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HAL Authorization

2 **Comparative effects of black soldier fly frass, poultry litter, and NPK fertilizer on plant growth, soil**
3 **microbial activity and earthworm biomass**

4 Authors (first name – last name):

5 Lucile Mirabello¹, Anne-Maïmiti Dulaurent¹, Céline Roisin¹, Benjamin Albert² and David Houben^{1*}

6 Affiliation :

7 ¹UniLaSalle, AGHYLE UP 2018.C101, 60000 Beauvais, France.

8 ²Innovafeed SAS, route de Chaulnes, lieudit « Les Trente », 80190 Nesle, France.

9 * Corresponding author:

10 David Houben ; E-mail: david.houben@unilasalle.fr; Phone: 0033 (0)3 44 06 93 45

11
12 **Abstract**

13 The increasing demand for protein has led to the development of insect farming as a sustainable and
14 circular solution. This generates large quantities of frass (insect excreta), which has potential as an
15 organic fertilizer. However, its effects on the soil–plant system remain underexplored. This study
16 investigates the potential of black soldier fly (BSF) (*Hermetia illucens* L.) frass as an alternative fertilizer
17 by assessing its impact on ryegrass (*Lolium multiflorum*) growth, nutrient uptake, and soil biota in
18 comparison to mineral NPK fertilizer and poultry litter. A greenhouse pot experiment was conducted
19 with four treatments: control, poultry litter and BSF frass applied at 5 t ha⁻¹, and NPK fertilizer applied
20 at an equivalent nutrient level to frass. Plant biomass production, nutrient uptake, microbial activity,
21 and earthworm biomass were measured across two harvests. At the first plant harvest, biomass
22 production was similar across frass, NPK, and poultry litter treatments. By the second harvest, NPK
23 fertilizer produced the highest biomass, followed by poultry litter and frass. Nitrogen uptake was
24 significantly higher in the frass treatment during the second harvest (1.5 times higher than in NPK
25 treatment), though phosphorus and potassium uptake were lower, likely limiting biomass production.
26 Frass-treated soils exhibited the highest microbial activity, likely due to the presence of chitin
27 stimulating chitinolytic microbial communities. Additionally, earthworm biomass was significantly
28 higher in frass-treated soils, possibly due to increased microbial activity and organic matter. Thus, BSF
29 frass can enhance soil health by stimulating microbial and earthworm activity but supplemental
30 nutrients may be required to optimize plant growth, especially for nutrient-demanding crops. This
31 study highlights the potential of frass as an organic fertilizer while identifying limitations that should
32 be addressed for its effective agricultural application.

33 **Keywords:** Black soldier fly frass; Poultry litter; Organic fertilizer; Soil microbial activity; Nutrient
34 cycling; Earthworm

35 **Introduction**

36 The ever-increasing global demand for agricultural production requires the development of sustainable
37 and circular practices to maintain soil fertility and crop yields. Conventional chemical fertilizers have
38 played a pivotal role in the dramatic increase in agricultural productivity over the past century. The
39 Haber-Bosch process, in particular, has been considered one of the most important technological
40 advances of the 20th century, contributing significantly to global food security by enabling large-scale
41 nitrogen fertilizer production (Erisman et al. 2008). However, the extensive use of these fertilizers also
42 poses significant environmental challenges (Tilman et al. 2002). In addition to requiring high energy
43 inputs for their production, the resources needed for phosphorus (P) or potassium (K) fertilizers are
44 mainly derived from finite and increasingly scarce rocks, raising concerns about the long-term
45 sustainability of these fertilizers (Dawson and Hilton 2011). These adverse effects highlight the urgent
46 need for complementary or alternative fertilization strategies that can sustainably enhance soil fertility
47 while mitigating environmental impacts (Faucon et al. 2015; Amadou et al. 2021).

48 One existing alternative involves the utilization of organic by-products as alternatives to conventional
49 fertilizers (Nobile et al. 2022). Organic materials such as poultry litter and, more recently, insect frass
50 have sparked attention for their potential to improve soil health and fertility. Poultry litter is rich in
51 essential nutrients and organic matter, contributing to improved soil fertility and microbial activity
52 (Bolan et al. 2010). Insect frass, particularly from mealworm (*Tenebrio molitor*) and black soldier fly
53 larvae (BSF; *Hermetia illucens*), is another emerging organic bioresource in industrialized countries due
54 to its nutrient-rich composition (Beesigamukama et al. 2020; Poveda 2021; Foscari et al. 2024). Frass is
55 defined as insect excrement, but in the context of the insect farming industry, it refers to a mixture of
56 mostly insect faeces and leftover exoskeletons (Barragán-Fonseca et al. 2022). The emergence of frass
57 as a potential fertilizer is closely linked to the development of insect farming which is gaining significant
58 interest as a sustainable and circular solution to meet the increasing demand for protein in animal feed

