et al., 2015).

A metagenomic analysis identified 753 unique bacterial amplicon sequence variants (ASVs) in the soil, 408 ASVs in BSF frass and 613 ASVs in ameliorant-treated soil. Our analysis of bacterial diversity and abundance indicated that soil and ameliorant-treated soil had greater diversity and more uniform distribution than BSF frass. Overall, the type of conditions present in the growth environment play a critical role in determining which types of bacteria grow (Gonzalez and Aranda, 2023). The evidence indicates; however, that untreated and ameliorant-treated soil host more ASVs, and reflect a more balanced microbial community. Conversely, when you consider total ASVs, BSF frass had a lower Simpson Index than soil or ameliorant-treated soil indicating a limited number of ASVs dominated BSF frass as associated with the conditions of the environment. When it comes to soil, it supported a more diverse microbial community that was more evenly distributed than BSF frass.

Bacterial ASV quantity and diversity were greater in soil (S) and ameliorant-treated soil (TS) than in BSF frass (BF). Bacterial abundance in soil represented a more uniform distribution whereas BSF frass abundance was represented by few bacterial species contributing to high abundance. These factors impacted by the distinct ecological conditions of soil and BSF frass such that soil represents a diverse microbial habitat, bacterial diversity includes all bacteria that play a possible role in nutrient cycling, decomposition of organic matter, and plant health. Bacterial diversity hinges on several factors such as soil pH, moisture, temperature, and organic matter content, but also interactions with higher life forms including plants and other microorganisms (Zhou et al., 2024; Zheng et al., 2019; Philippot et al., 2024). BSF frass, as previously stated, is a product of the ecosystem of decomposing organic material whereby a homogenous condition is created for specific bacteria to proliferate. Roller and Schmidt (2015) illustrated that when conditions were homogenous, organisms recognized as rapidly growing organisms were favourable.

Phylogenetic analysis identified BSF frass ASVs as a separate cluster from those found in soil. The unique environmental conditions created in BSF frass, which are mainly organic matter remnants, support the growth of organic matter degrading bacteria. When applied as an ameliorant, only a small subset of organic matter-degrading bacteria inhabit ameliorant-treated soil; this suggests that the degradation of organic matter by the soil bacteria faced challenges surviving in acidic soils with low organic carbon concentrations.

The Variable Importance in Projection (VIP) scores from the PLS-DA, shown in Figure 3b, highlight differences in the contribution of bacterial groups to group separation. For instance, *Alicyclobacillus* had a VIP score of 1.4, while *Acidothermus* scored 0.6. Microbes or species with high VIP scores typically play a key role in differentiating between groups and can be located within the phylogenetic tree (Figure 5). The BSF frass significantly altered the structure of the soil microbial community and the nutrient profile, as indicated by the treatment-specific clustering of bacterial ASVs in phylogenetic analysis. When these influential microbes cluster within a specific taxonomic clade, the grouping in the tree may reflect underlying biological trends—such as a particular clade being more abundant in one condition.

This study demonstrated that the combination of BSF frass (BF) with lime and AMF resulted in higher levels of exchangeable potassium (K^+), exchangeable magnesium (Mg^{2+}), and base saturation (BS), without significantly affecting soil pH. The bacterial community profile in the BSF frass differed markedly from that in the soil, with BF containing ASVs such as *Galbibacter*, *Brevibacterium,* members of *Celvibrioraceae, and Moheibacter*—reflecting a distinct microbial composition linked to the unique chemical characteristics of the frass. The results were obtained using BSF reared on restaurant waste. Frass was applied at 5 g, together with 5 g of lime and 2.5 g of AMF per 1,000 g of soil, and the mixture was incubated for three months at room temperature (30 °C) under field capacity moisture.

Conclusions

The findings of this study suggest that BSF frass has distinct chemical properties and microbial communities relative to untreated soils. The organic matter content in BSF frass supports the growth of many particular microbes involved in the decomposition of organic material. Ameliorant application to acidic soil significantly increased exchangeable potassium (K^+), magnesium (Mg^{2+}) and base saturation (relative to before treatment), as well as altered the composition and abundance soil microbial communities.

