Waste Management 119 (2021) 183–194

Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

Low-cost technology for recycling agro-industrial waste into nutrient-rich organic fertilizer using black soldier fly

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ARTICLE INFO

Article history:
Received 20 April 2020
Revised 23 September 2020
Accepted 24 September 2020

Keywords:
Black soldier fly larvae
Compost maturity
Nutrient retention in frass fertilizer
Substrate amendment
Waste recycling

ABSTRACT

Efforts to recycle organic waste using black soldier fly (BSF) larvae into high-quality alternative protein ingredients in animal feeds and organic fertilizers have gained momentum worldwide. However, there is limited information on waste manipulation to increase nutrient retention for enhanced larval performance and frass fertilizer quality. In the present study, brewer's spent grain with a carbon to nitrogen (C/N) ratio of 11 (control) was amended with sawdust to obtain substrates with C/N ratios of 15, 20, 25 and 30. The effects of substrate C/N ratios on BSF larval yield, waste degradation, biomass conversion efficiency, compost maturity and nutrient levels of frass fertilizer were evaluated. Substrates amended with sawdust did not significantly affect waste degradation efficiency and biomass conversion rates of BSF larvae. The wet and dried larval yields were significantly higher for substrates with C/N ratio of 15 compared to the other amended substrates. An amended substrate with C/N ratio of 15 enhanced nutrients uptake by BSF larvae, and increased nitrogen (N) and phosphorus retention in frass compost by 21 and 15%, respectively. Compost maturation time was shortened to five weeks, as indicated by the stable C/N ratios and high seed germination indices. This study has demonstrated that the amendment of the substrate with sawdust to C/N ratio of 15 could generate compost with desirable nutrients for use as high-quality fertilizer for organic farming.

1. Introduction

There is an increasing demand for the use of insects as high-quality protein ingredients in animal feeds (Makkar et al., 2014). Black soldier fly (*Hermetia illucens* L.) larvae have been widely advocated as an important source of proteins, fats, and vitamins in animal feeds (Shumo et al., 2019; Liu et al., 2017; van Huis, 2013). The black soldier fly (BSF) belongs to the family Stratiomyidae (Diclaro and Kaufman, 2012) and is native to tropical, subtropical and temperate regions of America (Caruso et al., 2014). The larvae of BSF can be grown on a range of decomposable organic waste streams including human feces (Banks et al., 2014; Lalander et al., 2013) a mixture of crop wastes (Jucker et al., 2017; Manurung et al., 2016; Supriyatna et al., 2016) and animal manure (Myers et al., 2008). The degradation efficiency of these decomposable organic wastes by BSF larvae ranges from 55 to 80% (Lalander et al., 2014; Diener et al., 2011).

The process of utilizing black soldier fly larvae to convert waste into high value fertilizer products have received little attention. The feed conversion ratios of BSF larvae are known to be superior to that of other insects (Oonincx et al., 2015). Compared to other insects, BSF survival rate, nitrogen and phosphorus compositions have been shown to largely depend on the substrate on which they are raised (Oonincx et al., 2015). The combination of their exuviae during the larval growth phase with the residual waste are anticipated to form a good fertilizer (Lalander et al., 2014; Webster et al., 2016), although little research has been conducted to support these findings. However, the main challenge with fertilizer from BSF frass is the low nutrient content and this is strongly supported

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https://doi.org/10.1016/j.wasman.2020.09.043
0956-053X/

by Lalander et al. (2014), who demonstrated that a large proportion of nitrogen (44%) is usually lost through evaporation as ammonia during the waste recycling process by BSF larvae. This ammonia evaporation is governed by the ammonium – ammonia equilibrium, which in turn depend on the pH and temperature of the substrate used (Sánchez et al., 2017; Bernal et al., 2009). Because of the pH and temperature variation, the evaporation of ammonia can become considerable in a well-ventilated system (Lalander et al., 2014; Bernal et al., 2009), which might lead to reduction of fertilizer value of the manure. The potential for commercialization of BSF larvae and frass fertilizer is relatively new yet has received a flurry of interest in the last couple of years within the United Nations (UN) and European Union (EU) (Oonincx et al., 2015). Generating frass fertilizer as a second value added product from BSF rearing would increase the profit margins of insect farmers as well as directly contribute to improving soil fertility and crop productivity.

Although the recycling process of organic by BSF larvae has been reported to produce reduced greenhouse gases (especially ammonia) compared to livestock production, it is feared that the negative human and environmental health impact involved (Palashikar et al., 2016) might cause BSF larvae farming to become less attractive business, both for potential young entrepreneurs as well as for authorities with the mandate to approve farming processes and policy formulations. Therefore, strategies to further conserve nitrogen during BSF larvae rearing through control of ammonia volatilization by using bulking agents to increase carbon to nitrogen ratios (C/N) of the substrates are needed (Sánchez et al., 2017; Bernal et al., 2009). For example, adjustment of substrate C/N ratio to a favourable range using carbon rich materials has been shown to improve nitrogen conservation during composting (Lim et al., 2009), thereby significantly reducing the impact of ammonia emissions. High carbon materials such as sawdust have been reported to enhance ammonia immobilization and binding onto phenolic compounds, which in turn reduce the amount of free ammonia, resulting in lower ammonia volatilization (Lim et al., 2017).

Composting of cattle manure with sawdust has been reported to reduce total nitrogen loss from 44.3% to < 35% and ammonia volatilization by up to 71% (Lim et al., 2017). Although, studies have demonstrated that a high C/N ratio (25–35) is crucial for the production of nutrient rich compost (Bernal et al., 2009), this might not be favourable for the growth performance of BSF larvae because, they require high nitrogen substrates (Lalander et al., 2019; Spranghers et al., 2017). However, information on the critical substrate C/N ratio for optimal BSF larval growth and frass nutrient conservation is limited. Therefore, further studies to understand the critical C/N ratios for optimal growth and better yields as well as frass quality are crucial. Composting has been reported as a common method widely used to recycle nutrients in organic wastes (Epstein, 1997; Bernal et al., 1998). However, information on appropriate time required to convert BSF larvae frass into mature and stable compost ready for field application is lacking. Therefore, the present study aimed to determine the critical substrate C/N ratio for optimal production of BSF larvae and production of nitrogen rich organic fertilizer from the frass.

2. Materials and methods

2.1. Black soldier fly colony maintenance

The stock colony of black soldier fly was established following the methodology described by Chia et al., (2018). The colony was initiated from eggs collected from wild adult BSF populations using a combination of 2-week-old fermented chicken and rabbit manure mixed with fruits (mango, pawpaw and orange peels) and household food wastes (rice, beans and posho) as baiting materials for adult flies. Wild adult BSF were collected in Kasarani, Nairobi County, Kenya (S 01° 13′ 14.6″; E 036° 53′ 44.5″, 1612 m above sea level). The baiting materials were placed inside 6-L plastic buckets designed with six openings, 5 cm from the lid to facilitate entry of adult flies for oviposition on the flutes of cardboard boxes attached on the sides of the containers. Thereafter, buckets containing the baits were hung on metallic stands under the shades around homesteads or close to garbage dump sites.