59 and human diets (Madau et al. 2020; van Huis 2020). The by-products of insect farming, including frass,
60 present a valuable opportunity to recycle nutrients and organic matter back into agricultural systems
61 (Houben et al. 2020). Although interest in the effects of frass within the soil-plant system is growing
62 (Watson et al. 2022), the available literature remains relatively limited compared to other organic
63 amendments. Studies suggest that it could have similar effect than other types of manures, especially
64 chicken manure and poultry litter (Chavez and Uchanski 2021; Borkent and Hodge 2021), only a few
65 studies have directly compared it to other organic fertilizers (Hénault-Ethier et al. 2023). In addition,
66 while most of research on the effects of frass has focused on plant responses, soil chemical properties
67 and to a lesser extent specific biological groups such as arbuscular mycorrhizal fungi (Salomon et al.
68 2025), its effects on soil biota and, especially, soil fauna, has been disregarded so far (Dulaurent et al.
69 2020).

70 Understanding the effects of these organic bioresources on soil organisms, particularly earthworms, is
71 crucial for evaluating their overall impact on soil fertility. Earthworms are key bioindicators and
72 ecosystem engineers in soil environments, playing key roles in nutrient cycling, soil aeration, and
73 organic matter decomposition (Blouin et al. 2013; Dulaurent et al. 2024). The interactions between
74 earthworms and organic amendments can significantly influence the efficacy of these fertilizers, as
75 earthworms can affect nutrient availability and soil structure through their burrowing and feeding
76 activities (Waqar et al. 2019; Honvault et al. 2023). On the other hand, studies report that organic
77 fertilizers generally increase earthworm abundance and biomass compared to mineral fertilizers or
78 unfertilized controls, mainly due to the additional organic matter and microbial stimulation they
79 provide (Brown et al. 2000; Blouin et al. 2013). Therefore, it is essential to investigate how organic
80 fertilizers such as poultry litter and insect frass impact earthworms.

81 This study aims to better understand the effects of frass on the soil-plant system, including soil biota,
82 and compare them to other fertilizers, namely conventional NPK fertilizers and poultry litter. Our
83 hypothesis is that black soldier fly frass improves soil biological activity and earthworm biomass to a
84 greater extent than mineral and poultry litter fertilizers, but its slower nutrient release may limit short-

85 term plant biomass production. Based on this hypothesis, the objectives of the study are to (1) compare
86 the effects of BSF frass, poultry litter, and mineral NPK fertilizer on ryegrass biomass and nutrient
87 uptake, and (2) assess their impacts on soil microbial activity and earthworm biomass. More
88 specifically, this study seeks to enhance our understanding of its role as a sustainable and circular
89 fertilizer alternative by not only addressing the potential of frass in promoting plant growth but also
90 considering its implications for soil health, offering valuable insights into its viability in sustainable
91 agriculture.

92

93 **Materials and Method**

94 *Soil and fertilizers*

95 The studied soil was sampled in Beauvais (Northern France) and was classified as a Haplic Luvisol. Soil
96 characterization was carried out by Houben et al. (2020) and revealed that the soil was a silt loam
97 (USDA classification) with 16% sand, 67% silt, and 17% clay. Frass (Hilucia™) from BSF (*H. illucens*) was
98 provided in the form of pellets by Innovafeed (Nesle, France), an industrial company farming this insect
99 at the large-scale. The flies were fed exclusively on raw materials authorized by French and European
100 regulations for farm animal feeds, which were in the present case a mix of beet and wheat co-products.
101 The frass was dried, pelletized then hygienized (70 °C, 60 minutes), and used as such, without any
102 chemical input, making it a fertilizer compatible with organic farming and not subject to any specific
103 restrictions. Poultry litter (poultry droppings with litter) was purchased from Solabiol (Ecully, France) as
104 a fertilizer permitted for organic agriculture. This commercial product was selected for its conformity
105 to organic standards (AFNOR NF U42-001), and although its chemical composition was verified, no
106 further microbiological characterization was conducted. The NPK composition was determined by
107 external laboratories following the French standards for organic fertilizer (AFNOR NF U42-001) and was
108 3-4-3 for frass and 4-3-2 for poultry litter.

109

110

111 *Treatments*

112 Frass and poultry litter were applied at a rate of 5 t ha⁻¹ in the soil. A mineral control adding the same
113 quantity of N, P and K as in the Frass treatment was achieved by mixing the soil with appropriate
114 amount of inorganic nutrients (NH₄NO₃, KH₂PO₄ and KCl) in powder (hereafter called “NPK”). Untreated
115 soil (hereafter called “control”) was also part of the experiment.

