PRODUCTION OF ORGANIC FERTILIZER FROM BLACK SOLDIER FLY FRASS FOR IMPROVED SOIL HEALTH AND MAIZE PRODUCTIVITY IN NAIROBI CITY COUNTY, KENYA

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DECLARATION

I Beesigamukama Dennis declare that this thesis is my original work and has not been presented for the award of a degree in any other university or any other award.

Signature……………………………… Date…………………………

SUPERVISORS' APPROVAL

We confirm that the work reported in this thesis was carried out by the candidate under our supervision and has been submitted with our approval as university supervisors.

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DEDICATION

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ABSTRACT

Use of organic wastes for Black soldier fly (BSF) farming presents an avenue that could contribute to improved crop yield and sustainable soil health. A major waste from BSF farming is frass which, however, generates organic fertilizer with low nutrient levels. In addition, and the agronomic performance of BSF frass fertilizer (BSFFF) is not known. This study aimed at producing high quality and cost-effective organic fertilizer from BSF frass for improved soil health and crop productivity. The study evaluated the effects of C:N ratio adjustment (C/N ratios of 11, 15, 20, 25 and 30) and substrate amendment with biochar (0, 5, 10, 15 and 20%) and gypsum (0, 5, 10 and 15%) on nutrient conservation in organic fertilizer. Comparative performance of BSFFF and commercial organic (SAFI) and mineral (urea) fertilizers on maize production was carried out under field conditions. The first experiment involved application of BSFFF and SAFI at sole rates of 0, 2.5, 5 and 7.5 t ha^{-1}., while in the second experiment, BSFFF, SAFI and urea were applied at rates equivalent to 0, 30, 60 and 100 kg nitrogen (N) ha^{-1}. Furthermore, field incubation studies involving BSFFF, and SAFI applied at 5 t ha-1 were also undertaken to determine synchrony of N mineralization for maize production. Finally, data obtained from BSF rearing, frass composting, and agronomic experiments were used to perform economic evaluation. The data obtained were analyzed using R software version 3.6.0. Results indicated that an amended substrate with C:N of 15 increased N and phosphorus (P) retention in frass compost by 21% and 15%, respectively compared to the unamended substrate, and did not cause significant decrease in larval yields. The highest frass compost yield and potassium (K) retention were achieved in amended substrate with a C:N ratio of 20. Amended substrate with biochar and gypsum enhanced N conservation in frass compost, but gypsum was more efficient than biochar. Amendment with 20% biochar significantly increased BSF larval yields (88%) relative to the control substrate while amendment with > 5% gypsum decreased larval yields. The inclusion of 20% biochar generated the highest frass compost yield, gave a 21% increase in N retention and significantly higher K content in frass fertilizer compared to the unamended substrate. Initial composting of organic wastes using BSF larvae significantly shortened compost maturity time to 5 weeks, compared to 8 – 24 weeks in conventional composting methods. Field experiments revealed that plots treated with 7.5 t ha^{-1} of BSFFF achieved 14% higher maize grain yields than plots treated with a similar rate of SAFI. There was a 27% and 7% increase in grain yields in plots treated with 100 kg N ha^{-1} of BSFFF compared to those treated with equivalent rates of SAFI and urea, respectively. The agronomic N use efficiency of maize grown using 30 kg N ha^{-1} of BSFFF was 27% and 116% higher than the values attained using equivalent rates of SAFI and urea, respectively. The BSFFF achieved higher nitrogen fertilizer equivalence values (105 – 229%) than SAFI. Application rates of 2.5 t ha^{-1} and 30 kg N ha^{-1} of BSFFF were found to be effective in improving maize yield, while double rates of SAFI would be required. Economic assessment revealed that generating organic fertilizer from BSF frass increased the net income from BSF farming by 5 – 15 times compared to sole larvae production. At equivalent rates, the benefit-cost ratios from maize grown using BSFFF was higher than those of SAFI. Also, the gross margins of maize grown using BSFFF (75 – 78%) were comparable to those of urea (76 – 78%). The findings of this study are crucial in reducing heavy reliance on the costly

mineral fertilizers, by adopting high quality organic fertilizers such as BSFFF for improved soil health and productivity.

CHAPTER ONE: INTRODUCTION

1.1 Background to the study

Improving food security requires strategies that can match future food demands with increasing population growth while conserving the soil resources. High levels of hunger and food insecurity dominate most regions of Africa, and the situation continues to worsen due to increasing soil degradation (FAO, 2017). Forty percent of soils in Sub-Saharan Africa (SSA) are low in nutrient stocks, with 25% affected by aluminum toxicity, 18% prone to leaching and 8.5% characterized by phosphorus fixation (Tully et al., 2015). Most soils in SSA are deficient in nitrogen (N), phosphorus (P) and potassium (K) (Cobo et al., 2010) while most farm lands in East Africa have negative macronutrient balances (Gachimbi et al., 2005; Ebanyat et al., 2010) and yet, very little ($\leq$ 10 kg ha^{-1}) or no mineral fertilizer is used (FAO, 2017). Even in the few farms where mineral fertilizers are used, low nutrient use efficiencies and crop yields have been reported (Ebanyat et al., 2010; Kihara et al., 2016; Liverpool-Tasie et al., 2017), mostly attributed to low soil organic matter levels, many micronutrient deficiencies and high acidity (Vanlauwe et al., 2015; Musinguzi et al., 2016).

Like in many SSA countries, most soils in Kenya are low in organic matter, with levels even below the critical value of 3% (Gachimbi et al., 2005), and exhibit high acidity (Keino et al., 2015). This has led to high P fixation, making P a limiting factor to crop production. Furthermore, uptake and utilization of macronutrient (mostly N, P and K) is affected by the low availability of secondary nutrients [calcium (Ca), magnesium (Mg) and sulphur (S)] and most micronutrients in soils (Tittonell et al., 2008a; Wortmann et al., 2019).

Past research efforts on soil fertility management in SSA have recommended combined application of mineral and organic fertilizers to improve and sustain soil and crop productivity (Tittonell et al., 2008b; Vanlauwe et al., 2014, 2015). When applied across farms in Kenya, combined organic and mineral fertilizers have improved crop yields, nutrient use efficiencies and soil fertility (Mucheru-Muna et al., 2007; Mucheru-Muna et al., 2014; Musyoka et al., 2017). Despite this situation, most farmers do not apply adequate quantities of organic matter, which could help to replenish some of the macro- and micro-nutrients into the soil through mineralization (Baligar et al., 2001; Grigatti et al., 2015; Ch'Ng et al., 2016) and at the same time improve the soil structure which is an important soil physical fertility property (Mucheru-Muna et al., 2014). The major challenge hindering the use of organic fertilizers is the limited sources of organic matter, since most organic resources have other competing uses such as feeding livestock on the farm (Rufino et al., 2011; Ndambi et al., 2019). Furthermore, most organic fertilizers have low nutrient levels (Ndambi et al., 2019; Ebanyat, 2009) which limit their effectiveness.

Insect frass has promising potential to provide alternative high-quality source of organic fertilizer. The increasing demand for animal feed has led to black soldier fly (*Hermetia illucens* L.) (BSF) mass rearing using organic waste (van Huis, 2013; Makkar et al., 2014), presenting an avenue for organic waste management that could also contribute to soil fertility improvement. The BSF larvae have a high waste degradation efficiency (65 – 79%) (Diener et al., 2011) and can significantly reduce pathogens present in the waste (Lalander et al., 2015).

The frass, which is a byproduct (a combination uneaten substrate, feaces, and exuviae) from BSF rearing contains substantial amounts of nutrients (Lalander et al., 2015; Oonincx et al., 2015) that could be useful in crop production if converted into organic fertilizer. The frass fertilizer generated would also increase income from insect farming through the sale of organic fertilizer as a second product from BSF rearing or save the farmer from incurring inorganic fertilizer purchase costs.

The effectiveness of organic fertilizers highly depends on the source, nutrient content, stage of mineralization and storage method (Rufino et al., 2007; Ebanyat, 2009; Ndambi et al., 2019). Nutrients availability in manure is greatly influenced by the source, mineralization status and C/N ratio (Grigatti et al., 2015; Musyoka et al., 2019a). In most homesteads, manure is heaped in open areas whereby most nutrients are lost through leaching and volatilization (Ebanyat, 2009; Ndambi et al., 2019). Manures with low nutrients require high application rates, which are not affordable by most farmers.

Application of immature and unstable compost causes nutrient immobilization, and phytotoxicity which affect seed germination, crop growth and yield (Emino & Warman, 2004; Teresa & Remigio, 2011; Luo et al., 2018; Musyoka et al., 2019a). Therefore, in addition to the quest for feasible sources, good handling and treatment practices are required to harness the full potential of manure in improving crop production and soil fertility. Conversion of BSF frass into quality organic fertilizer is a promising technology which has been established in this study.

1.2 Statement of the problem

The BSF frass generates organic fertilizer with low nutrient levels due to high N volatilization during larval feeding, which could reduce the agronomic potential of the fertilizer produced. Since most BSF farmers mainly target production of high larval biomass, substrates with low C/N ratio (< 25:1) are normally used (Lalander et al., 2017). A low C/N ratio encourages faster release (mineralization) of N from organic matter compared to the degradable carbon, causing an excess of N which may be lost in form of ammonia gas especially when the temperature (> 45 ºC) and pH (> 7.5) are high (Bernal et al., 2009). Furthermore, for optimal growth, BSF larvae require neutral to alkaline pH (7 and above) (Ma et al., 2018); yet, pH above 7 causes nitrogen loss through ammonia volatilization (Liang et al., 2006). This is because high pH has been reported to increase the dissociation of ammonium (NH_4^+) to ammonia gas (NH_3), thus shifting the equilibrium to NH_3 which eventually evaporates (Sánchez et al., 2017). This leaves the frass with low nutrients since in addition to the nutrients which are accumulated in the larval biomass, more N is lost through volatilization. Lalander et al. (2015) reported total N losses of 44% in form of ammonia volatilization while using the BSF larvae to compost dog food, pig manure and human feaces. Furthermore, Oonincx et al. (2015) demonstrated that 23 – 78% of substrate N from chicken, pig and cow manure is lost during BSF larvae-assisted composting.

On the contrary, the high C/N ratio (> 25) required for production of nutrient rich compost does not favour fast growth of BSF larvae. The low concentration of N, P and K left in the BSF frass lowers the quality of the organic fertilizer generated. The critical C/N ratio for optimal BSF larval growth and nutrient conservation in BSF rearing waste is not known. Information on organic or inorganic additives that can effectively control N volatilization through ammonium adsorption, absorption and precipitation (Sánchez et al., 2017) without being detrimental to BSF larvae growth is lacking. Also, it is not known whether by BSF larval harvesting stage (5th instar stage); the frass will have fully mineralized to release all the nutrients for crop use. There is inadequate information on the time needed to convert the BSF frass into mature and stable compost. Furthermore, the economic value of organic fertilizer production alongside BSF rearing is not known; and such information is useful in improving the adoption of BSF frass composting as a new concept of organic fertilizer production.

The use of BSF frass as organic fertilizer is a relatively new concept. Adoption of a new concept or product as fertilizer in any farming system requires information on its performance in terms of how it influences crop growth, yield, nutrient uptake, and nutrient use efficiency in comparison to existing fertilizers. Most research efforts on use of insect frass as a fertilizer have been conducted under controlled conditions (Kagata & Ohgushi, 2012; Poveda et al., 2019; Houben et al., 2020). Those that have involved BSF frass (Choi & Hassanzadeh, 2019) have been performed under potted conditions, without assessing the economic yield and nutrient utilization. Most research outputs from greenhouse or potted experiments cannot be directly transferred to field conditions without being tested due to variations in production environments.

Knowledge on the influence of BSF frass fertilizer on crop production in terms of nitrogen mineralization, nutrient uptake, and nitrogen use efficiency under field conditions is not known. The optimum application rates and comparative performance of BSF frass fertilizer in relation to existing commercial organic and mineral fertilizers are not documented. Furthermore, the nitrogen fertilizer equivalence of BSF frass fertilizer – an important index that would provide information on the amount mineral N fertilizer saved while using N from BSF frass fertilizer to achieve the same crop yield (Hijbeek et al., 2018) is largely unknown.

1.3 Justification

Black soldier fly rearing without frass fertilizer production is less profitable, especially if the frass is not good for other uses. Generating organic fertilizer from the BSF frass can increase profits and improve soil fertility, which is a major challenge to crop production. Furthermore, recent studies have recommended combination of organic and mineral fertilizers to reduce the effects of acidic, saline, and highly weathered soils to mineral fertilizer inputs (Tittonell et al., 2008a; Vanlauwe et al., 2015).

Composting process has been used to recycle nutrients in organic wastes into organic fertilizers. When the process is managed well, a nutrient-rich, mature and stable compost is generated (Epstein, 1997). Combining wastes with high-carbon substrates such as sawdust and crop straws help to achieve favourable C/N ratios that improve nitrogen conservation through the control of ammonia volatilization during composting (Sánchez et al., 2017; Bernal et al., 2009).

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 by nearly 35%, and ammonia volatilization by up to 71% (Lim et al., 2017).

Inclusion of materials such as biochar has been found to adsorb and absorb ammonium ions on the highly charged surfaces, thus preventing their loss as ammonia gas during composting (Awasthi et al., 2016, Awasthi et al., 2017a; Awasthi et al., 2017b; Sanchez-Monedero et al., 2018). These mechanisms reduce ammonium ion concentration in liquid phase and minimizes nitrogen loss as ammonia gas and other forms (Wang et al., 2018). It has been demonstrated that biochar inclusion during composting can reduce ammonia volatilization by up to 60% (Agyarko-Mintah et al., 2017), thus increasing total N content in the compost.

Conserving N in composting substrates can also be achieved through the addition of gypsum to stabilize ammonium ions through ammonium precipitation (Sánchez et al., 2017) to form stable compounds such as ammonium sulphate and ammonium phosphate (Wang et al., 2013; Li et al., 2018). Through reducing N diffusion and converting ammonium carbonate to ammonium sulphate (Sánchez et al., 2017), gypsum amendment aids in controlling ammonia volatilization during composting (Yang et al., 2015). Addition of gypsum to compost substrates has been found to improve P and ash contents, while reducing N loss by between 17% (Yang et al., 2015) and 94% (Tubail et al., 2008; Sheng, et al., 2015), thereby improving nutrient concentrations in the compost.

Unlike the conventional composting process which is mediated by microorganisms only (de Bartoldi, 1983), the use of BSF to compost organic waste amended with sawdust, biochar and gypsum is a new innovation. The effective rates of inclusion of these amendments for optimal growth of BSF larvae growth and yield, and nutrient conservation have not been documented. Therefore, before BSF frass fertilizer is integrated into existing farming practices, the challenge of nutrient losses and the knowledge gaps on its agronomic performance mentioned above should first be addressed.

1.4 Significance of the study

This study sought to develop strategies for obtaining both high quality larval biomass and organic fertilizer by recycling organic wastes using the BSF. Results from the study will provide recommendations for optimal fertilizer production from BSF frass, fertilizer application rates and efficient soil nutrient management. The BSF larval-based waste management technologies are crucial in converting the huge quantities of nutrients contained in waste streams into nutrient-rich larval biomass for incorporation as alternative protein additive in animal feeds, and high-quality organic fertilizer for improved soil health and crop productivity.

The integration of frass fertilizer into cropping practices would help to return nutrients back into the soil and increase farm productivity through reduced expenses in chemical fertilizer inputs. Consequently, the adoption of high-quality and affordable organic fertilizers such as the BSF frass fertilizer would sustainably increase farm productivity (Ndambi et al., 2019; Rufino et al., 2007) and lift farmers out of poverty.

This would go a long way in improving food and nutrition security among the vulnerable segments particularly women and children in the rapidly growing populations in most developing countries. With low initial capital investments, smallholder insect farmers have good opportunities to increase productivity, improve their livelihood and contribute to food security and a circular economy. The frass fertilizer can also be sold directly, thus increasing the household income of insect farmers. Therefore, BSF farming has the potential to create jobs for the women and youths involved in the insect production chain, while conserving the environment by using the BSF larvae as biowaste converters (Chia et al., 2020).

The development of inclusive business models involving organic fertilizer as additional value-added product from insect farming would significantly contribute to solving socio-economic and environmental problems in developing countries, aligning with the United Nations’ Sustainable Development Goals: namely, 1: Zero hunger through improved crop and animal yield, 2: Zero poverty through increased household income, 5: Gender equality by creating opportunities for women and girls, 6: Clean water and sanitation through waste recycling, 9: Industry, innovation and infrastructure through use of novel technologies for job creation, 11: Sustainable cities and communities through improved waste management, 12: Responsible consumption and production through cleaner production methods, 13: Climate action through climate change adaptation, and 15: Life on reducing land degradation and biodiversity loss.

1.5 General objective

To produce high quality and cost-effective organic fertilizer alongside BSF rearing without being detrimental to insect yields.

1.5.1 Specific objectives

i. To determine the critical carbon to nitrogen (C/N) ratio for optimal BSF larval growth and nutrient conservation in frass fertilizer.

ii. To evaluate the effectiveness of biochar and gypsum amendments on nutrient retention in BSF frass fertilizer.

iii. To compare the performance of BSF frass fertilizer and commercial fertilizers on the growth, yield, and nitrogen use efficiency of maize.

iv. To determine soil moisture storage, nitrogen fertilizer equivalence value and synchrony of nitrogen mineralization from BSF frass fertilizer and commercial organic fertilizer for maize production.

v. To determine the economic value of frass fertilizer production alongside BSF rearing and profitability of crop production using BSF frass fertilizer.

1.6 Hypotheses

1. Optimal growth and nitrogen conservation during BSF rearing is not significantly affected by the C/N ratios of the substrates.
2. Amending rearing wastes with ≥ 10% of biochar or gypsum significantly increases nutrient levels of BSF frass fertilizer.
3. The BSF frass fertilizer can perform equally good as commercial fertilizers in terms of maize yield and nitrogen use efficiency.
4. The BSF frass fertilizer has significantly higher soil moisture storage, nitrogen fertilizer equivalence values and better synchrony of nitrogen release for maize growth than the commercial organic fertilizer.

5. Generating frass fertilizer as a second product from BSF rearing significantly improves the profitability of BSF rearing and crop production.

1.7 Conceptual framework

The challenge of low agricultural productivity can be addressed by using the BSF larvae to recycle organic wastes in the urban areas of SSA into organic fertilizer for soil fertility improvement and using the BSF larvae as source of proteins in animal feeds (Figure 1). This strategy can save farmers the burden of expensive animal feeds and inorganic fertilizer inputs. However, this can only be achieved if the BSF rearing process is optimized to produce nutrient-rich organic fertilizer and high larval biomass. Integration of BSF frass fertilizer into existing farming practices and market structures requires information on its agronomic performance and the economic value on BSF farming and crop production. These knowledge gaps have formed the research focus of this study. The study was based on the hypothesis that in addition to BSF larvae for animal feeding, quality and cost-effective organic fertilizer can be generated from the BSF frass if nutrient losses are minimized during the rearing process.

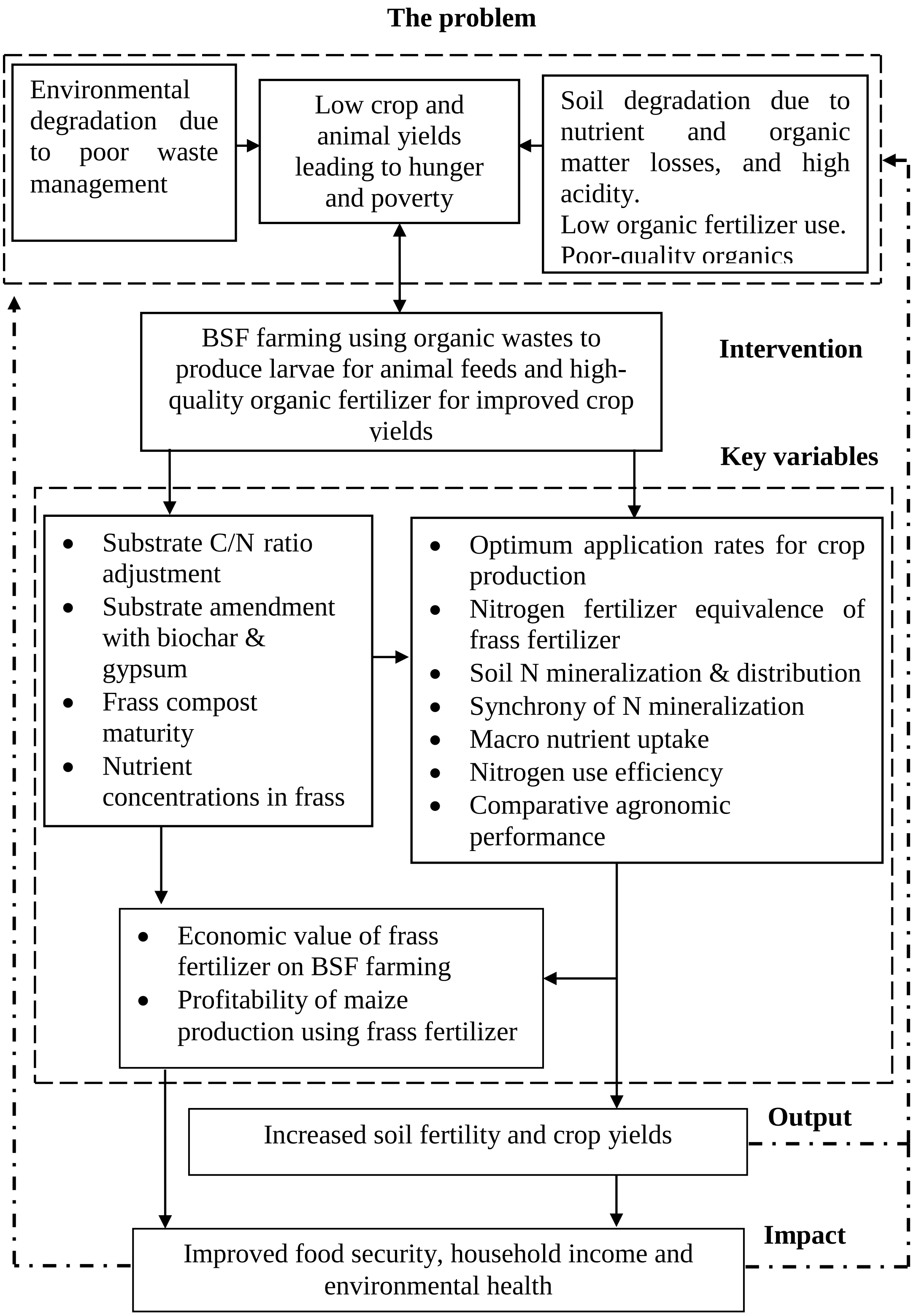

Figure 1: Conceptual framework.

CHAPTER TWO: LITERATURE REVIEW

2.1 The black soldier fly and its role in agriculture

The black soldier fly (BSF) (*Hermetia illucens* L.) is a two-winged black or blue fly with a wasp like appearance that belongs to the family Stratiomyidae (Diclaro & Kaufman, 2012). It is native to tropical, sub-tropical and temperate regions of America but has been dispersed by man to other parts of the world between 45 ºN and 40 ºS (Caruso et al., 2014). This non-pest and non-vector fly completes most of its life cycle in moist or decaying organic matter. The adult BSF mate three days after emergence and their life span ranges from 8 – 14 days (Caruso et al., 2014). The female black soldier fly lays pale yellow or creamy eggs near decaying wastes in batches of about 320 – 1000 (Caruso et al., 2014). At optimum conditions of temperature (20 – 30 ºC) and relative humidity (30 – 90%), the entire life cycle from egg to adult takes 40 – 43 days (Tomberlin et al., 2002). Eggs hatch into larvae in four days after which the larval stage stays for 22 – 24 days.

The BSF larval growth and yield are greatly influenced by the nature of rearing substrates/wastes (Oonincx et al., 2015; Chia et al., 2018a; Lalander et al., 2019). Waste materials with low proteins, carbohydrate levels and high level of chemical pollutants slow down BSF growth and yield (Diener et al., 2015; Cammack & Tomberlin, 2017). While the BSF pupae and adult flies do not feed and therefore not associated with decaying matter, the larvae stages have high feeding rate and hence the high waste degradation efficiency (40 – 80%) (Diener, 2010; Diener et al., 2011). The BSF larvae are rich in crude proteins, fats, and minerals (Caruso et al., 2014) and as feed, the BSF larval meal has been reported to improve the performance of pig (Makkar et al., 2014), poultry (Al-Qazzaz et al., 2016; Schiavone et al., 2017) and fish (St-Hilaire et al., 2007).

The BSF larval meal has thus been recommended as a potential replacement for conventional animal protein sources such as fish and soybean, that are not only expensive but also scarce and unsustainable (van Huis, 2013; Rana et al., 2015; van Huis & Oonincx, 2017). The black soldier fly larvae have high reduction efficiency of pathogens (Lalander et al., 2015), heavy metals (Diener et al., 2015), pesticides and antibiotics (Lalander et al., 2016) contained in organic wastes. Moreover, the BSF larvae do not accumulate these pathogens or chemical pollutants in their biomass, leaving them hygienically safe for animal feeding. This is because BSF larvae gut secretes antimicrobial agents (Park et al., 2014; Zdybicka-Barabas et al., 2017) and digestive enzymes (Kim et al., 2011) that are effective in reducing pathogens and chemical pollutants that may be present in organic wastes. The high waste degradation efficiency of BSF larvae mentioned above is not only useful in environmental cleanup but has also been utilized to recycle organic wastes into organic fertilizers (Oonincx et al., 2015; Lalander et al., 2015; Lalander et al., 2017; Choi & Hassanzadeh, 2019).

2.2 Organic wastes as a resource in agriculture

In most African cities, large volumes of wastes are produced which give rise to large quantities of organic wastes (Muniafu & Otiato, 2010; Okot-Okumu, 2012; Komakech, 2014; Friedrich & Trois, 2016 Somorin et al., 2017; Jambeck et al., 2018). Organic wastes constitute the highest percent of solid wastes collected in East Africa whereby 65, 71 and 77% are for Nairobi, Dar es Salaam and Kampala, respectively (Okot-Okumu, 2012).

Approximately, 4,788 metric tonnes of wastes are generated in Nairobi per day and 51% are decomposable organic wastes (von Blottnitz et al., 2010). Projections show that the volume of wastes generated in Nairobi city will be 8,000 metric tonnes per day by 2035 (von Blottnitz et al., 2010). These wastes have caused serious pollution problems where they have not been properly managed (Kimani et al., 2007; Nweke & Sanders, 2009). On the other hand, organic wastes have been used for crop production, biogas production and animal feeding (Tumuhairwe et al., 2009; Amoding et al., 2011; Komakech, 2014) .

Organic wastes are used as rearing substrates for insects that have been found to contribute to animal feed requirements such as the BSF larvae (Lalander et al., 2019). Land filling is the common waste disposal method in East Africa (Okot-Okumu, 2012) yet, this locks most of the nutrients that would have been used for crop production. Amoding (2007) established that about 30, 50 and 130 metric tonnes of N, P and K respectively, are likely to be locked in market crop wastes of Kampala city.

Several studies have investigated the methods of treating organic wastes for agricultural use. Tumuhairwe et al. (2009) recommended the above ground pile method for composting of market crop wastes while Diener et al. (2011) and Dortmans (2015) found the BSF larvae very efficient in waste valorization. Komakech et al. (2016) reported that vermicomposting as a waste management strategy could also contribute to animal feeds. When converted into organic fertilizers, organic wastes are useful in integrated soil fertility management to enhance utilization of mineral fertilizers (Zingore et al., 2007; Vanlauwe et al., 2015; Musyoka et al., 2017) and are sometimes applied singly by smallholder farmers who cannot afford synthetic fertilizers.

In Nairobi, organic wastes have been proven effective in supporting peri-urban farming (Onyango et al., 2012). Combined application of organics with synthetic fertilizers is already a necessity in East Africa since most soils have low levels of organic matter, micronutrients and high acidity (Gachimbi et al., 2005; Nkonya et al., 2008).

2.3 Composting

Composting is the biological decomposition and stabilization of organic substrates, under conditions that allow the development of thermophilic temperatures as a result of biologically produced heat, to produce a final product that is stable, free of pathogens, free of plant seeds and can be beneficially applied to the land (de Bartoldi, 1983; Epstein, 1997). Composting involves microorganisms such as heterotrophic bacteria, archaea, and fungi. The microorganisms break down the organic substrates using extracellular enzymes materials to release nutrients through the process of mineralization (Sylvia et al., 2005). Soil animals, such as worms may be involved in the breakdown of the raw organic materials piled for composting. Insects such as the BSF larvae have also been found useful in accelerating the composting process (Diener et al., 2011; Lalander et al., 2015; Ermolaev et al., 2019).

The composting process involves three stages: the mesophilic (15 – 40 ºC), thermophilic (45 – 80 ºC) and the curing phases (15 – 40 ºC) (de Bartoldi, 1983). The mesophilic phase involves oxidation of soluble substrates like proteins, starch, and sugars. Here, the composting process is mainly done by bacteria and fungi. However, because those microorganisms sparingly survive at high temperature, their population is reduced drastically as the temperatures approach the thermophilic limit (Epstein, 1997).

In the thermophilic phase, temperatures rise to about 70 – 80 ºC depending on the nature of the substrates, where energy in form of heat is produced. Some of the energy that is not biochemically trapped by microorganisms during catabolism is dissipated to the environment as heat (Epstein, 1997) and this is evidenced by increase in temperature of the compost pile. This high temperature is important in eliminating harmful microorganisms present in the pile (Wichuk & McCartney, 2007). Archaea and thermophilic bacteria dominate the thermophilic phase. The curing phase is characterized by a decrease in temperatures back to the mesophilic phase due to reduction in microbial population and activity because of exhaustion of readily oxidizable materials (Epstein, 1997).

2.4 Nutrient changes during composting

During the initial stage of composting, N losses occur as gaseous emissions (Barrington et al., 2002; Bernal et al., 2009) but as the process progresses, stable N compounds which are less susceptible to volatilization, denitrification and leaching are produced; thus realizing higher N levels during maturation as reported by Goyal et al. (2005). Composting the water hyacinth with cattle manure increased N, P, Na, Ca, and Mg levels of the compost (Chandra et al., 2012), and at the same time enhanced microbial activities and P mineralization. Likewise, small scale composting of bio-filtration waste with cow manure increased total N, P, K, Ca, and Na in the compost (Wilde et al., 2010).

Composting human feaces, pig manure and organic wastes using the BSF larvae significantly increased the total P content of frass compost by 45% but decreased N content (Lalander et al., 2015). In the same study, most of the N in the frass compost remained in the ammonium form. Nitrogen losses of 23 – 78% have been reported on composting chicken, pig, and cow manure using the BSF larvae (Oonincx et al., 2015). During the same study, the P content increased by 50% in frass compost. Most N losses during BSF rearing have been attributed to ammonia volatilization, arising from high pH and low C/N ratio of the rearing substrates (Lalander et al., 2015; Oonincx et al., 2015).

2.5 Effect of compost C/N ratio and pH on nitrogen transformation during composting

The adequate C/N ratio for composting ranges between 25 – 35 (Bernal et al., 2009). A C/N ratio higher than 30 results in N immobilization and the composting process takes a longer time to reach maturity (Guo et al., 2012). A low C/N ratio (< 20) encourages faster release (mineralization) of N from organic matter compared to the degradable (available) organic C; causing an excess of N which may be lost by volatilization in form of ammonia or denitrification of nitrates especially when the moisture capacity is more than 60% (Bernal et al., 2009; Guo et al., 2012). Lalander et al., (2015) reported 44% nitrogen loss through volatilization in BSF frass compost after using substrates with a low C/N ratio in this case 16.

The highest concentrations of ammonium ions occur during the first 2 – 3 weeks of composting when organic matter degradation is most intense (Sánchez-Monedero et al., 2001). During composting, N can be lost through leaching, volatilization and denitrification (Sánchez et al., 2017; Hao & Benke, 2008).

Volatilization is the most dominant pathway of N loss (Liang et al., 2006). In addition to reducing fertilizer value of manure, ammonia is harmful to human and environmental health. Exposure to ammonia gas can cause respiratory infections, eye irritation and dyspnea (Palashikar et al., 2016).

The nitrification process in which ammonium (NH_4^+) is oxidized to nitrates (NO_3^-) via nitrites acidifies the medium. By contrast, an increase in pH of a medium is caused by the ammonification process when organic N is converted into the ammonia (NH_3) gas that can be lost or reacts with water to form ammonium hydroxide (NH_4OH) which is an alkaline, thus increasing the pH (Sánchez-Monedero et al., 2001). The NH_4OH is not stable and can easily be oxidized to nitrates. Moisture abundance and limited oxygen supply promote denitrification (reduction of NO_3^- to N_2 gas) (Sánchez et al., 2017).

As a rule, and when the breakdown occurs on the surface, N is lost by volatilization as NH_3 gas (Sánchez-Monedero et al., 2001). Should the breakdown occur inside the pile, the NH_3 gas is converted into NH_4^+ as follows [$(NH_3 + H^+ = (NH_4)^+$] and the subsequent $(NH_4)^+$ is then bound to a negatively charged surface such as a nitrate, carbonate, chloride and sulphate, and retained in the substrate. Ammonium sulphate is more stable than ammonium carbonate and thus less susceptible to leaching or volatilization (Wang et al., 2013). Ammonia volatilization takes place when ammonium (NH_4^+) dissociates to ammonia gas (NH_3) and ranges from 13 – 70% (Hao & Benke, 2008). The rate of volatilization is controlled by temperature, pH and ammonium concentration (Hao & Benke, 2008; Wang & Schuchardt, 2010).

Most ammonia volatilization takes place during the early stages of composting at thermophillic temperatures and at high pH levels (> 7) (Sánchez-Monedero et al, 2001; Bernal et al., 2009). This is because, increase in pH increases dissociation of NH_4^+ to NH_3 gas and this shifts equilibrium to NH_3. Thermophillic temperature also increases dissolution of NH_4^+ to NH_3, where diffusion of NH_3 reduces solubility of NH_3; thus, increasing its evaporation. Ammonia emissions increase when moisture and initial C/N ratio are low (< 25) (Sánchez-Monedero et al., 2001) and when ambient temperatures and aeration are high (Hao & Benke, 2008).

2.6 Strategies for conserving nitrogen during composting

Strategies for conserving N during composting can be biological (addition of ammonia oxidizing enzymes and bacteria), chemical (precipitation, adsorption) or physical-chemical (regulation of temperature, pH, particle size and moisture) (Sánchez et al., 2017). Ammonia volatilization can be reduced by reducing the turning frequency, using smaller piles, covering the compost pile and using bulking agents to widen C/N of the composting materials (Ogunwande et al., 2008; Bernal et al., 2009; Sánchez et al., 2017).

