

COMPARATIVE EFFICACY OF BLACK SOLDIER FLY (*Hermetia illucens*) FRASS DERIVED FROM ANIMAL-BASED AND PLANT-BASED WASTES ON NITRIFICATION RATE AND NITRATE UPTAKE IN *Amaranthus dubius*

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ABSTRACT

Soil fertility plays a pivotal role in adopting intensive agricultural approaches, which are becoming increasingly popular and essential for feeding the ever-increasing population with limited available resources. The use of organic fertilizers offers a sustainable approach by improving soil fertility and reducing dependence on chemical fertilizers. We investigated the production level of frass, an organic fertilizer, by Black Soldier Fly (BSF) when fed with animal and plant-based substrates. While being commercial, plant-based compost fertilizer was used as a standard positive control and was labelled as CPC. Plant-based wastes reduce the decomposition rate for Organic Nitrogen (ON) ($0.057 \pm 0.007\%$), and recorded the lowest ON-NH₄⁺ rate ($0.50 \pm 0.002\%$) when used as a feeding substrate for Black Soldier Fly larvae (BSFL). On the other hand, it enhances the decomposition rate for total crude fibre (TCF) ($0.78 \pm 0.002\%$) and NO₃⁻ uptake rate ($0.93 \pm 0.001\%$). Contrary to this, we observed the highest decomposition rate for ON ($0.29 \pm 0.008\%$) and ON-NH₄⁺ rate ($0.77 \pm 0.002\%$), yet the lowest decomposition rate for TCF ($0.53 \pm 0.002\%$) and NH₄⁺-NO₃⁻ rate ($0.034 \pm 0.000\%$), along with the lowest NO₃⁻ uptake rate ($0.21 \pm 0.016\%$) by BSFL using animal-based wastes as a feeding substrate. Frass produced by black soldier fly when fed with plant-based substrate (BSFFP) recorded a balanced uptake ratio for NH₄⁺:NO₃⁻ (33:37), whereas Black Soldier Fly frass based on animal-feeding (BSFFA) recorded unbalanced uptake ratio for NH₄⁺:NO₃⁻ (40:08). Commercial plant-based compost fertilizer (CPC) used as the positive control, recorded higher NH₄⁺ uptake compared to NO₃⁻ uptake (25:17). Furthermore, the use of CPC showed the lowest rate of ON-NH₄⁺ ($0.36 \pm 0.002\%$) as well as lower NH₄⁺-NO₃⁻ rate ($0.60 \pm 0.003\%$) compared to BSFFP. This study highlighted the importance of both ON form and TCF decomposition rates as key determinants for effective nitrification (NH₄⁺-NO₃⁻) and NO₃⁻ uptake of BSFF frass-based fertilizer.

Keywords: Black soldier fly; plant-based substrate; animal-based substrate; polyphagous; frass; nitrification; nitrate uptake

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INTRODUCTION

Insect-based frass is gaining global value due to its alignment with circular bioeconomy principles. They also have a significantly lower water footprint than conventional ruminant-derived organic fertilizers (Abd Manan *et al.*, 2024). The Black Soldier Fly (BSF) (Diptera: Stratiomyidae) is among the most widely reared species in Malaysia. Its larvae are polyphagous, efficiently converting various animal- and plant-based wastes into valuable biomass. This bioconversion process yields refined organic fertilizers, supporting sustainable waste management (Abd Manan *et al.*, 2024; Bava *et al.*, 2019; Kinasih *et al.*, 2018; Parodi *et al.*, 2020; Siddiqui *et al.*, 2022). Its efficiency from plant-based waste in enhancing nitrification and nitrate uptake depends on factors such as substrate composition, frass maturity, soil type, and application rate (Bindraban *et al.*, 2015; Watson *et al.*, 2021). Beesigamukama *et al.* (2023) found that high-fiber, plant-based substrates produce frass with higher carbon-to-nitrogen (C/N) ratios, which may limit nitrogen availability. In contrast, nitrogen-rich substrates yield frass with lower C/N ratios, enhancing soil fertility. Similarly, Arabzadeh *et al.* (2022) stated that vegetable-based substrates produced frass with higher nitrogen content than fruit- or starch-based diets, highlighting the critical influence of substrate type on frass quality. Evaluations on the efficacy of BSF-derived frass as organic fertilizers recorded numerous successes, with comparable performance yet showed positive synergisms with synthetic inorganic fertilizers (Bortolini *et al.*, 2020; Čičková *et al.*, 2015; Gold *et al.*, 2018; Tanga *et al.*, 2022; van Huis and Oonincx, 2017). It is recommended especially for global crops without high sensitivity towards ammonium (NH₄⁺)-based fertilization and nutrition (Tanga *et al.*, 2022; Gärtling *et al.*, 2020). It is because the combination of the fecal matter, dead individual, and cuticular waste encompassed the total frass volume of the given insect-derived frass (Lopes *et al.*, 2022), directed towards high NH₄⁺ accumulation from glucosamine as the monomer of chitin (Dlamini *et al.*, 2020). Additionally, the fecal matter component is directly related to insect-derived nitrogenous wastes (Purkayastha and Khanal, 2024). Studies on the effectiveness of Black Soldier Fly Larvae Frass (BSFLF) in supplying and supporting ammonium (NH₄⁺) and nitrate (NO₃⁻) fertilization are still limited, particularly for short-cycle leafy vegetables that are typically sensitive to NH₄⁺ and have a low capacity to absorb it. (Jia *et al.*, 2020). Such non-NH₄⁺-sensitive crops increase the tendency of rapid NH₄⁺ uptake as the dominant inorganic-N available following the application of most insect-derived fertilizers (Beesigamukama *et al.*, 2022; Green and Popa, 2012). This also causes NH₄⁺ toxicity with stunted growth and increased physiological stress in amaranth (Munene *et al.*, 2017). Finally, it affects the