116

117 *Pot experiment*

118 Plastic plant pots (18-cm diameter, 14-cm height) were filled with 2 kg of each mixture in four
119 replicates. Before sowing, the pots were placed in a controlled dark room and the mixtures were
120 equilibrated during one week at 80% water holding capacity. The experiment was initiated in April
121 2023. After the equilibration period, the pots were transferred to a greenhouse and were arranged
122 according to a randomized design. Three earthworms (*Lumbricus terrestris* L.) were added in each pot,
123 representing a biomass of 11.57 ± 0.13 g, 11.56 ± 0.14 g, 11.43 ± 0.18 g and 11.64 ± 0.03 g in Control,
124 NPK, Frass and Poultry litter treatments, respectively, according to the recommendations by Dulaurent
125 et al. (2020). In each pot, 2.5 g seeds of Italian ryegrass (*Lolium multiflorum* Lam.) were sown. Ryegrass
126 was chosen as it is a fast-growing species frequently used as a bioindicator in soil fertility studies. Its
127 ability to rapidly absorb nutrients makes it well-suited to assess short-term fertilizer effects on plant
128 growth and nutrient uptake (Gómez-Suárez et al. 2020; Honvault et al. 2024). The trials were conducted
129 under controlled greenhouse conditions (temperature 18–25°C, 16-h photoperiod). Watering was daily
130 done by filling trays under the pots to allow capillary uptake and limit leaching while light surface
131 sprinkling was used to prevent drying at the surface. After 4 and 8 weeks, shoots were harvested by
132 cutting 1 cm above the soil with ceramic scissors, dried (60°C, 72 h), weighed and crushed. At the end
133 of the experiment, earthworms were extracted from pots, counted, and weighed.

134

135

136

137 *Elemental analyses of plant tissues*

138 After each harvest, phosphorus (P) and potassium (K) concentrations in shoots were directly measured
139 after fine grinding by X-ray fluorescence (XRF, S1 Titan, Bruker). Nitrogen concentration in shoots was
140 measured by elemental analysis by combustion (LECO FP628 Series, LECO Corporation). Nutrient
141 uptake was calculated by multiplying nutrient concentrations by the shoot dry biomass.

142 *Soil chemical and biological analyses*

143 After the second harvest, soils were kept for soil chemical and biological analyses. The soil was analyzed
144 for pH (5g of soil/25 ml of H₂O; NF ISO 10 390), organic C concentration by sulfochromic oxidation (NF
145 ISO 14235), total N concentration using the Dumas method (NF ISO 13 878), ammonium acetate
146 Metson cation exchange capacity (CEC) (NFX-31-130), available P (Joret-Hébert method; NFX 31-161),
147 and exchangeable K concentrations (ammonium acetate extraction; NFX 31-108).

148 Community-level physiological profiles (CLPPs) based on the ability of microorganisms to oxidize
149 various substrates were assessed using BIOLOG EcoPlates (BIOLOG Co., Hayward, USA) on fresh soil
150 samples. Each 96-well plate consisted of three replicates, each one comprising 31 sole C sources and a
151 water blank. Five grams of soil, collected at the end of the experiment, were shaken with 45 ml of
152 sterile 0.85% NaCl for 30 min at 200 rpm and then diluted to 1:1000. Each plate well was inoculated
153 with 150 µL of the dilution and the plates were incubated at 25 °C. Color development for each well
154 was obtained in terms of optical density (OD) at 590 nm using an automated plate reader. Kinetic curves
155 suggested that after 96 h incubation time, the wells with the most active microbial communities
156 reached the asymptote of color development. Therefore, this point was considered as the optimal
157 incubation time for further statistical analyses, as suggested by previous studies (Denier et al. 2022).
158 The C sources were grouped into six categories representing different substrate guilds (Sala et al. 2010;
159 Houben et al. 2021): amino acids (L-arginine, L-asparagine, L-phenylalanine, L-serine, glycyl-L-glutamic
160 acid, L-threonine,), amines (phenylethylamine, putrescine), carbohydrates (D-mannitol, glucose-1-
161 phosphate, D,L- α -glycerol-phosphate, β -methyl-D-glucoside, D-galactonic acid γ -lactone, erythritol, D-

162 xylose, N-acetyl-D-glucosamine, D-cellobiose, α -D-lactose), carboxylic acids (D-glucosaminic acid, D-
163 malic acid, itaconic acid, pyruvic acid methyl ester, D-galacturonic acid, α -ketobutyric acid, γ -
164 hydroxybutyric acid), phenolic compound (2-hydroxybenzoic acid, 4-hydroxy benzoic acid) and
165 polymers (Tween 40, Tween 80, α -cyclodextrin, glycogen). Substrate average well color development
166 (AWCD) values for each substrate category were calculated with the equation: $AWCD = \sum ODi/N$, where
167 ODi is the corrected OD value of the substrates within the substrate category and N is the number of
168 substrates in the category.

169

170 *Statistical analyses*

171 All recorded data were analyzed using descriptive statistics (mean \pm standard error) and normality was
172 determined using the Shapiro-Wilk test. One-way ANOVAs and Fisher's multiple comparison tests or
173 Kruskal-Wallis and Mann-Whitney tests were used to compare biomass and nutrient concentrations in
174 the shoot, available P and K concentration in soil, change in earthworm biomass and microbial enzyme
175 activity according to whether the distribution was normal or not, respectively. Principal component
176 analysis (PCA) was used to depict the general relationships between plant and soil parameters. All
177 statistical analyses were performed using XLSTAT (2023.1.6 version) software.