2.6.1 Regulation of pH

Reducing compost pH to values below 7 has been reported to reduce ammonia loss (Liang et al., 2006). Additives such as acids (sulphuric, hydrochloric, nitric and phosphoric) and base precipitating salts (chlorides and sulphates of Ca and Mg and aluminium) (Hao & Benke, 2008) can also be used. Acids are more efficient than bases, but they are limited by cost and hazards of use. Use of elemental sulphur and phosphor-gypsum has also been found effective (Hashemimajd et al., 2012; Li et al., 2018).

A dose of 0.5% (weight/weight) elemental sulphur was found effective for decreasing compost pH by one unit within two weeks and nitrogen conservation during composting of olive mill wastes (Roig et al., 2004). Adjusting initial pH to values below 7 using phosphate salts was capable of N retention in wheat straw and dairy manure compost by 82 – 90% (Liang et al., 2006).

Addition of 8% aluminium sulphate, and 4% aluminium chloride reduced ammonia volatilization from poultry litter by 94 and 92%, respectively and improved N levels in mature compost but increased electro conductivity to values higher than 10 mS/cm (Choi & Moore, 2008a). Use of 2.5% aluminium sulphate reduced ammonia emissions from slurry by 58% and also reduced extractable P levels (Lefcourt & Meisinger, 2010). While using aluminium salts, nitrogen conservation is achieved through pH lowering and formation of a foam on manure surface that act as a physical barrier to NH_3 volatilization. A 75% reduction in NH_3 loss was observed when the initial pH of broiler litter decreased from 8.3 to 6.6 using aluminium sulphate (Ekinci et al., 2000). Aluminium sulphate, therefore, has been found to be more effective in N conservation than calcium and magnesium chloride additives (Koenig et al., 2005).

2.6.2 Ammonium precipitation

Ammonium precipitation happens when salts are added to chemically combine with ammonium ions in composting and from stable compounds such as struvite ($MgNH_4PO_4.6H_2O$), ammonium sulphate and ammonium phosphate (Wang et al., 2013; Li et al., 2018). Addition of 17% gypsum to dairy cow manure improved P and ash contents, while it reduced ammonia volatilization and N loss to 6.4% (Tubail et al., 2008).

Use of 20% zeonite, biochar and flue gas desulfurization gypsum during chicken litter composting reduced ammonia loss by 68, 40 and 21%, respectively, but increased N, P, K, Ca, Mg and micronutrient levels in the compost (Sheng et al., 2015). Co-composting of kitchen waste with 10% phosphogypsum and super phosphate conserved N by 16.7 and 9.9%, respectively, and improved levels of P, K, Ca and Mg in final compost (Yang et al., 2015). The additives also reduced ammonia volatilization by 23.5 and 18.9% for phosphogypsum and super phosphate, respectively.

2.6.3 Ammonium adsorption and absorption

Ammonia adsorption happens when materials with high surface charge such as biochar, peat zeolite and clay adsorb ammonium ions and prevent further loss by reducing the activities of nitrifying bacteria (Xiao et al., 2017). The adsorption of ammonium on negatively charged materials reduces its concentration in liquid phase, and consequently potential for loss as ammonia gas and other forms (Wang et al., 2018).

Through the adsorption and absorption processes of ammonium into its pore spaces, biochar has been found to reduce ammonia volatilization by 44 and 60% during composting of poultry manure (Janczak et al., 2017) and poultry litter (Agyarko-Mintah et al., 2017), respectively. Use of 6.25% zeolite achieved 47% reduction in slurry ammonia emissions, through the adsorption process (Lefcourt & Meisinger, 2010). Such mechanisms described above reduce ammonium mineralization but later enhance conversion of ammonium into nitrates during the maturation stage, which improves N content in the compost (Sanchez-Monedero et al., 2018).

Addition of biochar has been reported to improve microbial activity by providing habitat and enhancing the physicochemical properties of compost (Sanchez-Monedero et al., 2018). It has been established that biochar also reduces heavy metal toxicity through adsorption, thereby reducing the mobility and bioavailability of heavy metals (Li et al., 2017). Addition of 2% co-composted biochar reduced N leaching and improved crop growth through increased nitrate capture in pores and sorption on anion exchange surfaces (Kammann et al., 2015). In addition to improving the agronomic value of compost, biochar also reduces greenhouse gas emissions (Sánchez-García et al., 2015; Awasthi et al., 2017a); thereby safeguarding the environment. Biochar doses of 5 – 10% have been found effective while rates higher than 20% have been found to negatively affect microbial activity by reducing enzymatic activity, thus slowing down the composting process (Sanchez-Monedero et al., 2018).

2.6.4 Adjustment of substrate C/N ratio

Adjustment of C/N ratio of composting materials to a favourable range (25 – 35) has been reported to improve N conservation in compost (Bernal et al., 2009; Wu et al., 2010). High carbon materials such as sawdust have enhanced NH_4^+ immobilization and binding onto phenolic compounds of sawdust, which in turn reduces the amount of free ammonia and thus occurrence of ammonia volatilization (Lim et al., 2009).

Composting of cattle manure with saw dust and phosphogypsum or zeolite has been reported to reduce total N loss from 44% to < 35% and ammonia volatilization by up to 71% (Lim et al., 2017).

2.7 Effect of organic amendments on soil nitrogen mineralization

The effectiveness of organic fertilizers for crop production largely depends on their nutrient content, especially N, and rate of nutrient release (Bowden et al., 2007; Cabrera et al., 2005; Musyoka et al., 2019a). Plant available N from organic fertilizers is highly dependent on their composition, organic N fractions, C/N ratio, appropriate timing, rate, and method of application (Li and Li, 2014; Cai et al., 2016). Organic materials with high levels of lignin and polyphenols are resistant to microbial decomposition (Rovira and Vallejo, 2002; Kleber, 2010), and consequently cause N immobilization (Kimetu et al., 2004; Musyoka et al., 2019a). Soils with low total N concentration have also been reported to stimulate N immobilization (Gómez-Muñoz et al., 2015), thereby, reducing the quantity of available N in soils.

Nitrogen mineralization rate is the single most important factor which determines the quantity and period in which N from organic fertilizers is available for plant uptake (Cabrera et al., 2005; Musyoka et al., 2019a). For optimal crop growth and yield, N mineralization patterns should match fluctuations in crop nutrient demands to cause synchrony, i.e., the balance between N supply and N demand (Johnson et al., 2012). The mineralization and synchrony of N from different organic resources have been widely studied (Bowden et al., 2007; Alizadeh et al., 2012; Li and Li, 2014; Musyoka et al., 2019). However, some studies have not incorporated the manure into the soil (Adin Yéton et al., 2019), thereby ignoring the soil factors that influence N mineralization (Friedel et al., 2000; Cai et al., 2016; Osterholz et al., 2017).

Mineralization studies which do not involve a crop make it difficult to know whether the N mineralized from BSF frass fertilizer can match the crop N demand, because, although the N content of the organic fertilizer could be high, its release could be hampered by soil factors such as pH, bacterial regime, and C/N ratio of the substrate after incorporation in the soil (Johnson et al., 2012; Musyoka et al., 2019a).

While using organic fertilizers, the likelihood of meeting crop N demand during critical growth stages depends on the ability of the crop to compete for the mineralized N with other consumption pathways such as immobilization by microbes (Osterholz et al., 2017), losses through volatilization, and leaching beyond the rooting system (Musyoka et al., 2019b). Therefore, knowledge of the dynamics and synchrony of N release from organic fertilizer for crop production in any cropping system is crucial in guiding recommendations on timing and rate of application for efficient N management (Musyoka et al., 2019a).

2.8 Nitrogen fertilizer equivalence of organic fertilizers

In addition to N mineralization, the quality of an organic resource is also assessed based on its performance as compared with a standard mineral fertilizer, also known as the N fertilizer equivalence (NFE) (Bowden et al., 2007; Lalor et al., 2011). Nitrogen fertilizer equivalence also known as nitrogen fertilizer replacement value, is the amount of mineral fertilizer N saved when using organic amendment to produce the same yield (Hijbeek et al., 2018). Nitrogen fertilizer equivalences of organic fertilizers have been found to vary with the type (Kimetu et al., 2004) and application rates (Hijbeek et al., 2018).

Higher NFE values have been reported while using organic fertilizers with higher total N such as tithornia and calliandra (Kimetu et al., 2004), lucerne (De Notaris et al., 2018), poultry litter and yard waste compost (Bowden et al., 2007), cattle manure (Lalor et al., 2011; Cavalli et al., 2016; De Notaris et al., 2018) and pig manure (van Middelkoop and Holshof, 2017).

In addition to nutrient supply, previous studies have reported additional benefits associated with certain types of organic fertilizers (Kagata and Ohgushi, 2012; Debode et al., 2016; Choi and Hassanzadeh, 2019; De Tender et al., 2019). For example, higher crop improved drought tolerance, disease suppression, and higher crop growth yield, while using insect frass fertilizer (Kagata and Ohgushi, 2012; Poveda et al., 2019; Houben et al., 2020). Furthermore, the chitin contained in insect frass fertilizers has been reported to improve plant health by stimulating disease resistance in crops (Quilliam et al., 2020).

2.9 Economic value of BSF farming and frass fertilizer

Insects such as the BSF are expected to contribute immensely to boosting agricultural productivity because of their multipurpose nature (Chia et al., 2019a; Dobermann et al., 2017; Makkar et al., 2014). In Kenya, socio-economic studies have shown that majority (70 – 80%) of poultry, fish and pig farmers are aware that insects can be used as a feed ingredient while 60 - 70% of fish farmers considered insects as a good feed ingredient (Chia et al., 2020), and 90% of the farmers were ready and willing to use insect-based feeds (Okello et al., 2021). The potential macroeconomic impact of adopting BSF larval meals in Kenya’s poultry sector has been estimated at between 16 and 687 million USD per year (Abro et al., 2020), not forgetting other benefits such as job creation and environmental sanitization.

The BSF frass fertilizer is a by-product that is gaining popularity due to its high nutrient content (Lalander et al. 2015; Oonincx et al. 2015) and potential for use as organic fertilizer. Abro et al. (2020), using a replacement cost approach estimated the macroeconomic gains of BSF frass fertilizer at 9 – 85 million USD in Kenya.

Previous studies have reported improvement in crop growth and yield while using the BSF frass fertilizer (Choi and Hassanzadeh, 2019; Quilliam et al., 2020). Although, information on the economics of using BSF frass fertilizer for crop production is still limited, it is expected to increase profits like any other organic fertilizer. For example, application of organic fertilizers has been reported to increase profitability of maize production in Central Kenya (Mucheru-Muna et al., 2014), with higher profits recorded in farming systems where manure is locally available on the farm (Adamtey et al., 2016).

In the long term, the residual benefits of organic fertilizers on soil fertility are expected to reduce the need for fertilizer application (Adamtey et al., 2016), thereby saving the farmer from incurring high fertilizer costs. Therefore, the use of organic fertilizers such as BSF frass fertilizer is envisaged as a sustainable way of increasing agricultural productivity in soils with multiple degradation challenges (Tilman et al., 2002; Vanlauwe et al., 2014).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Description of experimental sites

The black soldier fly (BSF) rearing and frass composting experiments were carried out at the animal rearing and quarantine unit of the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi Kenya (S 01° 13' 14.6"; E 036° 53' 44.5", 1612 m above sea level). The field experiments were carried out for two seasons (April – September 2019 and October 2019 – March 2020) at the Kenyatta University Teaching and Demonstration farm (1° 10′ 59″ S, 36° 55′ 34″ E), located in Nairobi County, Kenya at an elevation of 1580 m above sea level. Nairobi County receives bimodal rainfall with annual averages of 925 mm. The first rainfall season starts from March to June while the second season runs from October to December. The mean monthly temperatures of Nairobi ranges between 21 and 28 ºC (www.meteo.go.ke).

During the field experiments, daily temperatures and rainfall data were sourced from Kenyatta University weather station, located about 0.5 km from the experimental site. Cumulative rainfall totals of 252 and 281 mm were received during the short and long rain season, respectively (Figure 2a). Mean daily temperatures of 22 – 29 ºC and 22 – 28 ºC were recorded at the experimental site during the short and long rain season, respectively (Figure 2b). The long rain season received higher mean monthly rainfall (31 – 102 mm) than the short rain season (40 – 87 mm) but had lower average monthly temperatures (25 – 26 ºC) than the short rain season (25 – 27 ºC) (Figure 2c).

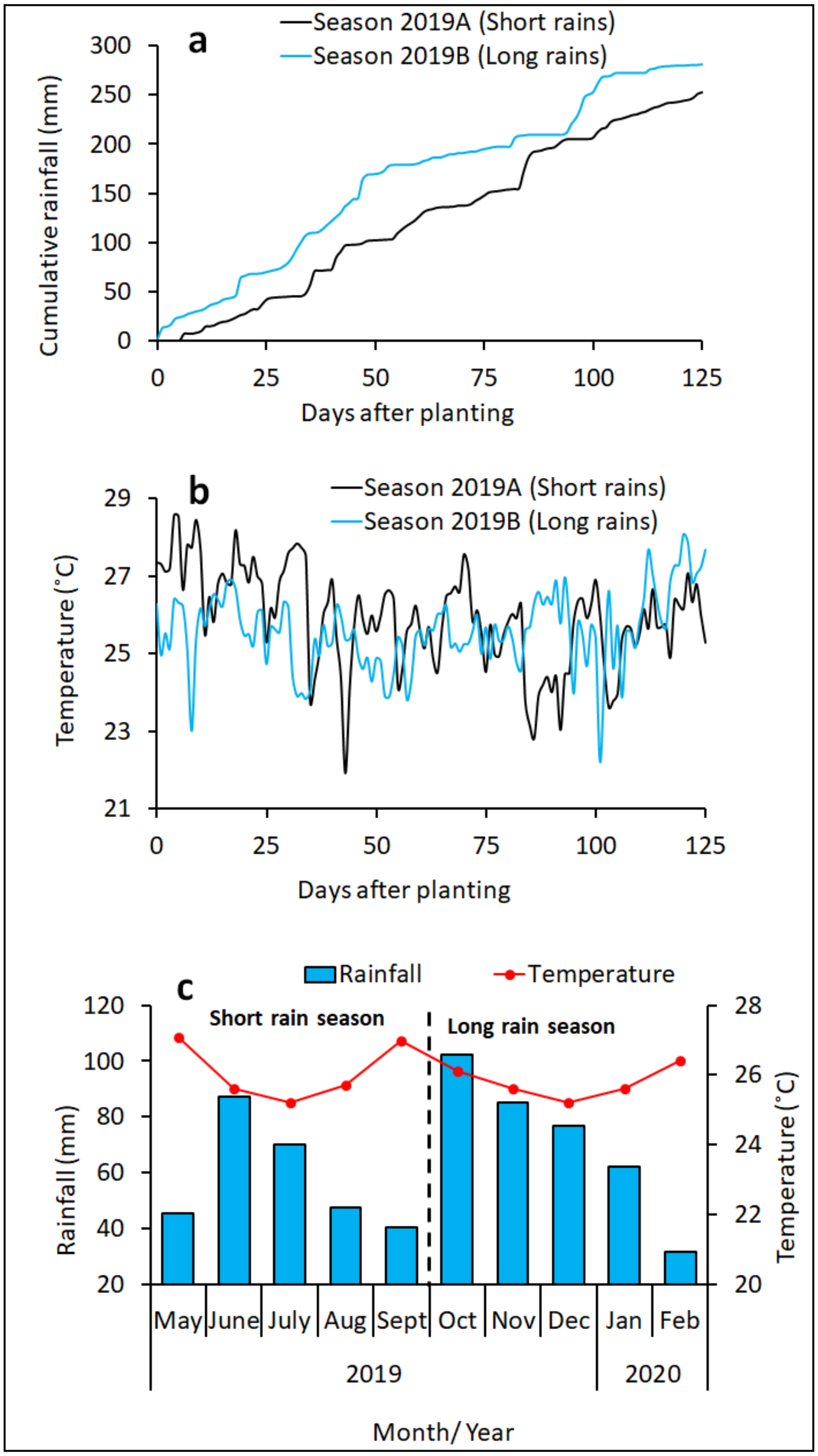

Figure 2: Cumulative daily rainfall (a), average daily temperature (b), cumulative monthly rainfall, and average monthly temperature (c) during field experiments at Kenyatta University Teaching and Demonstration farm.

Soils in the study site are Acric Ferralsols (Gachene & Kimaru, 2003), which are highly weathered soils characterized by shallow depths, low organic matter, low P, and low pH levels. Before field experiments, soil sampling (0 – 20 cm) was done for determination of total organic N, total organic carbon, available P, exchangeable cations (K, Ca and Mg), pH, electrical conductivity and soil texture using methods described in (Okalebo et al., 2002). Table 1 shows selected physical-chemical characteristics of the soils used in the experiment.

Table 1: Selected physical and chemical characteristics of the soil at the experimental site

Parameter	pH (1:2.5 water)	ρ_b (g cm^{-3})	Min. N (ppm)	TN	TOC	SOM	Av. P (ppm)	Exchangeable cations (cmol kg^{-1})			% sand	% clay	% silt	Textural class
				(%)				K	Ca	Mg				
Test value	5.9	1.35	1.81	0.04	1.3	2.3	9.7	2.07	0.91	0.07	63	20.3	16.7	Sandy loam
Critical value*	5.5	NA	NA	0.25	NA	3	15	0.22	4	0.2	NA	NA	NA	

*Source: Okalebo et al. (2002) Key: ρ_b = bulk density, Min.N = mineral nitrogen (ammonium and nitrate), TOC = total organic carbon, SOM = soil organic matter, Av. P = available phosphorus, NA = Not available.

3.2 Black soldier fly colony establishment and maintenance

The colony of BSF was established and maintained at the animal rearing and quarantine unit at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi Kenya following the methodology described by Chia et al. (2018b). 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 and household food wastes as baiting materials for adult flies.

Wild adult BSF were trapped at Kasarani, Nairobi County, Kenya. The baiting materials were placed inside 6-litre 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, the 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% relative humidity and a photoperiod of 12 hours of light and 12 hours of darkness. 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 were allowed to grow to pupal stages and the 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.

3.3 Experiments carried out

The study comprised of five experiments. The first experiment determined critical C/N ratio for optimal BSF growth, and nutrient conservation in frass fertilizer. In the second experiment, effectiveness of biochar and gypsum on nitrogen retention during BSF rearing was assessed. The third experiment compared the performance of the BSF frass fertilizers and commercial fertilizers on maize production and N use efficiency. In the fourth experiment determined the N fertilizer equivalence value and synchrony of N mineralization from BSF frass fertilizer for maize production. The fifth study assessed the economic value of organic fertilizer production alongside BSF rearing and profitability of crop production using BSF frass fertilizer.

3.3.1 Determining the critical C/N ratio for BSF larvae growth and nutrient conservation in frass fertilizer

3.3.1.1 Sources of experimental substrates

Before the start of the experiment, fresh brewery spent grains (BSGs) were sourced from Kenya Breweries Ltd. (Nairobi, Kenya), while sawdust (from blue gum tree, *Eucalyptus globulus* L. 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 as described in section 3.3.1.4. Results from the analyses are presented in Table 2.

Table 2: Selected characteristics of substrates used in black soldier fly rearing

Substrate	Moisture content (%)	pH (1:10 water)	EC (mS/cm)	TOC	Total N	Total P	Total K	Total Ca	Total Mg	C/N ratio
				(%)						
BSG	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

Based on the results presented in Table 2, a combination of BSGs and sawdust was formulated to obtain five different substrates with varying C/N ratios (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 was calculated using equation (1) (Richard & Trautmann, 1996). Table 3 shows the percent weight of sawdust and BSGs used to obtain the experimental substrates

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

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 brewery spent grain and sawdust, respectively

M_1 and M_2 represent moisture contents of brewery spent grain and sawdust, respectively

Q_1 - represents the amount of brewery spent grain (fresh weight) used

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

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

Table 3: Percentage weights of sawdust and brewery spent grain waste mixed to produce feedstocks of varying carbon to nitrogen ratios.

Feedstock Formulations	Brewery spent grain (%)	Sawdust (%)	Cost of brewery spent grain	Cost of sawdust	Total cost per kg of feedstock
			US dollar		
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

Exchange rate: 1USD = 100 Ksh.

3.3.1.2 Black soldier fly larval growth and yield on various substrates

The BSF larvae rearing facility was equipped with a wooden stand (180 cm high × 66 cm wide × 420 cm long). 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 long × 27.5 cm wide × 10 cm deep. The bottom of each tray measured 52 cm in length by 27.5 cm in width, which allowed for both edges of the tray to be inclined at an angle of 35º. 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 with three replicates. At the start of the experiment, two thousand (2000) 5-day old BSF larvae (equivalent to 10.7 g fresh weight) were obtained from the stock colony (section 3.2) and introduced into each tray containing 6.85 kg of the fresh formulated substrates. Lumpsum feeding and the recommended feeding rate of 125 mg (dry weight) per larva per day was used (Diener et al., 2009; Banks et al., 2014).

Each rearing substrate was hydrated to approximately 70.0 ± 2% moisture by weight according to the protocol described by Cammack & Tomberlin (2017) which was confirmed using a moisture sensor with two 12-cm-long probes (HydroSense CS620, Campbell Scientific, Logan, USA). Each experimental substrate was monitored 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. Environmental conditions in the experimental rearing room were maintained at 28 ± 2 °C, 60 – 70% RH and a photoperiod of L12:D12. The BSF larvae were harvested at fifth instar stage (13 days after hatching) as indicated by the appearance of cream-like color on the exoskeleton of the larvae (Dortmans et al., 2017). At harvesting, hand sieving with a 2 mm diameter sieve was used to separate the larvae from the frass. Thereafter, the larvae from each rearing tray were harvested and weighed to determine the yield per tray.

Larvae from each treatment was oven dried at 105 ℃° for 24 hours to determine the dry larval weight.

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

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

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

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

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.

3.3.1.3 Quality of frass and frass compost yield

After larval harvesting, the frass from each treatment was returned to respective trays for further composting until compost maturity attained. The moisture content of the frass from the different treatments after harvesting the BSF larvae was maintained at 70% as described in section 3.3.1.2. Compost maturity was monitored both during the larvae rearing and frass composting phases. During the larvae rearing and frass composting phases, changes in temperatures were carefully monitored 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 daily.

From each tray, 50g 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). The C/N ratio (< 20) (Goyal et al., 2005) of the frass was monitored on a bi-weekly basis, while seed germination index (> 80%) was determined at maturity (Teresa & Remigio, 2011).

At maturity, frass compost from each treatment was weighed and oven dried at 105 ^{o}C for 24 hours to determine the dry frass compost yield. In addition, 50 g of the frass compost were collected from each treatment, air dried for five days and ground into powder (< 0.25 mm) to determine total organic carbon, nitrogen, phosphorus, potassium, and C/N ratio at a different time of each experiment (weeks 1, 3 and 5).

3.3.1.4 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 minutes, 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 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 ºC for 30 minutes.

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 hour 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 colorimetrically 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 hour 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 while considering the extraction volume, sample weight and moisture content as described in Okalebo et al. (2002).

Total organic carbon was determined using the wet oxidation method (Nelson & sommers, 1982). Total N, P, K, Ca, and Mg were extracted using acid digestion methods as outlined in the procedures described by Okalebo et al. (2002). Total nitrogen in the digestate was determined using the Kjeldahl distillation method (Jackson, 1973).

Total P 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 hour 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 as described by Okalebo et al. (2002) while considering the sample weight and volume of the digest .

Total K concentration in the digested sample solution was determined using flame photometry (Okalebo et al., 2002). Standards for K 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, prepared by plotting transmission readings against K concentration. The sample weight and volume of the digest were used in calculating final K concentration (Okalebo et al., 2002).

The Ca and Mg 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 aspirated 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 as described by Okalebo et al. (2002).

3.3.1.5 Phytotoxicity test on mature frass compost extracts

The germination index (GI) was determined by growing cabbage seeds in each of the prepared compost extracts, obtained by dissolving 10 g of fresh frass compost in 100 ml of water. Thereafter, the mixtures were shaken for 30 minutes and filtered using Whatman No. 1 filter paper. Ten cabbage seeds were randomly selected and placed on petri dishes lined with filter paper moistened with 10 ml of 10% compost extracts for 96 hours at 25°C in a dark chamber. The same procedure was repeated using distilled water as a positive control. Germinated seeds were counted, and their radicle lengths measured. Cabbage seeds were selected due to their high photosensitivity (Teresa & Remigio, 2011).

Germination index (GI) was calculated using equation (4) (Emino & Warman, 2004). Frass composts with GI values below 50% were considered highly phytotoxic, values between 50% and 80% indicated moderate phytotoxicity; and values above 80% indicated the absence of phytotoxicity (Emino & Warman, 2004; Teresa & Remigio, 2011).

$$GI = \frac{(RSG\% \times RRG\%)}{100} \tag{4}$$

Where,

RSG (%) represents the relative seed germination calculated as:

$$RSG = \frac{number\ of\ seeds\ germinated\ in\ compost\ extracts}{number\ of\ seeds\ germinated\ in\ the\ control\ (distilled\ water)} \times 100$$

RRG (%) represents the relative root growth calculated as:

$$RRG = \frac{mean\ root\ length\ in\ compost\ extract}{mean\ root\ length\ in\ the\ control\ (distilled\ water)} \times 100$$

3.3.2 Evaluating the effectiveness of biochar and gypsum amendments on nitrogen retention in BSF frass fertilizer

3.3.2.1 Experimental diets

The brewery spent grains (BSG) was used as the rearing substrate. Biochar made from rice husks at pyrolysis temperatures of 350 °C was sourced from SAFI Organics Limited, located in Mwea, Kirinyaga County, Kenya. Gypsum was sourced from the Kenya Farmers’ Association stores, Nairobi. Detailed analyses of the nutrient levels (C, N, P, K, Ca, and Mg), moisture content, pH, EC of the BSG, biochar and gypsum were conducted using laboratory methods described in section 3.3.1.4. The results are presented in Table 4.

Based on the analyses described above, the BSG was amended with biochar at different inclusion rates of 5, 10, 15 and 20% dry weight (weight /weight) to obtain four treatment regimes, denoted as 5Biochar, 10Biochar, 15Biochar and 20Biochar, respectively. The BSG was also amended with gypsum at three different inclusion rates of 5, 10 and 15% dry weight (weight /weight), represented as 5Gypsum, 10Gypsum and 15Gypsum, respectively. The control treatment was neither amended with biochar nor gypsum.

Table 4: Selected characteristics of substrate and additives used in the experiment.

Substrate	Moisture content (%)	pH (1:10 water)	EC (mS/cm)	TOC	Total N	Total P	Total K	Total Ca	Total Mg	C/N ratio
				(%)						

BSG	71.4	3.5	0.86	33.9	2.65	0.54	0.38	0.48	0.20	12.7
Biochar	8.9	7.7	0.51	22.6	0.46	0.014	2.67	0.14	0.86	49.1
Gypsum	0.62	8.0	4.24	na	0.00	0.003	0.05	25.1	0.43	na

na = not applicable

3.3.2.2 Black soldier fly larval growth and yield on various substrates

The conditions of rearing facility, wooden stand and rearing trays were like those described in section 3.3.1.2. The wooden stand had three shelves separated from each other by 30 cm space, where the metallic trays used in rearing the BSF larvae were fitted. In the experimental room, 8 trays were fitted per shelf of the wooden stand, totaling to 24 trays arranged in a complete randomized design with three replicates.

At the start of the experiments, eggs of BSF were hatched as described in section 3.2 and larvae reared on each of the eight substrate treatments as described in section 3.3.2.1. After 5 days, 2,000 neonates (equivalent to 10.7 g fresh weight) from each treatment were collected and transferred directly to their respective treated substrates (7.10 kg) in metallic trays. The effect of substrate amendment with biochar and gypsum on BSF growth was monitored by determining wet and dried larval yields as described in section 3.3.1.2 and N accumulated by BSF larvae from each treatment. Data on waste reduction efficiency (equation 2) and biomass conversion rate (equation 3) were recorded at harvesting stage.

Samples of the dried BSF larvae from each treatment were ground into powder and used for determination of total N concentration (section 3.3.1.4). The fraction of initial N accumulated by BSF larvae (BSFL) biomass was determined using equation

(5), while the fraction of initial N retained in the raw frass and mature frass fertilizer were determined using equation (6).

$$\%N\ accumulated\ in\ BSFL\ biomass\ = \frac{\left(\frac{\%\ N\ in\ BSFL}{100}\right)\times Dry\ BSFL\ yield}{Initial\ N\ content\ in\ substrate}\times 100$$

(5)

$$\%N\ retained\ = \frac{\left(\frac{\%\ N}{100}\right)\times\ dry\ matter}{Initial\ N\ content\ in\ substrate}\times 100 \tag{6}$$

Where,

$$Initial\ N\ content\ in\ substrate = \frac{\%N\ in\ formulated\ substrate\ \times dry\ weight\ of\ substrate}{100}$$

Dry matter represents the dry weight of raw frass or mature frass compost

The effect of biochar and gypsum amendments on frass compost quality was determined by assessing compost maturity, nutrient concentrations and phytotoxicity of the frass composts generated (equation 4), following procedures described in sections 3.3.1.3, 3.3.1.4 and 3.3.1.5, respectively.

3.3.3 Determining the comparative performance of BSF frass fertilizer and commercial fertilizers on crop production

3.3.3.1 Source of fertilizers

The experiment involved three fertilizers: BSF frass fertilizer, commercial organic (SAFI), and urea fertilizer (45% N). The BSF frass fertilizer was produced as described in section 3.3.1. The SAFI fertilizer was sourced from Safi Organics Limited (http://safiorganics.co.ke/) located in Mwea town, Kirinyaga County, Kenya. It was a mixture of chicken manure, biochar, and rock phosphate. Urea was sourced from Kenya

Farmers' Association stores, Nairobi. Table 5 shows selected physical-chemical characteristics of the organic fertilizers used in the experiments.

Table 5: Chemical characteristics of the organic fertilizers used during experiments.

Parameters	Moisture (%)	pH	EC (mS/cm)	NH_4^+	NO_3^-	TOC	N	P	K	Ca	Mg	C/N ratio
				…. (mg/ kg) …		…………………		(%)		…………………..		
BSF frass	30.1	7.7	2.7	74.4	1.39	35.2	2.1	1.16	0.17	0.19	0.16	16.8
SAFI	29.8	6.4	6.1	39.4	92.3	45.1	3.0	1.23	1.49	0.29	0.43	15.0

Key: TOC = total organic carbon, EC = electrical conductivity, NH_4^+ = ammonium, NO_3^- = nitrate,
SAFI = commercial organic fertilizer.

3.3.3.2 Treatments and experimental set up

The study consisted of two experiments, each repeated for two seasons: (1) the first experiment involved the use of two organic fertilizers (BSF frass and SAFI organic) applied at sole rates in tonnes per hectare (t ha^{-1}) and (2) the second experiment involved the utilization of three fertilizers (BSF frass, SAFI and urea) applied to supply different levels of nitrogen. The two experiments were set side-by-side at the Kenyatta University teaching and demonstration farm, using the randomized complete block design (RCBD) with three replicates per treatment. Plots measured 4 x 4 m with border widths of 0.5 m and 1 m between the plots and blocks, respectively. The distance between experiments was 2 m.

In the first experiment, the BSF frass fertilizer and SAFI were applied singly at 2.5, 5.0 and 7.5 t ha^{-1} to determine the most effective rate for crop production (Table 6). These were denoted as control, 2.5BSF, 5BSF and 7.5BSF for BSF frass fertilizer, and 2.5SAFI, 5SAFI and 7.5SAFI for SAFI treatments. The control treatment consisted of unfertilized soil. This gave a total of seven treatments, which are referred to as sole organic fertilizer treatments in this document. In the second experiment, the organic fertilizers were applied at three rates equivalent to 30, 60 and 100 kg N ha^{-1} to determine the optimum N rate (Table 6). These were denoted as 30N BSF, 60N BSF and 100N BSF for BSF frass fertilizer treatments, and 30N SAFI, 60N SAFI and 100N SAFI for SAFI treatments. The synthetic N fertilizer (45% N urea) was applied at equivalent rates (30, 60 and 100 kg N ha^{-1}) as the organic fertilizers.

Table 6: Treatments considered during the study.

Experiment	Fertilizer	Fertilizer rates
Experiment 1	BSF frass	Each fertilizer applied at four uniform rates equivalent to 0, 2.5, 5 and 7.5 t ha^{-1}
	SAFI organic	
Experiment 2	BSF frass	Each fertilizer applied at four uniform rates equivalent to 0, 30, 60 and 100 0 kg N ha^{-1}
	SAFI organic	
	Urea	

To remove any limitation in the mineral N and organic N fertilizer treatments, inorganic P [supplied as triple super phosphate – TSP (46% P_2O_5)] and K [supplied as muriate of potash (60% K_2O)] were applied at uniform rates of 60 kg P ha^{-1} and 50 kg K ha^{-1} (Tittonell et al., 2008a). Table 7 shows the amounts of N, P and K supplied by the different organic fertilizer treatments. The control treatment consisted of unfertilized soil.

The second experiment, where fertilizers were applied in terms of N rates and supplemented with P and K consisted of seven treatments which are referred to as combined fertilizer treatments in this document. Maize hybrid H513, which is recommended for low and medium altitude areas of Kenya, was used as the test crop. The TSP fertilizer was applied at planting while urea and muriate of potash were applied in two splits: 50% at 4 weeks after planting and another 50% at 7 weeks after planting.

Table 7: Actual amounts of macro nutrients supplied by organic fertilizer treatments.

Organic fertilizer	Treatment	Amount applied (kg ha^{-1})	Nutrients supplied (kg ha^{-1})		
			Nitrogen	Phosphorus	Potassium
Black soldier fly - composted manure	30 kg N ha^{-1}	1,429	30	16.57	2.43
	60 kg N ha^{-1}	2,858	60	33.14	4.86
	100 kg N ha^{-1}	4,762	100	55.24	8.09
	2.5 t ha^{-1}	2,500	52.5	29	4.25
	5 t ha^{-1}	5,000	105.0	58	8.50
	7.5 t ha^{-1}	7,500	157.5	87	12.75
Safi organic fertilizer	30 kg N ha^{-1}	1,000	30	12.3	14.9
	60 kg N ha^{-1}	2,000	60	24.6	29.8
	100 kg N ha^{-1}	3,334	100	40.99	49.7
	2.5 t ha^{-1}	2,500	75	30.75	37.25
	5 t ha^{-1}	5,000	150	61.50	74.50
	7.5 t ha^{-1}	7,500	225	92.25	111.75

3.3.3.3 Maize growth, yield, and mineral nitrogen

Plant height and chlorophyll contents were measured from 10 plants per plot which were randomly selected and tagged for repeated measurements at early vegetative [35 days after planting (DAP)] (8 – 10 leaves), late vegetative (55 DAP) (13 – 16 leaves), tasseling (70 DAP) and silking stages (91 DAP). Plant heights were measured using a tape measure from the ground level up to the apex of the topmost leaf while chlorophyll content was measured using a SPAD meter placed on the fourth fully opened leaf from the top (Yuan et al., 2016). Biomass amounts and nitrogen uptake were determined at early vegetative, tasseling, silking and maturity (125 DAP) (harvesting) stages. Two plants were randomly selected from the two outer rows of each plot, cut at ground level and their fresh weights determined.