To prevent intruders (ants, reptiles, rats, and others) from accessing the content of the traps, the metallic stands holding the buckets were smeared with Tanglefoot (Tangle-trap; The Tanglefoot Company, Grand Rapids, MI, USA) insect barrier paste. The buckets were checked regularly (2–3 times/week) for deposited egg clusters in the cardboard flutes. Thereafter, the egg clusters were transferred into new containers with suitable substrates (kitchen waste, brewers' spent grain and pig manure) for rearing the newly hatched neonates. Conditions in the rearing room were maintained at 28 ± 2 °C, 60–70% RH and a photoperiod of L12:D12. Portable digital thermo-hygrometers were placed inside each of the rearing rooms to monitor temperature and relative humidity. The pre-pupal stages were harvested from the rearing trays and placed in transparent rectangular plastic containers (Kenpoly Manufacturer, Nairobi, Kenya) containing 60% moist wood shavings (sawdust). The prepupae grew to pupal stages and emerged flies were transferred into large outdoor cages (1 × 1 × 1.8 m) holding approximately 2000–2500 adult flies per cage. The adult flies were provided with 60% sugar solution on cotton wools and the colony was maintained at the animal rearing and quarantine unit (ARQU) at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi Kenya.

2.2. Sources of experimental substrates

Before the start of the experiment, fresh brewer's spent grains (BSGs) were sourced from Kenya Breweries Ltd. (Nairobi, Kenya), while sawdust (from blue gum tree, *Eucalyptus globulus* Labill. belonging to the family Myrtaceae) was collected from a local carpentry workshop located in Kasarani, Nairobi, Kenya. Detailed analyses of the nutrient levels, moisture content, pH, electrical conductivity, and C/N ratio of BSGs and sawdust were conducted using recommended laboratory methods (Okalebo et al., 2002). Results from the analyses are presented in Table 1.

Based on the results presented in Table 1, a combination of BSGs and sawdust was formulated to obtain five different substrates with varying C/N ratios of 15, 20, 25 and 30. The control treatment was not amended with sawdust. The amount of sawdust required to achieve the above C/N ratios were calculated using equation (1) (Richard and Trautmann, 1996). Table 2 shows the percent weight of sawdust and BSGs used to obtain the experimental substrates

$$Q_2 = \frac{Q_1 \times N_1 \times \left(R - \frac{C_1}{N_1}\right) \times (100 - M_1)}{N_2 \times \left(\frac{C_2}{N_2} - R\right) \times (100 - M_2)} \quad (1)$$

Where,

C_1 and C_2 - represent total organic carbon contents of brewer's spent grain and sawdust, respectively

N_1 and N_2 - represent total nitrogen contents of brewer's spent grain and sawdust, respectively

M_1 and M_2 represent moisture contents of brewer's spent grain and sawdust, respectively

Q_1 - represents the amount of brewer's spent grain (fresh weight) used

Table 1
Selected characteristics of substrates used in black soldier fly rearing.

Substrates	Moisture content (%)	pH (1:10 water)	EC (mS/cm)	TOC	Total N	Total P	Total K	Total Ca	Total Mg	C/N ratio
				(%)						
BSGs	68.5	4.2	1.52	30.5	2.76	0.44	0.11	0.45	0.19	11.1
Saw dust	21.3	5.4	0.45	53.3	0.18	0.016	0.043	0.14	0.024	296.1

BSGs = Brewer's spent grains, EC = Electrical conductivity, TOC = Total organic carbon, N = Nitrogen, P = Phosphorus, K = Potassium, Ca = Calcium, Mg = Magnesium.

Table 2
Percentage weights of sawdust and brewer's spent grain waste mixed to come up with feedstocks of varying carbon to nitrogen ratios.

Feedstock Formulations	Brewer's spent grain	Sawdust	Cost of brewer's spent grain	Cost of sawdust	Total cost per kg of feedstock
			US dollars		
Control (C: N 11)	100	0.0	0.36	0.0	0.180
C/N 15	91	8.5	0.33	0.003	0.167
C/N 20	80.2	19.8	0.29	0.008	0.149
C/N 25	68.5	31.5	0.25	0.012	0.131
C/N 30	56.1	43.9	0.20	0.018	0.109

Q_2 - represents the amount of sawdust (fresh weight) required to adjust C/N ratio of brewer's spent grain to the desired value

R - represents the desired C/N ratio of the rearing substrate (brewer's spent grain and sawdust mixture).

2.3. Black soldier fly larval growth and yield on various substrates

The BSF larvae rearing facility was equipped with a wooden stand (180 cm height × 66 cm width × 420 cm length). The stand had three shelves separated from each other by 30 cm space, where the metallic trays used in rearing the BSF larvae were fitted. Metallic trays used during the experiment measured 76 cm length × 27.5 cm width × 10 cm depth. The bottom of each tray measured 52 cm in length by 27.5 cm width, which allowed for both edges of the tray to be inclined at an angle of 35°. Both ends of each tray were provided with a collar of 5 cm long to allow it to sit smoothly on the edge of each trough. In the experimental room, one wooden stand was used, to accommodate 5 trays per shelf, totaling to 15 trays arranged in a complete randomized design.

At the start of the experiment, two thousand (2000) 5-day old BSF larvae were obtained from the stock colony and introduced into each tray containing 6.85 kg of the fresh substrates formulated. The recommended feeding rate per larva per day was 125 mg (dry weight) (Banks et al., 2014; Diener et al., 2009). Each rearing substrate was hydrated to approximately 70.0 ± 2% moisture by weight according to the protocol described by Cammack and Tomberlin (2017) and confirmed using a moisture sensor with two 12-cm-long probes (HydroSense CS620, Campbell Scientific, Logan, USA). Three replicates were conducted for each experimental substrate fed as often as necessary until the last larval stage (5th instar) was completed.

The experimental room was fitted with heater and subsequently heated using fast moving dry hot air at 28.0 ± 2 °C (using Xpelair heater: WH30, 3KW Wall Fan Heater, UK) with thermoregulators. Portable digital thermo-hygrometers were placed inside each of the rearing rooms to monitor temperature and relative humidity. Conditions in the experimental rearing room was maintained at 28 ± 2 °C, 60–70% RH and a photoperiod of L12:D12. The developmental time of larvae in each treatment was recorded. Thereafter, the larvae from each rearing tray was harvested and weighed to determine the yield per tray. At harvesting, hand sieving with a 2 mm diameter sieve was used to separate the larvae from the frass.

The amount of substrate consumed throughout the larval development phase and the frass was used to determine waste reduction efficiency on dry weight basis (Eq. (2)) (Diener et al., 2009)

$$\%WR = \frac{(\text{Initial weight} - \text{Final weight})}{\text{Initial weight}} \times 100 \quad (2)$$

Bioconversion rate (BCR) which indicates the efficiency of conversion of waste by BSF larvae into usable energy was also calculated using Eq. (3) (Dortmans, 2015).

$$\%BCR = \left[\frac{M_{pp} - M_i}{F_{in}}\right] \times 100 \quad (3)$$

Where,

Mi - represents the dry weight of the larvae at start of experiment,

M_{pp} - represents the dry weight of larvae at 5th instar,

F_{in} - represents the initial dry weight of the rearing wastes used at the start of experiment.