178

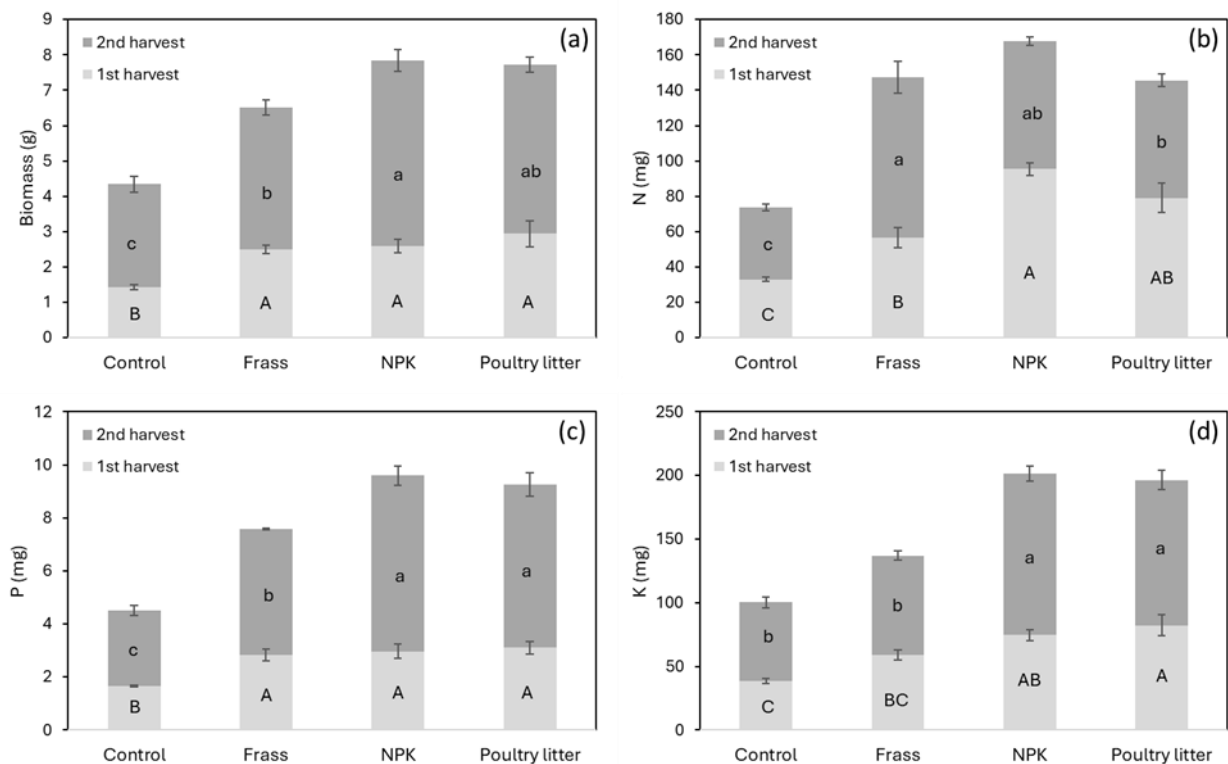
179 **Results and Discussion**

180 *Biomass production and nutrient uptake*

181 Plant biomass production was significantly influenced by the type of amendment applied to the soil
182 (Figure 1a). At the first harvest, the treatments receiving BSF frass, NPK, and poultry litter resulted in
183 similar biomass production, indicating that all three fertilizers were effective in promoting early growth
184 of ryegrass. As suggested by Menino et al. (2021) for similar frass, this might indicate that BSF frass
185 could provide sufficient nutrients to support plant growth initially, comparable to the immediate
186 availability from NPK.

187 However, at the second harvest, significant differences emerged among the treatments (Figure 1a). The
 188 NPK treatment resulted in the highest biomass production, followed by poultry litter and frass, with
 189 the control remaining the lowest. The higher biomass in the presence of mineral NPK at the second
 190 harvest can be attributed to the high initial availability of readily-soluble nutrients provided by the
 191 inorganic fertilizer at the start of the experiment, especially for ryegrass whose the growth depends on
 192 the N supply (Cordovil et al. 2007; Houben et al. 2021). This is supported by the high N amount taken
 193 up by plants in the presence of NPK treatment even though N uptake at the second harvest was the
 194 highest in the presence of frass (Figure 1b). However, despite this higher N uptake, the biomass
 195 production of plants grown in frass-treated soils was surprisingly lower than that in the NPK and poultry
 196 litter treatments. This indicates that N was not the only limiting factor for plant growth and that other
 197 nutrients might drive biomass production.