Plant subsamples were collected from each plot, and air dried for five days before oven drying at 60 ºC for 72 hours for complete drying without affecting nutritional quality. After cooling the dry weights were determined. The dried samples were ground into powder (< 0.25 mm) which was used for the determination of total N.

Grain yield data was collected at harvesting period from each plot area after all the ears had dried. Plants in the harvested area were cut at ground level and their ears threshed to determine grain weights and weight of residues using a weighing scale. Grain and stover samples were taken to the laboratory and air-dried to 12.5% moisture content for determination of grain and residues yields per plot and on a hectare basis (t ha^{-1}). Part of the grain and stover sample from each treatment was ground into powder for determination of N, P and K concentrations.

During plant sampling, soil samples were also collected at two depths (0 – 20 cm and 20 – 40 cm) to determine mineral N (nitrate-N and ammonium-N) content in the top- and sub-soil layers during crop growth. A soil auger was used to collect sub-samples from eight spots within the inner four rows of each plot. The subsamples were homogenized by the quarter sampling approach (Okalebo et al., 2002) to obtain representative samples. The soil samples were placed in air-tight polythene bags and carried to the laboratory using cool boxes containing ice blocks to reduce microbial activities during transportation.

3.3.3.4 Nitrogen use efficiencies

The following N use efficiencies were calculated: the total N uptake by plants as measured in plant tissues and grain, agronomic efficiency, apparent N recovery efficiency, agro-physiological N efficiency, and N harvest index. Part of the air-dried samples were ground using an analytical mill for analysis of N in grain and plant tissues, which were used to measure the amount of N in grain and plant residues per treatment (equation 7). Agronomic efficiency (AE_N), which is a measure of economic yield produced per unit N supplied from each treatment, was calculated using grain yields from each treatment (equation 8). Apparent N recovery efficiency (ANR_N) was calculated to determine the ability of the plant to acquire the N supplied from different treatments (equation 9).

Agro-physiological N efficiency (APE_N) was calculated to determine the economic yield per unit N accumulated from each fertilizer treatment (equation 10). Nitrogen harvest index (NHI) was calculated to determine the fraction of nitrogen accumulated in the grain (equation 11). All the N use efficiency indices were calculated according to Baligar et al. (2001).

$$N\ uptake\ (kg\ ha^{-1}) = \frac{(\%N \times dry\ matter)}{100} \tag{7}$$

$$AE_N(kg\ kg^{-1}) = \frac{(Yield_{\ F} - Yield_{\ C})}{Quantity\ of\ N\ applied} \tag{8}$$

$$ANR_N(\%) = \frac{(N\ uptake_{\ F} - N\ uptake_{\ C})}{Quantity\ of\ N\ applied} \times 100 \tag{9}$$

$$APE_N(kg\ kg^{-1}) = \frac{(Yield_{\ F} - Yield_{\ C})}{\left[\begin{pmatrix} N\ uptake\ in \\ grain\ and\ stover_{\ F}\end{pmatrix} - \begin{pmatrix} N\ uptake\ in \\ grain\ and\ stover_{\ C}\end{pmatrix}\right]} \tag{10}$$

$$NHI(\%) = \frac{N\ uptake\ in\ grain}{N\ uptake\ in\ grain\ and\ stover} \times 100 \qquad (11)$$

Where,

F represents plots that received fertilizer treatments,

C represents the control plots.

3.3.4 Determination of soil moisture storage, nitrogen fertilizer equivalence and synchrony of N mineralization from BSF frass and commercial organic fertilizers

3.3.4.1 Nitrogen fertilizer equivalence

The grain yield data obtained from the second field experiment where fertilizers were applied in terms of N rates (0, 30, 60 and 100 kg N ha^{-1}) (section 3.3.3) was used in the determination of the nitrogen fertilizer equivalence values (NFEs) of BSF frass and SAFI fertilizers. Nitrogen fertilizer equivalence value represents the amount mineral N fertilizer saved when using organic fertilizer N to achieve the same crop yield (Hijbeek et al., 2018). Nitrogen fertilizer equivalence value is also referred to as nitrogen fertilizer replacement value and is expressed as kg kg^{-1}, but mostly converted into percent for comparison purposes (Kimetu et al., 2004; Cavalli et al., 2016; van Middelkoop & Holshof, 2017). The grain yields from the urea fertilizer treatment were used to draw the N response curves (Figure 3) that were used to calculate the NFEs of the two organic fertilizers (equation 12) (Lalor et al., 2011).

$$NFE\ (kg\ kg^{-1}) = \frac{NA_{f=GY}}{NA_{org}} \qquad (12)$$

Where,

$NA_{f=GY}$ represents the mineral fertilizer N required to obtain grain yield equivalent to those of organic fertilizer treatments (kg ha^{-1}).

NA_{org} represents the total N supplied by organic fertilizer inputs (kg ha^{-1}).

The $NA_{f\,=\,GY}$ for each organic fertilizer treatment was estimated using the grain yield response curve specific to each season. Since the response assumed a quadratic function with the equation $Y = aNA_f^2 + bNA_f + c$, the equations were solved using the quadratic formula. The constants a, b and c were obtained from the N response curves.

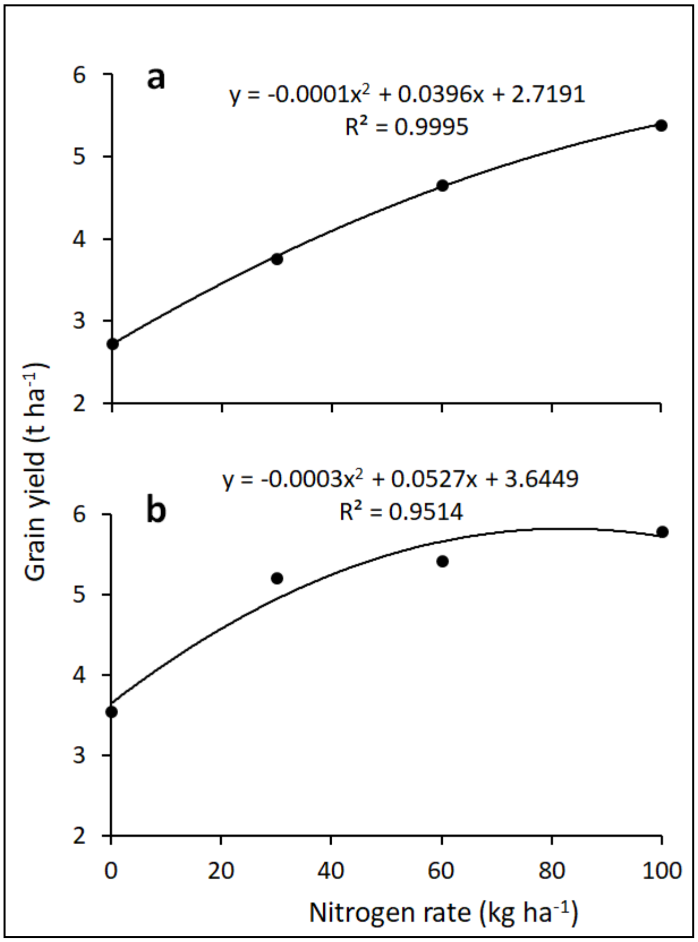

Figure 3: Maize grain yield response curves used to determine fertilizer equivalences of BSF frass and SAFI organic fertilizers during the short (a) and long rain (b) season experiments. Note: Grain yields for BSF frass fertilizer.

3.3.4.2 Synchrony of nitrogen mineralization for maize production

In this study, the BSF frass and SAFI fertilizers were applied at rates of 0 and 5 t ha^{-1}, a rate that has been previously used in central Kenya (Mucheru-Muna et al., 2014; Musyoka et al., 2017, 2019a). The establishment and management of experiments were done following procedures described in section 3.3.3. Data was collected on maize biomass amounts, N uptake at different maize growth stages (early seedling, tasseling, silking and maturity) and mineral N in bulk soil determined at early seedling, tasseling, silking and maturity stages, following procedures described in section 3.3.1.4.

3.3.4.3 Soil sampling and incubation

From each plot, soil was collected from 0 – 20 cm depth before application of organic fertilizers. The soil was manually sorted to remove objects, stones, and big clods bigger than 2 mm. The soil was then homogenized by hand mixing in a basin. The two organic fertilizers were mixed with the soil at same rates as mentioned in section 3.3.4.2 and the moisture content of the mixture (soil-organic fertilizer) was adjusted to 60% soil water holding capacity using distilled water. Two hundred grams of the mixture were then placed in an air permeable zip lock bag that was sealed to prevent water entry. The bags were then buried at 10 – 20 cm depth in respective plots. Similarly, 200 g of unamended soil from each plot (as control) were placed in zip lock bags and buried at the same depths (10 – 20 cm) in each of the respective plots.

Five bags were randomly buried per replicate, giving a total of 15 bags per treatment at the beginning of each cropping season. The bags were retrieved at 0, 35, 70, 91 and 125 days of the incubation period which corresponded to planting, early vegetative, tasseling, silking, and harvesting stages of the maize crop. The positions of the bags were marked using pegs to avoid disturbance during weeding and for ease of retrieval.

On each sampling date, the retrieved bags were labelled, placed in airtight polythene bags, and carried in a cool box containing ice blocks to maintain low temperatures and reduce microbial activities pending analysis. The samples were used for determination of mineral N content. Also, on each sampling date, other soil samples were collected in top (0 – 20 cm) and subsoil (20 – 40 cm) from the respective plots for the determination of soil moisture and mineral N content during crop growth (early seedling, tasseling, silking and maturity). A soil auger was used to collect sub-samples from eight spots within the inner four rows of each plot.

The subsamples were homogenized by quarter sampling approach to obtain representative samples. The soil samples were also placed in airtight polythene bags and carried to the laboratory using cool boxes containing ice blocks to reduce microbial activities during transportation. Soil moisture was determined using the gravimetric method (Okalebo et al., 2002).

3.3.4.4 Mineral nitrogen release

Mineral N released was calculated on per hectare basis (kg N ha^{-1}) by using bulk density and soil depth as shown in equation 13.

$$Mineral\ N\ content\ (kg\ N\ ha^{-1}) = \frac{mineral\ N\ concentration\ (mg\ kg^{-1}) \times mass\ of\ soil\ layer\ (kg)}{1{,}000{,}000} \quad (13)$$

Where,

$$mass\ of\ soil\ layer\ (kg) = soil\ bulk\ density\ (kg\ m^{-3}) \times volume\ of\ soil\ layer\ (m^3)$$

$$volume\ of\ soil\ layer\ (m^3) = area\ of\ one\ hectare\ (m^2) \times depth\ of\ soil\ layer\ (m)$$

The mineral N released from unamended soil and from soil amended with organic fertilizers at each sampling time during mineralization was determined using equations (14) and (15) (Musyoka et al. (2019a). The N release rate (kg N ha day^{-1}) was calculated by dividing mineral N released at each sampling date by the number of incubation days (Loecke et al., 2012).

$$Mineral\ N\ released\ (kg\ N\ ha^{-1})_{soil\ alone} = Mineral\ N_{t+k\ soil} - Mineral\ N_{ti\ soil} \quad (14)$$

$$Mineral\ N\ released\ (kg\ N\ ha^{-1})_{amended\ soil} = Mineral\ N_{t+k\ amended\ soil} - Mineral\ N_{ti\ amended\ soil} \quad (15)$$

Where,

t_i represents sampling times $i = 0, 1, 2, 3\ldots$

$t+k$ is t_i plus time k intervals where $k = 1, 2, 3, 4\ldots$

3.3.4.5 Synchrony of nitrogen mineralization and nitrogen uptake

To determine the N synchrony, N flux was calculated as the difference between plant N uptake and mineral N released from the amended soils per day (equation 16) according to Musyoka et al. (2019a). A positive N flux meant that the N released was larger than the crop's N demand while a negative N flux meant that mineral N released was insufficient to meet the crop's N demand.

$$N\ flux\ (kg\ N\ ha\ day^{-1}) = Mineral\ N\ released_{inputs} - Daily\ N\ uptake \tag{16}$$

Where,

$$Daily\ N\ uptake\ (kg\ ha\ day^{-1}) = \frac{(N\ uptake\ t_{\ i+k} - N\ uptake\ t_i)\ kg\ ha^{-1}}{(t_{i+k} - t_i)}$$

N uptake represents the quantity of N taken up at each maize growth stage (equation 7).

3.3.5 Economic value of frass fertilizer production alongside BSF rearing and profitability of crop production using BSF frass fertilizer

3.3.5.1 Economic value of frass fertilizer as a second product from BSF farming

A cost-benefit analysis of frass fertilizer as a by-product of BSF farming was carried out, where gross margins (equation 17), the benefit cost ratio (equation 18), and return on investment (equation 19) were calculated as shown in the three equations (17,18 &19) (Chia et al., 2019b).

$$Gross\ margin\ (\%) = \frac{Net\ income}{Gross\ income} \times 100 \tag{17}$$

$$Benefit\ cost\ ratio\ = \frac{Net\ income}{Total\ variable\ costs} \tag{18}$$

$$Return\ on\ investment\ = \frac{Net\ income}{Total\ variable\ costs} \times 100 \qquad (19)$$

Where,

$Net\ income\ = Gross\ income\ - Total\ variable\ costs$

For comparative purposes, the cost-benefit analysis considered two options for the outputs of BSF farming: (1) BSF farming solely for larvae production and (2) BSF farming for both larvae and frass fertilizer production. Under the first option, the BSF production process ends at the 5th instar larval stage when the larvae are usually harvested for animal feeding purposes. The variable costs of insect production included cost of transporting rearing substrates, water utilized, electricity consumed, and labour for egg collection, feeding and harvesting the BSF larvae (Table 8). Labour costs were calculated by recording the number of hours spent on each activity, at a wage rate of 1.25 USD per hour (Kenyan Government, 2019). Gross income was measured using the revenue generated from BSF larvae.

Under the second option, after larvae harvesting, frass was collected and composted to convert it into an organic fertilizer, which is referred to as frass fertilizer following procedures described in section 3.3.1 and 3.3.2 above. The additional costs incurred in transporting the frass to the composting site and turning the frass to convert it into an organic fertilizer were added to the fertilizer production costs (Table 8). The quantity of dry frass fertilizer and dry BSF larvae as obtained from each waste combination, were used to measure the gross income at current market prices in Kenya.

Table 8: Parameters used in calculating economic returns from frass fertilizer.

Parameter	Unit price
Cost of labour	1.25 USD hour^{-1}
Cost of sawdust	0.02 USD kg^{-1}
Cost of brewery waste	0.0522 USD kg^{-1}
Cost of biochar	0.3 USD kg^{-1}
Cost of gypsum	0.48 USD kg^{-1}
Price of dry BSF larvae	0.9 USD kg^{-1*}
Price of BSF frass fertilizer	0.3 USD kg^{-1}

Dollar exchange rate: 1USD = 100 Kshs
*Source: Sanergy limited (http://www.sanergy.com)

The costs of substrate amendments (sawdust, biochar, and gypsum) were included in insect production costs under the second option. After 14 days of rearing (5th instar stage), the BSF larvae were harvested, and quantity of dry larvae obtained per treatment was recorded.

The performance of BSF larvae on brewery spent grain is comparable with those of other wastes such as chicken manure and kitchen waste (Shumo et al., 2019) that can be readily available both on- and off-farm. Therefore, three scenarios of rearing substrate in establishing the economic benefits of frass fertilizer were considered: (1) BSF farming when the main rearing substrate is freely available but has to be transported to the farm which is the case currently; (2) BSF farming when the main rearing substrate is purchased and transported to the farm; and (3) BSF farming when the main rearing substrate is available on the farm, i.e., no purchase or transport costs incurred. In all options, it was assumed that the farmer is producing BSF farming for commercial purposes and the frass was not utilized in any sector e.g. crop fields where it could have served as an organic fertilizer.

3.3.5.2 Economic evaluation of maize production using BSF frass fertilizer

This study utilized the data collected from the two field experiments as described in section 3.3.3 to determine the economic return to using frass fertilizer for maize production. Cost benefit analysis was carried out by calculating gross margin, benefit-cost ratio and return on investment using equations 17 – 19 (section 3.3.5.1). The economic return to frass fertilizer was compared with that for mineral fertilizer (urea) and commercial organic fertilizer (SAFI).

Prices of fertilizers, and maize seed were obtained from Africa fertilizer (Africafertilizer.org) and Kenya Farmers' Association, respectively (Table 9). Labour expenses (for land preparation, planting, weeding, fertilizer application, and harvesting) were estimated by recording the time taken to perform each activity and valuing labour at the rate of 1.25 USD per working hour (Kenyan Government, 2019). Revenue was calculated using harvested maize grain and stover yields per fertilizer treatment (in kg per hectare). Price of maize grain was obtained from human data exchange (https://data.humdata.org). Since stover is used as an animal feed in Kenya, it was considered an additional output and valued at 22.1 USD per tonne (Mucheru-Muna et al., 2014).

Table 9: Parameters used to calculate economic returns for different fertilizer inputs.

Parameter	Unit cost
Price of urea (46% N)	0.54 USD kg^{-1a}
Price of Safi organic	0.3 USD kg^{-1}
Price of maize seed	1.8 USD kg^{-1}
Labour cost	1.25 USD hour^{-1}
Price of maize	0.37 USD kg^{-1b}

Dollar exchange rate: 1USD = 100 Ksh.
Source: [a]Africafertilizer.org, [b]https://data.humdata.org/

3.3.5.2.1 Economically optimum fertilizer rates for maize production

The values of grain yield were regressed against the fertilizer rates and response curves were drawn for each fertilizer treatment (Figure 4). Since the regression equations for N rates (Figures 4a – d) were quadratic in nature, a quadratic function equation (equation 20) was used to calculate the economically optimum fertilizer rates (N) for maize production using equation (21) (Naher et al., 2011).

$$Y = a + bN + cN^2 \qquad (20)$$

$$N = \frac{E_n - b}{2c} \qquad (21)$$

Where,

Y is maize grain yield in kg per hectare,

N is rate of nitrogen fertilizer (kg ha^{-1}),

a, b and c are parameters estimated by the quadratic function

E_n represents the ratio of fertilizer price (P_f) to maize price (P_y), $E_n = \frac{P_f}{P_y}$

P_f represents the price of fertilizer (urea, FF, and SAFI) per kg

P_y represents the price of maize per kg

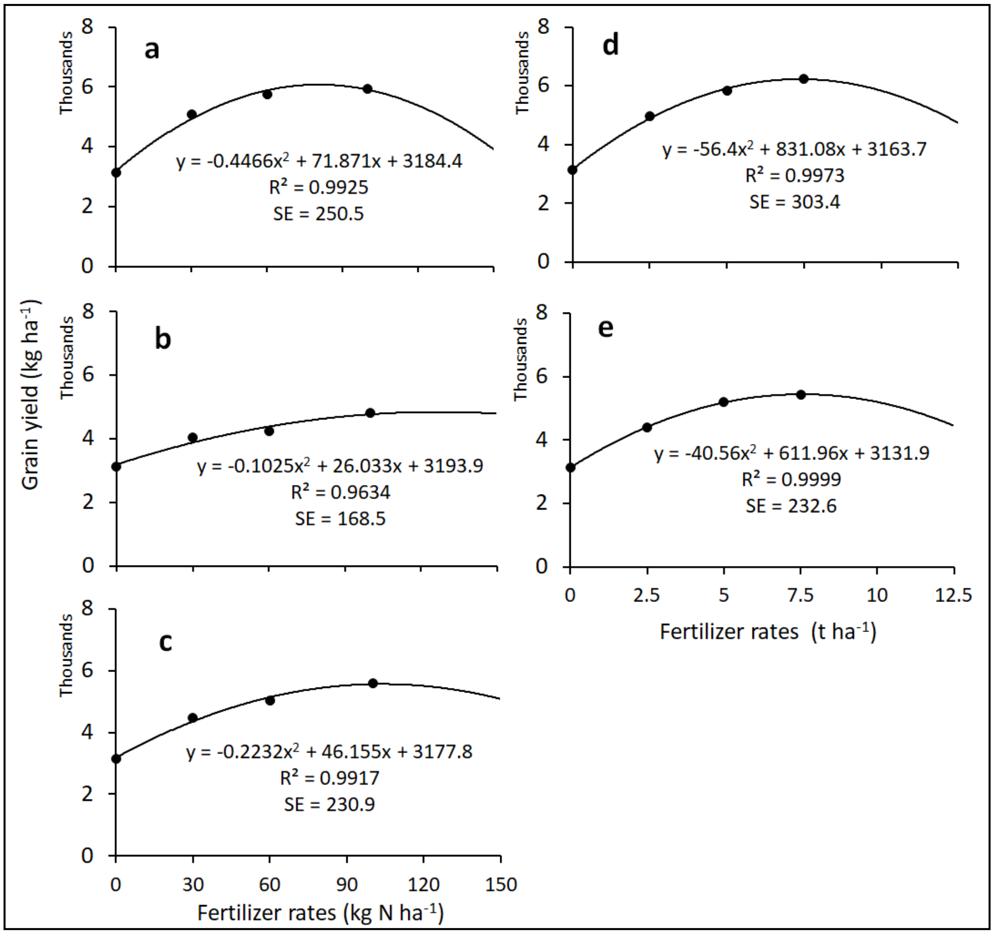

Figure 4: Yield response curves used to calculate economically optimum rates of BSF frass (a and d), urea (c) and SAFI (b and e) fertilizers for maize production.

3.3.5.2.2 Stochastic dominance analysis

Plots of cumulative density functions for grain yields obtained while using BSF frass fertilizer, SAFI organic fertilizer and urea fertilizer were constructed to compare yield distributions obtained using the three fertilizer treatments.

3.4 Data analysis

Prior to analysis, data was 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 a random effect. The effects of the substrate C/N ratios, biochar and gypsum amendments and composting time on pH, electrical conductivity, water soluble carbon, ammonium, nitrate, ammonium/nitrate ratio, total organic carbon, C/N ratio, total N, P and K were determined. The linear mixed-effect model was used for analysis of data collected from field experiments on plant height, chlorophyll content, nitrogen uptake, and mineral N content, whereby fertilizer treatments and maize growth stage were considered as fixed effects while replication was considered as a random effect.

Data on fresh and dry BSF larval yields, waste reduction, biomass conversion rate, frass compost yield, seed germination, germination index, initial substrate N content, nutrient uptake by BSF larvae, N retention in frass, maize grain and stover yields, P and K uptake and N use efficiency, and NFE were analyzed using one-way analysis of variance. Computation of least squares means was done using ‘lsmeans’ package. Significant means were separated using Tukey’s test at $p < 0.05$. For objective two (biochar and gypsum amendments) data was analyzed per experimental set. Also, data from field experiments (objectives 3 and 4) was analyzed per season.

For economic analysis, the two-sample parametric Kolmogorov-Smirnov test for first-order stochastic dominance was done to assess for the vertical distance between the cumulative density functions of the maize grain yield obtained. All the statistical analyses were conducted using R software version 3.6.0 (R Core Team, 2019).

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Introduction

This chapter is arranged in the following order. Section 4.2 presents results on the critical carbon to nitrogen (C/N) ratio for optimal black soldier fly (BSF) growth and nutrient conservation in frass fertilizer. Section 4.3 evaluates the effect of biochar and gypsum amendments on BSF larval performance and nitrogen retention in frass fertilizer. In both sections (4.2 and 4.3), the effects of BSF larvae on compost maturity and stability were determined by assessing temperature, pH, electrical conductivity, C/N ratio, water soluble carbon to nitrogen ratio and seed germination index. Section 4.4 describes results on the comparative performance of BSF frass fertilizer and commercial fertilizers on the growth, yield, and nitrogen use efficiency of maize. Section 4.5 presents results on the N fertilizer equivalence value and synchrony of N mineralization from BSF frass and commercial organic fertilizer for maize production. Section 4.6 assesses the economic value of frass fertilizer production alongside BSF rearing and profitability of crop production using BSF frass fertilizer.

4.2 Critical carbon to nitrogen (C/N) ratio for optimal BSF larval growth and nutrient conservation in frass fertilizer

4.2.1 Black soldier fly larvae yield

There was a significant effect of rearing substrates on the fresh ($p < 0.001$) and dry ($p = 0.001$) BSF larval yields (Figure 5a). 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 ratios of 30 and 25 had the least fresh and dry larval yields, respectively.

There was significant percentage reduction in fresh ($p < 0.001$) and dry ($p = 0.014$) BSF larval yield across the different substrates when compared with the control treatment (Figure 5b). 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 ratios of 25 and 30, respectively.

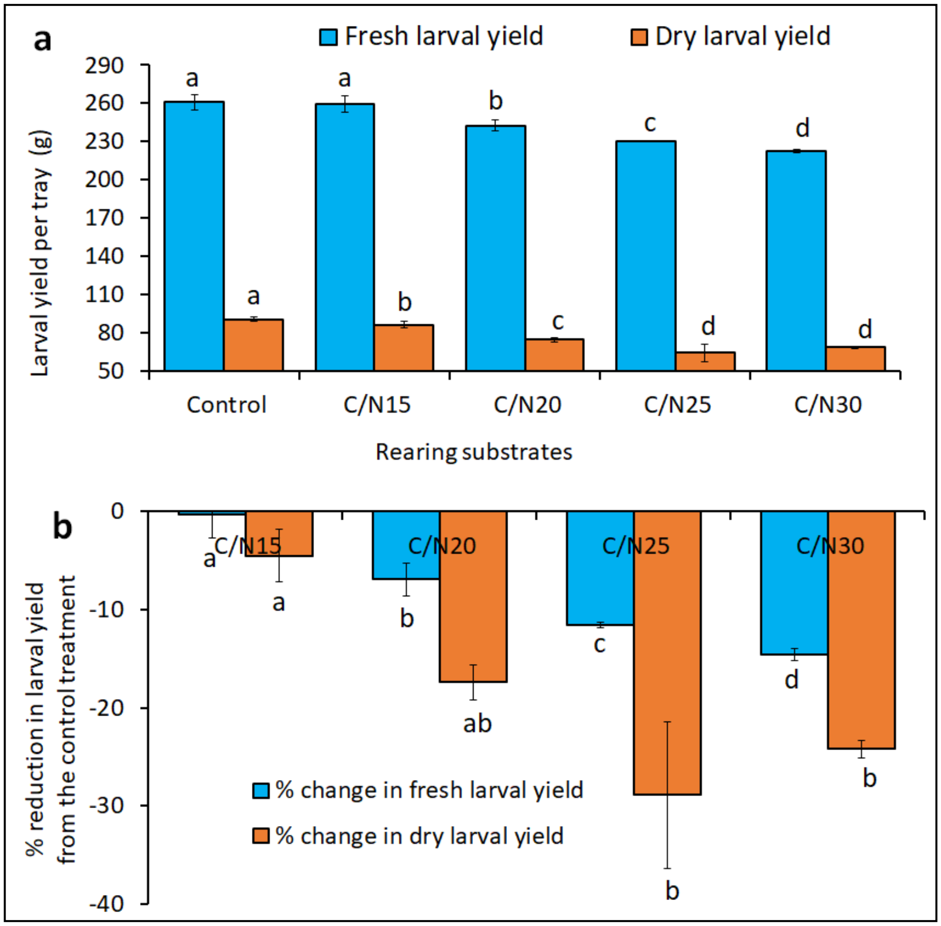

Figure 5a and b: Effect of substrate C/N ratio on black soldier fly larval yields.
Key: C/N = total organic carbon to total organic nitrogen ratio; Control = unamended substrate (brewery spent grain); C/N 15, C/N 20, C/N 25, and C/N 30 = amendment of brewery spent grain using sawdust to obtain substrates with C/N ratios of 15, 20, 25 and 30, respectively. For each parameter and panel, means (± standard error) followed by the same letters are not significantly different at $p \leq 0.05$.

The 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 (Figure 5a and 5b) could be attributed to higher nitrogen content of both substrates. Results from the current study 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. Furthermore, the findings of this study are supported by Jucker et al. (2017), who observed smaller larvae sizes from substrates with low nitrogen content.

4.2.2 Waste reduction and biomass conversion rate

The waste reduction ($p = 0.234$) and biomass conversion rates ($p = 0.178$) did not vary significantly across the different substrates (Figure 6). The waste reduction ranged between 24 and 34 % while biomass conversion rates varied between 9 and 14% (Figure 6). Results from the current study are consistent with those reported by Rehman et al. (2017) who found that substrates with high carbon to nitrogen ratios such as soybean residues resulted in low biomass conversion rates by BSF larvae, which is in accordance with the larval yields recorded from substrates with C/N ratio of 30. On the contrary, substrates with lower carbon to nitrogen ratios in the present study had higher biomass conversion rates (Figure 6). 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 (Shumo et al., 2019).

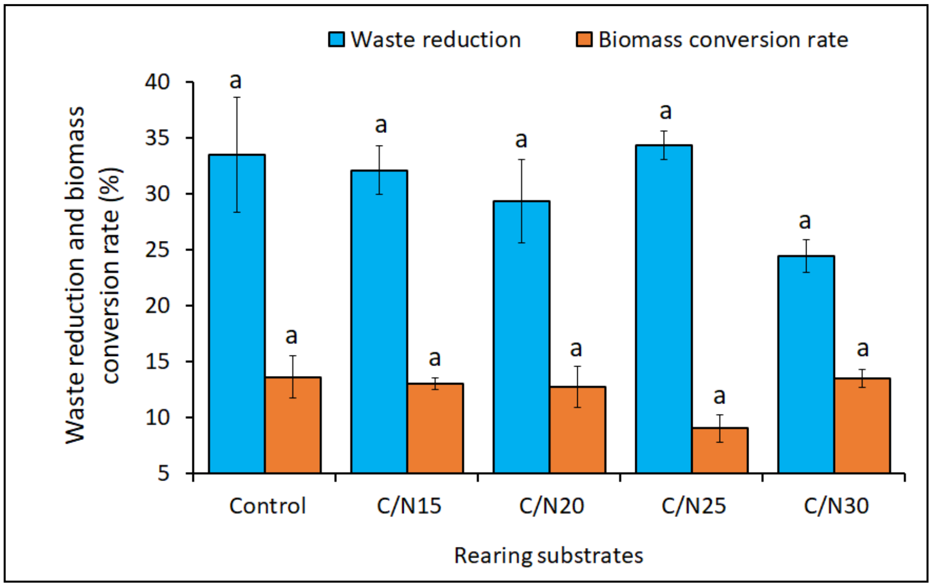

Figure 6: Effect of substrate C/N ratio on waste reduction and biomass conversion rates of black soldier fly larvae.

Key: C/N = total organic carbon to total organic nitrogen ratio; Control = unamended substrate (brewery spent grain); C/N 15, C/N 20, C/N 25, and C/N 30 = amendment of brewery spent grain using sawdust to obtain substrates with C/N ratios of 15, 20, 25 and 30, respectively. For each parameter and panel, means (± standard error) followed by the same letters are not significantly different at $p \leq 0.05$.

The waste degradation efficiencies obtained in the current study (24 – 34%) (Figure 6) could be due to the presence of recalcitrant carbon tissues such as lignin, cellulose and hemicelluloses that are difficult to be broken down (Huang et al., 2004). The findings of this study are comparable to the findings of earlier studies with rice straw (10 – 32%) (Manurung et al., 2016) and cassava peels (28%) (Supriyatna et al., 2016), which based their arguments on the presence of recalcitrant carbon tissues. For farmers, targeting organic fertilizer production using BSF larvae, the low waste reduction is advantageous since it would result into higher frass compost yields.

4.2.3 Frass compost yield

Significant differences were observed for both fresh (p = 0.002) and dry (p = 0.001) compost yields among the substrates used (Figure 7). Substrates with C/N ratio of 20 and above showed no significant variations in fresh frass compost yields. Also, the frass compost yields generated substrates with C/N ratio of 15 was not significantly different from that of the control. Substrates with C/N ratio of 20 generated frass compost with the highest weight of fresh (3 kg) and dry (0.89 kg) compost yields, followed by substrates with C/N ratios of 25 and 30. Substrates whose initial C/N ratios were adjusted to 20 and above produced significantly higher ($p < 0.05$) frass compost yields than the control.

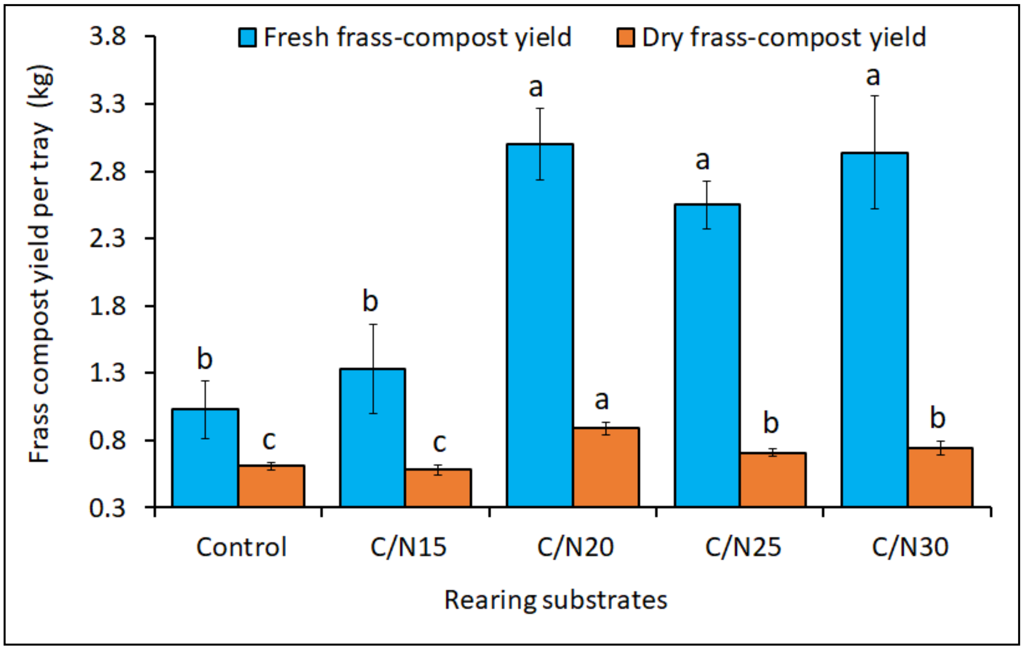

Figure 7: Effect of substrate C/N ratio on frass compost yields
Key: C/N = total organic carbon to total organic nitrogen ratio; Control = unamended substrate (brewery spent grain); C/N 15, C/N 20, C/N 25, and C/N 30 = amendment of brewery spent grain using sawdust to obtain substrates with C/N ratios of 15, 20, 25 and 30, respectively. For each parameter and panel, means (± standard error) followed by the same letters are not significantly different at $p \leq 0.05$.