2.4. Nutrient flows

The fraction of initial N, P and K accumulated in BSF larvae (BSFL) biomass were determined using Eq. (4), while the fraction of initial N, P and K retained in the frass fertilizer were determined using Eq. (5).

$$(\%)N,P,K \ \ accumulated \ in \ BSFL \ biomass = \frac{\left(\frac{\%N,P,K \ \ in \ \ BSFL}{100}\right) \times Dry \ \ BSFL \ \ yield \ \ (g)}{Initial \ \ N,P,K \ \ content \ \ in \ \ substrate \ \ (g)} \times 100 \quad (4)$$

$$(\%)N,P,K \ \ retained \ \ in \ \ FF = \frac{\left(\frac{\%N,P,K \ \ in \ \ FF}{100}\right) \times Dry \ \ weight \ \ of \ \ FF(g)}{Initial \ \ N,P,K \ \ content \ \ in \ \ substrate \ \ (g)} \times 100 \quad (5)$$

Where,

$$Initial \ \ N,P,K \ \ content \ \ in \ \ substrate \ \ (g) = \frac{\%N,P,K \ \ in \ \ \ substrate \times Dry \ \ \ weight \ \ of \ \ substrate \ \ (g)}{100}$$

FF represents frass fertilizer

2.5. Quality of frass and frass compost yield

The moisture content of the frass from the different treatments after harvesting the BSF larvae were maintained at 70% as described above and monitored daily to assess readiness for use as compost. During the larvae rearing and composting phases, changes in temperatures were carefully recorded daily using a composting digital thermometer. The metallic probe of the digital thermometer was inserted at a depth of 7 cm into the substrate and four readings from four different points in each tray were recorded weekly. From each tray, 50 g of the substrate was collected weekly for monitoring compost maturity. This process was repeated for each treatment and parameters for monitoring compost maturity such as water soluble carbon to total nitrogen ratio (0.55), pH (6–8), ammonium to nitrate ratio (<0.16) (Bernal et al., 2009), electrical conductivity (<4 mS cm^{-1}) (Huang et al., 2004) were determined. The C/N ratio (<20) (Goyal et al., 2005) of the frass was monitored on a bi-weekly basis, while seed germination index (>80%) (Teresa and Remigio, 2011) was determined at maturity. Compost maturity was monitored both during the larvae rearing and composting phases. At maturity, frass compost from each treatment was weighed and oven dried at 105 °C for 24 h to determine the dry frass compost yield. Also, 50 g of the frass compost was collected from each treatment, air dried for five days and ground into powder to determine total organic carbon, nitrogen, phosphorus, potassium and C/N ratio at a different time of each experiment (weeks 1, 3 and 5).

2.6. Laboratory analysis methods

Fifty (50) grams of fresh frass compost from each treatment were analyzed to determine their pH, electrical conductivity (EC) and water-soluble carbon using aqueous extracts of 1:10 (w/v) compost to distilled water (Teresa and Remigio, 2011). The content of each treatment was shaken for 30 min, and the pH and EC values were recorded directly using a pH meter (AD1000, Romania) and an EC meter (AVI, India), respectively. The solution was then filtered using a Whatman No. 1 filter paper and 2 ml of the filtrate was used for further analysis of water-soluble carbon by complete oxidation using potassium dichromate and concentrated sulphuric acid, which was heated at 150 °C for 30 min. Later, the solution was allowed to cool, and the un-neutralized potassium dichromate was titrated against ferrous ammonium sulphate and water-soluble carbon concentration was determined following standard procedures (Okalebo et al., 2002).

The nitrate and ammonium from compost of each treatment was determined using 0.5 M potassium sulphate at 1:10 (w/v). The entire content was shaken for 1 h using a reciprocating shaker (KOS – 3333/KCS – 3333, MRC, UK) and the solution was filtered through a Whatman No. 1 filter paper and the filtrate used for further analyses. Ammonium and nitrate concentrations were determined calorimetrically according to the methods described by (Okalebo et al., 2002). The ammonium in the filtrate was complexed with sodium hydroxide, sodium hypochlorite, sodium nitroprusside, sodium salicylate, sodium citrate and sodium tartrate while nitrate was complexed with sodium hydroxide and 5% salicylic acid. The complexed solutions were allowed to stand for 1 h to enable blue and yellow colour development for ammonium and nitrate, respectively. Standard solutions for ammonium and nitrate were prepared using ammonium sulphate and potassium nitrate, respectively. Reagent blanks and the standard series for ammonium and nitrate were complexed following the procedures described above for colour development.

The absorbencies of the standards, samples and blanks were read using the atomic absorbance spectrophotometer (G10S UV–Vis, USA) at 419 and 655 nm for nitrate and ammonium, respectively. Standard calibration curves of absorbance against concentration of ammonium and nitrate were plotted. Slopes from the calibration curves were used to calculate the ammonium and nitrate concentrations using the absorbance values obtained above. Thereafter, the final concentrations of ammonium and nitrate (on dry weight basis) in the samples were calculated using Eq. (6). The ammonium/nitrate ratio was determined by dividing the concentration of ammonium to that of nitrate, determined at each sampling period.

$$Ammonium/nitrate(mgkg^{-1}) = \frac{(a-b) \times v \times MCF}{w} \quad (6)$$

Where,

a - represents concentration of ammonium/nitrate in the sample solution

b - represents concentration of ammonium/nitrate in the blank

v - represents volume of the extract

w - represents the sample weight

MCF - represents moisture correction factor, determined by oven drying the samples at 105 °C for 24 h. MCF = dry sample weight/ fresh sample weight.

Total organic carbon was determined using the wet oxidation method (Nelson and Sommers, 1982). Total nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) were extracted using acid digestion methods as outlined in the procedures described by Okalebo et al. (2002). A sample weight of 0.3 g was used in the digestion. The nutrients were extracted using 10 ml of digestion mixture made by dissolving 0.42 g of selenium powder and 14 g of lithium sulphate in 350 ml of 30% hydrogen peroxide and 420 ml of concentrated sulphuric acid. The digestion process was carried out in 250 ml digestion tubes at temperatures of 360 °C for three hours to obtain a colourless solution (digestate) that was later used during the analysis of the different nutrients. Total nitrogen in the digestate was determined using the Kjeldahl distillation method (Jackson, 1973).

Total phosphorus was determined using the Ultraviolet–visible (UV–Vis) spectroscopy method by complexing the digestate with the solution containing sulphuric acid, ammonium molybdate, antimony potassium tartrate and ascorbic acid (Okalebo et al., 2002). The standard series for P were prepared using potassium dihydrogen phosphate. The complexed solutions were allowed to stand for 1 h for complete reaction (i.e. development of blue colour). Thereafter, the absorbances of the standard solutions, samples and blanks were read at 880 nm using atomic absorbance spectrophotometer (G10S UV–Vis, USA). Standard calibration curve of the absorbance versus concentration was plotted. Sample absorbances were converted into P concentration using the slope calculated from the standard curve. The final P concentration was calculated using equation (7).

$$TotalP(\%) = \frac{(a-b) \times v \times f}{w} \quad (7)$$

Where,

a - represents concentration of P in the sample solution

b - represents concentration of P in the blank

v - represents volume of the digest

f - represents dilution factor

w - represents the sample weight

Total potassium concentration in the digested sample solution was determined using flame photometry (Okalebo et al., 2002). Standards for potassium were prepared using potassium chloride. The K standards, digested sample solutions, and blanks were sprayed into flame photometer (410 flame photometer, UK) and the flame transmissions were recorded. The amount of K present in the sample was read from the standard calibration curve, pre-

pared by plotting transmission readings against K concentration. The final K concentration was calculated using Eq. (8).

Calcium and magnesium contents in the digested solutions were determined by atomic absorbance spectrometry (Okalebo et al., 2002). Standard solutions were prepared using calcium carbonate and magnesium sulphate for Ca and Mg, respectively. During Ca determination, 10 ml of 0.15% Lanthanum chloride were added in the standard solutions, sample solutions and the blanks to avoid ionic interference. Magnesium samples were prepared by diluting 5 ml aliquots of digested solution to 50 ml using distilled water in a 50 ml volumetric flask. The standard solutions, sample solutions and the blanks for Ca and Mg were sprayed into atomic absorption spectrophotometer (iCE 3000 series AA spectrometer, China) to obtain absorbances at 422.7 and 285.2 nm for Ca and Mg, respectively. The calibration curves of the standard series readings were constructed for both Ca and Mg, which were later used to determine the concentrations of the samples and the blanks. The final concentrations of Ca and Mg in the samples were calculated using equation (8).

$$TotalK, Ca, Mg(\%) = \frac{(a-b) \times v \times f \times 100}{1000 \times w \times 1000} \quad (8)$$

a - represents concentration of K, Ca, and Mg in the sample solution

b - represents concentration of K, Ca, and Mg in the blank

v - represents volume of the digest

f - represents dilution factor

w - represents the sample weight.