reduced uptake of another essential nutrient, such as potassium ion (K⁺) (Esteban *et al.*, 2016), dissimilar to NO₃⁻ fertilization with reduced risks of interruption and interference of K⁺ uptake (Rietra *et al.*, 2017). It was hypothesized that Black Soldier Fly frass from animal-based feeding substrates would have a greater nitrogen content and a lower C/N ratio than frass from plant-based substrates. This would improve nitrate absorption in *Amaranthus dubius* and increase soil nitrification rates. Types of feeding substrates fed to the farmed BSFL, either animal-based or plant-based wastes, are directly related to the resulting differential concentrations of nitrogenous wastes excreted and could play a crucial role in the balanced NH₄⁺ and/or NO₃⁻ fertilizations. The current study was conducted to specifically assess the influence of animal-based and plant-based wastes as feeding substrates of farmed BSFL on differential NH₄⁺ and NO₃⁻ fertilizations. The specific objectives were, (1) to evaluate the effect of the used substrate on the physicochemical properties of BSF frass, particularly its nitrogen content and C/N ratio; (2) to quantify the impact of frass application on soil nitrification dynamics and nitrate uptake efficiency in *Amaranthus dubius* (3) and agronomic potential of BSF frass derived from different substrates as a sustainable alternative to conventional fertilizers.

MATERIALS AND METHODS

Study sites: This study was carried out from January to March 2022 at the School of Biological Sciences (SBS) Plant House, L14 Building, Universiti Sains Malaysia (USM), Penang, Malaysia (5°21'26.4"N and 100°17'39.9"E). The soil at the study site was identified as medium to coarse-grained porphyritic muscovite-biotite granite containing microcline (Ahmad *et al.*, 2006; Kong, 1994). Its texture was classified as sandy clay loam, comprising 26.90% coarse sand, 38.90% fine sand, 28.10% clay, and 6.10% silt. For this study, the topsoil from a depth of 0-20 cm was used as the potting medium. The study was conducted using a pot-culture system. In this study, plant growth conditions were standardized across all treatments. Soil moisture was consistently maintained by applying 130 mL of deionized water twice daily to each *Amaranthus dubius* seedling, using veterinary-grade stainless steel syringes equipped with 80 mL needles. Important physico-chemical characteristics related to this study include: total nitrogen (703.35 ± 1.24 mg kg⁻¹), soil pH (6.41), organic nitrogen (1701.28 ± 7.21 mg kg⁻¹), total extractable NH₄⁺ (11.10 ± 0.05 mg kg⁻¹), total extractable NO₃⁻ (4.95 ± 0.06 mg kg⁻¹), total exchangeable K⁺ (16.15 ± 0.05 mg kg⁻¹), total organic matter (89059.22 ± 114.42 mg kg⁻¹), and total crude fiber (20958.75 ± 142.96 mg kg⁻¹).

Experimental materials and design: *Amaranthus dubius* (Amaranthaceae), a short-term annual leafy vegetable, was used as the trial crop in this study. Seedlings were first sown and transplanted in early January 2022. The plants were then grown under experimental conditions and harvested after approximately eight weeks, at the end of February 2022 (± 56 days). The experiment was set in a Completely Randomized Design (CRD) with five replicates per treatment, including a negative control with no fertilizer application. Each replicate consisted of 10 seedlings, follow-on in a total of 200 seedlings across all treatments. The selection of five replicates followed standard agronomic practices to ensure statistical reliability, minimize experimental error, and allow valid analysis of variance (ANOVA). This replication size provided sufficient power to detect treatment effects while accounting for biological variability

Crop establishment and agronomic practices: Soils used in this study were first temperature-treated at 60°C for 30 minutes to reduce the risks of possible soil pathogenic microbial infection to the *A. dubius* seedlings while maintaining the survival of the beneficial soil microbial community. The temperature-treated soils were sieved using a 1.0 mm stainless steel sieve. Three organic-based fertilizers were evaluated in this study; two were derived from Black Soldier Fly larvae (BSFL), based on the decomposing feeding substrates provided to the developing BSFL. The frass produced by the BSFL when fed with animal-based wastes (poultry wastes such as chickens' heads, intestines, and other unused discarded internal organs) as a feeding substrate was labelled as BSFFA. BSFL fed with plant-based wastes (from rotting or decomposing green leafy vegetables of both *Amaranthaceae* and *Brassicaceae*) produced frass labelled as BSFFP. The BSFFA and BSFFP were sieved via a 1.0 mm stainless steel sieve to separate the fecal component of the frass from non-fecal residues, including insect carcasses, exuviae, and undigested substrate particles. All fertilizers attained organic maturity and were tested after ± 50 days post-frass production.

In this study, only the fecal matter component of the frass was evaluated. Commercial plant-based compost fertilizer was used as a standard positive control, labelled as CPC. The fertilizers' proximate chemical characteristics were run under the department of Inorganic and Analytical Chemistry, School of Chemical Sciences, Universiti Sains Malaysia, following the standard analytical methods of AOAC 2005 (Kundu *et al.*, 1996). All averaged results (Mean \pm S.E.) involved 20 replicates of the following chemical analyses.

Total nitrogen (TN; mg kg⁻¹), fertilizers' pH, total organic nitrogen (TON; mg kg⁻¹), total extractable ammonium (total extr. NH₄⁺; mg kg⁻¹), total extractable nitrate (total extr. NO₃⁻; mg kg⁻¹), total

potassium (total K; mg kg⁻¹), total organic matter (TOM; mg kg⁻¹), and total crude fibre (TCF; mg kg⁻¹) are summarized in (Table 1). Total nitrogen and ammonia were analysed using the Kjeldahl method, while ammonium (NH₄⁺) was determined by the USP 36 method. Nitrate (NO₃⁻) levels were determined by the colorimetric method described by Okalebo *et al.* (2002). Ultraviolet-visible (UV-Vis) spectroscopy technique was used for the Phosphorus determination, while total potassium was analysed by using flame photometry. Similarly, pH was determined by using an aqueous medium of 1: 10 (weight/volume) sample to distilled water and was then read by using different meters for the measurement (AD1000, Adwa, Romania) (Okalebo *et al.*, 2002). The crude fiber was analysed by using a standard technique (A.O.A.C 2005)

Fertilizer Application: The fertilizer treatment in the current study was applied at the transplanting stage. According to the Malaysian Agricultural Research and Development Institute (MARDI) indorses a nitrogen (N) fertilizer rate of 60 kg N ha⁻¹ for edible amaranth (*Amaranthus* spp., Amaranthaceae). However, due to the use of a pot-culture system which confines root growth and lacks access to sub-soil nitrogen, this rate was doubled to 120 kg N ha⁻¹ to meet plant nitrogen requirements under limited conditions (Kundu *et al.*, 1996).