The control treatment and substrate with C/N of 15 had the lowest fresh (1.03 kg) and dry (0.58 kg) compost yields, respectively. The significantly higher fresh and dry frass compost yields produced from substrates with C/N ratios of 20 and 30 compared to the control and substrate with C/N of 15 can be attributed to reduced nitrogen availability for larval uptake, arising from increased sawdust as a carbon source in the various substrates (Huang et al., 2004).

4.2.4 Nutrients flow in BSF larvae and frass compost

Increasing substrate C/N ratios caused significant differences in the initial content of K in rearing substrates ($p = 0.022$) (Table 10). However, increasing C/N ratio did not significantly influence the initial contents of total N ($p = 0.075$) and P ($p = 0.160$) of the substrates. Initial total N contents ranged between 31 and 40 g while the initial total P was between 6.7 and 8.6 g. The highest K content was observed in substrate with C/N ratio of 15. The fraction of initial nitrogen ($p = 0.189$), phosphorus ($p = 0.312$) and potassium ($p = 0.340$) accumulated in BSF larval biomass did not vary significantly due to increasing substrate C/N ratio.

Increasing substrate C/N ratios caused significant differences ($p = 0.023$) in the fraction of phosphorus retained in frass composts (Table 10). Frass composts generated from substrates with C/N ratio of 20 retained significantly ($p = 0.023$) higher P than the frass composts generated from control substrates and substrates with C/N ratios of 30. The fraction of initial total nitrogen ($p = 0.056$) and potassium ($p = 0.058$) retained in the frass composts did not vary significantly (Table 10). The total N and K retention efficiencies of 12 – 19 g 100 g^{-1} and 20 – 36 g 100 g^{-1}, respectively, were recorded during the study.

Table 10: Effect of C/N ratio adjustment on nutrient accumulation by BSF larvae and nutrient retention in frass fertilizer

Rearing 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
Control	39.7 ± 2.7	8.6 ± 0.1	2.2 ± 0.0ac	6.8 ± 0.7	6.0 ± 1.0	22.2 ± 0.8	33.1 ± 4.6a	12.0 ± 0.4b	35.5 ± 4.4
C/N 15	30.9 ± 0.6	7.3 ± 0.6	3.07 ± 0.5a	9.0 ± 0.5	8.8 ± 2.0	23.0 ± 6.2	47.0 ± 4.4a	15.8 ± 1.1ab	20.0 ± 2.1
C/N 20	30.9 ± 2.4	7.5 ± 0.9	3. 05 ± 0.1ab	8.9 ± 0.6	5.5 ± 0.8	14.7 ± 1.2	45.6 ± 6.7a	19.1 ± 2.2a	29.1 ± 2.8
C/N 25	30.5 ± 2.3	6.7 ± 0.2	2.04 ± 0.2ac	7.4 ± 1.3	6.4 ± 0.3	20.5 ± 1.7	36.2 ± 2.3a	14.4 ± 1.4ab	28.4 ± 1.7
C/N 30	32.7 ± 0.8	6.9 ± 0.3	1.89 ± 0.2ac	7.4 ± 0.4	6.8 ± 0.4	21.7 ± 0.3	29.2 ± 0.8a	11.7 ± 1.5b	21.7 ± 4.9
	ns	ns	*	ns	ns	ns	ns	*	ns

Key: * $P < 0.05$, ns = not significant ($p \geq 0.05$), N = nitrogen, P = phosphorus, K = potassium. C/N = total organic carbon to total organic nitrogen ratio; Control = unamended substrate (brewery spent grain); C/N 15, C/N 20, C/N 25, and C/N 30 = amendment of brewery spent grain using sawdust to obtain substrates with C/N ratios of 15, 20, 25 and 30, respectively. In the same column, means (± standard error) followed by the same letters are not significantly different at $p \leq 0.05$.

The higher N, P and K uptake by larvae reared on substrates with C/N ratios of 15 and 20 (Table 10) imply that adjusting C/N ratio of rearing substrates could positively influence the nutritional value of BSF larvae obtained (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 wastes that dominate most cities of SSA (Okot-Okumu, 2012), this study has demonstrated that the larvae yield would largely depend on the substrate qualities such as nutrient content and C/N ratio.

4.2.5 Temperature, pH, and electrical conductivity during frass composting

The variation in room and substrate temperature during the larval rearing and composting phase (post-larval rearing phase), respectively, is presented in Figure 8a. The temperature of the various substrates during the larval rearing phase increased from initial values of between 26 and 31 ^{o}C to peak levels of between 26 and 43 ^{o}C on the third day. The highest initial temperature was recorded in substrate with C/N ratio of 20 (42 ^{o}C). During the composting phase, the room temperature was slightly above that of the substrate throughout the experiment.

The pattern of temperature observed during the study has been previously reported by (Tumuhairwe et al., 2009). The highest temperature achieved at the onset of composting could be due to high availability of energy sources from simple carbohydrates such as simple sugars coupled with amino acids and proteins that are easily broken down by microbes to release energy in form of heat that eventually causes rise in temperatures (Bernal et al. 2009).

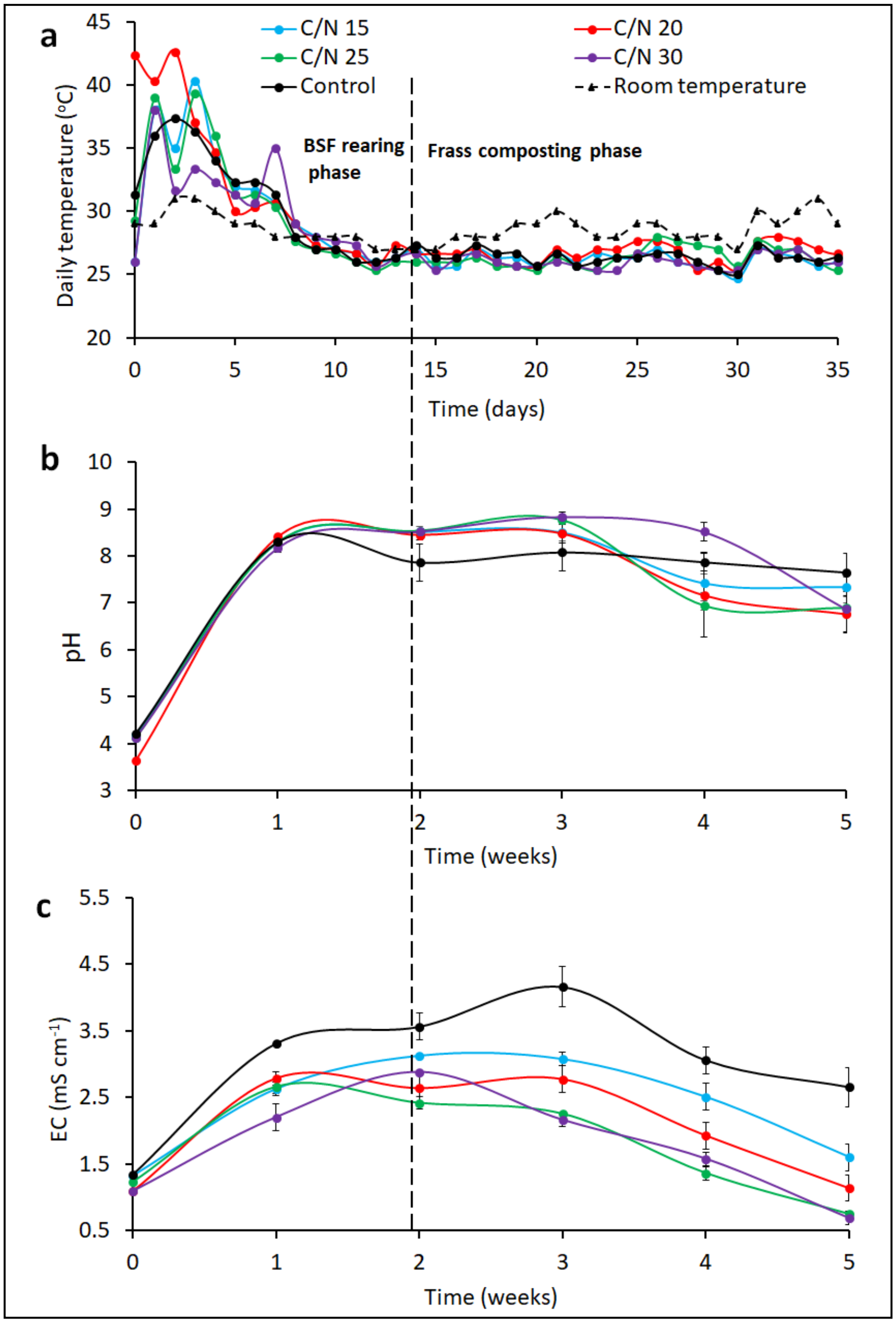

Figure 8: Effect of substrate C/N ratio on temperature (a), pH (b) and electrical conductivity (EC) (c), during BSF larvae rearing and frass composting phases.

Key: C/N = total organic carbon to total organic nitrogen ratio; Control = unamended substrate (brewery spent grain); C/N 15, C/N 20, C/N 25, and C/N 30 = amendment of brewery spent grain using sawdust to obtain substrates with C/N ratios of 15, 20, 25 and 30, respectively.

As the composting process progresses, the available sources of energy get depleted and microbial activities reduce, causing a decline in temperature as observed from the 10th day of experiments. Temperature evolution during composting is majorly determined by the nature of substrate, size of the compost heap/pile and moisture content of decomposing materials (Epstein, 1997).

A big compost heap/pile conserves temperatures within the heap due to self-insulating property and this causes rapid heating to thermophilic temperatures (Said-Pullicino et al. 2007). Therefore, the low temperatures (< 45 °C) observed in this study could partly be attributed to the small size of substrate heaps (≤ 10 cm deep and 50 cm wide) required in BSF rearing. Furthermore, since there was no covering of rearing trays, most of the heat could have been lost to the environment, thus leading to lower temperatures in the rearing substrates (Bernal et al., 2009). Nevertheless, the low temperatures (< 45 °C) observed in this study (Figure 8a) could have been beneficial in conserving ammonia (Figure 9a), which is highly volatilized at thermophilic temperatures (> 45°C) (Bernal et al., 2009).

The pH of the substrates during the larval rearing and composting phase varied significantly ($p = 0.015$) (Figure 8b). The pH reached peak values of between 8.1 and 8.8 in the third week, with the highest value recorded in substrates with C/N ratio of 30. At the end of the experiment, the control treatment had the highest pH of 7.6 while frass compost from substrates with C/N ratio of 30 had the lowest pH of 6.9. The electrical conductivity (EC) values of the substrates differed significantly during the experiment ($p < 0.001$) (Figure 8c). The EC increased to peak values of between 2.7 and 4.2 mS cm^{-1} in the first three weeks after which the values decreased up to the end of experiment.

The EC of the unamended (control) was significantly higher than those of the other substrates throughout the experiments. The increase in pH and electro conductivity at initial stages could be attributed to evolution of ammonium (Figure 8), resulting from rapid degradation of organic substrates (Said-Pullicino et al., 2007, Azim et al., 2018). During the ammonification process, organic N is converted into the ammonia gas which reacts with water to form ammonium hydroxide which is alkaline and thus high pH (Sánchez-Monedero et al., 2001).

During nitrification (an oxidation process), the hydrogen ions are formed and released into the compost matrix, and this leads to the decrease of pH as observed in the fourth and fifth weeks (Figure 8b). Oxidation of one molecule of ammonium to nitrate gives rise to two molecules of hydrogen ions which are the cause of pH reduction (Aparna et al., 2008; Sánchez-Monedero et al., 2001). More so, during the maturation phase, organic acids, such as humic acids are produced and these also contribute to reductions in compost pH (Epstein, 1997).

In the same phase (maturation), reduction in electro conductivity is mostly due to precipitation or adsorption of most cations on to humic substances; thus making the cations unavailable in compost matrix (Huang et al. 2004). Substrates with higher organic carbon content are most likely to produce more humic acids than those with low organic carbon levels. Therefore, the pH values of less than 7 and lower EC values observed in treatments C/N20, C/N25 and C/N30 (Figure 8b and 8c) could be attributed to higher nitrification and production of organic acids during the maturation phase of compost compared to control and C/N15 which experienced low nitrification.

The mature frass compost generated had pH (Figure 8b) and electrical conductivity (Figure 8c) values that were also within the recommended ranges (6 – 8 for pH and < 4 mS cm^{-1} for electrical conductivity) (Huang et al., 2004; Bernal et al., 2009).

4.2.6 Ammonium and nitrate concentrations, and ammonium nitrate ratio

Increasing substrate C/N ratios caused significant ($p < 0.001$) differences in ammonium concentration during the study (Figure 9a). The ammonium concentration recorded in the control treatment was significantly higher ($p < 0.05$) than those of the other frass composts, followed by that of substrates with C/N ratio of 15.

The ammonium concentrations increased steadily in the control experiment up to the fourth week and decreased slightly 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 (Figure 9a). The nitrate concentration changed significantly ($p < 0.001$) over time and was significantly ($p < 0.05$) influenced by the substrate treatments (Figure 9b). The nitrate concentration of substrates with C/N ratio 15 sharply increased after the fourth week and peaked at the fifth week (159.6 mg kg^{-1}); while for the control treatment, the nitrate concentration 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 substrates with C/N ratio of 25 (59.8 mg kg^{-1}).

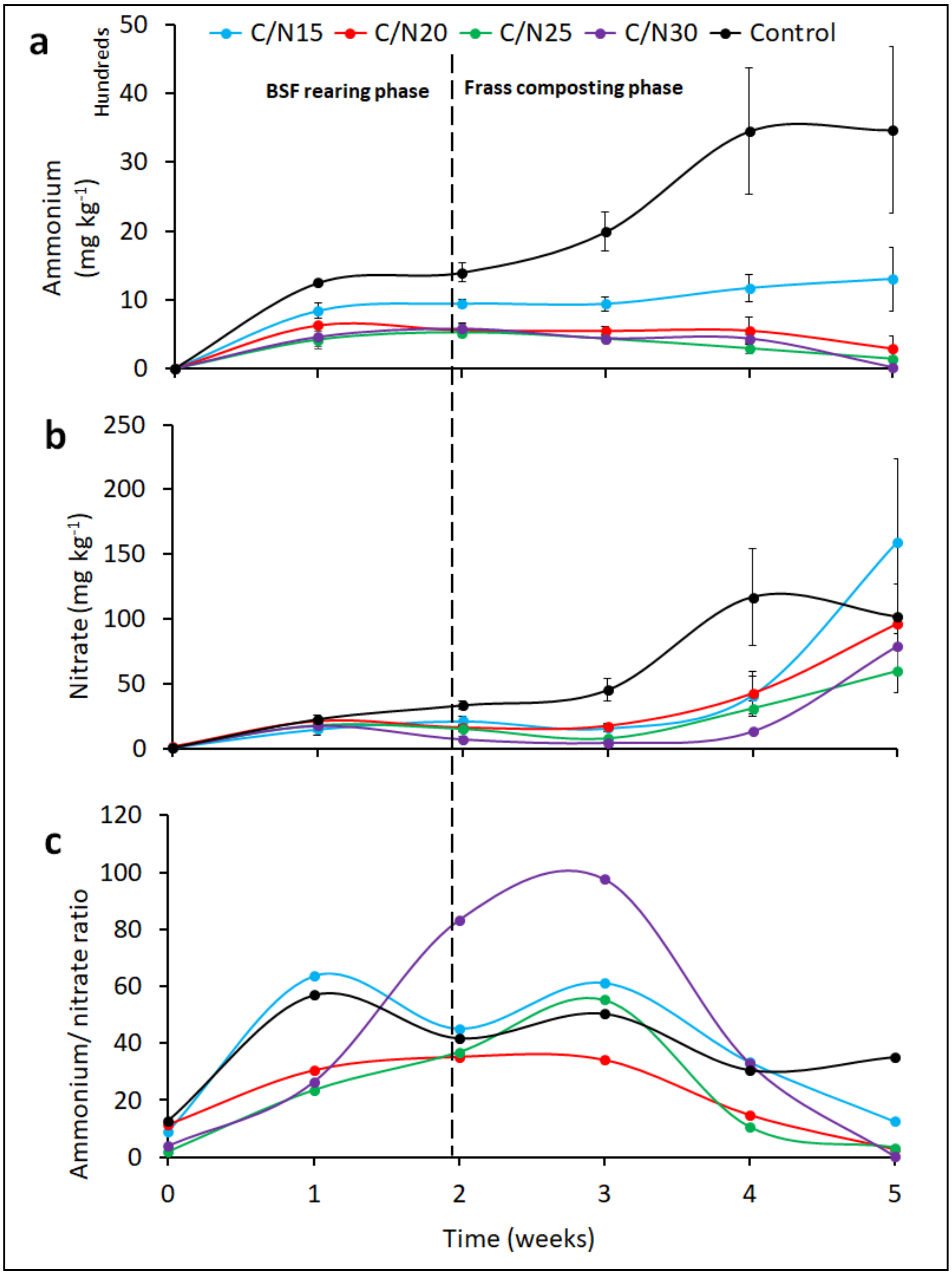

Figure 9: Effect of substrate C/N ratio on concentrations of ammonium (a), nitrate (b) and ammonium/nitrate ratio (c) during BSF larvae rearing and frass composting phases.

Key: C/N = total organic carbon to total organic nitrogen ratio; Control = unamended substrate (brewery spent grain); C/N 15, C/N 20, C/N 25, and C/N 30 = amendment of brewery spent grain using sawdust to obtain substrates with C/N ratios of 15, 20, 25 and 30, respectively.

The ammonium to nitrate ratios of compost generated from substrate with C/N ratio of 30 were the highest at weeks 2 and 3 (Figure 9c). There was an overall decline of ammonium-nitrate ratios among the various frass composts from the third week of the experiment. However, the ammonium to nitrate ratio of control treatment increased in the fifth week. Mature frass compost generated from substrates with a C/N ratio of 30 had the lowest ratio of ammonium/nitrate ratio (0.4), while the control treatment had the highest (35.2).

Through some studies, it has been revealed that nitrogen mineralization (ammonification and nitrification processes) is controlled by the substrate C/N ratio, availability of microorganisms, compost pH and temperature (Huang et al. 2004). The high ammonium concentration at initial stages (Figure 9a) could be attributed to the high pH (> 8) (Figure 8b) and higher temperature (Figure 8a) that prevailed during the early stages of the experiment. The low temperatures (< 45℃) observed in this study (Figure 8a) therefore, were beneficial in conserving ammonia (Figure 5d), which is highly volatilized at thermophilic temperatures (> 45℃) (Bernal et al., 2009).

As soon as the pH dropped below 8 (Figure 8b), nitrate concentration started increasing (Figure 9b), indicating the onset of the nitrification process. Nitrifying bacteria (*nitrosomonas and nitrobacter*) are mainly favoured by neutral pH range and mesophilic temperatures (Sánchez-Monedero et al., 2001) which dominated the process from the third week up to the end of experiment. Therefore, the surge in nitrate concentration observed in fourth and fifth weeks for all treatments where C/N ratio was increased could be attributed to favorable pH for nitrification process.

For example, the low pH and EC values observed in substrates with C/N ratio of 15 and 20 might have been responsible for enhancing the nitrification process, which could have had a direct effect on the total nitrogen retained in the frass compost (Table 9). Substrates with high C/N ratios are difficult to breakdown and this reduces the ammonium concentration released from such substrates (Huang et al., 2004). Therefore, the significantly low concentrations of ammonium observed in treatments whose C/N ratios were adjusted to 20 and above could be attributed to the high amount of resistant carbon tissues in form of lignin and cellulose from the added sawdust. Nevertheless, the rate of conversion of ammonium to nitrate nitrogen remained constantly low in this study (Figure 9c). Future studies should determine the population and species of nitrifying bacteria in BSF composted manures to devise microbe-based approaches for improving the nitrification process.

The ammonium to nitrate ratios (0.4 – 35) of the mature frass composts (Figure 9a) 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). The findings of this work are in line with other studies (Guo et al., 2012), which reported much higher ammonium to nitrate ratios (1 – 60) of mature compost.

4.2.7 Phytotoxicity of frass composts

Frass composts generated from the various substrates had significant influence on the percent seed germination (p = 0.022) and seed germination index (p = 0.033) (Table 11). The seed germination percent was above 80% for all substrates tested. The frass compost derived from control and substrates with C/N ratio of 20 had the highest seed germination rates, while the compost from substrate with C/N ratio of 15 had the lowest value (83%). The seed germination indices (GI) of frass composts generated from substrates with C/N ratios of 15 and 20 had significantly higher values than other composts. No significant differences in GI were observed between the compost derived from control substrate and that produced from substrates with C/N ratios of 25 and 30.

Table 11: Effect of substrate carbon to nitrogen (C/N) ratio adjustment on seed germination and seed germination indices of mature BSF frass composts.

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
p value	*	*

Key: *$p < 0.05$; C/N = total organic carbon to total organic nitrogen ratio; Control = unamended substrate (brewery spent grain); C/N 15, C/N 20, C/N 25, and C/N 30 = amendment of brewery spent grain using sawdust to obtain substrates with C/N ratios of 15, 20, 25 and 30, respectively. In the same column, means (± standard error) followed by the same letters are not significantly different at $p \leq 0.05$.

Germination index is used as a reliable indicator to evaluate the effect of compost extract on crop growth (Emino & Warman, 2004; Bernal et al., 2009). Germination index values below 50% indicate high phytotoxicity, while GI of 50 – 80% indicate moderate phytotoxicity and GI values above 80% indicate the absence of phytotoxicity. When the GI exceeds 100%, the compost is considered a phytonutrient or phytostimulant (Teresa & Remigio, 2011). The high percentage seed germination and germination index values recorded (Table 10) 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 & Remigio, 2011). On the other hand, the lowest germination index registered for compost generated from the control (C/N ratio of 11) (Table 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 & Remigio, 2011). The results of the germination indices reported in the current study for all the frass composts were within the ranges reported by several authors (Guo et al., 2012; Antil et al., 2013), which was achieved within five weeks.

4.2.8 Total organic carbon and total nitrogen

The total organic carbon concentration of the various treatments varied significantly ($p < 0.001$) throughout the experiment. The trends of total organic carbon concentration are presented in Figure 10a. The initial total organic carbon ranged between 31 (control treatment) and 49% in substrates with C/N ratio of 30.

The total organic carbon contents of all the experimental substrates declined considerably throughout the experiment. Frass compost generated from substrates with a C/N ratio of 30 had the highest (30%) total organic carbon content at the end of experiments while compost generated from the control substrate had the least (24%).

When organic matter is aerobically broken down, carbon is lost to the atmosphere in form of carbon dioxide gas while nitrogen and other nutrients are retained in the residual materials (Tumuhairwe et al., 2009). Such changes cause high carbon loss which give way to narrow C/N ratios of the decomposing materials. However, as the process goes on, the easily decomposable materials get depleted quickly effecting the reduction in microbial activities as indicated by minimal changes in organic carbon observed from third week up to the end of experiment (Epstein, 1997).

Adjustment of substrate C/N ratios also caused significant differences ($p = 0.005$) in total N concentrations. A consistent decline in total N concentrations in all the treatments was observed until the third week after which there was a gradual increase until the fifth week (Figure 10b). The control treatment maintained highest N concentration in the first three weeks, while total N concentration of substrate with a C/N ratio of 15 increased beyond that of the control in the 5th week. The total N concentration of mature frass compost generated from substrates with C/N ratio of 15 (2.5%) was significantly ($p < 0.001$) higher than those of frass composts derived from substrates whose C/N ratios were increased.

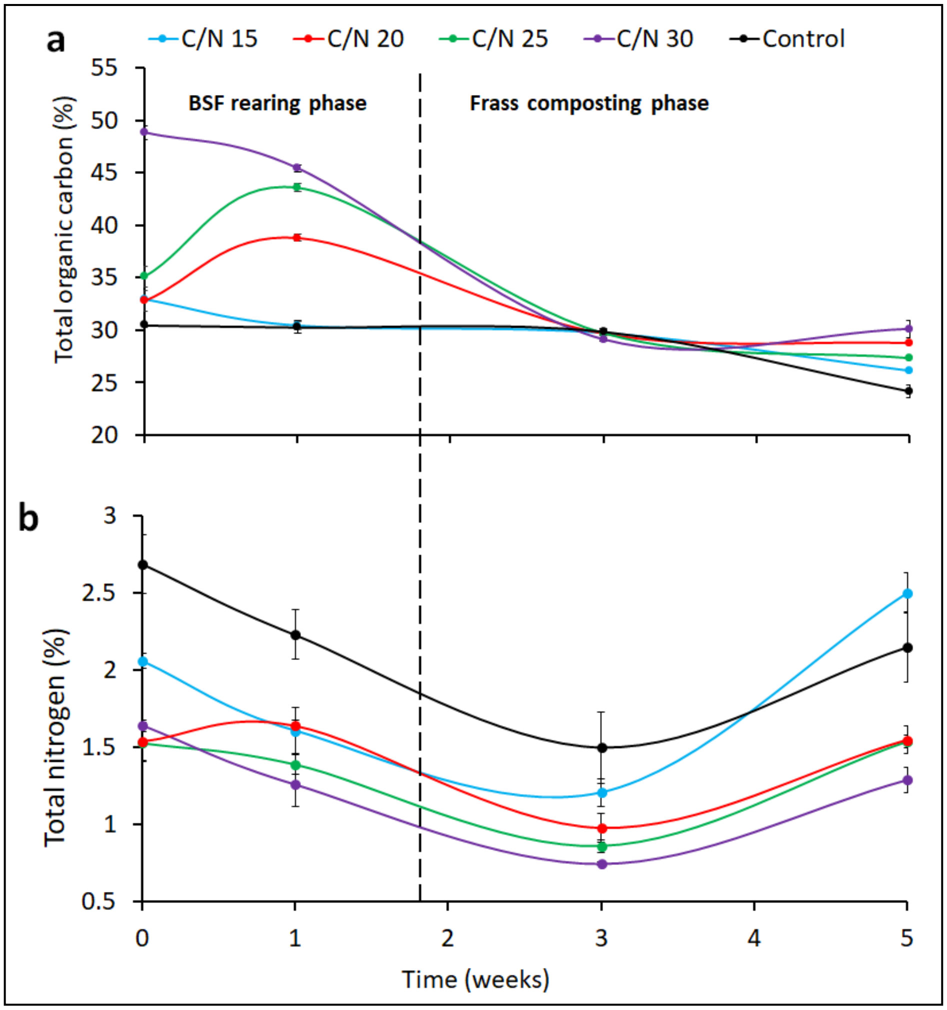

Figure 10: Effect of substrate C/N ratio on concentrations of total organic carbon (a), total nitrogen (b) during BSF larvae rearing and frass composting phases.

Key: C/N = total organic carbon to total organic nitrogen ratio; Control = unamended substrate (brewery spent grain); C/N 15, C/N 20, C/N 25, and C/N 30 = amendment of brewery spent grain using sawdust to obtain substrates with C/N ratios of 15, 20, 25 and 30, respectively.

There was a significant (p = 0.015) 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% higher 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 ratio of 30 (-21%) and the control (-19%).

The higher N retention in frass composts generated from substrates with C/N ratios of 15 and 20 compared to the control substrate (Table 10), indicate the effect of C/N adjustment on improving compost quality. Increase in total nitrogen during composting has been previously reported by Lalander et al. (2015) and indicate that the increase in total N in the frass could be due 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 labile ammonium ions (Lim et al., 2017).

Studies have also reported that nitrogen mineralization (ammonification and nitrification processes) during composting can be controlled by the substrate’s C/N ratio, compost pH and temperature (Huang et al., 2004; Ogunwande et al., 2008). Therefore, the low temperature (Figure 8a) and pH values (< 7.5) (Figure 8b) observed for most treatments in the fourth week of the experiment could have also been crucial in controlling ammonia volatilization; thereby conserving N in the frass compost.

High carbon materials like sawdust also release a lot of organic acids (Chefetz et al., 2010) that reduce compost pH during maturation phase, thereby deterring ammonia volatilization. In comparison however, the high pH in control treatment during maturation phase must have triggered ammonia volatilization, and consequently a reduction in total N of mature frass compost. Furthermore, frass compost derived from substrates with C/N ratio 15 had higher nitrate concentration in the fifth week than the control (Figure 9b), meaning that most of its ammonium was converted to nitrates as opposed to the control treatment, which might have led to more nitrogen accumulation in mature frass compost than the control treatment.

4.2.9 Total phosphorus and potassium

The total P concentration in the different frass composts was found to vary significantly ($p = 0.004$) during the composting period (Figure 11a). Phosphorus concentration was observed to decline significantly until the third week of the experiment, followed by a slight increase at fifth week. The P concentration of frass compost derived from substrate with C/N ratio of 15 was significantly ($p = 0.004$) higher than those 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 ($p < 0.001$) higher P concentration (2.0 g kg^{-1}) than those derived from substrates with higher C/N ratios. However, there was a significant ($p < 0.037$) percent reduction in total P concentration at end of the experiment in all the frass composts.

The lowest P reduction was observed in compost from substrate with a C/N ratio of 15 (-45%), while the highest percent reduction was recorded in the compost derived from substrate with a C/N of 30 (-68%). Total K concentrations were found to vary significantly (p = 0.029) among the different experimental substrates tested (Figure 11b). Substrates with C/N ratios of 20 and 15 had the highest initial total K concentrations while the substrate with a C/N ratio of 30 had the least.

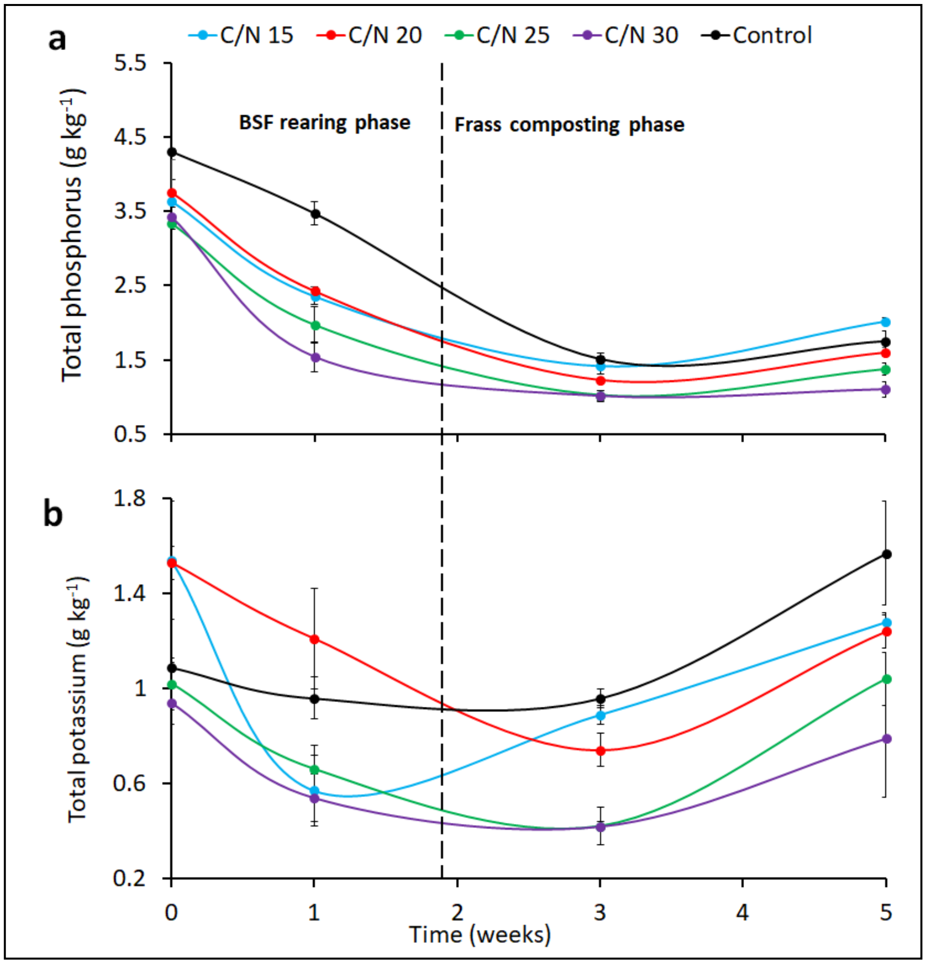

Figure 11: Effect of substrate C/N ratio on concentrations of total phosphorus (a) and potassium (b) during BSF larvae rearing and frass composting phases.

Key: C/N = total organic carbon to total organic nitrogen ratio; Control = unamended substrate (brewery spent grain); C/N 15, C/N 20, C/N 25, and C/N 30 = amendment of brewery spent grain using sawdust to obtain substrates with C/N ratios of 15, 20, 25 and 30, respectively.

Total K concentration reduced to minimum levels in the first three weeks and then increased from the third week up to the end of experiments. At the end of the composting phase, K concentration in frass compost generated from the control substrate (1.6 g k^{-1}) was higher ($p = 0.057$) than those of frass composts generated from amended substrates. Substrates with a C/N ratio of 30 generated frass compost with the least K concentration (0.8 g kg^{-1}).

The percentage change of K content at the end of the composting process (mature frass compost) was observed to vary significantly ($p = 0.043$) 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%) while the least reduction (-12%) in K concentration was recorded in frass compost derived from substrate with C/N ratio of 15.

The highest decrease in total K (-20%) was recorded in the compost generated from substrates with C/N ratio of 30 and this was slightly higher than that of compost generated from substrates with a C/N ratio of 10 (-18%). Unlike nitrogen, phosphorus (Figure 11a) and potassium (Figure 11b) concentrations showed minimal variation during the experiment. 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 easily lost from the compost matrix (Tumuhairwe et al., 2009).