2.7. Phytotoxicity test on mature frass compost extracts

The germination index was determined by growing cabbage seeds in the various frass fertilizers. Ten (10) cabbage seeds were randomly selected and placed on petri dishes lined with filter paper moistened with 10 ml of 10% compost extracts for 96 h at 25 °C in a dark chamber. The same procedure was repeated using distilled water as a positive control. Germination index (GI) was determined from: GI = (%G × %L)/100. Where, %G and %L are relative germination and root elongation, respectively, compared to distilled water. Compost with GI values below 50% was considered high phytotoxic, values between 50% and 80% indicated moderately phytotoxic; and values above 80% indicated the absence of phytotoxicity (Teresa and Remigio, 2011).

2.8. Data analysis

To quantify the effect of C/N ratio amendment on nutrient levels of mature compost, change in N, P and K contents were calculated as differences between the initial nutrient in the substrates formulated and nutrient concentration in mature frass compost generated. The changes in nutrient concentration were then expressed as percent of the initial nutrient in the substrates formulated. Data on pH was converted into hydrogen ion concentration before analysis and the output was transformed back to $-\log_{10}[H^+]$ = pH. Data were tested for normality using the Shapiro-Wilk test. Analysis of variances was performed using the linear mixed-effect model with 'lmer' function from the package 'lme4′ in R statistical software. The substrate and sampling time were kept as fixed effects, while replication was random effect. The effect of the substrate C/N ratios and the composting time on pH, electrical conductivity, ammonium, nitrate, ammonium/nitrate ratio, total organic carbon, C/N ratio, total N, P and K were determined. Data on fresh and dry BSF larval yields, waste reduction, biomass conversion rate, frass compost yield, seed germination, germination index, initial substrate N content, N uptake by BSF larvae, N retention in frass were analysed using one-way analysis of variance. Computation of least squares means was done using 'lsmeans' package. Significant means were separated using Student Newman Keul's (SNK) test at $p < 0.05$. All the statistical analyses were conducted using R software version 3.6.0 (R Core Team, 2019).

3. Results

3.1. Black soldier fly larval yield

There was a significant effect of rearing substrates on the fresh ($p < 0.05$) and dry ($p < 0.05$) BSF larval yields (Fig. 1A). Fresh larval weight gain of BSF reared on the control substrate (unamended) and substrate with C/N of 15 did not vary significantly. Rearing substrates with C/N ratio of 30 and 25 had the least fresh and dry larval yields, respectively. The development time of BSF larvae reared on the various substrates did not vary considerably. The total development time from hatching to fifth instar larvae was 13 days in each substrate.

There was significant percentage reduction in fresh ($p < 0.05$) and dried ($p < 0.05$) BSF larval yield across the different substrates when compared with the control treatment (Fig. 1B). The lowest percentage reduction for both dried and fresh BSF larvae was observed on substrates with C/N ratio of 15. While the highest percent reduction in dried and fresh larval yields were recorded on substrate with C/N ratio of 25 and 30, respectively.

3.2. Waste reduction and biomass conversion rate

The waste reduction and biomass conversion rates did not vary significantly ($p > 0.05$) across the different substrates (Fig. 1C). Waste reduction ranged between 24.4 and 34.3% for substrates with C/N ratios of 30 and 25, respectively. However, biomass conversion rates recorded varied between 9 and 13.6% for the substrate with a C/N ratio of 25 and the control treatment, respectively.

3.3. Frass compost yield

Significant differences were observed for both fresh ($p < 0.05$) and dried ($p < 0.05$) compost yields between the substrates used (Fig. 1D). Substrates with a C/N ratio of 20 and above showed no significant variation on fresh compost yields. Substrates with C/N ratio of 20 had the highest fresh and dried compost yields, followed by substrates with C/N ratios of 25 and 30. The control treatment and substrate with C/N of 15 had the lowest fresh and dried compost yields, respectively.

3.4. Nutrient flows in BSF larvae and frass compost

Adjustment of substrate C/N ratio caused significant ($p < 0.05$) differences in the initial total N concentrations of the substrates (Table 3). However, the initial concentrations of total P and K did not vary significantly ($p > 0.05$) due to C/N ratio adjustment. The control substrate had the highest initial concentrations of total N and P, while substrate with C/N ratio of 20 had the highest total K concentration.

The initial content of K in rearing substrates varied significantly ($p < 0.05$) due to C/N ratio adjustment (Table 3). However, the C/N ratio adjustment did not significantly ($p > 0.05$) influence the total N and P contents of the substrates. The control substrate had the highest contents of nitrogen and phosphorus concentrations while highest potassium content was achieved in substrate with C/N ratio of 15. The fraction of initial contents of nitrogen, phosphorus and potassium accumulated in BSF larval biomass did not vary significantly ($p > 0.05$) due to C/N ratio adjustment. However, larvae