198



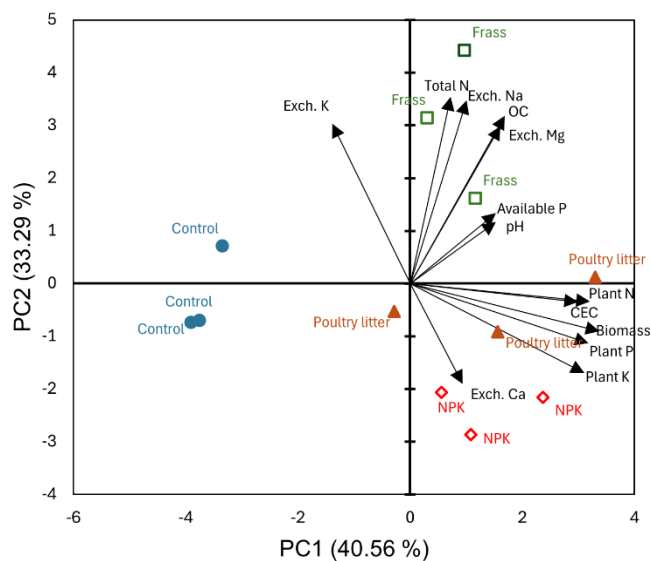
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200 **Figure 1. Biomass (a) and uptake of N (b), P (c) and K (d) at the first and second harvest. Values are average (n = 4) ±**

201 **standard error. Columns with same letter do not differ significantly at the 5% level.**

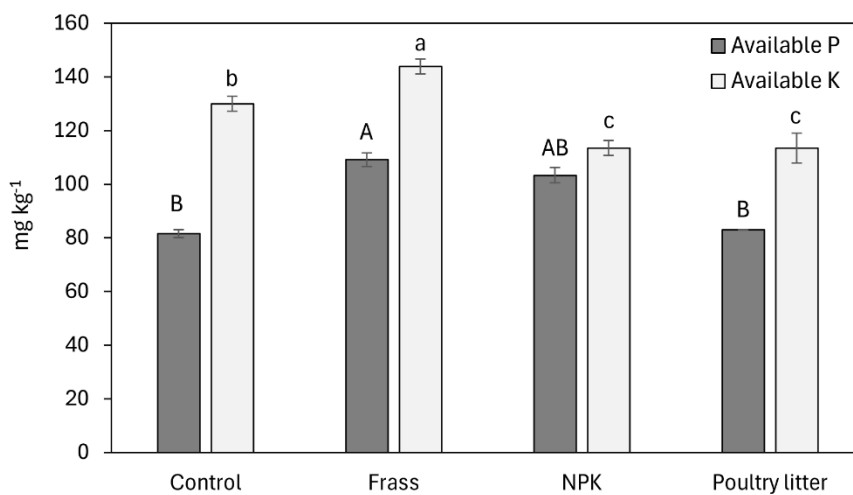
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203 According to PCA (Figure 2), plant biomass was positively correlated not only with N but also with P
204 and K uptake, suggesting that the lower biomass for plants grown in the presence of BSF frass might be
205 due to P and K limitation. This hypothesis was supported by the analysis of N:P ratios which was $14.3 \pm$
206 0.5 , 19.1 ± 2.0 , 11.0 ± 0.7 and 10.9 ± 0.8 for plants grown in the control and in the presence of frass,
207 NPK and poultry litter, respectively. Since it is generally admitted that N:P ratio below 12 reflects N
208 limitation while N:P ratio above 16 reflects P limitation (Koerselman and Meuleman 1996; He et al.
209 2014), our results indicate that plants grown in the presence of frass were P limited while plants grown
210 with NPK and poultry litter were N limited while plants grown in the control were N and P co-limited.
211 These results suggest therefore that, for some crops, BSF frass might not be as efficient as NPK and
212 poultry litter to sustain plant biomass production possibly due to microbial nutrient immobilization
213 occurring during the initial stages of frass decomposition. The high microbial activity in frass-treated
214 soils may have led to temporary drawdown of nutrients by microbes, limiting plant uptake and growth
215 during the early phase (Xu et al. 2020; Jindo et al. 2023). This hypothesis is confirmed by the lower
216 amount of P and K taken up by plants grown in the presence of frass compared to the other fertilized
217 treatments (Figure 1c, d) as well as by the higher concentrations of available P and K remaining in the
218 frass-treated soil at the end of experiment (Figure 3). This suggests that nutrient availability may have
219 been temporarily constrained by biological processes such as microbial immobilization, in addition to
220 potential slow-release dynamics of the frass itself. These findings indicate that BSF frass acts as a slow-
221 release fertilizer and that, as demonstrated by Houben et al. (2020) for mealworm frass, its application
222 could be coupled with addition of readily-available nutrients to avoid P and K limitation at the early
223 stage of growth. Overall PCA indicates that BSF frass had a distinct behavior compared to both NPK and
224 poultry litter. While NPK and poultry litter treatments were closely linked to plant-related parameters,
225 BSF frass showed a stronger association with most of soil chemical properties. This highlights that the
226 mode of action of BSF frass differs from that of NPK and poultry litter, suggesting that its application
227 primarily enhances soil chemical fertility, with its benefits on plant biomass or nutrient uptake likely
228 being more gradual.