In the mature frass compost, K concentration among the substrates did not vary significantly (Figure 11b). During composting, K is usually lost through leaching due to excessive moisture, which was not the case in the present studies because the moisture content was maintained at field capacity, that is between 40 and 65%. Therefore, the reduction in phosphorus and potassium (Figure 11b) observed were due to uptake by black soldier fly larvae (Table 10). However, much as treatments substrates with C/N ratio of 15 and control had the highest larvae yields, their final P and K contents were higher (Figure 11) because of the initially high levels in the substrates (Table 1). Potassium usually is not much in plant tissues because it is not one of the building units like carbon, hydrogen, oxygen, phosphorus, magnesium, iron, and silicon but is a nutrient that plays a big role in catalytic-enzymic reactions in the plant.

4.2.10 Ratios of carbon to nitrogen during experiment

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 12. The C/N 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 where values ranged 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 (1.0) ratios. The C/N ratios in mature frass compost ranged between 10.5 and 23.4 (Table 12), which is within the recommended range (< 25) for field application (Goyal et al., 2005). However, the ratios WSC/TN in all mature composts were above the recommended value of < 0.55 (Bernal et al., 1998). This could be partly attributed to the recalcitrant carbon introduced by sawdust amendment.

Table 12: Ratios of organic carbon and water-soluble carbon to total nitrogen at selected periods during the experiment.

Ratio	Time (weeks)	Rearing substrates				
		Control	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 ± 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

Key: C/N = total organic carbon to total organic nitrogen ratio; Control = unamended substrate (brewery spent grain); C/N 15, C/N 20, C/N 25, and C/N 30 = amendment of brewery spent grain using sawdust to obtain substrates with C/N ratios of 15, 20, 25 and 30, respectively. WSC/TN = water-soluble carbon to total nitrogen.

4.3 Effect of biochar and gypsum amendments on BSF larval performance and nitrogen retention in frass fertilizer

4.3.1 Black soldier fly larval yields and biomass conversion rates

There was a significant effect of rearing substrates on the fresh (experiment set 1: $p < 0.001$, set 2: $p < 0.001$) and dry (experiment set 1: $p = 0.003$, set 2: $p < 0.001$) yields of BSF larva (Figure 12a and 12b). Substrates amended with 20% biochar produced significantly higher fresh (experiment set 1: $p < 0.001$, set 2: $p < 0.001$) and dry (experiment set 1: $p < 0.001$, set 2: $p < 0.01$) larval yields than the gypsum and control treatments in both experiments.

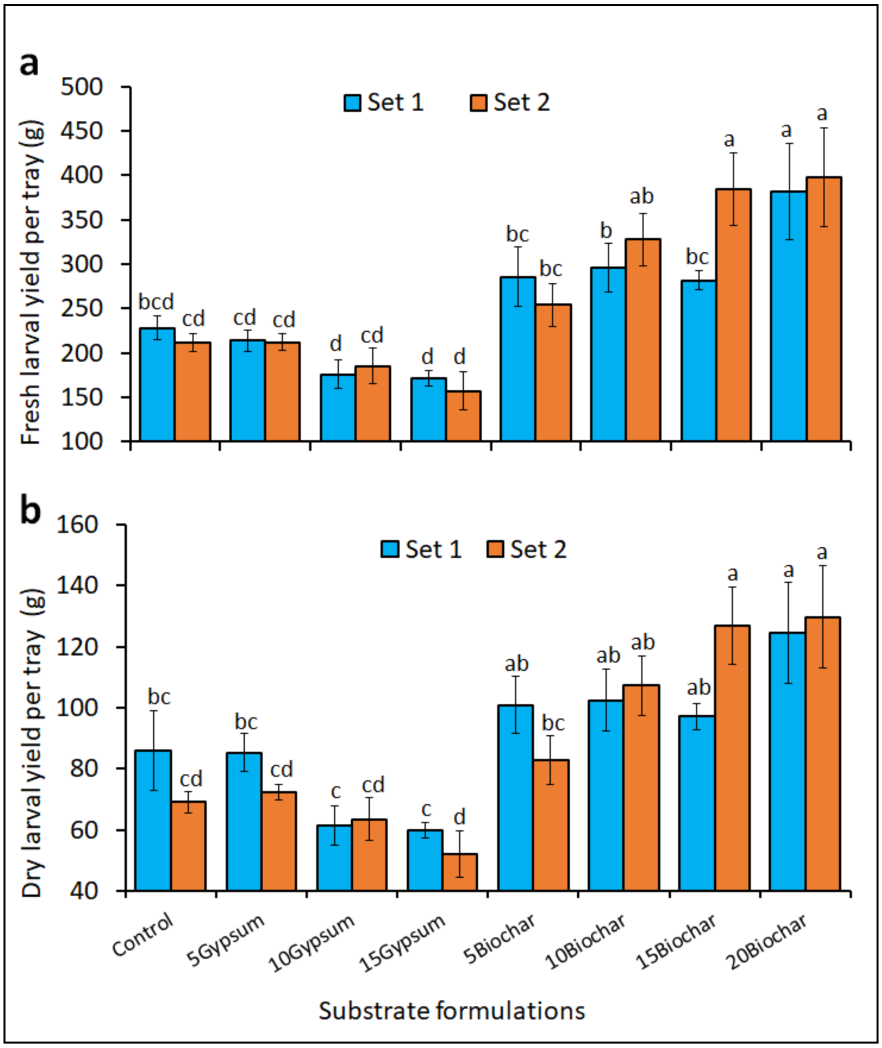

Figure 12: Effect of biochar and gypsum amendments on fresh (a) and dry (b) black soldier fly larval yields during experiments.

Key: Control = unamended substrate (brewery spent grain); 5Gypsum, 10Gypsum, 15Gypsum = substrate amended with 5, 10 and 15% gypsum, respectively; 5Biochar, 10Biochar, 15Biochar and 20Biochar = substrate amended with 5, 10, 15 and 20% biochar, respectively. For each parameter and panel, means (± standard error) followed by the same letters are not significantly different at $p \leq 0.05$.

Substrates amended with gypsum resulted in decreased larval yields, whereby dry larval yields decreased by 0.6 – 30% and 8 – 25% during experiment set 1 and 2, respectively. However, these differences were not statistically significant. The highest decline in dry larval yield was recorded on substrates amended with 15% gypsum (Figure 12a). Amendment of rearing substrate using biochar significantly increased the wet and dry BSF larval yields by up to 89 and 85%, respectively, compared to the control substrate. Larval yields increased with increase in the amount of biochar added, whereby substrates amended with 20% biochar produced the highest fresh (382 and 398 g for experiment set 1 and 2, respectively) and dry (125 and 130 g for experiment set 1 and 2, respectively) larval yields (Figure 12).

Significant differences were also observed in biomass conversion rates (BCR) among the various treatments in experiment set 1 ($p = 0.002$) and set 2 ($p < 0.001$) (Table 13). Larvae provided with substrates amended with 20% biochar had around double (15 and 24% for experiment set 1 and 2, respectively) the BCR recorded in the control. Larvae fed on substrates amended with 20% biochar had significantly (experiment set 1: $p = 0.002$, set 2: $p < 0.001$) higher BCR than those reared on the control substrate and substrates amended with gypsum, but not for larvae reared on substrates amended with 10 and 15% biochar in experiment set 2. The lowest BCRs (6.9%) were recorded from larvae fed on substrates treated with 10 and 15% gypsum.

The higher yields of fresh and dried BSF larvae and BCR (Figure 12a and 12b) from biochar-amended substrates, especially at inclusion levels of 15 and 20% could be attributed high availability of N for larval uptake. This is in line with results reported by Awasthi et al. (2016), who found that improved nitrogen conservation can be achieved through biochar amendment of substrates. Similarly, Lalander et al. (2019) demonstrated that BSF larvae fed on substrates with high nitrogen concentration developed faster and had higher biomass accumulation. Furthermore, Sanchez-Monedero et al. (2018) also showed that biochar amendment has the ability to improve water holding capacity and porosity of substrate, which enhances larvae mobility and feeding and thus resulting into high larval biomass.

Table 13: Waste degradation, biomass conversion rates and frass compost yield by BSF larvae reared on biochar and gypsum amended substrates during experiment set 1 and 2.

Substrate Formulations	Experiment set 1			Experiment set 2		
	Waste degradation efficiency (%)	Biomass conversion rate (%)	Frass compost yield per tray (kg)	Waste degradation efficiency (%)	Biomass conversion rate (%)	Frass compost yield per tray (kg)
Control	57.4 ± 1.1a	7.4 ± 1.08b	0.49 ± 0.02e	40.6 ± 0.31a	8.2 ± 0.5cd	0.75 ± 0.02d
5Gypsum	53.0 ± 1.9ab	8.0 ± 0.82b	0.58 ± 0.01de	36.0 ± 0.76ab	9.7 ± 0.3cd	0.89 ± 0.04cd
10Gypsum	44.0 ± 1.2bc	6.9 ± 0.78b	0.79 ± 0.03bc	30.4 ± 0.91bc	9.9 ± 0.9cd	1.03 ± 0.03abc
15Gypsum	42.7 ± 0.84bc	6.9 ± 0.21b	0.85 ± 0.01b	22.9 ± 1.60d	10.8 ± 1.4cd	1.12 ±0.02ab
5Biochar	52.8 ± 2.9abc	9.6 ± 1.40ab	0.59 ± 0.04de	37.2 ± 0.91ab	10.8 ± 1.1cd	0.81 ± 0.04d
10Biochar	45.0 ± 4.0bc	11.5 ± 1.61ab	0.63 ± 0.02cde	36.8 ± 0.56ab	14.2 ± 1.1bc	0.89 ± 0.03cd
15Biochar	47.9 ± 0.29abc	10.0 ± 0.43ab	0.68 ± 0.02bcd	32.9 ± 1.04bc	18.2 ± 1.4b	0.97 ± 0.01bc
20Biochar	42.2 ± 2.9c	14.6 ± 1.68a	1.13 ± 0.08a	26.3 ± 3.10cd	24.2 ± 1.3a	1.13 ± 0.04a
p value	***	**	***	***	***	***

Key: *** $p < 0.001$, **$p < 0.01$; Control = unamended substrate (brewery spent grain); 5Gypsum, 10Gypsum, 15Gypsum = substrate amended with 5, 10 and 15% gypsum, respectively; 5Biochar, 10Biochar, 15Biochar and 20Biochar = substrate amended with 5, 10, 15 and 20% biochar, respectively. In the same column, means (± standard error) followed by the same letters are not significantly different at $p \leq 0.05$.

Several authors using nitrogen-rich substrates such as dog food, poultry food, abattoir waste, food remains, chicken, pig and cow manures have also reported higher BSF larval yields (Oonincx et al., 2015; Lalander et al., 2019; Shumo et al., 2019). Despite its efficiency in nitrogen conservation in organic substrates (Table 14) (Ren et al., 2010), the low BSF larvae yields and BCR associated with gypsum amended substrates (Figure 12a and 12b) might be attributed to aggregation and drying properties of substrates induced by gypsum inclusion, which might have negatively influenced BSF growth performance. BSF larvae have been reported to thrive well in substrates with 70% moisture content (Cheng et al., 2017). Hence, the drying properties of substrates due to gypsum inclusion further explain the low larvae performance.

High EC of substrates amended with gypsum was observed in the current studies (Figure 15a and 15b). These high EC levels are known to be directly related to high salt content added through gypsum, which has been documented to reduce nutrient uptake and assimilation by insect larvae (Clark et al., 2004). However, further studies to evaluate the effects of high EC on BSF larval performance are warranted.

4.3.2 Waste degradation and frass compost yield

The waste degradation efficiency in experiment set 1 ($p < 0.001$) and 2 ($p < 0.001$) varied significantly (Table 13). The rate of waste degradation among the different treatments ranged between 23 and 57%. However, larvae fed on the control substrate had significantly higher (experiment set 1: $p < 0.001$, set 2 $p < 0.001$) waste degradation efficiency than those provided with substrates amended with > 5% gypsum and > 10% biochar, except for 15% biochar in experiment set 1.

The lowest waste degradation rates were recorded in substrates amended with 15% gypsum (43 and 23% for experiment sets 1 and 2, respectively) due to reduced larval feeding arising from the high electrical conductivity, and aggregation and drying of substrates amended with gypsum (Clark et al., 2004; Cheng et al., 2017).

The frass compost yields varied significantly in both experiments set 1 ($p < 0.001$) and 2 ($p < 0.001$) (Table 13). All biochar and gypsum treatments produced higher frass compost yields than the control where frass compost yields increased with increase in the amounts of biochar or gypsum added in the substrates. The highest frass compost yields (1.13 kg) were recorded from substrates amended with 20% biochar treatment. The same treatment produced significantly (experiment set 1: $p < 0.001$, set 2: $p < 0.001$) higher frass compost yields than other treatments, except for compost generated from substrates amend\ed with 10 and 15% gypsum in experiment set 2. Substrates amended with > 5% gypsum and > 10% biochar generated significantly (experiment set 1: $p < 0.001$, set 2: $p < 0.001$) higher frass compost yields than the control treatment.

The higher frass fertilizer yields achieved from substrates amended with biochar could be attributed to the lower values of waste degradation efficiency associated with these treatments (Table 13). This is consistent with previous studies reported by Manurung et al. (2016) and Supriyatna et al. (2016) when high recalcitrant carbon substrates with high C/N ratios such as rice straw and cassava peels were used for rearing BSF larvae. Khan et al. (2014) also demonstrated that substrates amended with carbon-rich materials such as sawdust and biochar were extremely difficult to be broken down.

The factors that influenced the low frass fertilizer yields in unamended substrates (control treatment) compared to those amended with gypsum and biochar are likely due to the high waste degradation efficiency observed in control treatment (Table 13). This makes biochar a better amendment for organic fertilizer production since it also enhances frass fertilizer yields in addition to improving larval performance.

4.3.3 Effect of biochar and gypsum on nitrogen retention and use by BSF larvae

At the start of the experiments, the initial total nitrogen content of substrate amended with biochar and gypsum were significantly different (experiment set 1: $p < 0.001$, set 2: $p < 0.001$) (Table 14). The control treatment had significantly (experiment set 1: $p < 0.001$, set 2: $p < 0.001$) higher initial total nitrogen content than substrates treated with various inclusion levels of biochar and gypsum. The quantity of nitrogen accumulated in larval biomass varied significantly both in experiment set 1 ($p = 0.002$) and set 2 ($p < 0.001$) (Table 14). Larvae fed on substrates amended with 20% biochar accumulated 2- and 3-times greater nitrogen (experiment set 1: $p = 0.002$, set 2: $p < 0.001$) than those fed on control substrates in experiment set 1 and 2, respectively.

The fraction of initial N retained in the raw frass varied significantly among the various substrates in experiment set 2 ($p < 0.001$) but not in experiment set 1 ($p = 0.053$) (Table 14). In experiment set 2, amendment of substrates with 15% gypsum retained significantly ($p < 0.001$) higher nitrogen in frass than in biochar and control treatments, except for 15% biochar. The fraction of initial N retained in mature frass compost also varied significantly (experiment set 1: $p < 0.001$, set 2: $p = 0.001$) (Table 14).

Table 14: Effect of biochar and gypsum amendments on nitrogen retention and uptake by BSF larvae during experiment set 1 and 2.

Substrate formulations	Experiment set 1				Experiment set 2			
	Initial N content in substrate per tray (g)	Fraction of initial N accumulated in larval biomass (g/100g)	Fraction of initial N retained in raw frass (g/100g)	N content in frass fertilizer as % of initial N content (g/ 100g)	Initial N content in substrates per tray (g)	Fraction of initial N accumulated in larval biomass (g/ 100g)	Fraction of initial nitrogen retained in raw frass (g/ 100g)	N content in frass fertilizer as % of initial N content (g /100g)
Control	52.0 ± 0.80a	4.8 ± 0.59b	37.1 ± 2.93	23.4 ±1.4d	43.1 ± 1.32a	4.8 ± 0.40c	55.0 ± 4.02c	33.6 ± 0.9c
5Gypsum	44.0 ± 0.75bc	6.9 ± 0.81b	49.0 ± 5.48	28.7 ± 1.6d	36.2 ± 0.64bc	6.1 ± 0.35bc	68.6 ± 2.89abc	47.5 ± 2.1ab
10Gypsum	43.1 ± 0.00c	4.8 ± 0.31b	50.7 ± 1.37	39.9 ± 3.4bc	35.2 ± 0.06bc	5.5 ± 0.64bc	73.9 ± 2.88ab	53.7 ± 2.8a
15Gypsum	39.8 ± 0.56d	5.1 ± 0.43b	56.1 ± 1.40	41.5 ± 1.7b	33.5 ± 0.72c	4.8 ± 0.58c	83.3 ± 2.88a	52.2 ± 3.0ab
5Biochar	46.4 ± 0.28b	7.2 ± 1.53ab	39.1 ± 0.95	25.2 ± 0.6d	38.4 ± 0.50ab	6.9 ± 1.05bc	54.2 ± 0.85c	40.7 ± 3.5abc
10Biochar	43.6 ± 0.53c	8.3 ± 0.95ab	51.5 ± 8.45	28.1 ± 0.4d	38.8 ± 0.18ab	6.1 ± 0.01bc	51.2 ± 0.84c	39.5 ±3.0bc
15Biochar	44.9 ± 0.64bc	6.4 ± 0.54b	43.3 ± 3.35	30.3 ± 1.4cd	32.6 ± 2.12c	9.4 ± 1.21ab	64.4 ± 6.97abc	48.1 ± 2.8ab
20Biochar	34.1 ± 0.16e	11.6 ± 1.45a	44.7 ± 2.28	56.2 ± 3.7a	36.3 ± 0.19bc	11.4 ± 1.13a	62.2 ± 5.37bc	49.4 ± 3.5ab
p value	***	**	ns	***	***	***	***	**

Key: *** $p < 0.001$, ** $p < 0.01$, ns = not significant ($p \geq 0.05$); Key: Control = unamended substrate (brewery spent grain); 5Gypsum, 10Gypsum, 15Gypsum = substrate amended with 5, 10 and 15% gypsum, respectively; 5Biochar, 10Biochar, 15Biochar and 20Biochar = substrate amended with 5, 10, 15 and 20% biochar, respectively. In the same column, means (± standard error) followed by the same letters are not significantly different at $p \leq 0.05$.

Frass compost from substrates amended with 20% biochar retained significantly ($p < 0.001$) higher N content than other treatments in experiment set 1. In experiment set 2, the fraction of initial N retained in mature frass composts from substrates amended with gypsum, and those amended with 15 and 20% biochar was significantly ($p < 0.01$) higher than that retained in the frass compost produced from the unamended substrate.

The findings of this study are in line with those reported by Awash et al. (2016, 2017) and Wang et al. (2018), who demonstrated that improved nitrogen conservation can be achieved through biochar amendment of substrates. Biochar amendments improve nitrogen conservation through mechanisms such as adsorption of ammonium on the negatively charged surface and absorption of ammonium ions into the pore spaces of biochar (Sanchez-Monedero et al., 2018). For gypsum amended frass compost, the high N retention could have been influenced by the lower pH values, given that pH levels of 7.5 and below do not favour formation of ammonia gas (Liang et al., 2006, Hao and Benke, 2008; Bernal et al., 2009) but chemically combines to form a more stable ammonium sulphate (Yang et al., 2015).

4.3.4 Effect of biochar and gypsum on temperature, pH, and electrical conductivity during frass composting

The variation in temperature during the BSF rearing and composting phases is presented in Figures 13a and 13b. The temperature during this period in both experiments peaked between 43 and 47 ºC on the third and fourth day of BSF rearing. The highest temperatures were recorded for substrates amended with 20% biochar (43 ºC) and 15% biochar (47 ºC) on the third day for experiment set 1 and 2, respectively.

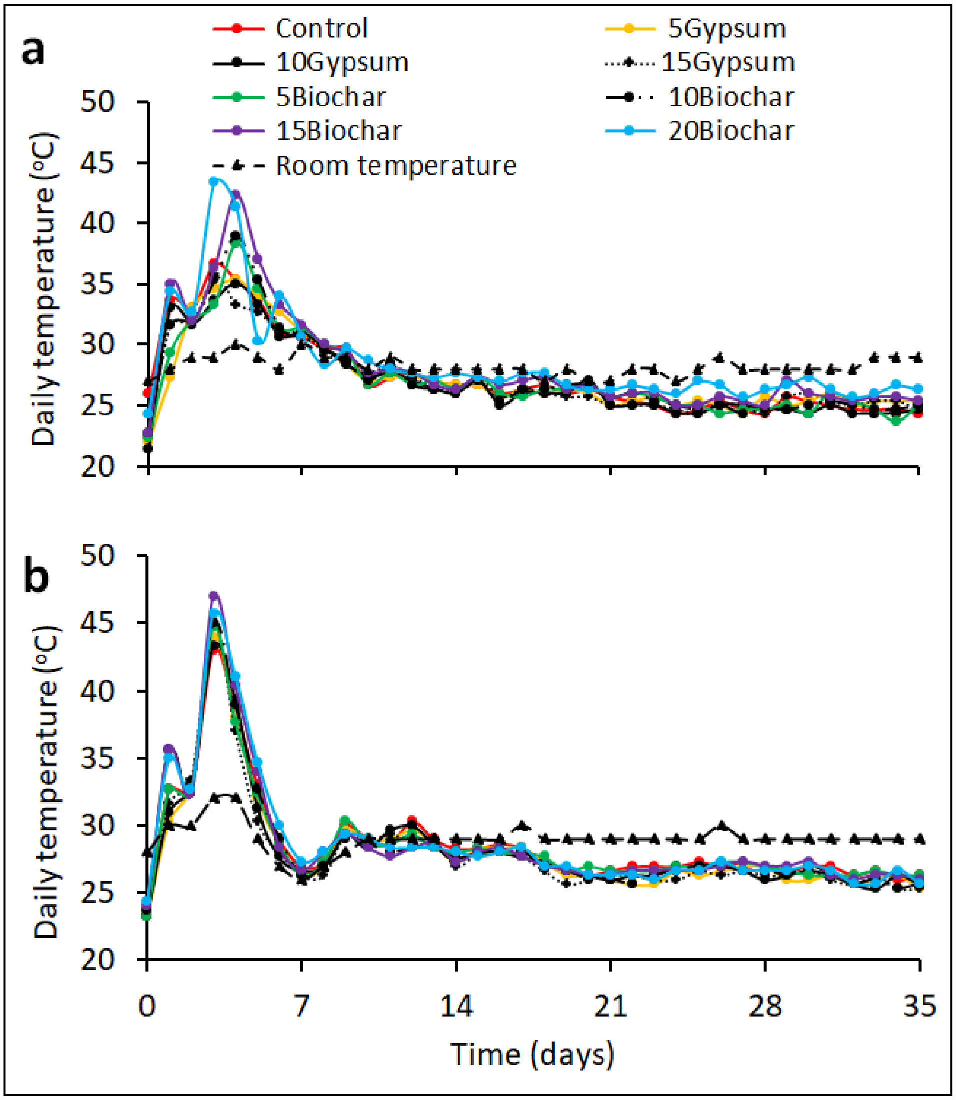

Figure 13: Effect of biochar and gypsum amendments on temperature during experiment set 1 (a) and set (2) of BSF frass composting.

Key: Control = unamended substrate (brewery spent grain); 5Gypsum, 10Gypsum, 15Gypsum = substrate amended with 5, 10 and 15% gypsum, respectively; 5Biochar, 10Biochar, 15Biochar and 20Biochar = substrate amended with 5, 10, 15 and 20% biochar, respectively.

A sharp decline of temperature was then observed up to the ninth day (28 – 30 ºC). Thereafter, temperature fluctuations were negligible until the end of the experiment. This study reports for the first-time room and substrate temperature patterns during BSF facilitated composting (Figure 13a and 13b). Most studies using BSF for waste degradation and composting have focused on monitoring the room temperature only (Lalander et al., 2015; Isibika et al., 2019), yet it is the temperature inside the substrate that greatly influences microbial activities and nitrogen dynamics (Bernal et al., 2009).

The mesophilic temperature (45 ºC and below) patterns were observed in the current study (Figure 13a and 13b) during the composting process and were found to be within the range as reported in previous studies (Dias et al., 2010). The mesophilic temperature ranges are crucial in reducing nitrogen volatilization during composting (Bernal et al., 2009). The highest temperature values observed during the first week could be attributed to the rapid breakdown of available simple protein and carbohydrate sources for energy by BSF larvae and associated micro decomposers (Tumuhairwe et al., 2009).

The pH was significantly (experiment set: $p < 0.001$, set 2: $p < 0.001$) affected by substrate amendments during experiments (Figure 14a and 14b). The pH significantly increased from initial values of between 3.6 and 5.0 and reached peak values on the third week of composting which ranged between 7.9 and 9.1. Substrate treated with gypsum had the lowest pH values (6.7 – 7.1) throughout the rearing and composting process in both experiments. On the other hand, substrates amended with 15% biochar inclusion had the highest pH value (7.8) at the end of composting process.

The low pH associated with gypsum amended substrates could be attributed to the acidity effect caused by sulphate ions in solution. When gypsum dissolves, the sulphate ions in solution combine with hydrogen ions to form sulphuric acid, that is responsible for all the apparent low pH values observed across all gypsum amended substrates (Wang et al., 2013).

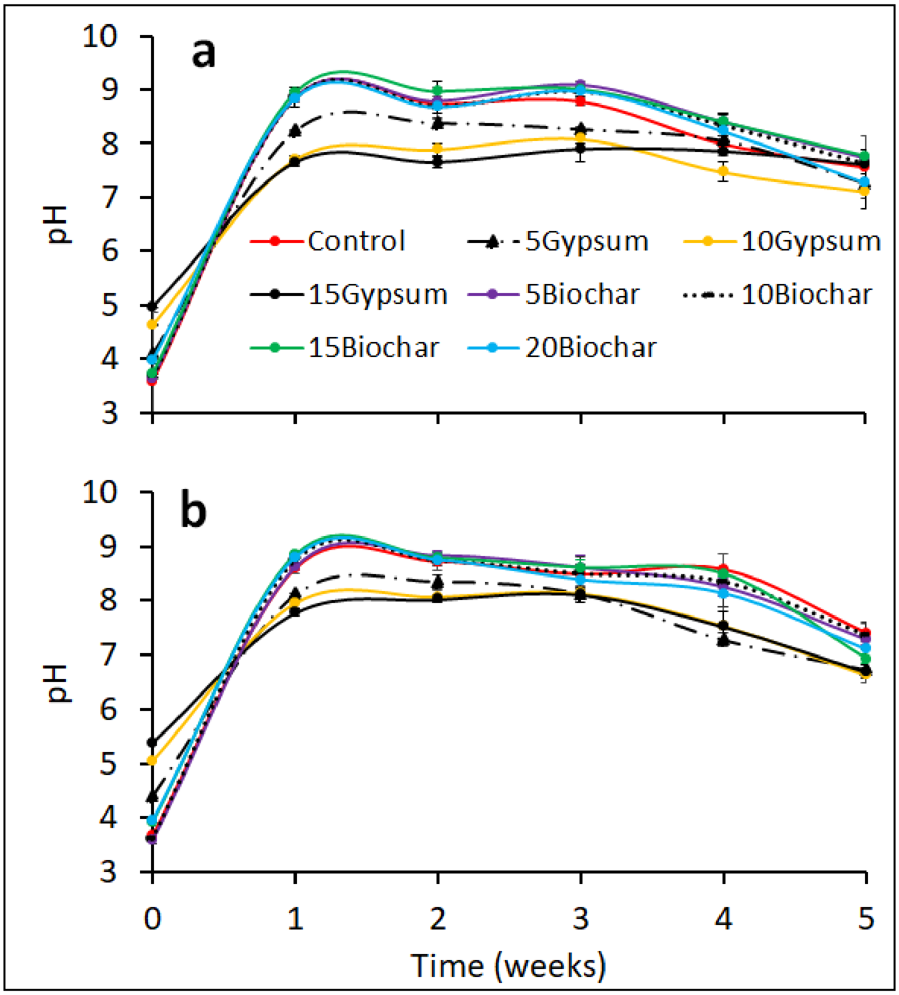

Figure 14: Effect of biochar and gypsum amendments on pH of BSF frass compost during experiment set 1 (a) and 2(b).

Key: Control = unamended substrate (brewery spent grain); 5Gypsum, 10Gypsum, 15Gypsum = substrate amended with 5, 10 and 15% gypsum, respectively; 5Biochar, 10Biochar, 15Biochar and 20Biochar = substrate amended with 5, 10, 15 and 20% biochar, respectively.

The EC of the various treatments also varied significantly (experiment set 1: $p < 0.001$, set 2: $p < 0.001$) during both experiments. The pattern of EC fluctuations throughout the experiments are shown in Figures 15a and 15b. Gypsum amended substrates had significantly ($p < 0.001$) higher EC values than biochar amended substrates throughout the experiment period.

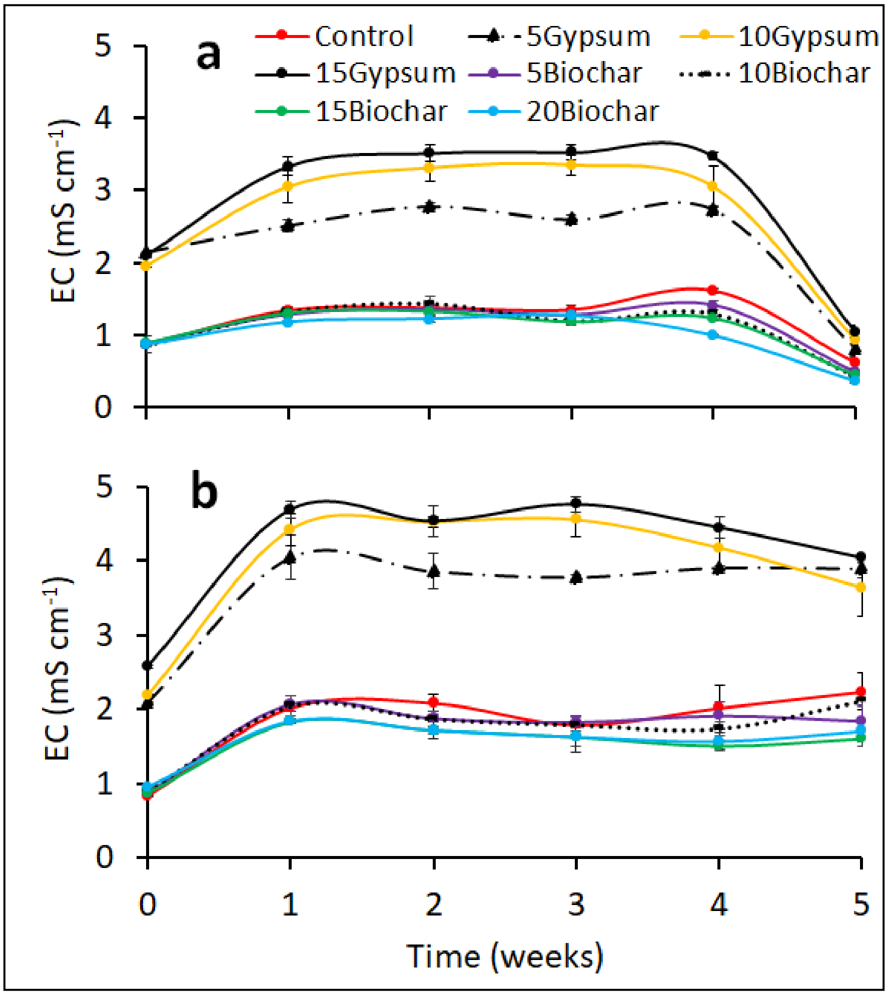

Figure 15: Effect of biochar and gypsum amendments on electrical conductivity (EC) of BSF frass compost during experiment set 1 (a) and 2(b).

Key: Control = unamended substrate (brewery spent grain); 5Gypsum, 10Gypsum, 15Gypsum = substrate amended with 5, 10 and 15% gypsum, respectively; 5Biochar, 10Biochar, 15Biochar and 20Biochar = substrate amended with 5, 10, 15 and 20% biochar, respectively.

The highest EC values were recorded in substrates amended with 15% gypsum in the third week (3.5 and 4.8 mS cm^{-1}) for experiment sets 1 and 2, respectively. In mature composts, substrates amended with 15% gypsum had the highest EC value (4 mS cm $^{-1}$), while those amended with 20% biochar had the lowest EC value (0.36 mS cm^{-1}). The decrease in EC observed in the fifth week could be largely attributed to precipitation of salts in solution during the compost maturation phase (Bernal et al., 2009).

The significantly high EC in substrate treated with gypsum during the experiments (Figure 15a and 15b) is in line with previous studies where the inclusion of salts of calcium, potassium and aluminium resulted in significant increase in EC values in composts (Choi & Moore, 2008; Yang et al., 2015; Wang et al., 2016). However, the EC values of mature compost for substrates amended with gypsum were also within the recommended range (< 4 mS cm^{-1}) for field application (Huang et al., 2004).

4.3.5 Ammonium and nitrate concentrations during frass composting

Ammonium concentration was significantly influenced by substrate amendments (experiment set 1: $p < 0.001$, set 2: $p < 0.001$), composting time (experiment set 1: $p < 0.001$, set 2: $p < 0.001$), but not their interactions (experiment set 1: $p = 0.089$, set 2: $p = 0.615$) (Figure 16a and 16b). The ammonium concentration in all the treatments had similar patterns of increase in both experiments, whereby significant ($p < 0.001$) increases were observed in the first week. Gypsum amended substrates retained higher ammonium concentration than biochar treatments. In mature frass composts, the ammonium concentration ranged between 588 and 4516 mg kg^{-1} in all the treatments.

However, the highest ammonium concentrations were observed in substrates amended with 15% gypsum (Figure 16a and 16b).