The corresponding fertilizer application rates for each treatment were as follows:

CPC (Chemical Fertilizer): 2.330 t N ha⁻¹

BSFFP (Black Soldier Fly Frass – Plant-based): 3.955 t ha⁻¹

BSFFA (Black Soldier Fly Frass – Animal-based): 2.805 t ha⁻¹

To confirm consistency, fertilizers were mixed into the soil using a multi-step serial dilution method before planting. The fertilizer-soil mixtures were thoroughly blended and packed into standard polybags (35 cm \times 35 cm \times 25 cm) with a maximum soil-holding capacity of approximately 5 kg per bag.

During the experimental period, soil moisture levels were maintained by applying 150 ml of deionized water twice daily to each *A. dubius* seedling. Watering was conducted using veterinary-grade stainless-steel syringes (80 ml capacity) fitted with 10 mm hypodermic stainless-steel needles to ensure precise and uniform application (Mesgaran *et al.*, 2021).

Data collection and post-harvest analysis: Growth parameters of *Amaranthus dubius* were recorded at 7-day intervals from day 7 to 56 after transplanting (~8 weeks). Shoot measurements, including plant height, leaf length, leaf width, stem diameter, and shoot diameter (all in mm), were taken using a 600 mm digital calliper (± 0.01 mm).

Plant height was measured from the lowest observable stem at soil level to the tip of the highest shoot. For leaf length and width, the first five fully developed leaves per seedling were marked and consistently measured throughout the study, with averages used for final analysis. At harvest, fresh biomass (g) was recorded for whole plants and individual parts (stem, shoot, leaf, and root). Samples were then oven-dried at 70 °C for 48 hours to determine dry biomass. Each treatment, BSF frass powder (BSFFP), BSF frass aqueous extract (BSFFA), commercial plant-based compost (CPC), and control (no fertilizer), included 10 seedlings per polybag replicate. Post-harvest plant tissues

were analysed for crude protein (CP; mg/kg) and total dietary potassium (K⁺; mg/kg) using standard proximate analysis procedures. were run under the Department of Food Technology, School of Industrial Technology, Universiti Sains Malaysia, following the standard analytical methods of AOAC (Latimer, 2016).

Data analysis: All the fertilizers' chemical elements, as summarized in Table 1, were analysed following the standard analytical methods of AOAC (Latimer, 2016).

Data regarding TOM, TCF, and ON decomposition rates for BSFFP, BSFFA, and CPC in mg kg⁻¹ were calculated using the following formula:

$$\frac{\text{Fertilizer } \frac{\text{TOM}}{\text{TCF}}}{\text{ON}} - \left(\frac{\left(\frac{\text{Initial Soil } \frac{\text{TOM}}{\text{TCF}}}{\text{ON}} - \text{Post-harvest Soil } \frac{\text{TOM}}{\text{TCF}} \right)}{\text{ON}} \right) \div \left(\frac{\left(\frac{\text{Fertilizer } \frac{\text{TOM}}{\text{TCF}}}{\text{ON}} - \text{Initial Soil } \frac{\text{TOM}}{\text{TCF}} \right)}{\text{ON}} \right)$$

The ammonification (ON-NH₄⁺) and nitrification (NH₄⁺-NO₃⁻) rates for BSFFP, BSFFA, and CPC in mg kg⁻¹ were based on the given formulas:

$$\text{Ammonification rate} = \text{Fertilizer } \text{NH}_4^+ - \text{ON} \div \text{ON}$$

$$\text{Nitrification rate} = \text{Fertilizer } \text{NO}_3^- - \text{NH}_4^+ \div \text{NH}_4^+$$

The rates of NH₄⁺, NO₃⁻, and K⁺ uptake for BSFFP, BSFFA, and CPC in mg kg⁻¹ were based on the following formula:

Fertilizer NH₄⁺/NO₃⁻/K - ((Initial Soil NH₄⁺/NO₃⁻/K⁺ - Post-harvest Soil NH₄⁺/NO₃⁻/K⁺)) ÷ (Fertilizer NH₄⁺/NO₃⁻/K - Initial Soil NH₄⁺/NO₃⁻/K⁺).

$$\frac{\text{Fertilizer } \frac{\text{NH}_4^+}{\text{NO}_3^-}}{\text{K}} - \left(\frac{\left(\frac{\text{Initial Soil } \frac{\text{NH}_4^+}{\text{NO}_3^-}}{\text{K}^+} - \text{Post-harvest Soil } \frac{\text{NH}_4^+}{\text{NO}_3^-} \right)}{\text{K}} \right) \div \left(\frac{\left(\frac{\text{Fertilizer } \frac{\text{NH}_4^+}{\text{NO}_3^-}}{\text{K}^+} - \text{Initial Soil } \frac{\text{NH}_4^+}{\text{NO}_3^-} \right)}{\text{K}^+} \right)$$

Data regarding shoot systems and post-harvest biomasses of fresh and dry weights (g) of each of *A. dubius* seedlings were recorded independently within the same polybag and among different polybags of similar fertilizer treatment applied. One-way Welch's ANOVA was run to evaluate the significant difference in the above-ground shoot (mm), as the data violated the

homogeneity of variances. Games-Howell post hoc tests were used for pairwise comparison, which is suitable for unequal variances. Similarly, all fresh and dry post-harvest weights (g) data were analysed following One-Way ANOVA with Tukey's HSD post hoc tests, to determine the significant differences and influences among the applied fertilizers as the source of variations.

All statistical analyses were conducted at a significance level of $p < 0.05$.