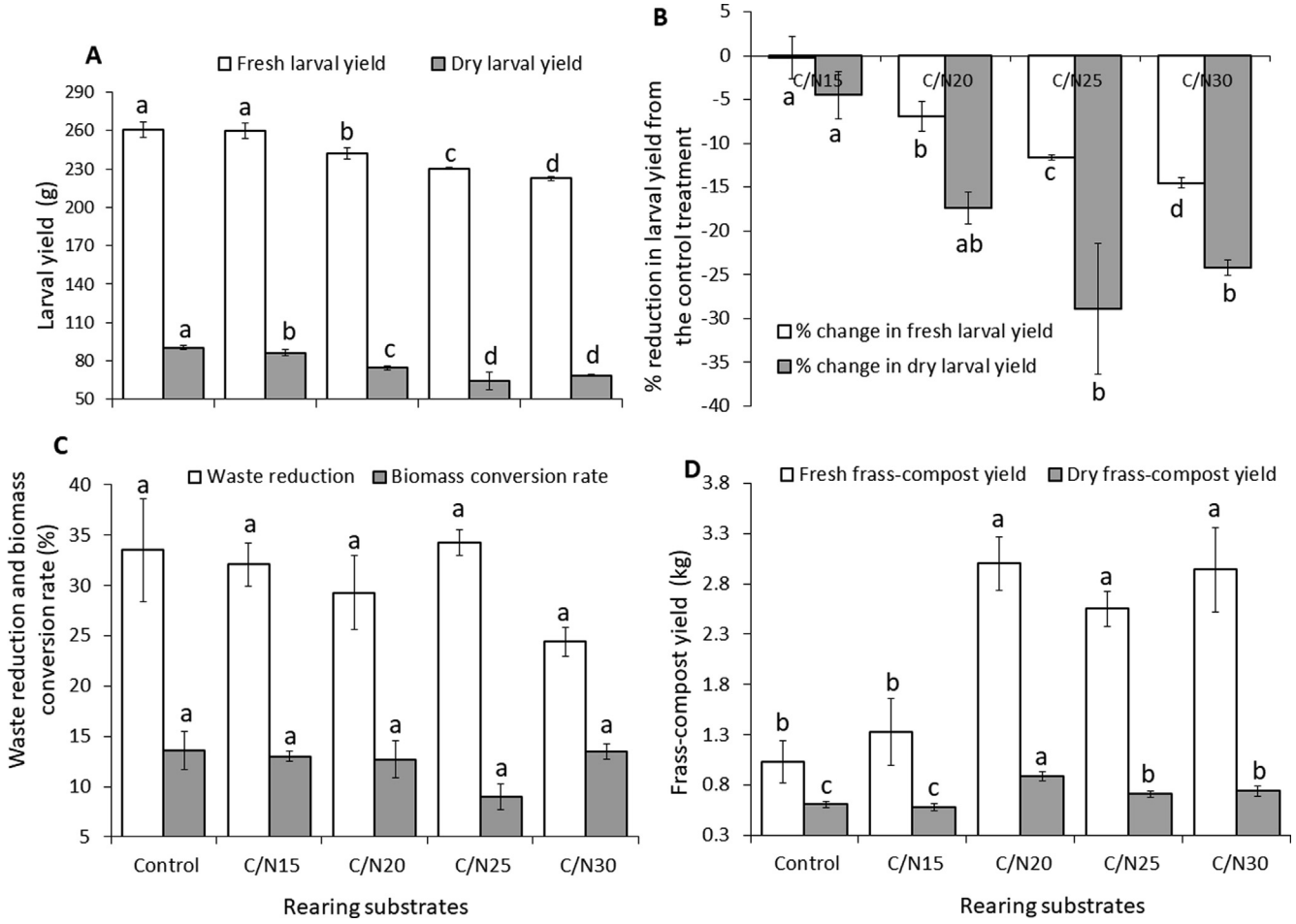

Fig. 1. Effect of substrate C/N ratio on black soldier fly larval yields (A and B), waste reduction and biomass conversion rates (C), and frass compost yield (D).

Table 3
Effect of substrate C/N ratio on nutrient accumulation by BSF larvae and nutrient retention in frass fertilizer.

Rearing substrates	Initial nutrient concentrations in substrates			Initial nutrients content per tray (g)			Fraction of initial nutrients content accumulated by BSF larvae (g 100 g^{-1})			Fraction of initial nutrients content retained in frass fertilizer (g 100g^{-1})		
	N (%)	P	K	N	P	K	N	P	K	N	P	K
		(g kg^{-1})										
Control	2.69 ± 0.2a	4.30 ± 0.0a	1.09 ± 0.0a	39.7 ± 2.7a	8.6 ± 0.1a	2.2 ± 0.0ac	6.8 ± 0.7a	6.0 ± 1.0a	22.2 ± 0.8a	33.1 ± 4.6a	12.0 ± 0.4b	35.5 ± 4.4a
C/N 15	2.06 ± 1b	3.64 ± 0.3a	1.54 ± 0.3a	30.9 ± 0.6ab	7.3 ± 0.6a	3.07 ± 0.5a	9.0 ± 0.5a	8.8 ± 2.0a	23.0 ± 6.2a	47.0 ± 4.4a	15.8 ± 1.1ab	20.0 ± 2.1a
C/N 20	1.54 ± 0.1b	3.76 ± 0.4a	1.53 ± 0.1a	30.9 ± 2.4ab	7.5 ± 0.9a	3. 05 ± 0.1ab	8.9 ± 0.6a	5.5 ± 0.8a	14.7 ± 1.2a	45.6 ± 6.7a	19.1 ± 2.2a	29.1 ± 2.8a
C/N 25	1.53 ± 0.1b	3.34 ± 0.1a	1.02 ± 0.1a	30.5 ± 2.3ab	6.7 ± 0.2a	2.04 ± 0.2ac	7.4 ± 1.3a	6.4 ± 0.3a	20.5 ± 1.7a	36.2 ± 2.3a	14.4 ± 1.4ab	28.4 ± 1.7a
C/N 30	1.64 ± 0.0b	3.43 ± 0.1a	0.94 ± 0.1a	32.7 ± 0.8ab	6.9 ± 0.3a	1.89 ± 0.2ac	7.4 ± 0.4a	6.8 ± 0.4a	21.7 ± 0.3a	29.2 ± 0.8a	11.7 ± 1.5b	21.7 ± 4.9a

N = nitrogen, P = phosphorus, K = potassium

reared on substrates with C/N ratio of 15 accumulated higher N, P and K contents than those provided with the control substrate.

There were significant differences ($p < 0.05$) in the fraction of phosphorus retained in frass composts due to C/N ratio adjustment (Table 3). Frass composts generated from substrates with C/N ratio of 20 retained significantly ($p < 0.05$) higher P than the frass composts generated from control substrate and substrate with C/N ratio of 30. The fraction of total nitrogen and potassium retained in the frass composts did not vary significantly ($p > 0.05$) (Table 3). However, frass composts generated from substrates with C/N ratios of 15, 20 and 25 retained higher N than the frass compost derived from the control substrate, which achieved the highest K retention.

3.5. Temperature, pH, and electrical conductivity evolution during the experiment

The variation in room and substrate temperature during the larval rearing and composting phase (post-larval rearing phase), respectively, is presented in Fig. 2A. The temperatures of the various substrates during the larval rearing phase increased and peaked on the third day with temperatures ranging between 26 and 43 °C. The highest initial temperature was recorded from substrate with C/N ratio of 20 (42 °C). During the composting phase, the room temperature was slightly above that of the substrate throughout the experiment.

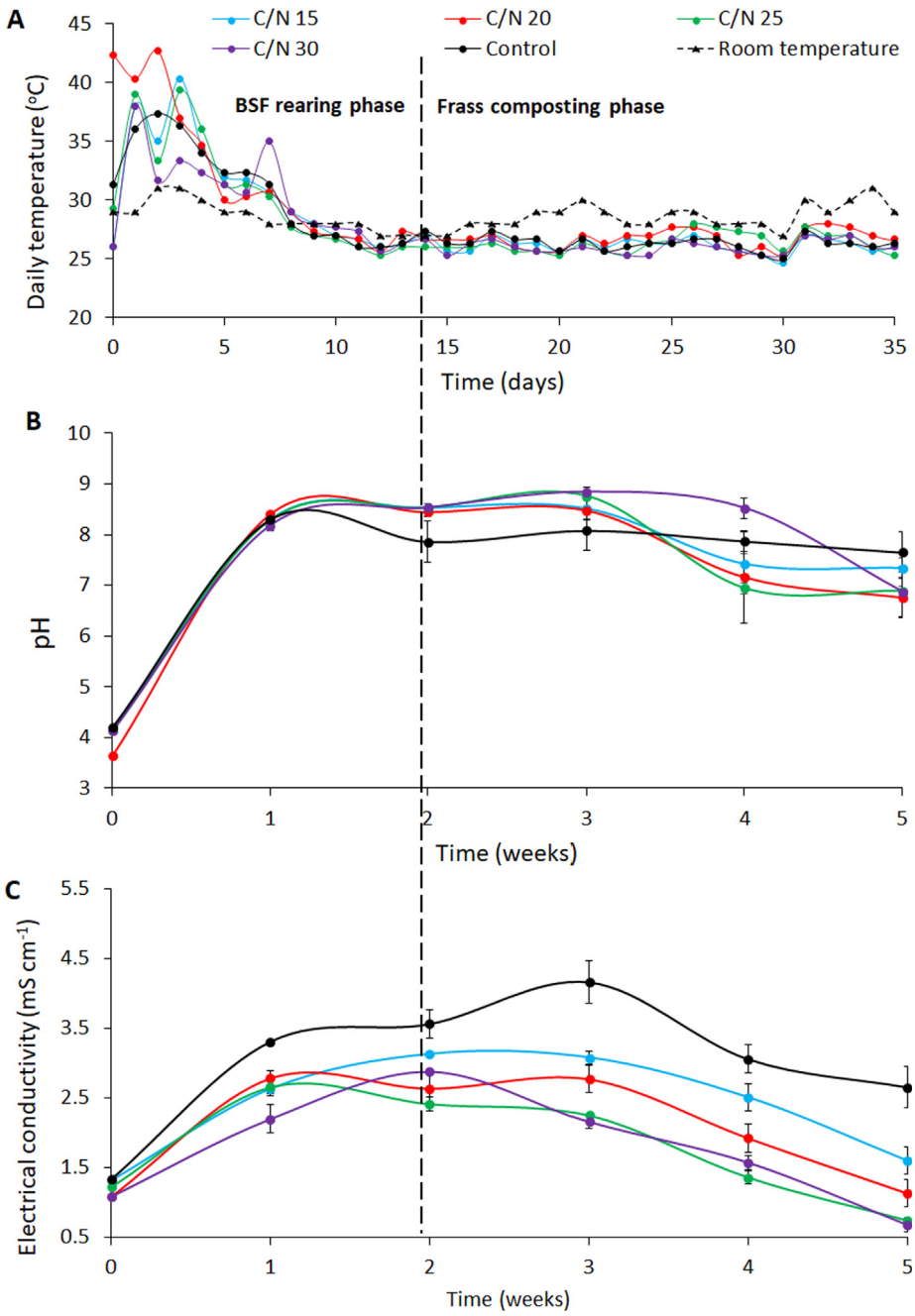

Fig. 2. Effect of substrate C/N ratio on temperature (A) pH (B) and electrical conductivity during BSF larvae rearing and frass composting phases.