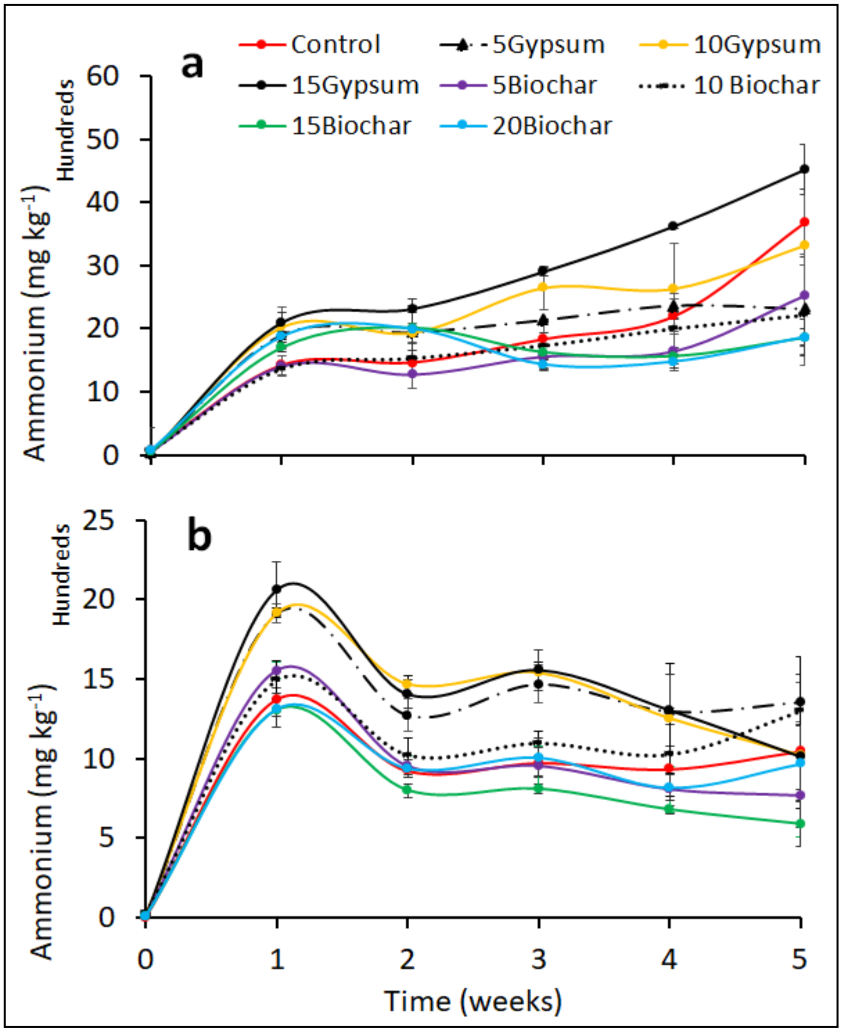

Figure 16: Effects of biochar and gypsum amendments on concentrations of ammonium during experiment set 1 (a) and 2(b) of frass composting.

Key: Control = unamended substrate (brewery spent grain); 5Gypsum, 10Gypsum, 15Gypsum = substrate amended with 5, 10 and 15% gypsum, respectively; 5Biochar, 10Biochar, 15Biochar and 20Biochar = substrate amended with 5, 10, 15 and 20% biochar, respectively.

In the case of nitrate, the concentrations varied significantly due to substrate amendments with biochar and gypsum in experiment set 1 ($p = 0.03$) and composting time in both experiments (experiment set 1: $p < 0.001$, set 2: $p < 0.001$). The interaction effect of substrates and composting time was significant in experiment set 2 only ($p < 0.001$) (Figure 17a and 17b).

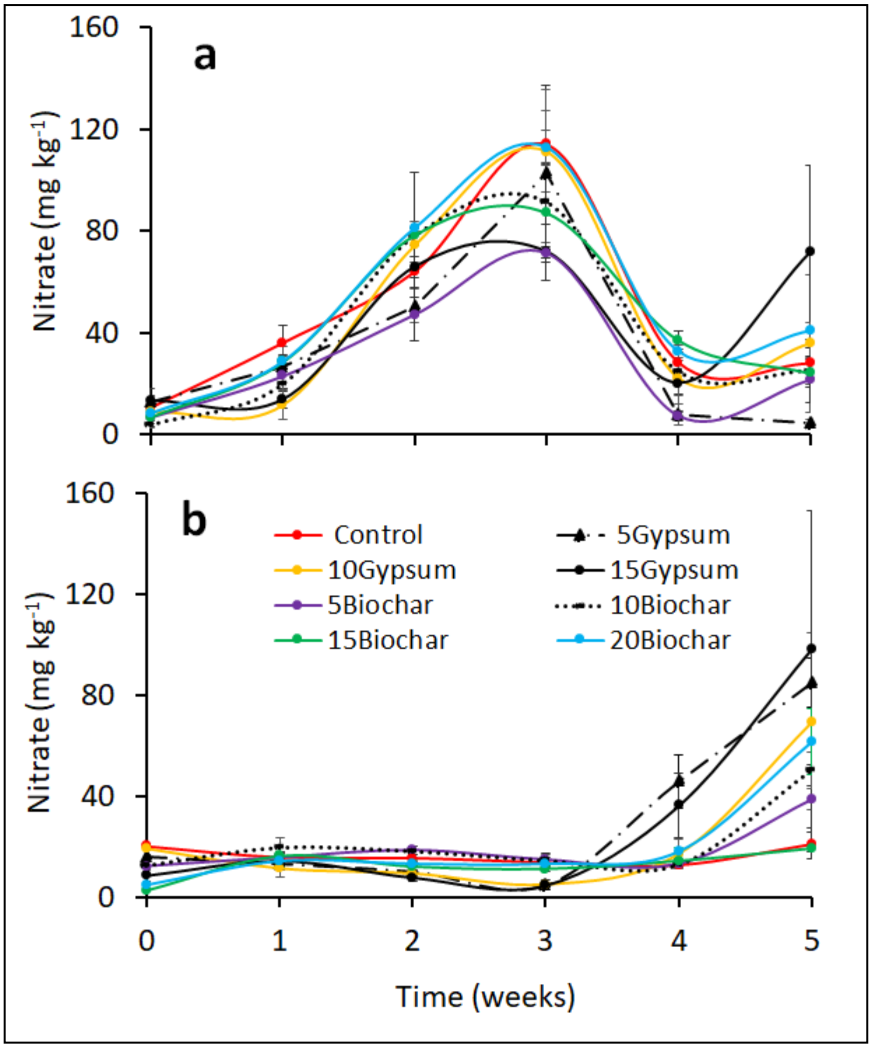

Figure 17: Effects of biochar and gypsum amendments on concentrations of nitrates during experiment set 1 (a) and 2(b) of frass composting.

Key: Control = unamended substrate (brewery spent grain); 5Gypsum, 10Gypsum, 15Gypsum = substrate amended with 5, 10 and 15% gypsum, respectively; 5Biochar, 10Biochar, 15Biochar and 20Biochar = substrate amended with 5, 10, 15 and 20% biochar, respectively.

The peak concentrations of nitrate (71 – 114 mg kg^{-1}) were observed in the 3rd and 5th weeks of the experiment set 1 ($p < 0.001$) and 2 ($p < 0.001$), respectively. For both experiments (set 1 and 2), the highest concentration of nitrates (72 mg kg^{-1}) in the mature frass composts was recorded in substrates amended with 15% gypsum.

During experiment set 2, all treatments the ammonium concentration from the first week up to the end of experiment, indicating the onset of the nitrification process. The fairly high ammonium concentration in gypsum amended substrates could be attributed to the lower pH values associated with gypsum amended substrates (Figure 14a and 14b) that could have reduced ammonium loss by inhibiting formation of ammonia gas through shifting the chemical equilibrium towards ammonium form (Wang et al., 2013).

In addition to shifting the chemical equilibrium towards ammonium nitrogen, gypsum also chemically combined with ammonium ions to form ammonium sulphate (equation 22), a more stable from that is less prone to losses unlike ammonium carbonate (Wang et al., 2013, 2016; Yang et al., 2015).

$$CaSO_4 . 2H_2O + (NH_4)_2CO_3 \rightarrow (NH_4)_2SO_4 + CaCO_3 + H_2O \qquad (22)$$

On the other hand, the higher ammonium retention in the biochar amended substrates could be attributed to the high cation exchange capacity, that could have caused ammonium adsorption. Furthermore, the high surface area of biochar has been reported to enhance absorption of ammonium ions in the pores (Sánchez et al., 2017; Sanchez-Monedero et al., 2018). Other studies have also reported higher ammonium retention due to amendment of substrates such as poultry litter with biochar (Agyarko-Mintah et al., 2017).

On the other hand, the nitrification process in which ammonium is converted to nitrate nitrogen was rather slow during the experiments. The low nitrate concentration observed during the study (Figure 17a and 17b) could be attributed to high ammonium retention by biochar and gypsum additives, whereby high ammonium has been found to inhibit the enzymatic activities of nitrifying bacteria (Agyarko-Mintah et al., 2017). On the contrary, the sharp decline in nitrate concentration observed in the fourth week during experiment set 1 (Figure 17a) could be largely attributed to nitrogen immobilization.

4.3.6 Effect of biochar and gypsum on total organic carbon and total nitrogen during frass composting

There was a significant impact of substrate amendments (experiment set 1: $p < 0.001$, set 2: $p < 0.001$) on total organic carbon during frass composting. Initial total organic carbon concentration ranged between 34 and 40%, whereby substrates amended with 20 and 5% biochar had highest carbon concentrations during experiment set 1 and 2, respectively (Figure 18a and 18b). There was a gradual decrease in total organic carbon concentration in compost treatments throughout both experiments. Significant decreases ($p < 0.001$) in total organic carbon were observed in the first and third weeks of experiments.

Mature frass composts generated from substrates amended with 15% gypsum had the lowest carbon concentration (26%) while those generated from substrates amended with 5% biochar had the highest (35%) (Figure 18a and 18b). The trends of carbon concentration observed during the study are normal and have been reported in other studies (Tumuhairwe et al., 2009).

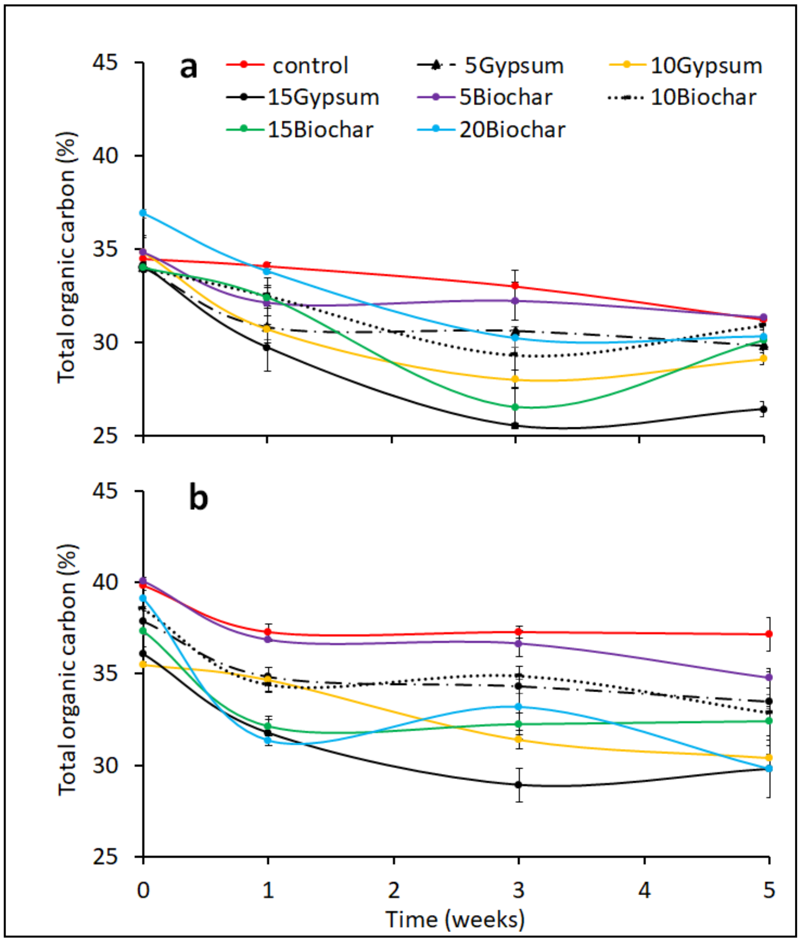

Figure 18: Effect of biochar and gypsum amendments on total organic carbon during experiment set 1(a) and 2 (b) of BSF frass composting.

Key: Control = unamended substrate (brewery spent grain); 5Gypsum, 10Gypsum, 15Gypsum = substrate amended with 5, 10 and 15% gypsum, respectively; 5Biochar, 10Biochar, 15Biochar and 20Biochar = substrate amended with 5, 10, 15 and 20% biochar, respectively.

The rapid decrease in concentrations of total organic carbon observed in the first three weeks could be attributed to rapid breakdown of organic matter by the BSF larvae and microbial decomposers (Epstein, 1997; Tumuhairwe et al., 2009). The total N varied significantly due to substrate treatments (experiment set 1: $p < 0.001$, set 2: $p < 0.001$)

and composting time (experiment set 1: $p < 0.001$, set 2: $p < 0.001$) during experiments but the interaction effect was significant during experiment set 2 only ($p < 0.001$) (Figure 19a and 19b).

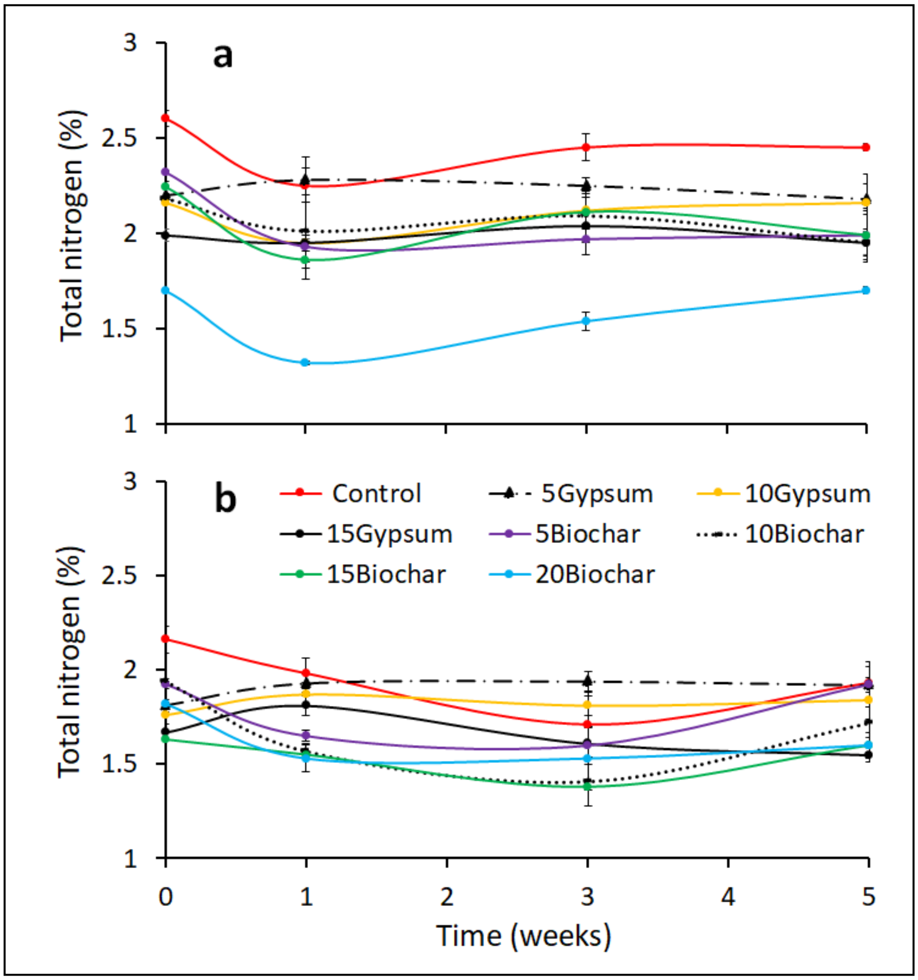

Figure 19: Effect of biochar and gypsum amendments on total nitrogen during experiment set 1(a) and 2 (b) of BSF frass composting.

Key: Control = unamended substrate (brewery spent grain); 5Gypsum, 10Gypsum, 15Gypsum = substrate amended with 5, 10 and 15% gypsum, respectively; 5Biochar, 10Biochar, 15Biochar and 20Biochar = substrate amended with 5, 10, 15 and 20% biochar, respectively.

Initial N concentration ranged between 1.6 and 2.6%, whereby the control treatment had the highest while substrate amended with 20% biochar had the lowest.

The total N concentration for most treatments decreased in the first and third week during experiment set 1 and 2, respectively.

The control treatment generated mature frass compost with significantly higher (p = 0.003) total N concentration (2.5%) than composts generated from substrates amended with 15% gypsum, and 10 and 20% biochar in experiment set 1 (Table 15). In experiment set 2, the total N concentrations of mature frass composts generated from the control substrate and substrates amended with 5% biochar and 5% gypsum were significantly (p = 0.01) higher than the total N concentrations of frass composts amended with 15% gypsum, and 15 and 20% biochar.

Mature frass composts generated from substrates amended with 20% biochar and 15% gypsum had the lowest total N concentrations during experiment set 1 (1.7%) and 2 (1.6%), respectively. A remarkable increase in N retention was observed in frass generated from substrates amended with biochar and gypsum (Table 14). For gypsum treated frass, N retention could have been influenced by the lower pH values (Figure 14a and 14b), given that pH levels of 7.5 and below do not favour formation of ammonia gas (Liang et al., 2006; Hao & Benke, 2008; Bernal et al., 2009) but chemically combines to form a more stable ammonium sulphate (Yang et al., 2015).

Nitrogen conservation in biochar amended frass compost (Table 14) can be attributed to both adsorption and absorption mechanisms of ammonium ions as discussed in section 4.2.5 (Sánchez et al., 2017). However, frass compost generated from substrate amended with 20% biochar had the lowest N (Figure 19a and 19b) and P concentrations (Figures 20a and 20b, Table 14), which could be partially attributed to high bio-conversion efficiency (Table 13) and nitrogen uptake of larvae reared on this substrate (Table 14) (Lalander et al., 2019; Shumo et al., 2019).

Table 15: Effects of biochar and gypsum amendments on the concentrations of nutrients in mature BSF frass composts during experiment set 1 and 2.

	Experiment set 1			Experiment set 2		
Substrate formulations	Nitrogen (%)	Phosphorus ($g\ kg^{-1}$)	Potassium ($g\ kg^{-1}$)	Nitrogen (%)	Phosphorus ($g\ kg^{-1}$)	Potassium ($g\ kg^{-1}$)
Control	2.45 ± 0.02a	11.03 ± 0.34a	4.56 ± 0.62bc	1.94 ± 0.087a	10.58 ± 0.37a	0.82 ± 0.004bc
5Gypsum	2.18 ± 0.08ab	10.36 ± 0.29ab	2.89 ± 0.62c	1.92 ± 0.044a	8.74 ± 0.14b	0.69 ± 0.00c
10Gypsum	2.16 ± 0.15abc	8.44 ± 0.75bc	1.75 ± 0.37c	1.84 ± 0.102a	8.16 ± 0.36bc	0.67 ± 0.069c
15Gypsum	1.95 ± 0.05bc	6.79 ± 0.42c	1.55 ± 0.44c	1.55 ± 0.042a	7.24 ± 0.18c	0.56 ± 0.013c
5Biochar	1.99 ± 0.14abc	10.54 ± 0.58ab	4.82 ± 1.51bc	1.92 ± 0.120a	9.33 ± 0.25ab	1.12 ± 0.075ab
10Biochar	1.95 ± 0.07bc	9.42 ± 0.66ab	7.46 ± 1.26ab	1.72 ± 0.084a	9.11 ± 0.17b	1.35 ± 0.097a
15Biochar	1.99 ± 0.13abc	8.65 ± 0.10bc	9.63 ± 0.62a	1.60 ± 0.074a	7.17 ± 0.31c	1.31 ± 0.168a
20Biochar	1.70 ± 0.02c	8.34 ± 0.07bc	10.99 ± 0.56a	1.60 ± 0.069a	7.15 ± 0.29c	1.35 ± 0.085a
p value	**	***	***	**	***	***

Key: *** $p < 0.001$, ** $p < 0.01$; Control = unamended substrate (brewery spent grain); 5Gypsum, 10Gypsum, 15Gypsum = substrate amended with 5, 10 and 15% gypsum, respectively; 5Biochar, 10Biochar, 15Biochar and 20Biochar = substrate amended with 5, 10, 15 and 20% biochar, respectively. In the same column, means (± standard error) followed by the same letters are not significantly different at $p \leq 0.05$.

4.3.7 Total phosphorus and potassium during frass composting

Total P concentration varied significantly due to substrate amendments during both experiments (experiment set 1: $p < 0.001$, set 2: $p < 0.001$) (Figure 20a and 20b). There were significant ($p < 0.001$) increases in total P in the first and third weeks of the experiment set 1, and throughout experiment set 2.

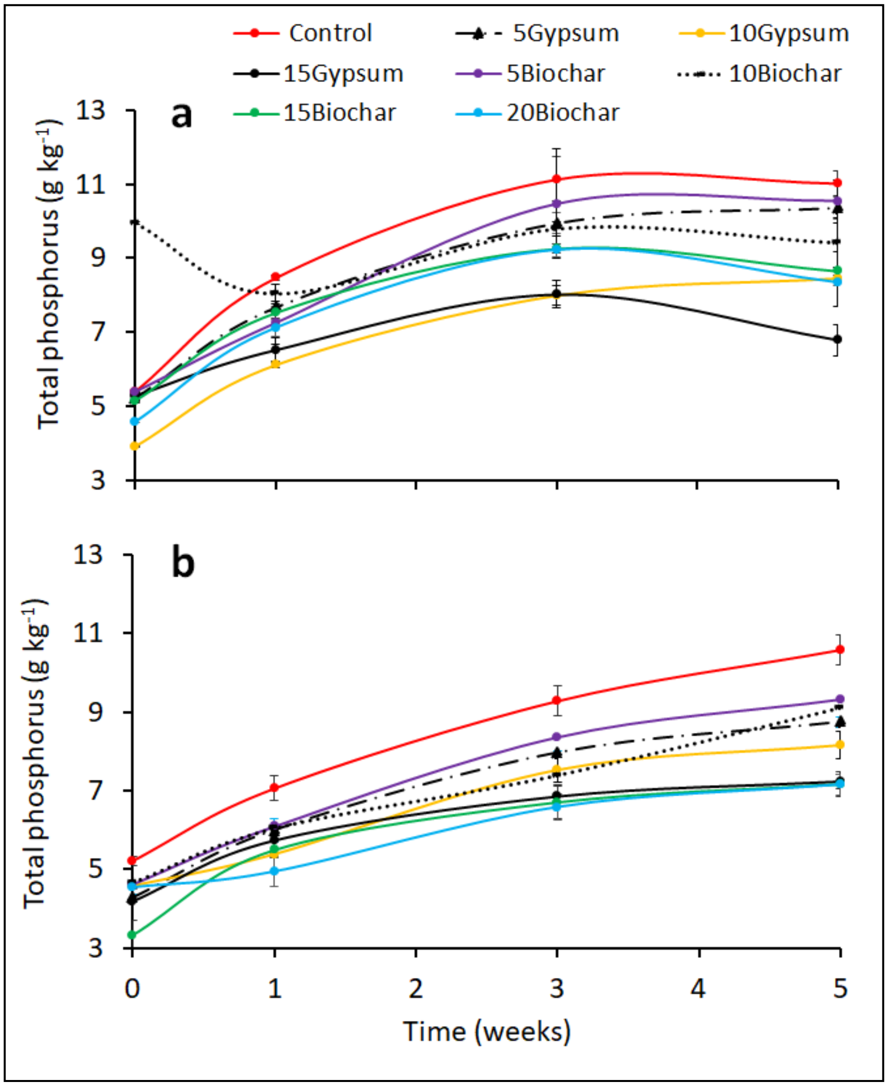

Figure 20: Effect of biochar and gypsum amendments on total phosphorus of BSF frass compost during experiment set 1(a) and 2(b).

Key: Control = unamended substrate (brewery spent grain); 5Gypsum, 10Gypsum, 15Gypsum = substrate amended with 5, 10 and 15% gypsum, respectively; 5Biochar, 10Biochar, 15Biochar and 20Biochar = substrate amended with 5, 10, 15 and 20% biochar, respectively.

Amendment of substrates with 5% of either biochar or gypsum produced frass composts with higher total P content than the other amended treatments. The frass compost generated from the control substrate had significantly (experiment set 1: $p < 0.001$, set 2: $p < 0.001$) higher total P concentration than other treatments, except for frass composts amended with 10 and 5% gypsum, and 10 and 10% biochar in experiment set 1, and 5% biochar in experiment set 2 (Table 15).

Total K concentrations of the different compost treatments were also significantly affected by substrate amendments ($p < 0.001$, set 2: $p < 0.001$) and frass composting time (experiment set 1: $p < 0.001$, set 2: $p = 0.023$) during experiments. The interaction effect of substrate amendments and composting time was only significant in experiment set 1 ($p = 0.006$) (Figure 21a and 21b).

Biochar amended composts had higher K concentrations than the control as well as gypsum treated composts. Substrates amended with 15% biochar had the highest initial K concentration (10 g kg^{-1}) while those amended with 15% gypsum had the lowest (0.7 g kg^{-1}). The concentrations of potassium in the frass composts were observed to increase with increased biochar rates, while it was the opposite with gypsum treatments, whereby potassium concentrations decreased with increased gypsum inclusion rates (Figure 21a and 21b). The mature frass composts generated from substrates amended with 15 and 20% biochar had significantly ($p < 0.001$) higher K concentrations than the other treatments, except for compost amended with 10% biochar in experiment set 1 (Table 15).

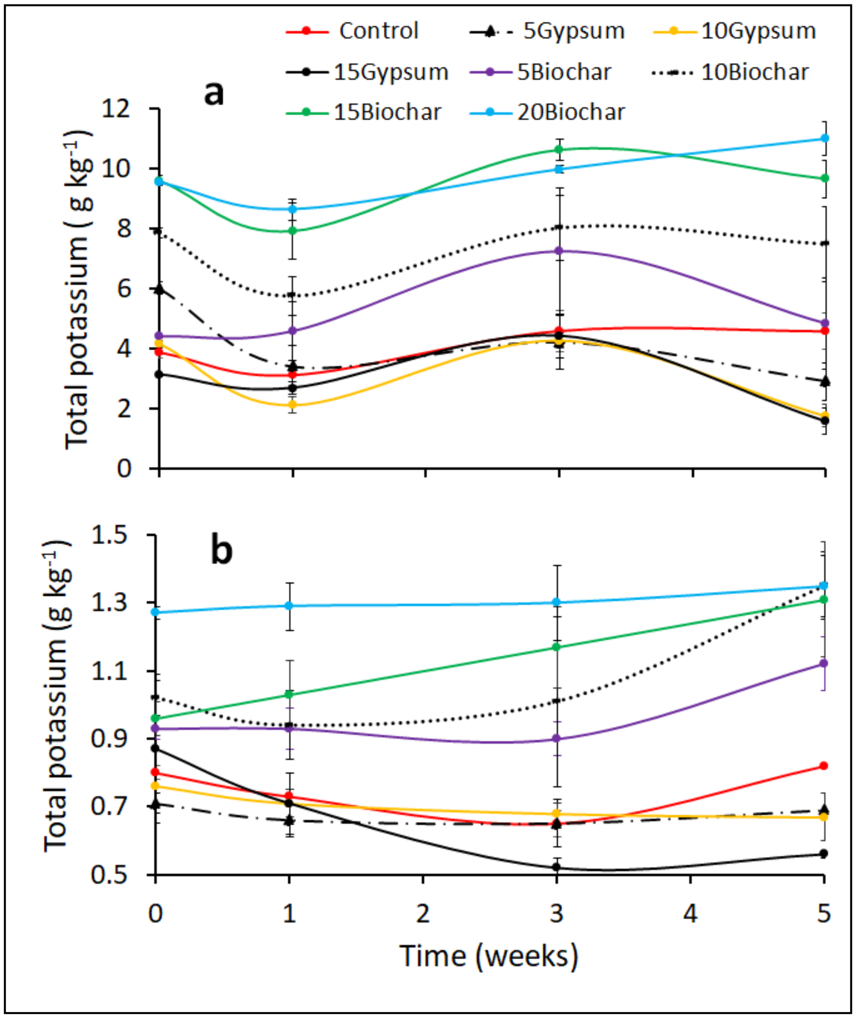

Figure 21: Effect of biochar and gypsum amendments on total potassium of BSF frass compost during experiment set 1(a) and 2(b).

Key: Control = unamended substrate (brewery spent grain); 5Gypsum, 10Gypsum, 15Gypsum = substrate amended with 5, 10 and 15% gypsum, respectively; 5Biochar, 10Biochar, 15Biochar and 20Biochar = substrate amended with 5, 10, 15 and 20% biochar, respectively.

In experiment set 2, frass composts derived from substrates amended with 10 – 20% biochar achieved significantly ($p < 0.001$) higher K levels than those generated from the control and gypsum amended substrates. The highest K concentration (11 g kg^{-1}) was recorded from compost generated from substrates amended with 20% biochar while compost generated from substrates amended with 15% had the lowest K (0.56 g kg^{-1}) (Table 15).

The considerable variation in the trend in K concentration observed throughout the experiments (Figure 21a and 21b) is consistent with previous studies using chicken, pig and cow manure as rearing substrates for BSF larvae (Lalander et al., 2015; Oonincx et al., 2015). However, the highest K concentrations were achieved at 20% inclusion of biochar (Table 15), which can be attributed to the initial high concentrations of K introduced through biochar amendment (Table 3) (Sanchez-Monedero et al., 2018). Therefore, biochar amendment is also effective for production of potassium-rich organic fertilizer.

The results of this study indicate that the N concentration in frass compost generated from all amended substrates (Table 15) were within the recommended standards of > 1% N according to the Kenya Bureau of Standards (KEBS) guidelines for optimal SAFI (Kenya Bureau of Standards, 2017). Also, the P and K concentrations in the frass compost (Table 15) and level of compost maturity achieved meet the required international standards and guidelines for compost quality (Brinton, 2000).

4.3.8 Effect of biochar and gypsum on ratios of carbon to nitrogen during frass composting

Ratios of total organic carbon to total nitrogen (C/N) and water-soluble carbon to total nitrogen (WSC/TN) decreased from the start up to the end of experiment (Table 16). At the start of the experiments, substrates treated with 15% biochar had the highest

C/N ratio (23.1) while the control substrate had the lowest C/N ratio (13.3). Minimal reduction in C/N ratio and WSC/TN ratio were noted across all treatments tested.

Table 16: Ratios of carbon (C/N) and water-soluble carbon (WSC/N) to total nitrogen of amended BSF frass composts.

	Time	Experiment set 1							
Ratios	(weeks)	Control	5Gypsum	10Gypsum	15Gypsum	5Biochar	10Biochar	15Biochar	20Biochar
C/N	0	13.3 ± 0.33	15.4 ± 0.27	16.2 ± 0.43	17.1 ± 0.23	15.0 ± 0.43	15.6 ± 0.19	15.1 ± 0.33	21.7 ± 0.07
	1	15.2 ± 0.48	13.6 ± 0.49	15.7 ± 0.09	15.3 ± 0.65	16.7 ± 1.23	16.4 ± 1.32	17.5 ± 0.95	25.6 ± 0.12
	3	13.5 ± 0.67	13.6 ± 0.30	13.3 ± 0.79	12.5 ± 0.52	16.4 ± 1.07	14.2 ± 1.41	12.6 ± 0.72	19.7 ± 0.33
	5	12.8 ± 0.12	13.7 ± 0.10	13.6 ± 1.09	13.6 ± 0.52	15.8 ± 1.06	15.9 ± 0.62	15.2 ± 1.02	17.9 ± 0.15
WSC/TN	0	0.50 ± 0.02	0.78 ± 0.05	0.72 ± 0.03	0.75 ± 0.02	0.53 ± 0.01	0.58 ± 0.04	0.57 ± 0.01	0.99 ± 0.03
	1	0.53 ± 0.06	0.45 ± 0.19	0.66 ± 0.07	0.53 ± 0.21	0.42 ± 0.11	0.39 ± 0.10	0.55 ± 0.06	1.05 ± 0.03
	3	0.47 ± 0.11	0.47 ± 0.10	0.52 ± 0.11	0.59 ± 0.02	0.52 ± 0.08	0.49 ± 0.12	0.37 ± 0.07	0.54 ± 0.06
	5	0.72 ± 0.15	0.47 ± 0.19	0.66 ± 0.27	0.66 ± 0.05	0.49 ± 0.14	0.55 ± 0.14	0.46 ± 0.03	0.56 ± 0.02
		Experiment set 2							
C/N	0	18.5 ± 0.69	20.9 ± 0.07	20.2 ± 0.03	21.6 ± 0.72	20.9 ± 0.15	19.9 ± 0.26	23.1 ± 1.54	21.5 ± 0.23
	1	18.8 ± 0.66	180 ± 0.49	18.6 ± 0.62	17.6 ± 0.55	22.3 ± 0.46	21.9 ± 0.21	20.9 ± 0.55	20.6 ± 0.80
	3	22.2 ± 2.28	17.7 ± 0.66	17.4 ± 0.75	18.0 ± 1.23	23.1 ± 0.95	25.4 ± 3.85	23.4 ± 1.17	22.6 ± 4.00
	5	19.3 ± 1.28	17.4 ± 0.48	16.6 ± 0.54	19.3 ± 1.02	18.2 ± 0.93	19.3 ± 1.24	20.3 ± 1.08	18.7 ± 0.83
WSC/TN	0	0.59 ± 0.02	0.75 ± 0.01	0.87 ± 0.01	0.88 ± 0.02	0.83 ± 0.06	1.14 ± 0.02	1.14 ± 0.07	1.16 ± 0.05
	1	0.84 ± 0.07	0.45 ± 0.06	0.46 ± 0.08	0.62 ± 0.06	1.11 ± 0.07	0.75 ± 0.10	0.43 ± 0.09	0.32 ± 0.03
	3	0.38 ± 0.12	0.25 ± 0.08	0.21 ± 0.05	0.23 ± 0.04	0.18 ± 0.02	0.40 ± 0.10	0.36 ± 0.02	0.27 ± 0.05
	5	0.22 ± 0.08	0.06 ± 0.02	0.07 ± 0.02	0.09 ± 0.03	0.11 ± 0.04	0.15 ± 0.03	0.07 ± 0.03	0.21 ± 0.10

Key: Control = unamended substrate (brewery spent grain); 5Gypsum, 10Gypsum, 15Gypsum = substrate amended with 5, 10 and 15% gypsum, respectively; 5Biochar, 10Biochar, 15Biochar and 20Biochar = substrate amended with 5, 10, 15 and 20% biochar, respectively.

In mature frass composts, biochar amended substrates had higher C/N ratios than gypsum amended and control treatments. At the end of the composting process, substrates amended with 15% biochar had the highest C/N ratio (20.3), while the control treatment had the lowest value (12.8). In comparison however, the control treatment had the highest ratio of WSC/TN in mature frass compost (0.77) while frass compost generated from substrates amended with 5% gypsum had the lowest (0.06). This was closely followed by frass compost derived from substrates amended with 10% gypsum and 15% biochar, with equal WSC/TN ratios of 0.07 (Table 16). The C/N ratios and WSC/TN of mature frass composts generated from the various substrates (Table 16) are within the range (< 20) recommended by Goyal et al. (2005) and Guo et al. (2012) for mature and stable composts.