RESULTS

Physicochemical Properties of BSF-Derived Fertilizers and Commercial Compost: In the current study, BSFFA, BSFFP, and CPC showed statistically significant differences for all the assessed chemical attributes. For nitrogen-based attributes, total nitrogen, TN, and CPC recorded the highest value ($51511.37 \text{ mg kg}^{-1}$), followed by BSFFA ($42774.73 \text{ mg kg}^{-1}$), and the lowest recorded by BSFFP ($30342.70 \text{ mg kg}^{-1}$). Contrary to TN values, total organic nitrogen (TON) values showed BSFFA recorded the highest TON ($2329.17 \text{ mg kg}^{-1}$), followed by BSFFP ($1964.43 \text{ mg kg}^{-1}$), and the lowest by CPC ($1267.71 \text{ mg kg}^{-1}$). Similar patterns were observed as of TON with Total Extractable NH_4^+ , where BSFFA recorded the highest value ($10304.51 \text{ mg kg}^{-1}$), followed by BSFFP ($3963.43 \text{ mg kg}^{-1}$), and the lowest by CPC ($458.33 \text{ mg kg}^{-1}$). Contrarily, for total exchangeable NO_3^- , BSFFP recorded the highest value ($869.95 \text{ mg kg}^{-1}$), followed by BSFFA ($347.73 \text{ mg kg}^{-1}$), and unexpectedly the lowest value by CPC ($184.35 \text{ mg kg}^{-1}$).

For pH values of the evaluated fertilizers, acidic pH was recorded for CPC (6.72), followed by BSFFA with moderate alkaline pH (8.33), and the most alkaline pH was recorded by BSFFP (8.99). Total potassium (K) showed that CPC recorded the highest value ($10820.23 \text{ mg kg}^{-1}$), followed by BSFFP ($8531.07 \text{ mg kg}^{-1}$), and BSFFA ($4078.98 \text{ mg kg}^{-1}$). Total organic matter (TOM) unexpectedly showed that BSFFA recorded the highest value ($894790.03 \text{ mg kg}^{-1}$), followed by CPC ($829145.38 \text{ mg kg}^{-1}$), and the lowest value recorded by BSFFP ($784189.92 \text{ mg kg}^{-1}$). Total crude fiber (TCF) showed that CPC recorded the expected highest value ($387421.26 \text{ mg kg}^{-1}$), followed by BSFFP ($221275.57 \text{ mg kg}^{-1}$), and the lowest value recorded by BSFFA ($27801.17 \text{ mg kg}^{-1}$) (Table 1).

Post-Harvest Soil Properties Following Fertilizer Application: In our study, BSFFP showed the highest remaining total extractable NH_4^+ ; (Total Extr. NH_4^+) ($695.28 \text{ mg kg}^{-1}$), compared to BSFFA with the second lowest remaining extractable NH_4^+ (74.15 mg kg^{-1}). Similar patterns of corresponding and complementary NO_3^- uptake were observed, with BSFFP recorded as the lowest remaining total extractable NO_3^- ; Total Extr. NO_3^- (73.03 mg kg^{-1}), reflecting the highest NO_3^- uptake, compared to the other two assessed fertilizers, where BSFFA recorded as the highest remaining total extr. NO_3^- ($279.81 \text{ mg kg}^{-1}$), followed by CPC ($110.85 \text{ mg kg}^{-1}$), reflecting lower NO_3^- uptake.

Higher exchangeable ionic potassium (K^+) uptake was also observed for BSFFP, with the remaining total exchangeable potassium Ion (K^+); Total Exch. K^+

was the lowest ($71.65 \pm \text{mg kg}^{-1}$), reflecting the highest K^+ uptake, followed by CPC ($309.49 \text{ mg kg}^{-1}$), and BSFFA with the highest remaining Total Exch. K^+ ($649.53 \text{ mg kg}^{-1}$), reflecting the expected lowest K^+ uptake. For the remaining total crude fiber (TCF), CPC recorded the highest remaining TCF ($177202.51 \text{ mg kg}^{-1}$), followed by BSFFP ($49087.89 \text{ mg kg}^{-1}$), and BSFFA recorded the lowest remaining TCF ($13006.44 \text{ mg kg}^{-1}$). From these observations, BSFFA exhibited the lowest rate of TCF decomposition, yet the highest TON decomposition rate compared to both CPC and BSFFP (Table 2).

Organic Matter Decomposition and Nutrient Dynamics Following Fertilizer Application: BSFFP recorded the highest TCF decomposition rate, affecting a lower ON- NH_4^+ rate, while BSFFA recorded the highest ON decomposition rate, and the subsequent highest ON- NH_4^+ rate. Unexpectedly, a higher ON- NH_4^+ rate was not a pre-determined factor for a higher NH_4^+ - NO_3^- rate, as observed in BSFFA, where the highest ON- NH_4^+ rate (0.77) resulted in the unexpectedly lowest NH_4^+ - NO_3^- rate (0.034). Similarly, a moderately low ON- NH_4^+ rate (0.50) resulted in the highest NH_4^+ - NO_3^- rate (0.78) as observed for BSFFP, followed by CPC with similar patterns of the lowest ON- NH_4^+ rate (0.36) and of moderate NH_4^+ - NO_3^- rate (0.60), as summarized in Table 3.

From these observations of varying rates between ON- NH_4^+ and NH_4^+ - NO_3^- , it was suggested that TCF levels and the resulting TCF decomposition rates in the assessed fertilizers regulated the effective nitrification (NH_4^+ - NO_3^-), where higher total nitrogen (TN) and total organic nitrogen (TON) were not predeterminant factors for effective NH_4^+ - NO_3^- , as contrarily observed between BSFFP and BSFFA. Similarly, low TCF levels and low TCF decomposition rate affected higher NH_4^+ uptake as observed in BSFFA fertilizer, and opposed to BSFFP with lower NH_4^+ uptake, yet the highest NO_3^- uptake.

Unexpectedly, CPC fertilizer with the highest TCF level recorded moderately lower NO_3^- uptake compared to BSFFP, suggesting the composition and the type of TCF also regulated the rate of NH_4^+ - NO_3^- , since comparatively, higher NH_4^+ uptake was recorded compared to NO_3^- uptake, following the highest TCF level of CPC fertilizer. BSFFP fertilizer recorded a balanced uptake between NH_4^+ and NO_3^- , with higher NO_3^- uptake, compared to BSFFA with higher NH_4^+ uptake and very low NO_3^- uptake. Higher TCF level and higher TCF decomposition rates observed in both BSFFP and CPC recorded positive effects on K^+ uptake rate, where both assessed fertilizers recorded nearly similar K^+ uptake rate, though significantly different, while rapid and the highest NH_4^+ uptake observed for BSFFA adversely affected the lowest K^+ uptake rate (Table 3).