The pH of the substrates during the larval rearing and composting phase varied significantly ($p < 0.05$) (Fig. 2B). The pH reached peak values (8.1–8.8) in the third week, at the end of the experiment, the control treatment had the highest pH (7.6) while frass compost from substrates with C/N ratio of 30 had the highest pH (6.9). The electrical conductivity (EC) values of the substrates differed significantly through the experiment ($p < 0.05$) (Fig. 2C). The EC of the unamended (control) was significantly higher compared to that of the other substrates throughout the experiments.

3.6. Ammonium and nitrate concentrations

The ammonium concentration recorded in the control experiment was significantly higher ($p < 0.05$) compared to that of the other frass composts, followed by that of substrates with C/N ratio of 15 (Table 4). The ammonium concentrations increased steadily in the control experiment up to the fourth week and slightly declined in the fifth week, while those of composts from substrates with C/N ratio of 15 increased from the second week up to the end of the experiment.

The nitrate concentration changed significantly ($p < 0.05$) over time and was significantly ($p < 0.05$) influenced by the substrate (Table 4). The nitrate concentration of substrates with C/N ratio of 15 sharply increased after the fourth week and peaked at the fifth week (159.6 mg kg^{-1}) while those of control treatment decreased in the fifth week of experiment. At the end of the fifth week, the lowest concentration of nitrate was recorded from frass compost derived from substrate with C/N ratio of 25 (59.8 mg kg^{-1}). The ammonium to nitrate ratio of compost generated from substrate with C/N ratio of 30 was the highest at weeks 2 and 3

Table 4
Concentrations of ammonium and nitrate, and ammonium/ nitrate ratio during the experiments.

Parameter	Time (weeks)	Rearing substrates				
		Control (C/N11)	C/N15	C/N20	C/N25	C/N30
Ammonium (mg kg^{-1})	0	11 ± 1.0ab	10 ± 0.6ab	14 ± 3.6a	1 ± 0.5c	5 ± 0.5bc
	1	1253 ± 34.9a	845 ± 114.8ab	635 ± 11.0b	423 ± 125.0b	464 ± 145.5b
	2	1400 ± 137.4a	945 ± 62.9b	563 ± 5.0bc	529 ± 0.6c	583 ± 71.1bc
	3	1987 ± 281.5a	944 ± 105.0b	554 ± 60.2b	437 ± 26.9b	444 ± 34.8b
	4	3448 ± 921.1a	1173 ± 193.6b	559 ± 194.7b	297 ± 71.1b	443 ± 46.4b
	5	3466 ± 201.7a	1304 ± 457.5ab	301 ± 185.7b	146 ± 36.8b	25 ± 11.9b
Nitrate (mg kg^{-1})	0	1.0 ± 0.3a	1.1 ± 0.2a	1.4 ± 0.4a	0.7 ± 0.0a	1.2 ± 0.2a
	1	22.8 ± 2.9a	15.2 ± 4.6a	21.1 ± 1.8a	18.0 ± 0.4a	17.8 ± 5.1a
	2	33.5 ± 3.1a	21.5 ± 3.1b	16.1 ± 1.2bc	15.2 ± 2.4bc	7.5 ± 1.6c
	3	45.2 ± 8.8a	15.9 ± 2.8b	17.4 ± 2.3b	7.9 ± 0.3b	4.8 ± 0.7b
	4	116.8 ± 37.3a	41.3 ± 14.7ab	42.3 ± 17.3ab	31.0 ± 6.1ab	13.5 ± 0.7b
	5	101.6 ± 25.2a	159.6 ± 64.3a	96.0 ± 7.4a	59.8 ± 16.6a	79.3 ± 19.1a
Ammonium/Nitrate ratio	0	11.0 ± 2.9	9.1 ± 1.7	10.0 ± 3.6	1.4 ± 0.6	4.2 ± 0.7
	1	55.0 ± 7.6	55.6 ± 15.8	30.1 ± 2.3	23.5 ± 6.8	26.1 ± 5.0
	2	41.8 ± 2.5	44.0 ± 4.8	34.9 ± 6.0	34.8 ± 6.6	77.7 ± 16.8
	3	44.0 ± 17.6	59.4 ± 7.5	31.8 ± 8.7	55.3 ± 4.4	92.3 ± 19.6
	4	29.5 ± 1.7	28.4 ± 9.5	13.2 ± 6.2	9.6 ± 3.6	32.8 ± 4.3
	5	34.1 ± 12.0	8.2 ± 2.3	3.1 ± 1.6	2.4 ± 1.8	0.3 ± 0.1

Note: In the same row, means with the same letter are not significantly different at $p < 0.05$.

(Table 4). There was an overall decline of ammonium-nitrate ratios among the various frass composts from the third week of the experiment, except for frass composts generated from substrates with C/N ratio of 30.

3.7. Seed germination and germination index

Frass compost generated from the various substrates had a significant influence on the percent seed germination ($p < 0.05$) and seed germination index ($p < 0.05$) (Table 5). The seed germination percent was above 80% for all substrates tested. The control and substrate with C/N ratio of 20 had the highest seed germination rates, while the substrate with C/N ratio of 15 had the lowest value (83.3%). The seed germination indices of substrates with C/N ratios of 15 and 20 had significantly higher values compared to other substrates. No significant differences were observed between the control and substrates with C/N ratios of 25 and 30.

3.8. Total organic carbon, total nitrogen, phosphorus, and potassium

The total organic carbon and nitrogen concentrations of the various treatments varied significantly ($p < 0.05$) throughout the experiment. The trends of total organic carbon and nitrogen concentration are presented in Fig. 3A and B. The total organic carbon contents of all the experimental substrates declined considerably throughout the experiment.

A consistent decline in total N concentrations in all the treatments were observed until the third week, followed by a gradual increase until the fifth week. The total nitrogen level in the frass compost generated from substrates with C/N ratio of 15 was significantly ($p < 0.05$) higher than those frass composts derived from substrates whose C/N ratios were adjusted. There was significant ($p < 0.05$) variation in the percent change of nitrogen across the different treatments. The substrate with C/N ratio of 15 was the most efficient in nitrogen conservation, as indicated by a 21% increase in N concentration of frass compost generated compared to the control. However, there was an increased reduction of nitrogen content in frass compost generated from substrates with C/N ratios of 30 (−21%) and the control (−19.43%).

The total phosphorus (P) in the different frass composts was found to vary significantly ($p < 0.05$) during the experiment (Fig. 3C). Phosphorus concentration was observed to decline significantly until the third week of the experiment, followed by a slight increase in the fifth week.