4.3.9 Phytotoxicity of mature frass compost extracts

The germination rate (experiment set 1: $p = 0.649$, set 2: $p = 0.255$) and germination index (GI) (experiment set 1: $p = 0.899$, set 2: $p = 0.076$) of the cabbage seeds were not significantly influenced by the compost treatments for both experiments (Table 17). Percent seed germination ranged between 63 and 100%, whereby frass compost generated from substrates amended with 15 gypsum had the lowest value while compost generated from substrates amended with 5% biochar had the highest seed germination rate.

Frass composts generated from biochar amended substrates had higher seed germination indices than the frass composts derived from the unamended substrate (Table 17). Similarly, frass fertilizers from substrates amended with biochar had higher seed germination rates and GI than those amended with gypsum in experiment set 2.

Table 17: Seed germination and germination indices of mature BSF frass composts amended with biochar and gypsum.

	Experiment set 1		Experiment set 2	
Substrate formulations	Germination rate (%)	Germination index (%)	Germination rate (%)	Germination index (%)
Control	93.3 ± 3.3	90.2 ± 26.9	90 ± 5.8	232.2 ± 92.6
5Gypsum	90 ± 5.8	115.1 ± 13.8	93.3 ± 6.7	56.2 ± 7.4
10Gypsum	83.3 ± 6.7	120.8 ± 37.0	86.7 ± 6.7	101.1 ± 45.7
15Gypsum	86.7 ± 3.3	123 ± 19.5	63.3 ± 23.3	68.4 ± 29.2
5Biochar	83.3 ± 12.0	98.3 ± 29.9	100 ± 0.0	273.5 ± 51.8
10Biochar	93.3 ± 3.3	133.7 ± 15.5	90 ± 5.8	158.1 ± 29.8
15Biochar	93.3 ± 3.3	108.1 ± 11.6	100 ± 0.0	165.4 ± 61.4
20Biochar	96.7 ± 3.3	114.1 ± 9.5	90 ± 5.8	132 ± 30.2
p value	ns	ns	ns	ns

Key: ns = not significant ($p \geq 0.05$); Control = unamended substrate (brewery spent grain); 5Gypsum, 10Gypsum, 15Gypsum = substrate amended with 5, 10 and 15% gypsum, respectively; 5Biochar, 10Biochar, 15Biochar and 20Biochar = substrate amended with 5, 10, 15 and 20% biochar, respectively.

The highest GI was recorded in frass compost generated from substrates amended with 5% biochar (274%) while frass compost derived from substrates amended with equivalent rate of gypsum had the lowest GI value (56%). This study revealed that biochar amendment of substrates did not influence the compost maturity period as indicated by the high cabbage seed germination rates (> 90%) and germination index values (> 80%) in mature compost extracts for all the treatments (Table 17). This implies that mature compost from substrates amended with biochar were free of any phytotoxic substances such as organic acids, salts and ammonia (Teresa & Remigio, 2011), thus ready for field application after only five weeks of composting.

The low seed germination rates and germination indices observed for frass compost generated from substrate amended with 5 and 15% gypsum (Table 17) implies moderate phytotoxicity, which might be probably attributed to the high EC (Figure 15a and 15b). Moderate phytotoxicity of mature compost has been reported to affect seed germination and radical elongation (Emino & Warman, 2004; Teresa & Remigio, 2011). This study demonstrated that the composting time of substrates amended with gypsum and biochar can be drastically reduced to 5 weeks using BSF larvae for initial breakdown compared to the conventional composting process of 8 – 24 weeks (Tumuhairwe et al., 2009 Dias et al., 2010; Awasthi et al., 2017a).

4.4 Comparative performance of BSF frass fertilizer and commercial fertilizers on the growth, yield, and nitrogen use efficiency of maize

4.4.1 Plant height

The different sole organic and combined fertilizer treatments showed significant differences in maize plant heights at different growth stages during the short (sole organic: $p < 0.001$, combined: $p < 0.001$) (Figure 22) and the long rain seasons (sole organic: $p < 0.001$, combined: $p < 0.001$) (Figure 23). Plant heights significantly ($p < 0.001$) increased from early seedling stage (27 – 94 cm) (35 DAP) to highest values (173 – 264 cm) at silking stage (91 DAP) with the smallest increases in plant heights observed at silking stage (Figure 22 and 23). Among sole organic fertilizer treatments, plots treated with 5 and 7.5 t ha^{-1} of SAFI produced the tallest plants during the short rain (215 cm) ($p < 0.001$) (Figure 22a and 22b) and long rain (264 cm) ($p = 0.26$) season, respectively (Figure 23a and 23b).

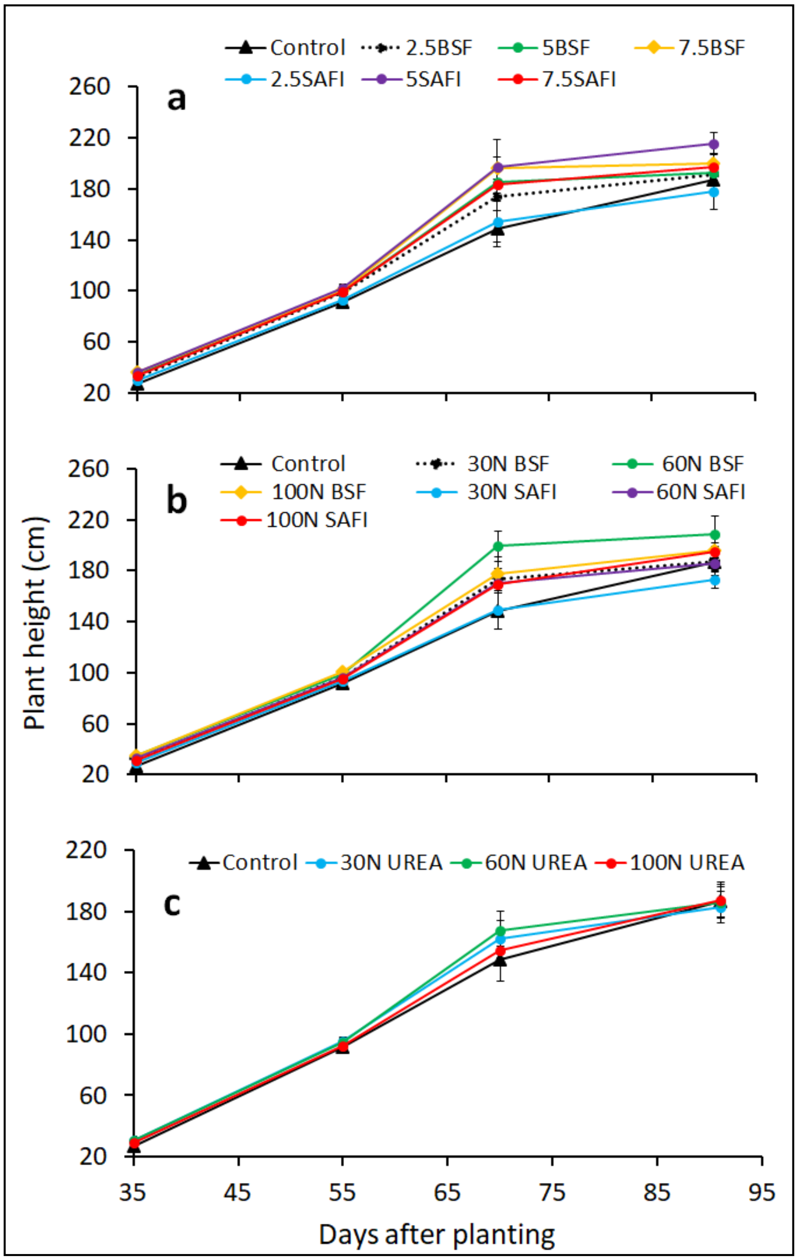

Figure 22: Effects of BSF frass fertilizer (a and b), commercial organic (SAFI) (a and b) and mineral (urea) (c) fertilizers on maize plant height during the short rain season experiments.

Key: Control = unfertilized soil; 2.5BSF, 5BSF and 7.5BSF = application BSF frass fertilizer at rates of 2.5, 5 and 7.5 t ha^{-1}, respectively; 2.5SAFI, 5SAFI and 7.5SAFI = application SAFI organic fertilizer at rates of 2.5, 5 and 7.5 t ha^{-1}; 30N BSF, 60N BSF and 100N BSF = application of BSF frass fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; 30N SAFI, 60N SAFI and 100N SAFI = application of SAFI organic fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; 30N UREA, 60N UREA and 100N UREA = application of urea fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; BSF = Black soldier fly frass fertilizer; SAFI = commercial organic fertilizer.

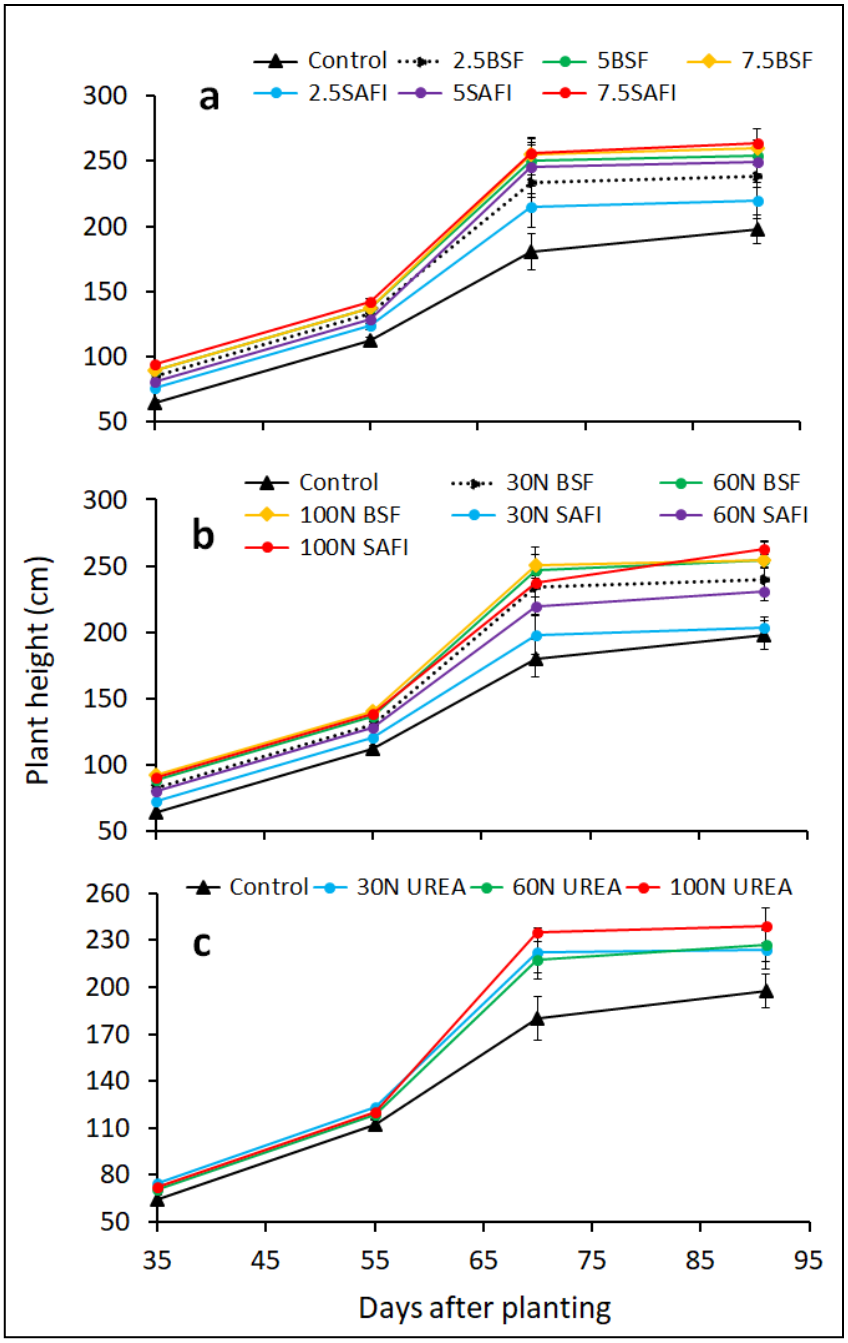

Figure 23: Effects of BSF frass fertilizer (a and b), commercial organic (SAFI) (a and b) and mineral (urea) (c) fertilizers on maize plant height during the long rain season experiments.

Key: Control = unfertilized soil; 2.5BSF, 5BSF and 7.5BSF = application BSF frass fertilizer at rates of 2.5, 5 and 7.5 t ha^{-1}, respectively; 2.5SAFI, 5SAFI and 7.5SAFI = application SAFI organic fertilizer at rates of 2.5, 5 and 7.5 t ha^{-1}; 30N BSF, 60N BSF and 100N BSF = application of BSF frass fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; 30N SAFI, 60N SAFI and 100N SAFI = application of SAFI organic fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; 30N UREA, 60N UREA and 100N UREA = application of urea fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; BSF = Black soldier fly frass fertilizer; SAFI = commercial organic fertilizer.

For combined fertilizer treatments, plots treated with organic fertilizers produced taller plants than urea treated plots (Figure 22a – c and 23a – c). Application of 60 kg N ha^{-1} supplied as BSF frass fertilizer produced the tallest plants (208 cm) that were 7 and 11% ($p = 0.53$) taller than those produced using equivalent rates of SAFI organic and urea fertilizers, respectively, during the short rain season (Figure 22a – c).

Plots treated with 100 kg N ha^{-1} suppled using SAFI produced the tallest plants (263 cm) during the long rain season, which were 3 and 10% ($p < 0.001$) taller than those grown using equivalent rates of BSF frass fertilizer and urea fertilizer, respectively (Figure 23a – c). The control treatment and plots treated with 2.5 t ha^{-1} as well as 30 kg N ha^{-1} supplied using the SAFI produced the shortest plants during the two seasons of the experiments.

The higher maize plant height associated with BSF frass fertilizer could be attributed to high growth rate arising from the high rate of nutrient release (Adin Yéton et al., 2019). Previous studies using frass fertilizer have reported the presence of growth hormones (Poveda et al., 2019), microbial strains and enzymes responsible for soil nitrogen cycling and plant defense against pests and diseases (Debode et al., 2016; Quilliam et al., 2020). The siderophores and chitinase contained in insect frass increase nutrients availability, acquisition, and metabolism through ammonia oxidization and nitrification (De Tender et al., 2019), thus the higher plant height observed of maize growth using BSF frass fertilizer.

4.4.2 Chlorophyll concentration

The maize leaf chlorophyll concentration varied significantly at different growth stages due to different sole organic fertilizer treatments during the short ($p = 0.032$) (Figure 24) and long rain seasons ($p = 0.003$) (Figure 25). However, the chlorophyll concentration was significantly influenced by the different combined fertilizer treatments (short rain season: $p < 0.001$, long rain season: $p < 0.001$) and maize growth stages (short rain season: $p < 0.001$), long rain season: $p < 0.001$) only. The interaction effects of combined fertilizer treatments and maize growth stage were not significant (short rain season: $p = 0.589$, long rain season: $p = 0.059$).

The highest chlorophyll concentrations (45 – 61 SPAD values) were achieved at late seedling stage (55 DAP) during the short rain season (Figure 24a – c). In the short rain season, chlorophyll concentrations for all treatments significantly decreased and reached their lowest values at silking stage. During the long rain season, however, a significant increase in chlorophyll concentrations was observed at both late seedling and silking stages (Figure 25a – c).

Plots treated with BSF frass fertilizer applied at 5 and 7.5 t ha^{-1}, 60 and 100 kg N ha^{-1} as well as urea fertilizer applied at 100 kg N ha^{-1} achieved highest chlorophyll concentrations (83 – 93 SPAD values) at silking stage compared to other treatments which peaked at late seedling stage. Plots treated with BSF frass fertilizer at 7.5 t ha^{-1} produced plants with the highest chlorophyll concentrations, which were 18% ($p = 0.032$) and 20% ($p = 0.003$) higher than those achieved using equivalent rates of the SAFI in the short and long rain seasons, respectively (Figure 25a and 25b).

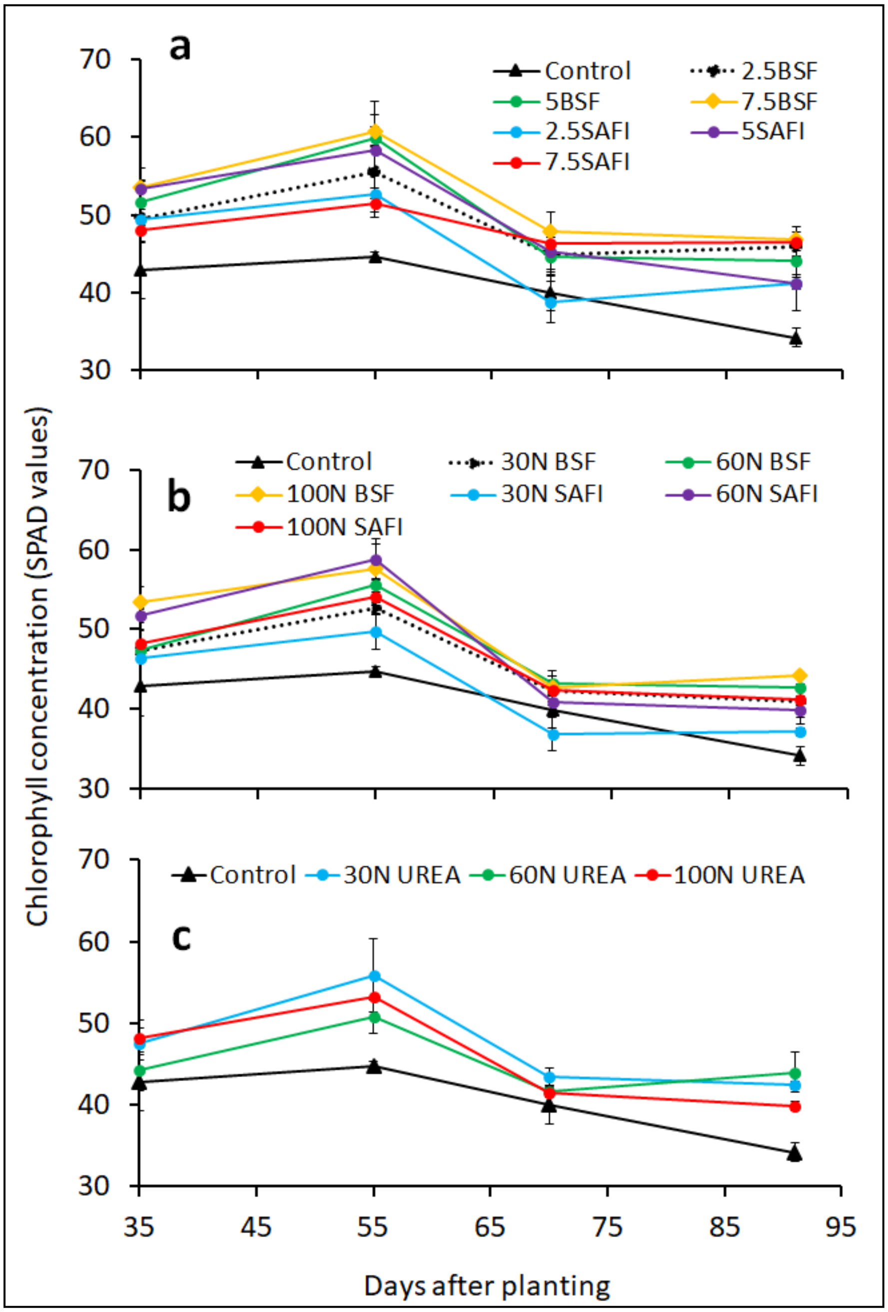

Figure 24: Effect of BSF frass fertilizer (a and b), commercial organic (SAFI) (a and b) and mineral fertilizer (urea) (c) on maize leaf chlorophyll concentration during the short rain season experiments.

Key: Control = unfertilized soil; 2.5BSF, 5BSF and 7.5BSF = application BSF frass fertilizer at rates of 2.5, 5 and 7.5 t ha^{-1}, respectively; 2.5SAFI, 5SAFI and 7.5SAFI = application SAFI organic fertilizer at rates of 2.5, 5 and 7.5 t ha^{-1}; 30N BSF, 60N BSF and 100N BSF = application of BSF frass fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; 30N SAFI, 60N SAFI and 100N SAFI = application of SAFI organic fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; 30N UREA, 60N UREA and 100N UREA = application of urea fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; BSF = Black soldier fly frass fertilizer; SAFI = commercial organic fertilizer.

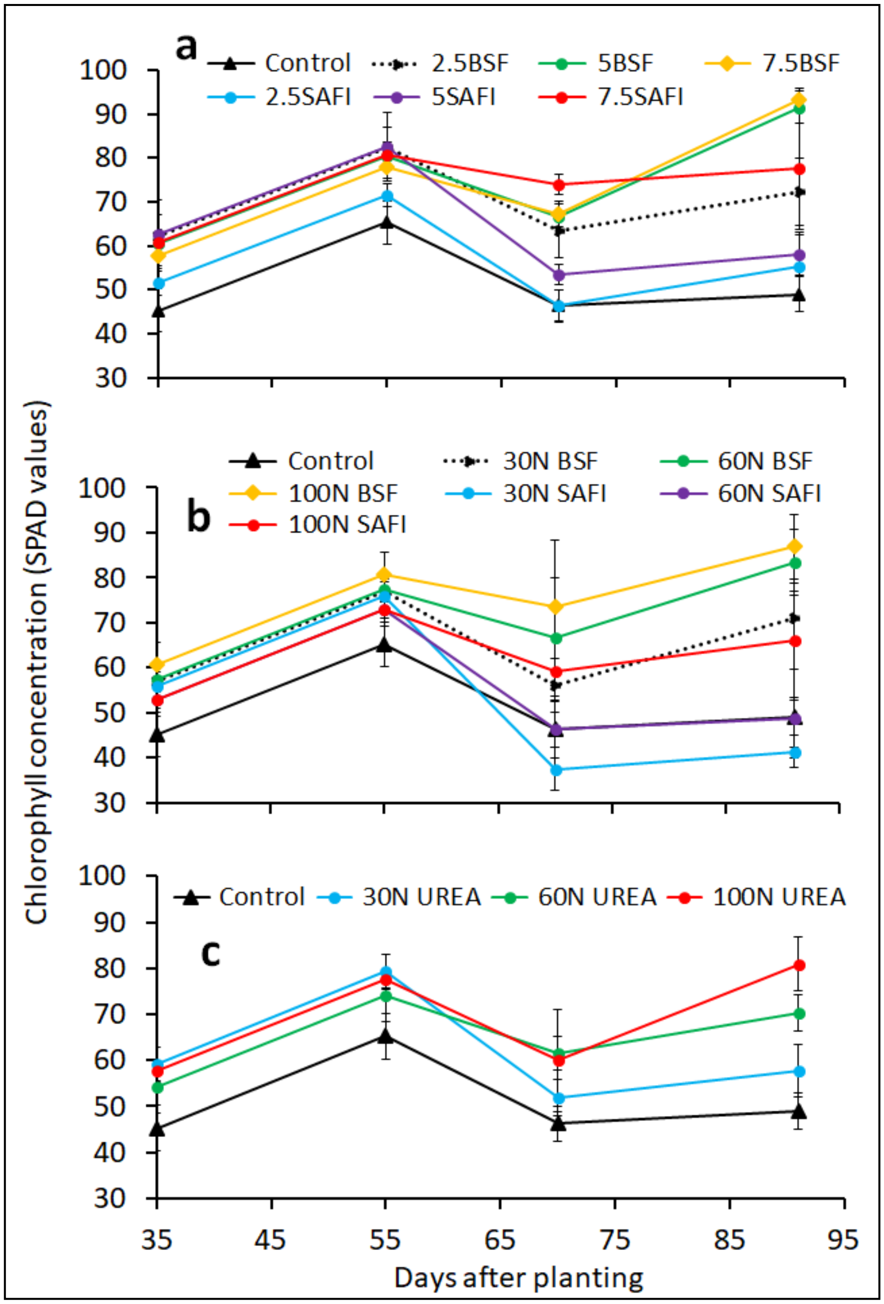

Figure 25: Effect of BSF frass fertilizer (a and b), commercial organic (SAFI) (a and b) and mineral (urea) (c) fertilizers on maize leaf chlorophyll concentration during the long rain season experiments.

Key: Control = unfertilized soil; 2.5BSF, 5BSF and 7.5BSF = application BSF frass fertilizer at rates of 2.5, 5 and 7.5 t ha^{-1}, respectively; 2.5SAFI, 5SAFI and 7.5SAFI = application SAFI organic fertilizer at rates of 2.5, 5 and 7.5 t ha^{-1}; 30N BSF, 60N BSF and 100N BSF = application of BSF frass fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; 30N SAFI, 60N SAFI and 100N SAFI = application of SAFI organic fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; 30N UREA, 60N UREA and 100N UREA = application of urea fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; BSF = Black soldier fly frass fertilizer; SAFI = commercial organic fertilizer.

During the long rain season, the highest chlorophyll concentration among the combined fertilizers was recorded from plants treated with 100 kg N ha^{-1} supplied as BSF frass fertilizer, and this was 32 and 7% ($p < 0.001$) higher those from plants treated with equivalent rates of SAFI (Figure 25b) and urea fertilizer (Figure 25c), respectively. The higher maize plant height (Figure 18), chlorophyll concentration (Figure 19) and N (Figure 20) and P uptake (Table 20) observed in BSF-treated plots than SAFI organic and urea fertilizer treated plots could be due to the better supply and availability of nutrients from this organic fertilizer. The high release of nutrients resulting from the high mineralisation rate of BSF frass fertilizer (Adin Yéton et al., 2019) might have led to better synchrony of nutrients supply with plant growth and chlorophyll formation of plants supplied with BSF frass fertilizer. High quality frass fertilizers have been associated with improved nutrient availability and plant growth as reported by Kagata & Ohgushi (2012).

On the other hand, high amount of recalcitrant carbon in the biochar used to make SAFI organic fertilizer could have caused nitrogen immobilisation (Fornara et al., 2011; Musyoka et al., 2019a), hence, limiting the amounts of N available for plant growth Further, the beneficial microbes associated with insect frass fertilizer have been found to promote plant growth and protect the pant against biotic and abiotic stresses (Choi & Hassanzadeh, 2019; Poveda et al., 2019; Quilliam et al., 2020). Such additional advantages of BSF frass fertilizer could have enhanced higher plant growth and nutrients acquisition than to plants treated with SAFI organic fertilizer and urea fertilizers. The higher chlorophyll concentration and nutrient uptake observed during the long rain season could be largely attributed to residual effect of fertilizers applied during the short rain season and higher rainfall (Figure 2) received during the long rain season.

4.4.3 Soil mineral nitrogen content

4.4.3.1 Sole organic fertilizer treatments

At 0 – 20 cm soil depth, the mineral N content during the short and long rain seasons of maize growth was significantly influenced by different sole organic fertilizer treatments (short rain season: $p < 0.001$, long rain season: $p < 0.05$) and maize growth stages (short rain season: $p < 0.001$, long rain season: $p < 0.001$) (Table 18). The interaction effect of fertilizer treatments and maize growth stages was significant during the short rain season only ($p < 0.001$).

The mineral N content significantly increased to highest levels at the early seedling stage (6 – 36 kg N ha^{-1}), before decreasing to significantly minimum values at tasseling stage. Thereafter, slight changes in mineral N were observed from tasseling to maturity stage. At the early seedling stage, the mineral N content in plots treated with 7.5 t ha^{-1} of BSF frass fertilizer was 4 and 6 times higher than those achieved at equivalent rates of SAFI during the short ($p = 0.004$) and long rain ($p = 0.003$) seasons, respectively. Plots treated with 5 t ha^{-1} of BSF frass fertilizer had 2.4 times higher ($p < 0.05$) mineral N content than those treated with equivalent rate of SAFI at tasseling stage during the short rain season (Table 17). Increase in mineral N contents was observed at the maturity stage during the long rain season for treatments consisting 5 and 7.5 t ha^{-1} of BSF frass fertilizer, and 7.5 t ha^{-1} of SAFI.

Table 18: Mineral nitrogen distribution in the top 40 cm of soil treated with sole BSF frass and SAFI fertilizers.

Soil depth (cm)	Rate (t ha^{-1})	Soil mineral N content (kg N ha^{-1})									
		SEASON 2019A (Short rains)					SEASON 2019B (Long rains)				
		Time (days after planting)									
		0	35	70	91	125	0	35	70	91	126
0 – 20	0	4.1 ± 1.1b	9.9 ±2.8b	2.4 ±0.8b	2.9 ±2.2a	6.3 ±0.8	39.0 ±3.8	5.7 ±1.0b	26.1 ±3.1	14.4 ±0.5	31.5 ± 0.8
	2.5 BSF	4.1 ±1.5b	8.8 ±3.2b	5.1 ±1.6ab	3.5 ±2.5a	6.4 ±1.5	39.0 ±3.8	14.5±1.9ab	63.3±9.2	16.5±2.1	32.4±7.9
	5 BSF	13.3±4.7ab	28.9±10ab	12.2 ±2.7a	11.3 ±2.4a	8.6 ±0.6	44.8±6.4	8.6±2.0b	107.4±71	20.7±3.3	51.6±21.9
	7.5 BSF	15.7 ±2.3a	35.7 ±5.8a	8.1 ±3.5ab	9.5 ±0.5a	7.6 ±1.1	40±3.3	38.3±13a	37±6.5	19.5±0.7	44.6±14.1
	2.5 SAFI	2.8 ±0.6b	6.0 ±1.2b	1.6 ±0.1b	5.8 ±0.6a	8.8 ±2.6	38.2±8.3	3.9±0.1b	26.2±4.5	16.4±1.1	40.7±16.6
	5 SAFI	3.4 ±1.4b	7.3 ±3.0b	5.0 ±2.1ab	3.7 ±2.1a	5.9 ±1.2	39.1±4.4	3.8±0.03b	18.8±3.2	17.2±0.4	21.2±6.2
	7.5 SAFI	3.7 ±1.1b	8.0 ±2.5b	5.4 ±0.8ab	4.8 ±1.6a	6.9 ±0.5	24.8±2.3	6.8±2.8b	27.8±8.2	18.2±2.3	41.9±14
	p value	**	**	*	*	ns	ns	**	ns	ns	ns
20 – 40	0	3.4 ±1.3	7.8 ±2.9	6.4 ±1.7	5.9 ±0.8	3.6 ±0.8ab	16.0 ± 6.3	19.1± 8.7b	35.9± 15	11.6±3.6	12.3±1.2
	2.5 BSF	2.1 ±0.7	4.1 ±1.3	9.0 ±2.0	6.6 ±1.0	3.5 ±1.3ab	24.7 ± 4.4	46.1±0.5b	29.7±16.9	14±4.3	11.3±2.5
	5 BSF	3.2 ±0.3	6.2 ±0.5	12.2 ±2.0	7.8 ±1.2	5.3 ±0.9ab	32.3 ± 21.5	41.5±12b	18.6±5.0	10.6±0.3	17.9± 4.1
	7.5 BSF	3.7 ±0.6	7.4 ±1.0	10.8 ±1.1	6.7 ±0.4	8.2 ±2.2a	29.7 ± 13.4	119±18.5a	43.3±6.4	18±3.9	13.4±5.8
	2.5 SAFI	1.2 ±0.4	2.4 ±0.7	9.0 ±1.8	5.0 ±1.1	4.8 ±0.4ab	22.7 ± 4.5	17.2±7.1b	37.7±1.1	10.1±3.5	6.9±0.4
	5 SAFI	1.6 ±0.1	3.3 ±0.2	9.5 ±1.7	5.3 ±0.6	3.4 ±0.7ab	15.3 ± 3.5	11±1.5b	31.9±9.1	18.4±0.4	20±2.5
	7.5 SAFI	2.6 ±0.5	5.0 ±1.0	8.5 ±2.0	6.0 ±0.8	2.3 ±0.4b	19.1 ± 2.5	32±11.5b	58.9±24.9	10±4.2	24.5±8.5
	p value	ns	ns	ns	ns	*	ns	***	ns	ns	ns

Key: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$; ns = not significant ($p \geq 0.05$); 0 = control (unfertilized soil); 2.5BSF, 5BSF and 7.5BSF = application of BSF frass fertilizers at rates of 2.5, 5 and 7.5 t ha^{-1}, respectively; 2.5SAFI, 5SAFI and 7.5SAFI = application of SAFI organic fertilizer at rates of 2.5, 5 and 7.5 t ha^{-1}; BSF = Black soldier fly frass fertilizer; SAFI = commercial organic fertilizer. In the same column, means (± standard error) followed by the same letters are not significantly different at $p \leq 0.05$.

At soil depth of 20 – 40 cm, the mineral N content was also significantly influenced by fertilizer treatments (short rain season: $p < 0.001$, long rain season: $p < 0.001$) and crop growth stage (short rain season: $p < 0.001$, long rain season: $p < 0.001$) (Table 18). The interaction effect of fertilizer treatments and maize growth stages was significant ($p < 0.001$) during the long rain season only. Peak mineral N levels during the short rain season were achieved at tasseling stage (70 DAP) with the highest recorded in plots treated with 7.5 t ha^{-1} of BSF frass fertilizer. The same treatment had significantly ($p = 0.044$) higher mineral N content at maturity stage than to plots treated with 7.5 t ha^{-1} of SAFI. During the long rain season, peak mineral N contents were attained at early seedling and silking stages for BSF frass fertilizer and SAFI organic fertilizer treatments, respectively. Soil treated with 7.5 t ha^{-1} BSF frass fertilizer had significantly ($p < 0.001$) higher mineral N contents than other treatments at the early seedling stage (Table 18).

4.4.3.2 Combined fertilizer treatments

For combined fertilizer treatments, mineral N content in the topsoil depth (0 – 20 cm) varied significantly at different maize growth stages during the long rain season only ($p = 0.014$) (Table 19). The mineral N significantly increased to peak levels between early seedling and silking stages before decreasing gradually to the end of the short rain season. However, no significant differences in mineral N content were observed between the treatments. During the long rain season, mineral N significantly decreased from initial values to lowest levels at early seedling stage. Plots treated with 60 kg N ha^{-1} supplied as BSF frass fertilizer and urea fertilizer had the highest mineral N contents in topsoil at tasseling and silking stages, respectively.