Comparative Decomposition and Nutrient Uptake Ratios for BSF and Commercial Fertilizers:

Based on differential multiplicative ratios of analyzed chemical characteristics, ammonification (ON-NH₄⁺) rate was influenced by TCF decomposition rate compared to TOM decomposition rate, where BSFFA recorded a 2.28 times higher ON-NH₄⁺ rate in respect to TCF decomposition rate compared to BSFFP and a 2.31 times higher than CPC, and only a 1.02 times difference between BSFFP and CPC, paralleled with the results in (Table 2) where the highest significant ON decomposition rate and ON-NH₄⁺ rate were recorded for BSFFA. On the other hand, the TOM decomposition rate influenced the ON-NH₄⁺ rate for a 1.06 times difference between BSFFA and BSFFP, a 1.05 times difference between BSFFA and CPC, and a 1.11 times difference between BSFFP and CPC. Opposing patterns were observed for nitrification (NH₄⁺-NO₃⁻) rate, where TOM decomposition rate influenced NH₄⁺-NO₃⁻ rate higher than TCF decomposition rate, with a 50.68 times higher NH₄⁺-NO₃⁻ rate for BSFFP compared to BSFFA, a 50.79 times higher for CPC compared to BSFFA, and a 1.00 times difference between BSFFP and CPC, while for TCF decomposition rate, BSFFP recorded a 21.00 times higher NH₄⁺-NO₃⁻ rate than BSFFA, CPC with a 22.90 times higher than BSFFA, and a 1.09 times difference of NH₄⁺-NO₃⁻ rate between BSFFP and CPC (Table 4).

External shoot system measurements of *A. dubius* (heights, shoot diameter, stem diameter, leaf length, leaf width, and number of leaves) exhibited no significant differences between BSFFP and CPC fertilizers, and both BSFFP and CPC were significantly different from BSFFA fertilizer. BSFFA fertilizer, on the other hand, was not significantly different from the negative control treatment, depicting the high possibility of NH₄⁺ toxicity due to the highest NH₄⁺ uptake compared to CPC and BSFFP. Similarly, the root system (root length and root width) recorded no significant

differences between CPC and BSFFP fertilizers, and both were significantly different from the BSFFA fertilizer.

Shoot system post-harvest biomass difference showed that BSFFP recorded the highest total dry biomass (9.94g), significantly different from CPC (5.77g) and BSFFA (4.09g). Correspondingly, BSFFP also significantly recorded the highest shoot dry biomass (4.32g), followed by CPC (2.51g) and BSFFA (1.77g). Stem dry biomass followed a similar pattern, with BSFFP significantly recording the highest stem dry mass (4.39g), followed by CPC (2.56g), and BSFFA (1.81g). Leaf dry biomass showed a different pattern where both BSFFP (2.77g) and CPC (1.60g) were not significantly different, and both were significantly different from BSFFA (1.14g). Overall, for post-harvest shoot system dry biomass, BSFFA showed the lowest post-harvest dry biomass compared to both BSFFP and CPC.

Growth and Nutritional Yield of *Amaranthus dubius* Under Different Fertilizer Treatments:

Root system post-harvest biomass difference showed a similar pattern as the shoot system post-harvest biomass, with BSFFP recorded as the highest root dry mass (1.24g), and being significantly different from CPC (0.72g) and BSFFA (0.51g). Consistent observed highest Crude Protein (29717.57 ± 9.30 mg kg⁻¹) and total accumulated dietary K⁺ ions (5072.93 mg kg⁻¹) of post-harvest *A. dubius* attributed to BSFFP fertilizer, corresponding to the highest dietary K⁺ ions uptake as in Table 2 and Table 3, and balanced with the least ratio difference between the uptake rates of NH₄⁺ and NO₃⁻ as observed in (Table 3). Significant reduced growths for both shoot and root dry post-harvest biomass of *A. dubius* observed following BSFFA fertilization was attributed to the rapid higher NH₄⁺ uptake and lowest NO₃⁻ uptake, where NH₄⁺ becoming the sole highest precipitated available inorganic-N, proposing the occurrences of NH₄⁺ toxicity, with the lowest detectable Crude Protein (CP) and lowest cumulative Dietary Potassium ions (K⁺) (Table 5).

Table 1. Mean ± S.E of Physicochemical Properties of BSF-Derived Fertilizers and a Commercial Plant-Based Fertilizer.

Parameters	BSFFP	BSFFA	CPC
TN (mg kg ⁻¹)	30342.70 ± 22.55 ^a	42774.73 ± 18.24 ^b	51511.37 ± 46.45 ^c
pH	8.99 ± 0.03 ^a	8.33 ± 0.02 ^b	6.72 ± 0.08 ^c
TON (mg kg ⁻¹)	1964.43 ± 6.82 ^a	2329.17 ± 19.05 ^b	1267.71 ± 3.03 ^c
Total Extr. NH ₄ ⁺ (mg kg ⁻¹)	3963.43 ± 11.01 ^a	10304.51 ± 18.56 ^b	458.33 ± 2.39 ^c
Total Extr. NO ₃ ⁻ (mg kg ⁻¹)	869.95 ± 6.29 ^a	347.73 ± 3.66 ^b	184.35 ± 0.76 ^c
Total K (mg kg ⁻¹)	8531.07 ± 26.85 ^a	4078.98 ± 16.41 ^b	10820.23 ± 6.47 ^c
TOM (mg kg ⁻¹)	784189.92 ± 22.44 ^a	894790.03 ± 22.57 ^b	829145.38 ± 51.26 ^c
TCF (mg kg ⁻¹)	221275.57 ± 48.09 ^a	27801.17 ± 10.31 ^b	387421.26 ± 66.74 ^c

Different alphabetic labels referred to statistically significant differences ($\alpha = 0.05$).

Abbreviations: TN = Total Nitrogen; TON = Total Organic Nitrogen; Extr. = Extractable; TOM = Total Organic Matter; TCF = Total Crude Fibre.