The P concentration of frass compost derived from substrate with C/N ratio of 15 was significantly higher compared to that of the other treatments until the third week. However, the P conservation in frass compost generated from substrate with C/N ratio of 30 remained low throughout the experiment compared to the other composts. At the end of the composting process frass compost derived from substrates with C/N ratio of 15 had significantly ($p < 0.05$) higher P concentration than those derived from substrates with higher C/N ratios. However, there was a significant ($p < 0.05$) percent reduction in total P concentration at end of the experiment in all the frass composts generated. The lowest P reduction was observed for compost from substrate with a C/N ratio of 15 (−45%), while the highest percent reduction was recorded from compost derived from substrate with a C/N of 30 (-67.8%).

Total K concentrations were found to vary significantly ($p < 0.05$) among the different experimental substrates tested (Fig. 3D). Substrates with C/N ratios of 20 and 15 had the highest initial total K levels while substrates with C/N ratios of 30 had the least concentration. At the end of the composting phase, K concentration in frass compost generated from the 'control' substrate was higher ($p > 0.05$) than that in frass compost generated from substrates with C/N ratio of 30 compost from substrate with C/N ratios of 15. The percentage change of K content at the end of the composting process (mature frass compost) was observed to vary significantly ($p < 0.05$) among the different treatments. Increase in K concentration was observed in composts from the control substrate (44%) and compost derived from substrates with C/N ratio of 25 (1.5%). The least reduction (12%) in K concentration was recorded in compost derived from substrate with C/N ratio of 15 while the highest (20%) was recorded from compost generated from substrates with C/N ratio of 30.

Table 5
Effect of substrate carbon to nitrogen (C/N) ratio adjustment on seed germination and seed germination indices of matured black soldier fly composted manures.

Rearing substrates	Seed germination (%)	Seed germination index (%)
Control (C/N 11)	100 ± 0.0a	62.4 ± 39.5b
C/N15	83.3 ± 3.3b	160.3 ± 47.3a
C/N20	100 ± 0.0a	190.7 ± 26.2a
C/N25	93.3 ± 3.3a	71.5 ± 12.9b
C/N30	93.3 ± 3.3a	81.6 ± 23.6b

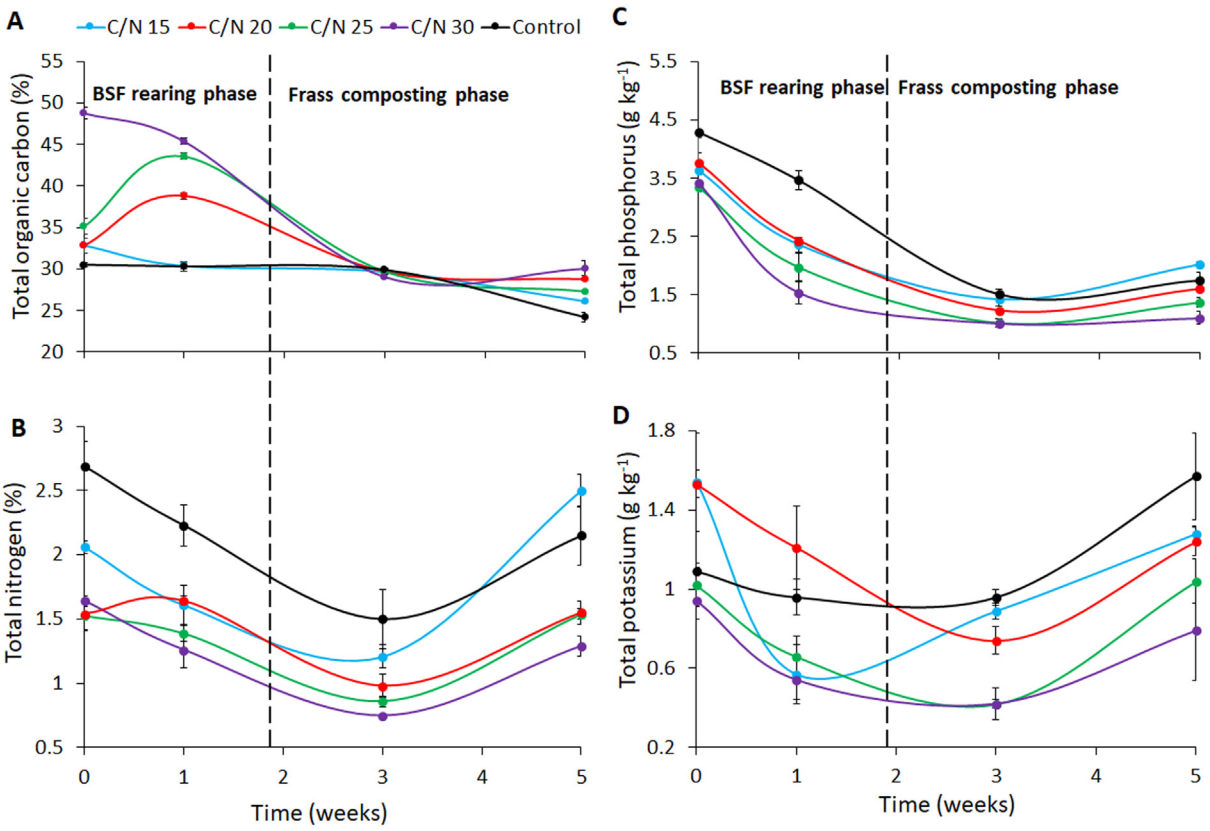

Fig. 3. Effect of substrate C/N ratio on concentrations of total organic carbon (A), nitrogen (B), phosphorus (C) and potassium (D) during BSF larvae rearing and frass composting phases.

3.9. Ratios of carbon to nitrogen

The ratios of total organic carbon to total nitrogen (C/N ratio) and water-soluble carbon to total nitrogen (WSC/TN) are presented in Table 6. The C/N ratios and WSC/TN ratios were observed to decrease progressively with composting time in all the substrates tested. The highest C/N ratios were recorded from substrate with C/N ratio of 30 with values ranging between 23 and 39. At the end of experiments, frass composts generated from substrates with C/N ratio of 15 had the lowest C/N (10.5) and WSC/TN ratios (1.0).

4. Discussion

4.1. Effects of substrate carbon to nitrogen ratios on BSF larval yields

The present study revealed significantly higher fresh and dry BSF larval yields in the control treatment with C/N ratio of 11 and the substrate with C/N ratio of 15. These findings can be attributed to higher nitrogen content of both substrates. Our results are consistent with those reported by Lalander et al. (2019) and Spranghers et al. (2017), who demonstrated that high nitrogen content of rearing substrates was one of the major factors capable of enhancing faster growth rates of BSF larval and biomass accumulation. Our findings are further supported by Jucker et al. (2017), who observed smaller larvae sizes from substrates with low nitrogen content. Studies have also shown that substrates with high carbon to nitrogen ratios such as soybean residues result in low biomass conversion rates by BSF larvae (Rehman et al., 2017), which is in accordance with the larval yields recorded from substrates with C/N ratio of 30. Contrarily, substrates with lower carbon to nitrogen ratios in the present studies had higher biomass conversion rates. This implies that substrates with lower carbon to nitrogen ratios or substrates with higher nitrogen should be used in BSF rearing to achieve higher larval biomass conversion rates

Table 6
Ratios of organic carbon and water-soluble carbon to total nitrogen at selected periods during the experiment.