Metagenomic sequencing confirmed clear differences in bacterial diversity and composition of bacterial communities from each sample. A total of 753 amplicon sequence variants (ASVs) were identified in soil (S), 408 in BSF frass (BF), and 613 in treated soil (TS). Bacterial ASVs were dominated by JG30-KF-AS9, *Acidothermus* and *Xanthobacteraceae* family in the soil sample. These bacterial ASVs in the treated soil were similar but differed in relative abundance (JG30-KF-AS9, *Alicyclobacillus*, *Acidothermus* and 1921-2), where *Alicyclobacillus* had a significant increase from 1% relative abundance to 14% relative abundance from the untreated soil (S) to the treated soil (TS).

Interestingly, the bacterial community profile that we found in BSF frass was different from that found in the soil samples. BF included ASVs of *Galbibacter*, *Brevibacterium*, *Celvibrioraceae* and *Moheibacter*, which was a distinct community profile representing a different chemical composition of the frass.

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Ethical Approval Statement

We ensure that all research conducted respects participant confidentiality, follows ethical guidelines, and contributes to the academic community with honesty and integrity.

Contribution of Authors

Darwati I, Ginting RCB, Yusron M, Wardhana AH, Sopha GA & Trisilawati O: Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, software, writing, review and editing.

Rusmin D, Garvita RV, Gunadi N, Suryadi R, Lestari IP & Haryati Y: Data curation, funding acquisition, investigation, writing, review and editing.

Hamdan KK, Cartika I, Marpaung AEB: Funding acquisition, writing, review and editing.

Adiwijaya HD & Lusiana: Methodology, writing, review and editing.

All authors read and approved the final draft of manuscript.

References

Adhikari K, Lalitha M, Dharumarajan S, Kaliraj S, Chakraborty R and Kumar N, 2024. Chapter 1 - Introduction to soils: soil formation, composition, and its spatial distribution, pp. 3-11. In S. Dharumarajan, S. Kaliraj, K. Adhikari, M. Lalitha, N. Kumar (eds). Remote Sensing of Soils, Elsevier.

Beesigamukama D, Mochoge B, Korir NK, Berresaw MK, Muriithi B, Kidoido M, Kirscth H, Diiro G, Ghemoh CJ, Subramanian S, Nakimbugwe D, Musyoka M, Ekesi S and Tanga C, 2022. Economic and ecological values of frass fertiliser from black soldier fly agroindustrial waste processing. J. Insects Food Feed. 8(3): 245-254.

Bellemain E, Carlsen T, Brochmann C, Coissac E, Taberlet P and Kauserud H, 2010. ITS as 2. an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. BMC Microbiol. 10: 1-9.

Berrios L, Yeam J, Holm, L, Robinson, W, Pellitier, PT, Chin ML, Henkel, TW and Peay KG, 2023. Positive interactions between mycorrhizal fungi and bacteria are widespread and benefit plant growth. Curr. Biol. 33: 2878-2887.

Bevilacqua A, Corbo MR and Sinigaglia M, 2025. Chapter 6 - *Alicyclobacillus* spp.: a spore-forming and spoiling microorganism with thermoacidophilic traits. pp. 145-155. In A. Bevilacqua, M.R. Corbo, and M. Sinigaglia (eds). Woodhead Series in Food Science, Technology and Nutrition, The Microbiological Quality of Food (Second Edition), Elsevier Science Ltd.

Bohm K, Hatley GA, Robinson BH, and Gutiérrez-Ginés MJ, 2023. Analysis of chemical and phytotoxic properties of frass derived from black soldier fly-based bioconversion of biosolids. Sustainability. 15(15): 11526.

Bray RH and Kurtz LT, 1945. Determination of total organic and available forms of phosphorus in soils. Soil Sci. 59: 39-45.

Bremner JM and Mulvaney CS, 1982. Nitrogen-Total. In: Methods of soil analysis. Part 2. Chemical and microbiological properties, pp. 595-624. A.L. Page, R.H. Miller and D.R. Keeney (eds.). Am. Soc. Agron., Soil Sci. Soc. Am. Madison, Wisconsin,

Bugg TD, Ahmad M, Hardiman EM and Singh R, 2011. The emerging role for bacteria in lignin degradation and bio-product formation. Curr. Opin. Biotechnol. 22(3): 394-400.

Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V and Henrissat B, 2008. The Carbohydrate-Active EnZymes database (CAZy): an expert resource for Glycogenomics. Nucleic Acids Res. 37(Database issue): D233-D238.

Ciuffreda E, Bevilacqua A, Sinigaglia M and Corbo MR, 2015. *Alicyclobacillus* spp.: New insights on ecology and preserving food quality through new approaches. Microorganisms 3: 625-640.

Dariah A, Nurida NL, Salma S, Nurjaya and Santi LP, 2021. The use of soil ameliorants to improve soil quality and crop productivity of degraded semi-arid upland in Gunung Kidul. Yogyakarta. Indonesia. IOP Conf. Ser. Earth Environ. Sci. 648: 012159.

Davies GJ and Henrissat B, 1995. Structures and mechanisms of glycosyl hydrolases. Structure 3(9): 853-859.

Gonzalez JM and Aranda B, 2023. Microbial growth under limiting conditions-future perspectives. Microorganisms. 11(7): 1641.

Guan N and Liu L, 2020. Microbial response to acid stress: mechanisms and applications. Appl. Microbiol. Biotechnol. 104: 51-65.

Herawati A, Syamsiyah J, Mujiyo M, Rochmatulloh M, Susila AA and Romadhon MR, 2021. Mycorrhizae and a soil ameliorant on improving the characteristics of sandy soil. ST-JSSA 18(1): 73-80.

Ismail SM, Almulhim N, Sedky A, El-Cossy SAN and Mahmoud E, 2025. Impact of soil ameliorants on soil chemical characteristics, sugar beet water productivity, and yield components in sandy soils under deficit irrigation. Sustainability. 17(4): 1513.

Jin M, Zhao Q, Zhou Z, Zhu L, Zhang Z and Jiang L, 2020. Draft genome sequence of a potential organic phosphorus-degrading bacterium *Brevibacterium frigoritolerans* GD44, isolated from radioactive soil in Xinjiang, China. Curr. Microbiol. 77: 2896-2903

Kalsoom A, Batool R and Jamil N, 2020. An integrated approach for safe removal of chromium (vi) by *Brevibacterium* sp. Pak. J. Sci. 72(1): 18-24.

Kiledal EA, Keffer JL and Maresca JA, 2021. Bacterial communities in concrete reflect its composite nature and change with weathering. mSystems. 6(3). Doi: 10.1128/msystems.01153-20.

Kirk TK and Farrell RL, 1987. Enzymatic 'combustion": the microbial degradation of lignin. Annu. Rev. Microbiol. 41: 465-501.

Li L, Yang X, Tong B, Wang D, Tian X, Liu J, Chen J, Xiao X and Wang S, 2023. Rhizobacterial compositions and their relationships with soil properties and medicinal bioactive ingredients in *Cinnamomum migao.* Front. Microbiol. 14: 1078886.

Lin W, Liu L, Liang J, Tang X, Shi J, Zhang L, Wu P, Lan S, Wang S, Zhou Y, Chen X, Zhao Y, Chen X, Wu B and Guo L, 2022. Changes of endophytic microbial community in *Rhododendron simsii* roots under heat stress and its correlation with leaf physiological indicators. Front. Microbiol. 13: 1006686.

Liu N, Liu Z, Wang K, Zhao J, Fang J, Liu G, Yao H and Pan J, 2024. Comparison analysis of microbial agent and different compost material on microbial community and nitrogen transformation genes dynamic changes during pig manure compost. Bioresour. Technol, 395: 130359.

Li X, Chen D, Carrión VJ, Revillini D, Yin S, Dong Y, Zhang T, Wang X and Delgado-Baquerizo M, 2023. Acidification suppresses the natural capacity of soil microbiome to fight pathogenic Fusarium infections. Nat. Commun. 14: 5090.

Lopes IG, Yong JWH and Lalander C, 2022. Frass derived from black soldier fly larvae treatment of biodegradable wastes. A critical review and future perspectives. Waste Manag. 142: 65-76.

Martin M, 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet. J. 17(1): 10-12.

Maswar, Firmansyah A, Haryati U and Irawan, 2021. The effect of ameliorant on peat soil properties and shallots productivity in peatlands. IOP Conf. Ser. Earth Environ. Sci. 648: 012057.