Table 2. Mean \pm S.E of Post-Harvest Soil Properties Following Fertilizer Application.

Parameters	BSFFP	BSFFA	CPC	Control
TN (mg kg ⁻¹)	6326.89 \pm 22.27 ^a	2699.25 \pm 15.45 ^b	9298.41 \pm 3.03 ^c	836.47 \pm 14.56 ^d
pH	9.26 \pm 0.01 ^a	6.82 \pm 0.02 ^b	7.76 \pm 0.02 ^c	6.25 \pm 0.01 ^d
TON (mg kg ⁻¹)	1852.99 \pm 13.41 ^a	1652.56 \pm 15.47 ^b	1109.94 \pm 5.15 ^c	1909.29 \pm 8.10 ^d
Total Extr. NH ₄ ⁺ (mg kg ⁻¹)	695.28 \pm 4.89 ^a	74.15 \pm 1.26 ^b	191.85 \pm 0.62 ^c	0.92 \pm 0.01 ^d
Total Extr. NO ₃ ⁻ (mg kg ⁻¹)	73.03 \pm 1.03 ^a	279.81 \pm 4.39 ^b	110.85 \pm 0.36 ^c	2.61 \pm 0.06 ^d
Total Exch. K ⁺ (mg kg ⁻¹)	71.65 \pm 0.54 ^a	649.53 \pm 3.11 ^b	309.49 \pm 4.06 ^c	12.16 \pm 0.09 ^d
TOM (mg kg ⁻¹)	360623.51 \pm 2406.66 ^a	97931.52 \pm 298.54 ^b	479128.48 \pm 137.32 ^c	87277.51 \pm 141.29 ^d
TCF (mg kg ⁻¹)	49087.89 \pm 126.78 ^a	13006.44 \pm 31.22 ^b	177202.51 \pm 122.44 ^c	25462.98 \pm 134.84 ^d

Different alphabetic labels referred to statistically significant differences ($\alpha = 0.05$).

Abbreviations: TN = Total Nitrogen; TON = Total Organic Matter; Extr. = Extractable; Exch. = Exchangable; TOM = Total Organic Matter; TCF = Total Crude Fibre.

Table 3. Organic Matter Decomposition and Nutrient Dynamics Following Fertilizer Application.

Parameters	BSFFP	BSFFA	CPC
TOM decom. Rate	0.54 \pm 0.004 ^a	0.89 \pm 0.000 ^b	0.42 \pm 0.000 ^c
TCF decom. Rate	0.78 \pm 0.001 ^a	0.53 \pm 0.002 ^b	0.54 \pm 0.001 ^c
ON decom. Rate	0.057 \pm 0.007 ^a	0.29 \pm 0.008 ^b	0.12 \pm 0.005 ^c
ON-NH ₄ ⁺ rate	0.50 \pm 0.002 ^a	0.77 \pm 0.002 ^b	0.36 \pm 0.002 ^c
NH ₄ ⁺ -NO ₃ ⁻ rate	0.78 \pm 0.002 ^a	0.034 \pm 0.000 ^b	0.60 \pm 0.003 ^c
NH ₄ ⁺ uptake rate	0.83 \pm 0.001 ^a	0.99 \pm 0.000 ^b	0.62 \pm 0.002 ^c
NO ₃ ⁻ uptake rate	0.93 \pm 0.001 ^a	0.21 \pm 0.016 ^b	0.44 \pm 0.003 ^c
K ⁺ uptake rate	0.99 \pm 0.000 ^a	0.85 \pm 0.001 ^b	0.97 \pm 0.000 ^c

Different alphabetic labels referred to statistically significant differences ($\alpha = 0.05$).

Abbreviations: TOM = Total Organic Matter; TCF = Total Crude Fibre; ON = Organic Nitrogen; NH₄⁺ = Ammonium; NO₃⁻ = Nitrate; K⁺ = Potassium Ion; decom. = decomposition. Rates are represented as percentages (%).

Table 4. Comparative Decomposition and Nutrient Uptake Ratios for BSF and Commercial Fertilizers.

Parameters	BSFFP	BSFFA	CPC
TOM: ON	22:02	36:12	17:05
TOM: TCF	22:31	36:21	17:22
TCF: ON	31:02	21:12	22:05
TOM: ON-NH ₄ ⁺	22:20	36:31	17:14
TCF: ON-NH ₄ ⁺	31:20	21:31	22:14
TOM: NH ₄ ⁺ -NO ₃ ⁻	22:31	36:01	17:24
TCF: NH ₄ ⁺ -NO ₃ ⁻	31:31	21:01	22:24
ON-NH ₄ ⁺ : NH ₄ ⁺ -NO ₃ ⁻	20:31	31:01	14:24
NH ₄ ⁺ : NO ₃ ⁻	33:37	40:08	25:17
NH ₄ ⁺ : K ⁺	30:40	40:34	25:39
NO ₃ ⁻ : K ⁺	37:40	08:34	17:39

Abbreviations: TOM = Total Organic Matter; ON = Organic Nitrogen; TCF = Total Crude Fibre; NH₄⁺ = Ammonium; NO₃⁻ = Nitrate; K⁺ = Potassium Ion.

Table 5. Mean \pm S.E. Mean of *Amaranthus dubius* shoot system parameters, post-harvest biomass (fresh and dry weights (g)), and cumulative crude protein (CP) and dietary potassium (K⁺).