229

230 **Figure 2. Principal component analysis (PCA) of plant parameters and soil properties in each treatment. CEC is cation**
 231 **exchange capacity, OC is organic carbon concentration, Exch. K, Na, Mg and Ca are exchangeable K, Na, Mg and Ca,**
 232 **respectively.**



233

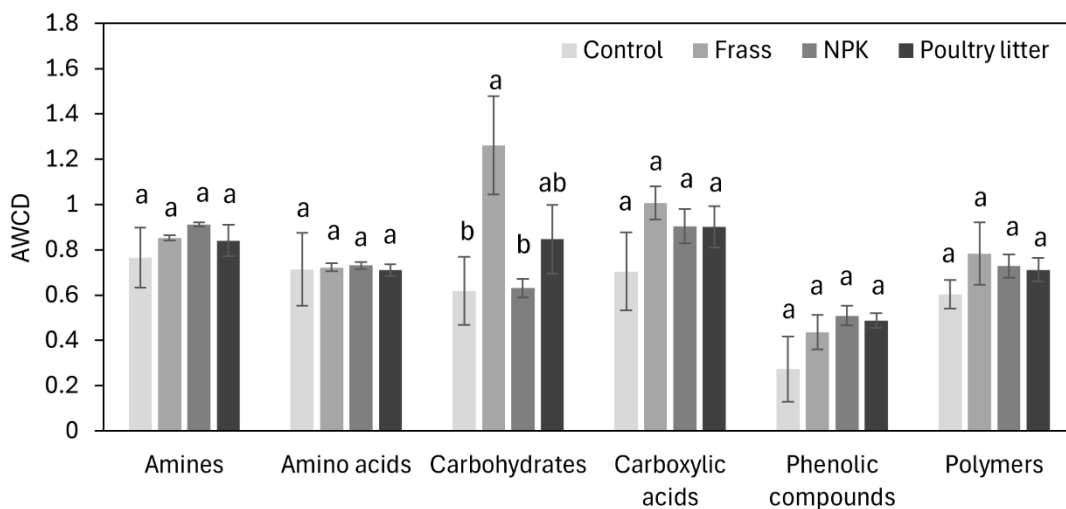
234 **Figure 3. Available P and K concentrations in soil at the end of the experiment. Values are average (n = 4) ± standard error.**
 235 **Columns with same letter do not differ significantly at the 5% level.**

236

237 *Microbial activity and earthworm biomass*

238 Microbial metabolic activity was assessed through the measurement of substrate AWCD in BIOLOG
 239 EcoPlate (Figure 4). As a source of available energy for soil microorganisms, organic amendments

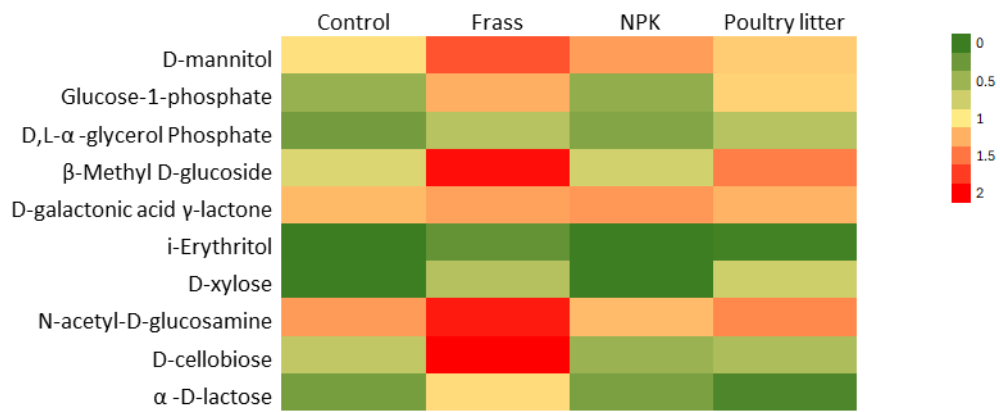
240 generally stimulate microbial activity compared to conventional inorganic fertilizer, which can be
 241 reflected by an increase of AWCD (Gomez et al. 2006; Cesarano et al. 2017). Here, our results indicate
 242 that substrate utilization was not affected by the addition of organic amendments except for
 243 carbohydrates which were more degraded in the presence of frass (Figure 4). Carbohydrates are the
 244 primary component of dissolved organic C and they provide energy and substrates for microorganisms
 245 (Kalbitz et al. 2003). As a result, enhanced degradation of carbohydrates after frass addition suggests
 246 that frass acts as a substrate that promotes a more active and diverse microbial community capable of
 247 degrading organic matter. As microbes degrade carbohydrates, they release not only C compounds but
 248 also nutrients such as N, which can be taken up by plants. This therefore might explain why N uptake
 249 was significantly higher in the frass treatment during the second harvest (Figure 1b), as the microbial
 250 breakdown of carbohydrates likely contributed to the steady release of N.