Matfu'ah E, Nurzakiah S, Sulaeman Y and Lestari Y, 2023. Use of Humic and Silica Materials as Soil Ameliorant to Improve the Chemical Properties of Acid Sulphate Soil. IOP Conf. Ser. Earth Environ. Sci. 1162: 012002.

Mon WW, Toma Y and Ueno H, 2024. Combined Effects of Rice Husk Biochar and Organic Manures on Soil Chemical Properties and Greenhouse Gas Emissions from Two Different Paddy Soils. Soil Syst. 8: 32.

Mulatu G and Bayata A, 2024. Vermicompost as Organic Amendment: Effects on Some Soil Physical. Biological Properties and Crops Performance on Acidic Soil: A Review. Front. Environ. Microbiol. 10(4): 66-73.

Murtiningsih R, Sopha GA, Marpaung AE, Tan SS, Cartika I, Lestari IP, Amisnaipa, Rawung JBM, Hamdani KK, Gunadi N, Indrasti R, Haryati Y, Handayani T, Khaririatun N and Waluyo N, 2023. Organic materials to enhance climate change resilience on shallot production: a review. IOP Conf. Ser. Earth Environ. Sci. 1165: 012036.

Oulas A, Pavloudi C, Polymenakou P, Pavlopoulos GA, Papanikolaou N, Kotoulas G, Arvanitidis C and Iliopoulos I, 2015. Metagenomics: tools and insights for analyzing next-generation sequencing data derived from biodiversity studies. Bioinform. Biol. Ins. 5(9): 75-88.

Pedrinho A, Mendes LW, Merloti LF, Andreote FD and Tsai SM, 2020. The natural recovery of soil microbial community and nitrogen functions after pasture abandonment in the Amazon region, FEMS Microbiol. Ecol. 96(9): fiaa149.

Pei G, Liu J, Peng B, Gao D, Wang C, Dai W and Jiang P, 2019. Edith Baia Nitrogen, lignin, C/N as important regulators of gross nitrogen release and immobilization during litter decomposition in a temperate forest ecosystem. Forest. Ecol. Manage. 440: 61-69.

Philippot L, Chenu C, Kappler A, Rillig MC and Fierer N, 2024. The interplay between microbial communities and soil properties. Nat. Rev. Microbiol. 22: 226-239.

Poveda J, Jim´enez-G´omez A, Saati-Santamaría Z, Usategui-Martín R, Rivas R and García-Fraile P, 2019. Mealworm frass as a potential biofertilizer and abiotic stress tolerance-inductor in plants. Appl. Soil Ecol. 142: 110-122.

Ragel P, Ródenas R, García-Martínez JL and Andrés Z, 2019. Role of potassium transport systems in plant responses to abiotic stress. Int. J. Mol. Sci. 20: 2279.

Raza T, Qadir MF, Khan KS, Eash NS, Yousuf M, Chatterjee S, Manzoor R, ur Rehman S and Oetting JN, 2023. Unraveling the potential of microbes in decomposition of organic matter and release of carbon in the ecosystem. J. Environ. Manage. 344: 118529.

Roller B and Schmidt T, 2015. The physiology and ecological implications of efficient growth. ISME J. 9: 1481–1487.

Sarma J, Sengupta A, Laskar MK, Sengupta S, Tenguria S and Kumar A, 2023. Chapter 13 - Microbial adaptations in extreme environmental conditions, pp. 193-206. In A. Kumar, and S. Tenguria (Eds). Developments in Applied Microbiology and Biotechnology, Bacterial Survival in the Hostile Environment, Academic Press.

Séneca J, Söllinger A, Herbold CW, Pjevac P, Prommer J, Verbruggen E, Sigurdsson BD, Peñuelas J, Janssens IA, Urich T, Tveit AT and Richter A, 2021. Increased microbial expression of organic nitrogen cycling genes in long-term warmed grassland soils. ISME Commun. 1: 69.

Shi Y, Li Y, Yang T and Chu H, 2021. Threshold effects of soil pH on microbial co-occurrence structure in acidic and alkaline arable lands. Sci. Total Environ. 800: 149592.