Variables	BSFFP	BSFFA	CPC	Control
Height. (mm)	190.74 \pm 18.41 ^a	117.32 \pm 11.33 ^b	159.90 \pm 15.33 ^a	95.37 \pm 9.21 ^b
Shoot Diameter(mm)	3.19 \pm 0.33 ^a	1.96 \pm 0.20 ^b	2.66 \pm 0.27 ^a	1.59 \pm 0.17 ^b
Stem Diameter. (mm)	3.75 \pm 0.43 ^a	2.31 \pm 0.26 ^b	3.13 \pm 0.36 ^a	1.88 \pm 0.21 ^b
Leaf Length. (mm)	53.95 \pm 3.94 ^a	33.18 \pm 2.42 ^b	44.95 \pm 3.28 ^a	26.97 \pm 1.97 ^b
Leaf Width. (mm)	29.89 \pm 3.16 ^a	18.38 \pm 1.95 ^b	24.90 \pm 2.63 ^a	14.94 \pm 1.58 ^b
Root Length. (mm)	90.48 \pm 5.87 ^a	55.19 \pm 3.48 ^b	75.10 \pm 5.07 ^a	45.24 \pm 2.98 ^b
Root Width. (mm)	9.30 \pm 0.86 ^a	5.68 \pm 0.52 ^b	7.72 \pm 0.71 ^a	4.65 \pm 0.43 ^b
No. of leaves	7.80 \pm 0.34 ^a	6.66 \pm 0.24 ^b	7.15 \pm 0.32 ^a	6.46 \pm 0.24 ^b
Total Frass weight. (g)	25.56 \pm 3.11 ^a	15.72 \pm 1.92 ^b	21.29 \pm 2.59 ^a	12.79 \pm 1.56 ^b
Total Dry weight(g)	9.94 \pm 1.21 ^a	4.09 \pm 0.50 ^b	5.77 \pm 0.70 ^b	2.81 \pm 0.34 ^b
Shoot fresh. weight. (g)	11.11 \pm 1.46 ^a	6.83 \pm 0.90 ^b	9.26 \pm 1.22 ^a	5.55 \pm 0.74 ^b
Shoot Dry weight. (g)	4.32 \pm 0.57 ^a	1.77 \pm 0.24 ^b	2.51 \pm 0.33 ^b	1.22 \pm 0.16 ^b
Stem Fresh Weight. (g)	11.30 \pm 1.02 ^a	6.95 \pm 0.64 ^b	9.41 \pm 0.85 ^a	5.65 \pm 0.52 ^b
Stem Dry. Weight (g)	4.39 \pm 0.40 ^a	1.81 \pm 0.17 ^b	2.56 \pm 0.23 ^b	1.25 \pm 0.11 ^b
Leaf Fresh. Weight (g)	7.10 \pm 1.05 ^a	4.36 \pm 0.65 ^b	5.91 \pm 0.88 ^a	3.55 \pm 0.53 ^b
Leaf Dry. Weight (g)	2.77 \pm 0.41 ^a	1.14 \pm 0.17 ^b	1.60 \pm 0.24 ^a	0.78 \pm 0.12 ^b
Root Fresh. Weight (g)	3.18 \pm 0.69 ^a	1.95 \pm 0.43 ^b	2.64 \pm 0.58 ^a	1.59 \pm 0.35 ^b
Root Dry. Weight (g)	1.24 \pm 0.27 ^a	0.51 \pm 0.11 ^b	0.72 \pm 0.16 ^b	0.35 \pm 0.08 ^c
CP (mg kg ⁻¹)	29717.57 \pm 9.30 ^a	23655.34 \pm 6.14 ^b	27097.41 \pm 1.77 ^c	22109.25 \pm 7.06 ^d
Total K ⁺ (mg kg ⁻¹)	5072.93 \pm 6.90 ^a	2481.08 \pm 0.83 ^b	3271.93 \pm 1.83 ^c	2654.62 \pm 2.44 ^d

Different alphabetic labels referring to statistically significant differences ($\alpha = 0.05$), Abbreviations: CP = Crude Protein; K⁺ = Potassium Ion.

DISCUSSION

This study highlighted the optimal balance between the availability of a suitable concentration of inorganic-N (NH₄⁺) and the concentration of freely available soil oxygen (O₂) for effective nitrification (NH₄⁺-NO₃⁻; chemical conversion of ammonium to nitrate) in the evaluated fertilizers, hence subsequently affecting effective higher NO₃⁻ uptake (van Rooyen *et al.*, 2021). Although the TCF decomposition rate was observed to influence the ON-NH₄⁺ rate compared to the TOM decomposition rate, it was important to highlight the actual significance of the TCF decomposition rate affecting both rates of ON-NH₄⁺ and NH₄⁺-NO₃⁻. This is because both TCF and ON were the components of TOM, and TCF protected TOM from microbial attack and decomposition (Shahbuddin *et al.*, 2023a), directly affecting the ON decomposition rate. Since both BSFFP and CPC were plant-derived fertilizers, it could be proposed that TCF rich in cellulosic elements had effectively protected TOM decomposition, hence slowing ON decomposition and subsequent ON-NH₄⁺ decomposition rate. Similarly, high cellulosic TCF protected TOM from microbial attack and decomposition. It reduced the rapid consumption of freely available soil O₂ for TOM decomposition, hence increasing the rate of success for NH₄⁺-NO₃⁻, to be evident for both BSFFP and CPC, but was not observed in low cellulosic animal-derived TCF.

In addition to the high cellulosic plant-derived TCF for lowering TOM decomposition rate, referring to both BSFFP and CPC, another important aspect of this study was the form of organic nitrogen in influencing ammonium NH₄⁺ availability. The BSFFA and BSFFP contained organic nitrogen primarily in the simplified non-protein nitrogen, naturally degraded from the organic nitrogenous wastes of BSFL in the form of urea, uric acid, and allantoinic acid (Green and Popa, 2012; Britto and Kronzucker, 2002). In contrast, the plant-based compost (CPC), the ON present was in the form of plant-based complex proteins and amino acids, requiring more extensive microbial degradation and oxidative process for ammonification (Greenfield, 1972; Kelley and Stevenson, 1995; Quastel and Scholefield, 1949). This compositional difference is the key, as NPN compounds such as urea undergo hydrolysis and rapid ammonification, which release NH₄⁺ more rapidly than the complex proteins (Unggang *et al.*, 2023; Shahbuddin *et al.*, 2023b), which require sequential enzymatic steps (Diener *et al.*, 2015). This study aligns the animal-based BSFF (BSFFA), which produced higher nitrification rates due to faster NH₄⁺ production, ensuring the effect of ON form on NH₄⁺ to NO₃⁻ conversion. This study confirms findings by Beesigamukama *et al.* (2020) and Gold *et al.* (2020), who reported that BSF frass derived from protein-rich substrates improves nitrogen mineralization and soil fertility more efficiently than conventional fertilizers. Although high cellulosic TCF slows down the TOM decomposition, subsequently affecting slower ON decomposition and the availability of NH₄⁺. In addition to

the ON-NH₄⁺ process, simplified ON form such as urea could rapidly increase the availability of NH₄⁺ compared to plant-based complex proteins and amino acids, which required additional decomposition via oxidation of complex proteins and amino acids for effective availability of free NH₄⁺, compared to urea to ammonia (Green and Popa, 2012; Britto and Kronzucker, 2002), highlighting the importance of ON form for effective NH₄⁺-NO₃⁻ (Farzadfar *et al.*, 2021).