251
 252 **Figure 4. Average well-color development (AWCD) for each group of substrates metabolized in BIOLOG EcoPlate. Values**
 253 **are average (n = 3) ± standard error. Columns with same letter do not differ significantly at the 5% level.**

254
 255 The elevated microbial activity related to carbohydrate degradation in the presence of frass can be
 256 explained by the composition of frass. BSF frass contains chitin (0.9% according to the supplier), a
 257 polysaccharide found in insect exuviae, which is a primary target for microbial degradation (Elissen et

258 al. 2023; Jasso et al. 2024). Chitin-degrading microbes, such as chitinolytic bacteria and fungi, are
259 particularly active in frass-treated soils, and their metabolic activity leads to the breakdown of chitin
260 into simpler sugars, which serve as energy sources for other microbes in the soil (Barragán-Fonseca et
261 al. 2022). This microbial cascade enhances the overall microbial diversity and function, particularly in
262 relation to carbohydrate metabolism. Interestingly, the heatmap (Figure 5) highlights that, among the
263 carbohydrates, β -Methyl D-glucoside, D-cellobiose, N-acetyl-D-glucosamine were the most degraded
264 substrates. N-acetyl-D-glucosamine being a monomer of chitin, its increased utilization in frass-treated
265 soils might indicate therefore higher chitin degradation by soil microbes. In addition, higher microbial
266 activity related to the degradation of N-acetyl-D-glucosamine in frass-treated soils has also implications
267 for nutrient cycling. Chitin, being rich in N, serves as a dual-purpose substrate by providing both C and
268 N to the soil microbial community (Hui et al. 2020). The degradation of chitin releases not only C for
269 microbial growth but also N, making it available for plant uptake (Sharp 2013). This process likely
270 contributed to the increased N uptake observed in the frass treatment during the second harvest
271 (Figure 1b). By enhancing the microbial degradation of chitin, it could be thus expected that frass
272 provides a slow but sustained release of N over time. This observation is overall consistent with studies
273 on BSF frass (Beesigamukama et al. 2020) but also on mealworm frass (Houben et al. 2021; Watson et
274 al. 2021) who emphasized the importance of microbial processes in the mineralization of N from frass.
275 Altogether, our findings support a broader picture in which frass, due to its richness in labile carbon
276 substrates (e.g., carbohydrates, chitin), strongly stimulates microbial activity in the early stages of soil
277 incorporation, as reported by other studies (Houben et al. 2021; Rummel et al. 2021; Lopes et al. 2022).
278 This microbial activation may have temporarily immobilized nutrients such as nitrogen, phosphorus,
279 and potassium during microbial proliferation, explaining the lower nutrient uptake by plant at the first
280 harvest. As microbial turnover progresses, nutrients may have been gradually released and became
281 available again for plant uptake (Malik et al. 2013). This mechanism could explain the lower biomass
282 production observed at the first harvest and the significantly higher nitrogen uptake measured at the
283 second harvest in the frass treatment.



284

285 **Figure 5. Heatmap representing the degradation of each carbohydrate present in the Biolog Ecoplate. High optical density**
 286 **(red color) indicates high degradation while low optical density (green color) indicates low degradation.**

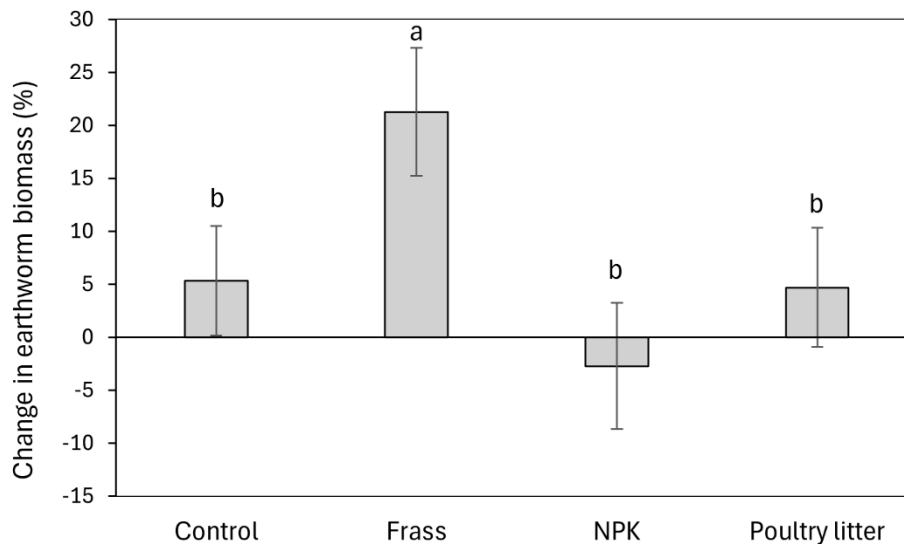
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288 Nevertheless, it is important to note that microbial activity was assessed only once, at the end of the
 289 experiment. As a result, we may have missed earlier phases of intense microbial activity following frass
 290 application, including the initial peak in microbial respiration and nutrient immobilization observed in
 291 previous studies (e.g., Rummel et al. 2021). This temporal limitation restricts our ability to fully
 292 characterize the dynamic microbial responses that likely occurred during the experiment.