Ratio	Time (weeks)	Rearing substrates				
		Control (C/N 11)	C/N15	C/N20	C/N25	C/N30
C/N	0	11.5 ± 0.95	15 ± 0.35	20.4 ± 0.99	25 ± 1.53	29.8 ± 0.32
	1	13.7 ± 0.68	19.3 ± 2.2	23.7 ± 0.75	31.4 ± 1.51	36.9 ± 3.74
	3	21.0 ± 3.62	24.8 ± 1.85	30.9 ± 2.85	34.6 ± 1.42	38.9 ± 0.44
	5	11.5 ± 1.47	10.5 ± 0.58	18.7 ± 0.98	17.7 ± 0.64	23.4 ± 1.72
WSC/TN	0	4.8 ± 0.57	5.2 ± 0.16	3.5 ± 0.11	4.2 ± 0.37	4.6 ± 0.06
	1	5.2 ± 0.88	5.2 ± 1.04	5.4 ± 0.18	6.1 ± 0.48	6.1 ± 0.59
	3	4.2 ± 1.16	3.0 ± 0.80	3.3 ± 0.22	1.8 ± 0.67	3.0 ± 1.16
	5	1.2 ± 0.25	1.0 ± 0.07	1.7 ± 0.24	1.6 ± 0.29	1.7 ± 0.24

WSC/TN = Water soluble carbon to total nitrogen.

(Shumo et al., 2019). However, substrates with higher carbon to nitrogen ratios will require higher feeding rates to achieve similar results (Manurung et al., 2016). Although, BSF larvae can successfully grow and thrive on a wide range of organic waste that dominate most cities of Sub - Saharan Africa (Okot-Okumu, 2012), our studies have demonstrated that the larvae yield would largely depend on the substrate quality.

4.2. Effects of substrate carbon to nitrogen ratios on frass compost yield and quality

Substrates with C/N ratios between 20 and 30 produced significantly higher fresh and dry compost yields compared to the control and substrate with C/N of 15. The high compost yield obtained in this study can be attributed to increased sawdust as a carbon source in the various substrates. The waste degradation efficiencies obtained in the current study (24–34%) are comparable to that of earlier studies with rice straw (10–32%) (Manurung et al., 2016) and cassava peels (27.9%) (Supriyatna et al., 2016), which could be due to the presence of recalcitrant carbon tissues such as lignin, cellulose and hemicelluloses that are difficult to be broken down.

Studies have revealed that nitrogen mineralization (ammonification and nitrification processes) during composting can be controlled by the substrate's C/N ratio, compost pH and temperatures (Ogunwande et al., 2008; Huang et al., 2004). The low temperatures (<45 ℃) observed in this study were beneficial in conserving ammonia, which is highly volatilized at thermophilic temperatures (>45 ℃) (Bernal et al., 2009). The increase in pH and electrical conductivity in the various substrates could be attributed to the production of ammonium and other ions, which could have resulted from the rapid degradation of organic substrates as reported by Said-Pullicino et al. (2007) and Azim et al. (2018). At pH<8, the nitrification process is faster and there is less ammonia volatilization (Bernal et al., 2009; Sánchez-Monedero et al., 2001). For example, the lower pH and EC values observed in substrates with C/N ratio of 15 and 20 in the fourth and fifth weeks might have been responsible for enhancing the nitrification process, which has a direct effect on the total nitrogen retained in the frass fertilizer.

Apart from the control and substrate with C/N ratio of 30, the other substrates experienced slight increase in total N content in the frass fertilizer. Increase in total nitrogen during composting has been previously reported by Lalander et al. (2014) and could be attributed to reduced N losses during the compost maturation phase. There was reduced loss of nitrogen in all the substrates with C/N ratios ranging between 15 and 30 as a result of enhanced retention of ammonium nitrogen through microbial immobilization (Huang et al., 2004) and the binding of ammonium onto phenolic compounds of sawdust which in turn reduces amount of free ammonia (Lim et al., 2017).

Unlike nitrogen, phosphorus (P) and potassium (K) showed minimal variation during the study. This is because phosphate ions are usually bound onto organic matter or chemically precipitated to form calcium and magnesium complexes at high pH levels (>8) and therefore not usually lost from the compost matrix (Tumuhairwe et al., 2009). In the mature frass compost (fertilizer), potassium content did not vary significantly. During composting, potassium is usually lost through leaching due to excessive moisture, which was not the case of the present studies because the moisture content was maintained between 40 and 65%.

4.3. Effects of substrate carbon to nitrogen ratios on frass compost maturity

Another important parameter for assessing organic fertilizer quality is whether the compost generated is free of toxic substances that may inhibit seed germination and plant growth (Teresa and Remigio, 2011; Bernal et al., 2009). In this study the ammonium to nitrate ratios (0.4–35) of the mature frass composts were above the recommended value of < 0.16 (Bernal et al., 1998). High ammonium concentration might have reduced the action of nitrifying bacteria by inhibiting possible enzymatic activities (Agyarko-Mintah et al., 2017). Our work is in line with other studies (Guo et al., 2012), which reported much higher ammonium to nitrate ratios (1–60) of mature compost. Furthermore, the C/N ratios in mature frass compost ranged between 10.5 and 23.4, which is within the recommended range (<25) for field application (Goyal et al., 2005). The mature frass compost generated had pH and electrical conductivity values that were also within the recommended ranges (6–8 for pH and < 4 mS cm^{-1} for electrical conductivity) (Bernal et al., 2009; Huang et al., 2004). Germination index was used as a reliable indicator to evaluate the effect of compost extract on crop growth. The high percentage seed germination and germination index results recorded is an indication that all the composts generated from substrate with C/N ratios of 15–30 were free of phytotoxic substances. The germination indices of above 100% for substrates with C/N ratios of 15 and 20 were a clear indication of complete frass compost maturity and stability (Teresa and Remigio, 2011). On the other hand, the lowest germination index registered for compost generated from the control (C/N ratio of 11) is an indicator of moderate phytotoxicity, probably due to high ammonium concentration and electrical conductivity, that have been reported to hinder root growth (Teresa and Remigio, 2011). The results of the germination indices reported in our study for all the frass composts were within the ranges demonstrated by several authors (Antil et al., 2013; Guo et al., 2012), which was achieved within five weeks.

5. Conclusions

Findings from this study have shown that in terms of larval yields and nutrient-rich frass compost, rearing substrate with C/N ratio of 15 is the most suitable for BSF larvae production. The use of BSF larvae during the initial composting phase shortened the compost maturity period to five weeks as opposed to over 3 months in conventional composting. The high germination index and stable C/N ratios obtained in the current studies indicate that all the composts generated from substrates with C/N ratios of 15 – 30 were free of phytotoxic substances and suitable for field application as organic fertilizer. Therefore, BSF rearing on amended waste substrates and organic fertilizer production from BSF frass remains a promising technology, worth integrating in low income countries of Sub - Saharan Africa, where agricultural production is highly affected by expensive animal feeds and unaffordable mineral fertilizers.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The senior author Dennis Beesigamukama was financially supported by a German Academic Exchange Service (DAAD) In-Region Postgraduate Scholarship. We remain indebted to all the technical staff for their substantial contribution during the data collection process of the study.

Funding

This research was financially supported by the Canadian International Development Research Centre (IDRC) and the Australian Centre for International Agricultural Research (ACIAR) (INSFEED—Phase 2: Cultivate Grant No: 108866-001), the Netherlands Organization for Scientific Research, WOTRO Science for Global Development (NWO-WOTRO) (ILIPA–W 08.250.202), and The Rockefeller Foundation (SiPFeed—2018 FOD 009) through the International Centre of Insect Physiology and Ecology (ICIPE). We also gratefully acknowledge the support of Kenyatta University and ICIPE core funding provided by United Kingdom's Foreign, Commonwealth & Development Office (FCDO); the Swedish International Development Cooperation Agency (Sida); the Swiss Agency for Development and Cooperation (SDC); the Federal Democratic Republic of Ethiopia; and the Government of the Republic of Kenya. The views expressed herein do not necessarily reflect the official opinion of the donors.

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