This was further evident for both BSFFA and BSFFP with the alkaline nature of the fertilizers; the ON was present in the form of ammonia from the simplified urea. The alkaline nature of BSFFA deviated from the initial assumption for BSFFA to record a more acidic pH, further proving the presence of simplified ON form (urea) in BSFFA, since most animal-based waste decompositions produced lactic acids (Hayek and Ibrahim, 2013; Masood *et al.*, 2011; Miranda *et al.*, 2021). CPC recorded slightly acidic pH, where most plant-based wastes related to plant-based complex proteins and amino acids' decompositions produced acetic acids (Lynch and Audus, 1976; Lynch, 1977). BSFFA could have been estimated to produce the highest NH₄⁺-NO₃⁻ rate, with the highest available NH₄⁺, but due to the high consumption of free available O₂ for rapid TOM decomposition, reducing the probability for effective NH₄⁺-NO₃⁻ rate compared to BSFFP with both essential factors of simplified ON (urea) and with high cellulosic TCF, slowing TOM decomposition, generating the highest NH₄⁺-NO₃⁻ rate. CPC, on the other hand, similarly to BSFFP with high cellulosic TCF, reduces TOM decomposition; however, due to the ON existing in CPC in the form of complex plant-based proteins and amino acids, requiring additional oxidation-based decomposition to simplified ammonia, hence consuming free available soil O₂, reducing the efficiency for effective NH₄⁺-NO₃⁻ rate.

It was initially postulated based from previous studies where nitrate uptake was to be hindered due to the relatively high clay portion of the soil type used in this study (> 25% clay; sandy clay loam) which many studies proved that high clay soil promoted denitrification; conversion of already available NO₃⁻ to NO₂ gas, with the accumulation of denitrifying bacteria in the top soil (D'Haene *et al.*, 2003; Luo *et al.*, 1998; Parkin and Meisinger, 1989), high cellulosic TCF decomposition produced elevated CO₂, slowing ON mineralization, increasing C/N ratio, higher organic carbon (OC) deposition, all produced positive effect on NO₃⁻ uptake rate, to be positively correlated with the increased growth rate of nitrifying bacteria (Jie *et al.*, 2009; Jones and Paskins, 1982). From this notion, BSFFA could not produce optimal nitrification due to low production of CO₂ from the decomposition of low cellulosic components of animal-based TCF. The relationship between carbon-rich amendments and boosting microbial

activity (Khair *et al.*, 2024) exhibited that increased CO₂ from high- cellulose material decomposition enhances the C/N ratio and organic carbon levels that extensively correlate with nitrification rate and nitrifier growth (Rieke *et al.*, 2022).

Analogous to other species of the short-term annual vegetable of the family *Amaranthaceae*, including *A. dubius*, showed the plasticity in terms of the uptake for the most available and the highest precipitated inorganic nitrogen (NH₄⁺ or NO₃⁻) in the applied fertilizers (Munene *et al.*, 2017). BSFFP fertilizer application induced comparable yet higher uptake of NO₃⁻ compared to NH₄⁺, while for both BSFFA and CPC, NH₄⁺ was the dominant inorganic nitrogen, with BSFFA recording 5.00 times higher NH₄⁺ uptake rate compared to NO₃⁻ uptake rate. Higher uptake of NO₃⁻ has been proven to induce simultaneous higher uptake of NH₄⁺, hence improving the efficient N acquisition under the application of both NH₄⁺ and NO₃⁻, compared to either sole NH₄⁺ or NO₃⁻ (Kronzucker *et al.*, 1999a). On the other hand, the initial increased uptake of NH₄⁺ also had an inhibitory effect on the allosteric-biochemical and physiological levels on the subsequent uptake of NO₃⁻ as proposed by most studies (Ayling, 1993; Boudsocq *et al.*, 2012; Kronzucker *et al.*, 1999b), evident for both BSFFA and CPC. Significantly reduced growth and biomass of *A. dubius* observed following BSFFA fertilization was attributed to the rapid NH₄⁺ uptake as the sole highest precipitated inorganic-N, proposed to be the result of NH₄⁺ toxicity, recorded among the lowest concentration of crude protein and lowest dietary potassium nutritional value (Table 3). Increased uptake of NH₄⁺ created uptake antagonism on the uptake of K⁺, since both cations have similar valence properties and ionic sizes (Xie *et al.*, 2021; Wang *et al.*, 1996).

Conclusions and Limitations of the Study: Rapid ammonification (ON-NH₄⁺) rate and high ON decomposition rate, where both processes produced higher extractable NH₄⁺ as the precursor for nitrification (NH₄⁺-NO₃⁻) was not the primary determinants for effective NH₄⁺-NO₃⁻ of insect-derived frass as new generation organic fertilizers. The integration or incorporation of both factors of extractable NH₄⁺ and available soil O₂ to produce effective NH₄⁺-NO₃⁻, dependent on high volume TCF, which affected the high TCF decomposition rate. Additionally, high cellulosic TCF protected TOM from microbial attack and decomposition, hence slowing TOM decomposition rate, reducing the consumption of freely available soil O₂ during TOM decomposition, related to plant-based wastes as the feeding substrate of BSFL. ON in the form of simplified non-protein nitrogen, such as urea, rather than complex proteins and amino acids, was also another important determinant for effective NH₄⁺-NO₃⁻ that reduced the consumption of free available soil O₂ during

ON decomposition. This study was conducted under controlled pot conditions, which may not fully reflect field performance. Additionally, the tested crop was only a short-term vegetable, *Amaranthus dubius*, and the results may not apply to other crops, while focused on short-term effects and did not evaluate long-term soil fertility and NH_4^+ -sensitive perennial crops, which require further studies.

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