Siddiqui SA, Ristow B, Rahayu T, Putra NS, Yuwono NW, Nisa' K, Mategeko B, Smetana S, Saki M, Nawaz A and Nagdalian A, 2022. Black soldier fly larvae (BSFL) and their affinity for organic waste processing, Waste Manage. 140: 1-13.

Sopha GA, Murtiningsih R, Cartika I, Hamdani KK, Handayani T, Lestari IP, Haryati Y, Indrasti R, Marpaung AE Br., Rawung JBM, Gunadi N, Amisnaipa A, Adi EBM, Priadi D, Tan SS and Surono S, 2025. Improve yield and quality of tropical vegetables, shallot, and red hot chilli peppers. Int. J. Hortic. Sci. Technol. 12 (2): 479-490.

Stackebrandt E, 2014. The Family *Alicyclobacillaceae* . In: E. Rosenberg, E.F. DeLong, S. Lory, E. Stackebrandt, F. Thompson, (eds) The Prokaryotes. Springer, Berlin, Heidelberg.

Tay TKY, Tan GS, Lee SH, Sam XX, Lim TH, Kiat Ng JW, Tan DSW and Lim TKH, 2024. Comparison of an amplicon-based large panel next generation sequencing (NGS) assay with conventional testing methods for MET and HER2 amplification in lung and breast cancers. Pathology 56(3): 325-333.

Thomas GW, 1982. Exchangeable cations. pp. 159-165. In A.L. Page (eds.), Methods of Soil Analysis, Part 2, 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Tyfa A, Kunicka-Styczyńska A and Zabielska J, 2015. Evaluation of hydrophobicity and quantitative analysis of biofilm formation by *Alicyclobacillus* sp. Acta Biochim. Pol. 62(4): 785-790.

Walkley AJ and Black IA, 1934. Estimation of soil organic carbon by the chromic acid titration method. Soil Sci. 37: 29-38.

Wang L, Wang LA, Zhan X, Huang Y, Wang J and Wang X, 2020. Response mechanism of microbial community to the environmental stress caused by the different mercury concentration in soils. Ecotoxicol. Environ. Saf. 188: 109906.

Wei L, Wang J, Liu X, Lai Q, Li G, Fu X, Li G, Shao Z and Dong C, 2023. *Galbibacter pacificus* sp. nov., isolated from surface seawater of the western Pacific Ocean and transfer of *Joostella marina* to the genus *Galbibacter* as *Galbibacter orientalis* nom. nov. and emended description of the genus *Galbibacter*. Int J. Syst. Evol. Microbiol. 73(20). doi: 10.1099/ijsem.0.006078.

Withers SG, 2001. Mechanisms of glycosyl transferases and hydrolases. Carbohydr. Polym. 44(4): 325-337.

Xu Q, Li L, Guo J, Guo H, Liu M, Guo S, Kuzyakov Y, Ling N and Shen Q, 2024. Active microbial population dynamics and life strategies drive the enhanced carbon use efficiency in high-organic matter soils. mBio 15: e00177-24.

Zhang S, Zhu Q, de Vries W, Ros GH, Chen X, Muneer MA, Zhang F and Wu L, 2023. Effects of soil amendments on soil acidity and crop yields in acidic soils: A world-wide meta-analysis. J. Environ. Manage. 345: 118531.

Zhang X, Gao G, Wu Z Wen X, Bian F and Yang C, 2020. Biochar-based organic fertilizer application rates for *Tetrastigma hemsleyanum* planted under Moso bamboo. J. Forest. Res. 31: 1813–1821.

Zhao J, Wang X, Liu Z, He L, Jiang H, Yao H, Fang J and Liu, G, 2024. Metagenomics analysis of the impact of protein-degrading functional microbial agents on composting of chicken manure from cereal hulls. Agronomy 14(8): 1675.

Zheng Q, Hu Y, Zhang S, Noll L, Böckle T, Dietrich M, Herbold GW, Eichorst SA, Woebken D, Richter A and Wanek W, 2019. Soil multifunctionality is affected by the soil environment and by microbial community composition and diversity. Soil Biol. Biochem. 136: 107521.

Zhou X, Tahvanainen T, Malard L, Chen L, Pérez-Pérez J and Berninger F, 2024. Global analysis of soil bacterial genera and diversity in response to pH. Soil Biol. Biochem. 198: 109552.