293 In addition to enhancing soil microbial activity, the addition of frass was also beneficial for earthworms.
 294 The biomass of earthworms significantly increased in the frass treatment compared to the Control, NPK
 295 and poultry litter treatments (Figure 6). This suggests that frass provided a more conducive
 296 environment for earthworm growth than inorganic fertilizers. The positive effect of BSF frass on
 297 earthworm biomass could be attributed to several factors related to its composition, which directly
 298 provides food for earthworms, but also, indirectly, stimulates microbial activity (Dulaurent et al., 2020).
 299 As microbial communities break down carbohydrates, they produce simple organic compounds that
 300 serve as food for higher trophic levels, including soil fauna such as earthworms (Blouin et al. 2013). On
 301 the other hand, it must be noted that higher earthworm activity is also known to significantly enhance
 302 soil microbial activity, suggesting possible positive feedbacks between microorganisms and
 303 earthworms with the presence of frass. As earthworms ingest soil and organic matter, they fragment
 304 and process organic material in their digestive systems, releasing it back into the soil as nutrient-rich

305 casts (Waqar et al., 2019). These casts are hotspots for biological activity, providing ideal conditions for
306 microbial communities (Blouin et al. 2013). Additionally, earthworm activity may create a more
307 favorable environment for microbial processes by increasing soil oxygen levels and releasing mucus
308 within the soil, which enhances the metabolic activity of aerobic microbes involved in organic matter
309 decomposition and nutrient cycling (Binet et al. 1998; Brown et al. 2000). On the other hand, the
310 greater abundance of earthworms in the frass treatment might suggest that some nutrients have been
311 temporarily immobilized within earthworm biomass and casts. Earthworms are known to uptake and
312 redistribute nutrients such as N, P and K, either by incorporating them into their tissues or by
313 concentrating them in casts. For instance, García-Palacios et al. (2014) demonstrated that earthworms
314 can reduce plant nitrogen capture in heterogeneous soils by preemptively utilizing nutrient-rich
315 patches. These mechanisms may have contributed to the reduced nutrient availability for plants at the
316 first harvest in the frass treatment, even though most studies report that, over the long term,
317 earthworms generally stimulate nutrient mineralization and plant growth by enhancing the
318 decomposition of organic matter and residue. In particular, van Groenigen et al. (2014) conducted a
319 meta-analysis showing that earthworms increase aboveground biomass by 23% on average, primarily
320 by promoting nitrogen mineralization from organic residues and soil organic matter. Their results
321 suggest that earthworm activity is especially beneficial in low-input systems where nitrogen availability
322 limits crop production. This highlights that the net effect of earthworms on nutrient availability and
323 plant performance depends on both temporal dynamics and the balance between nutrient
324 immobilization and mineralization.

325



326

327 **Figure 6. Change in earthworm biomass over the experiment. Values are average (n = 4) ± standard error. Columns with**
 328 **same letter do not differ significantly at the 10% level.**

329

330 **Conclusion**

331 This study demonstrates that black soldier fly (BSF) frass has significant potential as an alternative
 332 organic fertilizer, providing benefits not only for plant growth but also for soil health, microbial activity,
 333 and earthworm biomass. While mineral NPK fertilizer led to the highest biomass production during the
 334 second harvest due to its immediate availability of nutrients, frass provided a steady release of
 335 nitrogen, enhancing nitrogen uptake in plants, and stimulated microbial activity, likely through its high
 336 content in labile carbon and chitin. In particular, frass stimulated the degradation of carbohydrates,
 337 such as N-acetyl-D-glucosamine, a key component of chitin, indicating that frass support might a
 338 diverse microbial community capable of decomposing complex organic matter, contributing to
 339 sustained nutrient cycling over time. The positive effect of frass on earthworm biomass also highlights
 340 its role in fostering a healthy soil environment, as earthworms may further stimulate microbial activity
 341 and improve soil structure through their burrowing and feeding behavior.

342 Our findings also suggest that the short-term limitation in plant biomass in the frass treatment may not
 343 be solely due to slower nutrient release, but also to temporary nutrient immobilization caused by
 344 increased microbial and earthworm activity. This nutrient immobilization could explain the reduced

345 biomass at the first harvest, followed by higher nutrient uptake at the second possibly due to microbial
346 turnover. These interactions underscore the importance of considering temporal dynamics and
347 biological processes in the evaluation of organic fertilizers.

348 These findings highlight the value of BSF frass both as an organic fertilizer and a soil improver that
349 enhances biological processes within the soil, making it a promising tool for sustainable agricultural
350 practices and a potential substitute for mineral and organic fertilizers such as poultry litter. To fully
351 capture its agronomic potential, future research should include time-resolved assessments of microbial
352 activity and nutrient availability, as well as quantification of nutrient flows within the soil–plant–fauna
353 continuum. Future research should investigate the long-term effects of BSF frass on soil functioning
354 and crop performance across diverse soils, climates, and plant species to better assess its agronomic
355 potential.

356

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358

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