Table 19: Mineral nitrogen distribution in the top 40 cm of soil treated with combined fertilizers

Soil depth (cm)	Rate (kg N ha^{-1})	Soil mineral N content (kg N ha^{-1})									
		SEASON 2019A (Short rains)					SEASON 2019B (Long rains)				
		Days after planting									
		0	35	70	91	125	0	35	70	91	125
0 - 20	0	4.1 ± 1.1	9.9 ±2.8	2.4 ±0.8	2.9 ±2.2	6.3 ±0.8	39.0 ±3.8	5.7 ±1.0	26.1 ±3.1a	14.4 ±0.5	31.5 ± 0.8
	30N BSF	3.2 ±1.6	6.9 ±3.6	5.7 ±1.4	5.4 ±2.4	6.7 ±1.3	42.1±4.2	11.4±5.2	37±8.7a	19.9±3.3	23.5±16.5
	60N BSF	2.2 ±0.1	4.8 ±0.2	3.4 ±1.5	8.1 ±1.0	6.3 ±1.0	43.2±7.7	23±9.9	63.0±14.5a	15.4±1.4	23.4±13.1
	100N BSF	4.2 ±1.1	9.3 ±3.0	4.2 ±1.6	2.5 ±2.2	6.7 ±0.6	36.2±7.9	6.4±3.1	50.1±6.1a	27.5±4.7	54.4±7.1
	30N SAFI	1.9 ±1.1	4.4 ±2.6	5.1 ±1.9	6.0 ±0.9	5.8 ±1.0	38.1±4.9	4.0±0.1	28.9±8.5a	17.9±2.0	36.6±4.5
	60N SAFI	1.8 ±0.2	4.0 ±0.2	11.3 ±5.7	0.82 ±0.1	5.4 ±1.8	69.6±10.2	3.9±0.0	62.7±15.0a	12.6±5.6	59.2±10.3
	100N SAFI	5.7 ±1.7	12.9 ±3.4	7.2 ±1.0	4.8 ±1.8	5.9 ±1.6	46.5±23.3	5.4±1.6	47.1±7.1a	16.0 ±1.1	31.6±1.8
	30N UREA	1.7 ±0.8	3.7 ±1.9	7.3 ±1.2	5.5 ±2.2	7.1 ±2.1	46.2±1.3	6.8±1.6	23.6±4.7a	14.5±2.4	41.5±3.5
	60N UREA	2.6 ±1.3	5.9 ±3.1	6.1 ±0.7	8.1 ±0.7	7.5 ±1.2	25.4±5.0	5.2±1.6	45.1±8.3a	32±15.5	49.5±23.0
	100N UREA	6.1 ±0.5	13.3 ±0.7	6.6 ±1.1	8.2 ±3.9	13.0 ±3.9	39.9±11.4	13.3±2.9	51.1±7.8a	24.4±5.5	62.6±7.6
	p value	ns	ns	ns	ns	ns	ns	ns	*	ns	ns
20 - 40	0	3.4 ±1.3	7.8 ±2.9	6.4 ±1.7	5.9 ±0.8ab	3.6 ±0.8a	16.0 ± 6.3	19.1± 9b	35.9± 15	11.6±3.6	12.3±1.2ab
	30N BSF	2.0 ±1.1	3.9 ±2.0	11.4 ±0.4	5.0 ±0.4b	3.1 ±0.4a	17.5±4.2	27.9±11ab	44.5±16.4	15.2±6.2	9.1±2.6b
	60N BSF	1.2 ±0.4	2.5 ±0.7	6.2 ±0.8	5.9 ±0.5ab	3.1 ±0.6a	18±11.2	42.5±19ab	37.5±10.8	17.1±6.8	9.1±2.0b
	100N BSF	2.1 ±0.1	4.1 ±0.1	7.8 ±3.3	5.3 ±0.2ab	4.6 ±0.6a	19±4.4	34.5±11ab	49.6±8.1	11.0±2.7	15.9±5.0ab
	30N SAFI	1.2 ±0.4	2.5 ±0.7	7.5 ±1.6	4.5 ±0.3b	3.0 ±0.2a	32.5±12	16.5±8.3b	65.6±1.5	10.2±0.4	7.5±1.0b
	60N SAFI	0.8 ±0.4	1.8 ±0.7	7.4 ±1.6	5.1 ±0.2ab	3.6 ±0.5a	38.9±15.4	32.4±14ab	42±8.3	11.1±6.8	9.4±1.6b
	100N SAFI	2.4 ±0.9	4.6 ±1.7	7.3 ±2.8	6.4 ±0.6ab	2.8 ±1.3a	13.4±6.0	8.2±2.1b	35.9±11.2	12.4±4.6	6.5±0.1b
	30N UREA	2.1 ±0.9	4.0 ±1.6	12.1 ±2.5	5.1 ±0.1ab	5.9 ±0.8a	9.6±2.8	12.4±2.1b	36.8±13.8	16.1±6.4	20.5±4.1ab
	60N UREA	1.1 ±0.3	2.3 ±0.5	9.9 ±2.4	7.5 ±1.3ab	3.9 ±0.5a	19.7±8.7	89.5±22ab	48.1±14	12.7±3.5	26.1±5.5a
	100N UREA	2.4 ±1.0	4.6 ±1.8	15.5 ±5.2	8.9 ±1.6ab	6.2 ±0.5a	35.6±6.5	149.3±69a	108.2±41	26.2±6.9	20.6±1.5ab
	p value	ns	ns	ns	*	*	ns	*	ns	ns	**

Key: ** $p < 0.01$, * $p < 0.05$; ns = not significant ($p \geq 0.05$); 0 = Control (unfertilized soil); 2.5BSF, 5BSF and 7.5BSF = application BSF frass fertilizer at rates of 2.5, 5 and 7.5 t ha^{-1}, respectively; 2.5SAFI, 5SAFI and 7.5SAFI = application SAFI organic fertilizer at rates of 2.5, 5 and 7.5 t ha^{-1}; 30N BSF, 60N BSF and 100N BSF = application of BSF frass fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; 30N SAFI, 60N SAFI and 100N SAFI = application of SAFI organic fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; 30N UREA, 60N UREA and 100N UREA = application of urea fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; BSF = Black soldier fly frass fertilizer; SAFI = commercial organic fertilizer. Means (± standard error) followed by the same letters are not significantly different at $p \leq 0.05$.

In the subsoil depth (20 – 40 cm), mineral N content in soil was significantly affected by combined fertilizer treatments (short rain season: $p < 0.001$, long rain season: $p < 0.001$) and maize growth stage (short rain season: $p < 0.001$, long rain season: $p < 0.001$) (Table 19). The interaction effect of combined fertilizer treatments and maize growth stage was significant during the long rain season only ($p = 0.003$). The soil mineral N content significantly increased to peak levels of between 6.2 and 15.5 kg N ha^{-1} at tasseling stage, before gradually decreasing towards the end of the short rain season. Plots treated with 100 kg N ha^{-1} supplied as urea had significantly ($p = 0.018$) higher mineral N contents at silking stage of the short rain season than where 30 kg N ha^{-1} from SAFI and 60 kg N ha^{-1} supplied as BSF frass fertilizer were applied (Table 19). Similarly, plots treated with 100 kg N ha^{-1} supplied as urea achieved significantly ($p = 0.017$) higher mineral N content at the early seedling stage during the long rain season than the equivalent rate of SAFI.

At maturity stage (125 DAP), plots treated with 60 kg N ha^{-1} applied as urea fertilizer had significantly ($p = 0.001$) higher mineral N content than BSF frass and SAFI treatments, except for plots where 100 kg N ha^{-1} of BSF frass fertilizer was applied. The high soil mineral N content observed in plots treated with BSF frass fertilizer (Table 18 and 19) could be majorly attributed to the higher mineralization rate of this organic fertilizer (Adin Yéton et al., 2019). The low mineral N observed in urea amended plots could be attributed to losses through denitrification and volatilization (Bernal et al., 2009), thus reducing the quantity of N available for plant uptake, especially during the short rain season when temperatures were high (Figure 2).

On the other hand, biochar was one of the components of SAFI, yet biochar consists of recalcitrant organic carbon which causes delays in N release, thus low soil mineral N (Kleber, 2010; Rovira & Vallejo, 2002). Biochar consists of compounds such as lignin and polyphenols (Sanchez-Monedero et al., 2018) which have been reported to cause N immobilization (Kimetu et al., 2004; Musyoka et al., 2019b). Furthermore, the high nitrate concentration associated with SAFI organic fertilizer could have facilitated N leaching, especially during the long rain season, when soil moisture content was high (Loecke et al., 2012). High nitrate leaching (80% of total N), up to 1 m down the soil profile, has been reporting in maize cropping systems of Kenya (Musyoka et al., 2019ba) and could be attributed to the high sand content and low organic matter levels of the experimental soil.

4.4.4 Nitrogen uptake by maize

4.4.4.1 Sole organic fertilizer treatments

The quantity of nitrogen accumulated in maize biomass was significantly influenced by the different sole organic fertilizer treatments (short rain season: $p < 0.001$, long rain season: $p < 0.001$) and maize growth stages (short rain season: $p < 0.001$, long rain season: $p < 0.001$) (Figures 26 and 27). The interaction of sole organic fertilizer treatments and growth stages was significant during the long rain season only ($p = 0.012$) (Figure 27). Nitrogen uptake significantly ($p = 0.012$) increased from 9 – 16 kg N ha^{-1} at early seedling stage (35 DAP) to highest values (40 – 139 kg N ha^{-1}) at tasseling (70 DAP) and silking stages (91 DAP). Thereafter, N uptake levels decreased gradually to maturity stage.

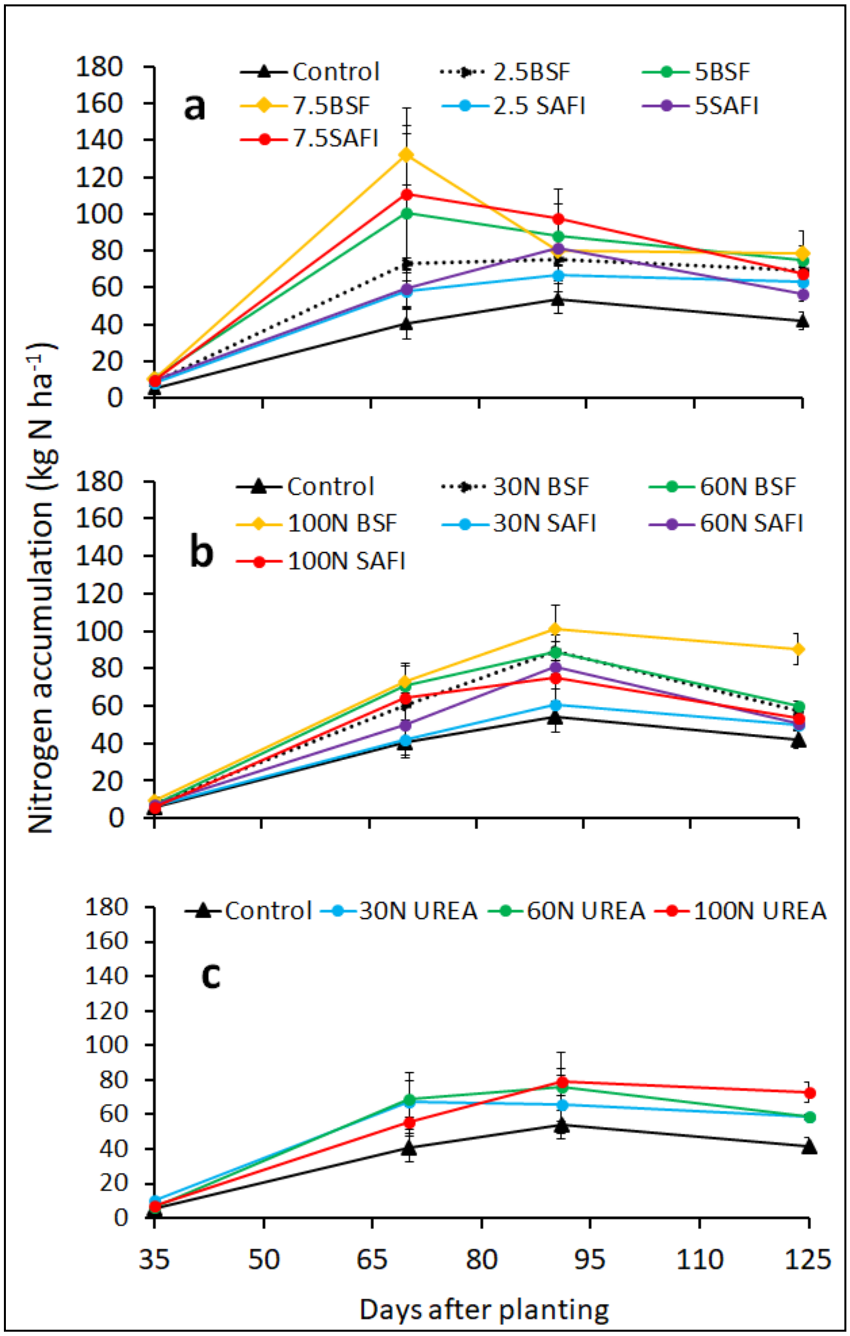

Figure 26: Effects of BSF frass fertilizer (a and b), commercial organic (SAFI) (a and b) and mineral (urea) (c) fertilizers on maize nitrogen uptake during the short season experiments.

Key: Control = unfertilized soil; 2.5BSF, 5BSF and 7.5BSF = application BSF frass fertilizer at rates of 2.5, 5 and 7.5 t ha^{-1}, respectively; 2.5SAFI, 5SAFI and 7.5SAFI = application SAFI organic fertilizer at rates of 2.5, 5 and 7.5 t ha^{-1}; 30N BSF, 60N BSF and 100N BSF = application of BSF frass fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; 30N SAFI, 60N SAFI and 100N SAFI = application of SAFI organic fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; 30N UREA, 60N UREA and 100N UREA = application of urea fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; BSF = Black soldier fly frass fertilizer; SAFI = commercial organic fertilizer.

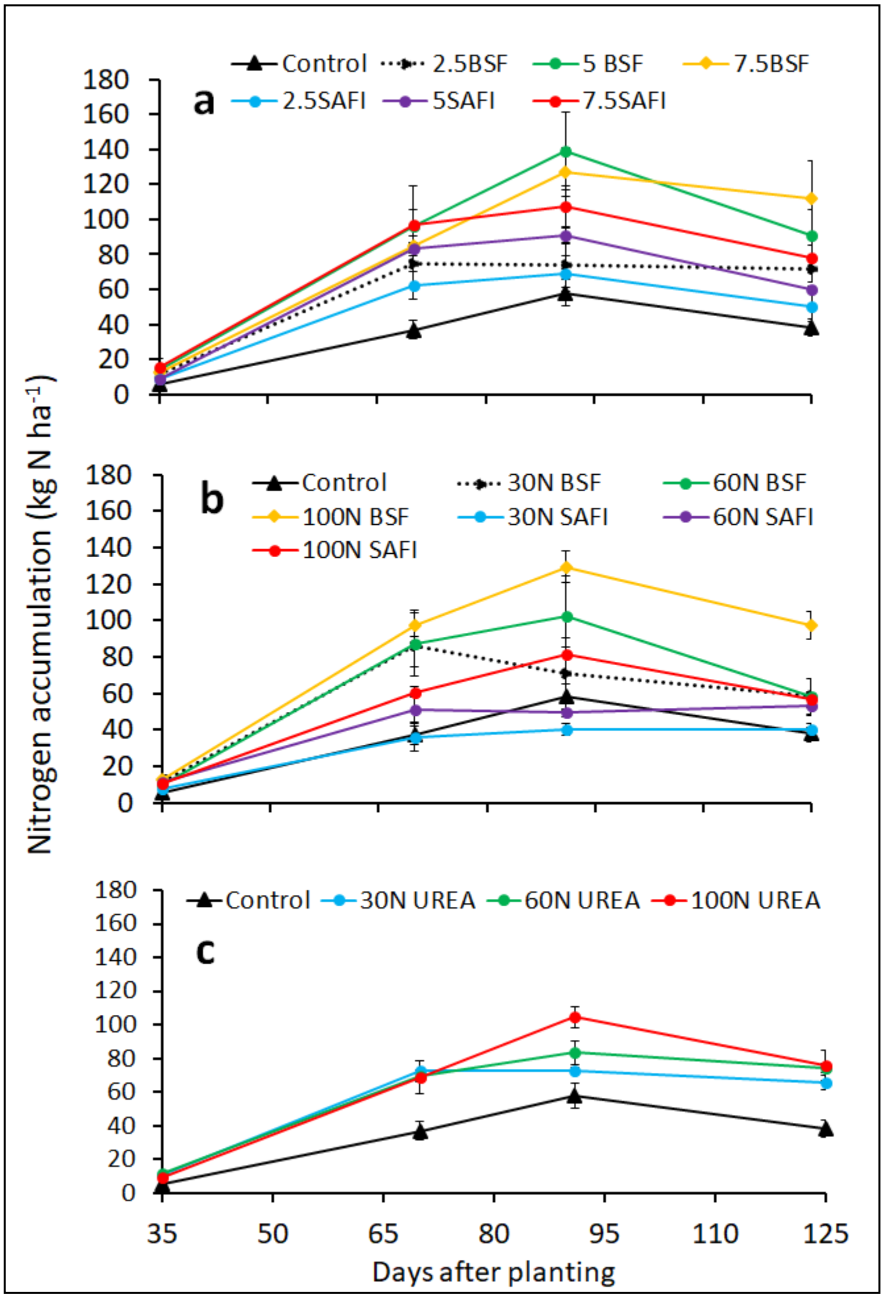

Figure 27: Effects of BSF frass fertilizer (a and b), commercial organic (SAFI) (a and b) and mineral (urea) (c) fertilizers on maize nitrogen uptake during the long rain season experiments.

Key: Control = unfertilized soil; 2.5BSF, 5BSF and 7.5BSF = application BSF frass fertilizer at rates of 2.5, 5 and 7.5 t ha^{-1}, respectively; 2.5SAFI, 5SAFI and 7.5SAFI = application SAFI organic fertilizer at rates of 2.5, 5 and 7.5 t ha^{-1}; 30N BSF, 60N BSF and 100N BSF = application of BSF frass fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; 30N SAFI, 60N SAFI and 100N SAFI = application of SAFI organic fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; 30N UREA, 60N UREA and 100N UREA = application of urea fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; BSF = Black soldier fly frass fertilizer; SAFI = commercial organic fertilizer.

Maize grown using 7.5 t ha^{-1} of BSF frass fertilizer and 7.5 t ha^{-1} of SAFI accumulated the highest N levels at tasseling stage of the short ($p = 0.021$) and long rain ($p = 0.2$) season experiments, respectively (Figure 26). At the silking stage of the short rain season, maize grown in plots treated with 5 t ha^{-1} of BSF frass fertilizer accumulated significantly ($p = 0.004$) higher N at the silking stage than those where 2.5 t ha^{-1} of either BSF frass or SAFI was applied (Figure 26a). At maturity stage, the N accumulation (38 – 112 kg ha^{-1}) varied significantly among the treatments (short rain season: $p = 0.012$, long rain reason: $p = 0.006$). The N accumulated in maize treated with 7.5 t ha^{-1} of BSF frass fertilizer was 17 and 44% higher than N accumulated in maize treated with equivalent rates of SAFI during the short (Figure 26a) and long rain seasons, respectively (Figure 27a), but the differences were not statistically significant.

4.4.4.2 Combined fertilizer treatments

The different combined fertilizer treatments significantly influenced the N uptake in maize biomass during the short and long rain seasons (short rain season: $p < 0.001$, long rain season: $p < 0.001$) (Figure 26 and 27). Like with sole organic fertilizers (section 4.4.4.1), N uptake also varied significantly at different maize growth stages (short rain season: $p < 0.001$, long rain season: $p < 0.001$). The interaction effect of combined fertilizer treatments and growth stages was significant during the long rain season only ($p < 0.001$). The N accumulation in maize tissues at early seedling stage (5 – 13 kg N ha^{-1}) significantly ($p < 0.001$) increased to highest values at tasseling and silking stages (40 – 129 kg N ha^{-1}). A significant ($p < 0.001$) decrease in N uptake was observed at maturity stage, except for plots treated with 30 and 60 kg N ha^{-1} supplied using SAFI organic fertilizer during the long rain season (Figure 26b and 27b).

Maize grown in plots treated with 60 and 100 kg N ha^{-1} supplied as BSF frass fertilizer accumulated higher N levels at tasseling (short rain season: $p = 0.181$, long rain season: $p < 0.001$) and silking stages (short rain season: $p = 0.197$, long rain season: $p < 0.001$) than equivalent rates of SAFI (Figure 26 and 27). The N accumulated in maize treated with 100 kg N ha^{-1} of BSF frass fertilizer at tasseling stage of the long rain season was significantly ($p < 0.001$) higher than those of maize grown using 30 and 60 kg N ha^{-1} supplied using SAFI organic fertilizer (Figure 27b). Similarly, application of 100 kg N ha^{-1} as BSF frass fertilizer caused significantly ($p < 0.001$) higher N uptake at silking stage of the long rain season compared to where 30 kg N ha^{-1} of all fertilizers, and 60 kg N ha^{-1} of SAFI organic fertilizer were applied.

Application of 100 kg N ha^{-1} supplied as BSF frass fertilizer during the short and long rain seasons effected significantly (short rain season: $p < 0.001$, long rain season: $p < 0.001$) higher N accumulation than other treatments at harvesting stage, except for maize grown using 100 kg N ha^{-1} of urea fertilizer in both seasons, and 60 kg N ha^{-1} of urea during the long rain season (Figure 26 – 27). The higher mineral N content and N uptake (Figure 26 – 27) associated with BSF frass fertilizer compared to other treatments could be attributed to higher nutrient release (Kagata & Ohgushi, 2012; Houben et al., 2020), better timing of application and climatic conditions during experiments.

It should be noted that for organic fertilizer treatments, the entire dose was applied at planting, while for urea two splits were administered, whereby the second split of urea was applied when rainfall was low during the short rain season (63 DAP) (Figure 2a – c). The low moisture in soils could have reduced available N concentrations in solution for plant uptake, while the high temperatures could have triggered N loss through volatilisation (Bernal et al., 2009) and thereby reducing N use by plants. Unfortunately, this coincided with tasselling stage, when there was high N demand for ear and grain formation by the plant (Figure 26 and 27).

Similarly, the higher rainfall in second season (Figure 2c) could have triggered N loss in mineral fertilizer treatments through leaching and denitrification due to excess soil moisture (Musyoka et al., 2019b). These N losses were responsible for the lower agronomic N use efficiency in mineral fertilizer than those of BSF frass fertilizer treatments (Table 21). On the other hand, organic inputs are effective in moisture conservation which consequently improve nutrient availability through minimising leaching losses (Baligar et al., 2001; Vanlauwe et al., 2015).

Since there was enough nitrogen at critical growth stages like tasselling and silking (Figure 20), a better crop yield was obtained from BSF frass fertilizer treatments (Figure 28 and 29). Findings of the study correspond with Musyoka et al. (2017) who also reported higher apparent nitrogen recovery and agronomic nitrogen use efficiencies in experiments treated with high quality organic inputs. Furthermore, the high nitrate-N contained in SAFI organic fertilizer (Table 5) could have been lost through leaching especially during the long rain season (Musyoka et al., 2019b), thereby leaving little mineral N for plant uptake.

4.4.5 Phosphorus and potassium uptake at harvesting

4.4.5.1 Sole organic fertilizer treatments

There were significant differences in the quantity of P accumulated in maize biomass at harvesting stage among the different sole organic fertilizer treatments during the short rain ($p = 0.032$) and long rain seasons ($p = 0.006$) (Table 20). Total P accumulated in maize grown in plots treated with 5 and 7.5 t ha^{-1} of BSF frass fertilizer were significantly higher than those of the control treatment in both seasons. The total P accumulated in plots treated with 7.5 t ha^{-1} of BSF frass fertilizer was 15 and 27% higher than the P accumulated by maize grown using similar rates of SAFI organic fertilizer during the short and long rain seasons, respectively.

Accumulated K also varied significantly in both seasons due to different sole organic fertilizer treatments (short rain season: $p = 0.019$, long rain season: $p = 0.044$) (Table 20). Maize grown using 7.5 t ha^{-1} of SAFI organic fertilizer accumulated significantly higher K contents in both seasons than maize grown in the control treatments. On the other hand, the total K accumulated in plots treated with 7.5 t ha^{-1} of SAFI organic fertilizer was 44 and 23% higher than that accumulated using equivalent rates of BSF frass fertilizer during the short and long rain seasons, respectively.

Table 20: Effects of BSF frass fertilizer, commercial organic and mineral (urea) fertilizers on phosphorus, and potassium uptake at maturity.

Fertilizer treatments	**SEASON 2019A (short rains)**		**SEASON 2019B (long rains)**	
	Phosphorus	Potassium	Phosphorus	Potassium
	(kg ha^{-1})			
	Sole organic fertilizer rates (t ha^{-1})			
Control	4.2 ± 0.22b	77.6 ± 10.3b	5.4 ± 1.3b	63.6 ± 13.7b
2.5 BSFF	12.7 ± 2.1a	128.5 ± 25ab	12.7 ± 1.3ab	122.0 ± 14.8ab
5 BSFF	12.9 ± 1.9a	160.5 ± 15.3ab	16.3 ± 2.5a	163.8 ± 34.5ab
7. 5 BSFF	13.3 ± 1.2a	118.9 ± 10.9ab	18.6 ± 2.8a	173.4 ± 37.0ab
2.5 SAFI	10.7 ± 2.2ab	97.4 ± 18.7ab	10.2 ± 1.5ab	123.5 ± 32.5ab
5 SAFI	11.1 ± 1.2ab	144.0 ±22.8ab	11.8 ± 1.8ab	110.1 ± 21.6ab
7.5 SAFI	11.6 ± 2.1ab	171.2 ± 10.6a	14.6 ± 1.8ab	212.5 ± 33.5a
p value	*	*	**	*
	Combined fertilizer rates (kg N ha^{-1})			
30N BSFF	10.6 ± 0.9ab	104.9 ± 20.9ab	11.7 ± 0.6abc	125.1 ± 21.6ab
60N BSFF	13.6 ± 1.8ab	109.4 ± 12.8ab	14.6 ± 2.4ab	115.5 ± 1.8ab
100N BSFF	16.4 ± 2.0a	179.0 ± 37.5a	17.4 ± 1.0a	189.2 ± 30.9a
30N SAFI	8.7 ± 0.6ab	83.3 ± 14.1ab	7.2 ± 0.9bc	69.0 ± 13.5b
60N SAFI	7.2 ± 1.1bc	98.0 ± 19.9ab	8.1 ± 0.4bc	94.1 ± 16.8b
100N SAFI	10.8 ± 1.3ab	119.0 ± 4.4ab	11.8 ± 1.3abc	124.7 ± 2.7ab
30N UREA	10.6 ± 0.8ab	143.2 ±28.5ab	12.7 ± 1.1abc	113.3 ± 7.3ab
60N UREA	10.0 ± 2.6ab	91.6 ± 11.3ab	12.6 ± 3.3abc	129.5 ± 5.5ab
100N UREA	12.6 ±2.3ab	121.4 ± 11.1ab	13.3 ± 2.9abc	118.3 ± 13.6ab
Control	4.2 ±0.2c	77.6 ± 10.3b	5.4 ± 1.3c	63.6 ± 13.7b
p value	**	*	**	***

Key: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$; 2.5BSF, 5BSF and 7.5BSF = application BSF frass fertilizer at rates of 2.5, 5 and 7.5 t ha^{-1}, respectively; 2.5SAFI, 5SAFI and 7.5SAFI = application SAFI organic fertilizer at rates of 2.5, 5 and 7.5 t ha^{-1}; 30N BSF, 60N BSF and 100N BSF = application of BSF frass fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; 30N SAFI, 60N SAFI and 100N SAFI = application of SAFI organic fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; 30N UREA, 60N UREA and 100N UREA = application of urea fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; BSF = Black soldier fly frass fertilizer; SAFI = commercial organic fertilizer. In the same column, means (± standard error) followed by the same letters are not significantly different at $p \leq 0.05$.

4.4.5.2 Combined fertilizer treatments

The quantity of phosphorus accumulated in maize biomass at maturity stage (4 – 17 kg P ha^{-1}) was significantly influenced by different combined fertilizer treatments (short rain season: $p = 0.002$, long rain season: $p = 0.004$) (Table 20). There was significantly higher accumulation of P in maize grown using 100 kg N ha^{-1} supplied as BSF frass fertilizer than in maize grown using 60 kg N ha^{-1} of SAFI organic fertilizer and control treatment in both seasons and 30 kg N ha^{-1} of SAFI organic fertilizer during the long rain season. Similarly, application of 100 kg N ha^{-1} supplied as BSF frass fertilizer caused significantly higher K accumulation in maize than the control treatment in both seasons (short rain season: $p = 0.048$, long rain season: $p < 0.001$), and where 30 and 60 kg N ha^{-1} of SAFI organic fertilizer were applied during the long rain season (Table 20).

The increased uptake of phosphorus in the study might have been partly responsible for high nitrogen accumulation observed in maize grown in plots treated with BSF frass fertilizer (Tittonell et al., 2008b). This can be attributed to the role of phosphorus in energy transfer as a component of adenosine phosphates (Fageria, 2001). Although, potassium has been equally reported to be important in nitrogen absorption and metabolism as well as plant growth (Fageria, 2001), it was taken up in sufficient quantities across all treatments. The observations described above are in line with the report by Kagata and Ohgushi (2012), who also demonstrated that frass fertilizer is of good quality and capable of improving soil nutrient availability, and growth and yield of *Brassica rapa* L. var. rapa. Additional benefits of insect frass fertilizer on soil health for improved drought and salt tolerance, disease suppression, higher crop growth and yield have also been documented by Choi and Hassanzadeh (2019) and Houben et al. (2020).

4.4.6 Nitrogen use efficiency

4.4.6.1 Agronomic nitrogen efficiency (AE_N)

The various treatments of sole organic fertilizers (short rain season: $p = 0.004$, long rain season: $p = 0.012$) and combined fertilizers (short rain season: $p = 0.005$, long rain season: $p < 0.001$) had significant effects on the AE_N of maize during experiments (Table 21). Application of 2.5 t ha^{-1} of BSF frass fertilizer increased the AE_N of maize by 2.4 and 2.2 times compared to the equivalent rate of SAFI organic fertilizer during the short and long rain season, respectively. Furthermore, maize grown using 2.5 t ha^{-1} of BSF frass fertilizer had significantly higher AE_N than other treatments, except for plots treated with 5 and 7.5 t ha^{-1} of the same fertilizer and 2.5 t ha^{-1} of SAFI organic fertilizer during the long rain season.

During the short rain season, the AE_N of maize treated with 30 kg N ha^{-1} of BSF frass fertilizer was significantly ($p = 0.004$) higher than those of maize treated with 100 kg N ha^{-1} of all fertilizers, and 60 kg N ha^{-1} of SAFI organic fertilizer (Table 21). Also, the AE_N in plots treated with 30 kg N ha^{-1} of BSF frass fertilizer was significantly ($p < 0.001$) higher than those achieved by all SAFI organic fertilizer treatments and 100 kg N ha^{-1} of urea during the long rain season.

4.4.6.2 Apparent nitrogen recovery (ANR_N)

The ANR_N of maize was significantly influenced by the different sole organic (short rain season: $p < 0.001$, long rain season: $p = 0.028$) and combined fertilizer treatments (short rain season: $p < 0.001$, long rain season: $p < 0.001$) as shown in Table 21.

Table 21: Effects of BSF frass fertilizer, commercial organic and mineral (urea) fertilizers on nitrogen use efficiency of maize.

Fertilizer treatments	SEASON 2019A (Short rains)				SEASON 2019B (Long rains)			
	AE_N (kg kg^{-1} N)	ANR_N (%)	APE_N (kg kg^{-1} N)	NHI (%)	AE_N (kg kg^{-1} N)	ANR_N (%)	APE_N (kg kg^{-1} N)	NHI (%)
Sole organic fertilizer treatments								
2.5 BSF	43.2 ± 10.8a	52.8 ± 2.4a	81.2 ± 19.6b	52.9 ± 3.3ab	33.0 ± 4.2a	63.8 ± 15.0a	55.5 ± 9.2	55.4 ± 1.9
5 BSF	18.5 ± 1.8b	31.7 ± 7.0ab	62.5 ± 9.6b	51.3 ± 5.4ab	29.7 ± 2.7ab	50.3 ± 13.5a	69.9 ± 23.2	60.9 ± 4.6
7.5 BSF	16.7±1.8b	23.5 ± 7.7b	80.9 ± 15.1b	53.4 ± 1.4ab	22.9 ± 3.8ab	46.7 ± 13.7a	56.2 ± 12.5	52.4 ± 6.1
2.5 SAFI	18.4 ± 1.0b	28.0 ± 1.9b	66.5 ± 7.6b	42.6 ± 4.0b	15.3 ± 7.8ab	15.5 ± 11.1a	35.7 ± 13.8	54.4 ± 9.4
5 SAFI	15.8 ± 1.9b	9.9 ± 2.8b	177.7 ± 36.9a	67.5 ± 5.6a	11.6 ± 2.1b	14.6 ± 1.1a	81.1 ± 17.5	65.7 ± 5.0
7.5 SAFI	18.3 ± 1.9b	11.3 ± 1.5b	72.3 ± 6.4b	42.7 ± 2.4b	12.1 ± 0.4b	17.6 ± 3.3a	74.6 ± 15.1	51.9 ± 6.9
Control	na	na	na	55.2 ± 2.0ab	na	na	na	51.6 ± 5.8
p value	**	***	**	**	*	*	ns	ns
Combined fertilizer treatments								
30N BSF	74.4 ± 7.1a	50.9 ± 9.5a	153.4 ± 22.3ab	54.3 ± 5.9	55.0 ± 10.7a	68.2 ± 3.4ab	79.9 ±13.8	55.4 ± 7.0
60N BSF	47.7 ± 6.7ab	30.2 ± 3.7ab	165.5 ± 34.8ab	52.2 ± 7.0	40.0 ± 4.3ab	32.9 ± 6.3bcd	82.4 ± 10.4	58.6 ± 3.3
100N BSF	29.9 ± 3.2b	48.7 ± 8.3a	64.6 ± 12.0b	62.8 ± 2.2	26.1 ± 1.0abc	59.2 ± 7.5abc	45.2 ± 4.5	62.9 ± 0.8
30N SAFI	58.8 ± 17.4ab	27.0 ± 10.7ab	234.7 ± 43.3a	50.3 ± 2.0	4.4 ± 1.3c	7.5 ± 2.7d	98.4 ± 58.2	64.6 ± 1.5
60N SAFI	21.3 ± 7.4b	14.0 ± 5.3b	156.2 ± 14.7ab	56.0 ± 1.6	15.6 ± 4.6bc	25.1 ± 8.7bcd	66.7 ± 18.0	58.9 ± 5.7
100N SAFI	17.8 ± 1.5b	11.4 ± 4.0b	182.0 ± 38.8ab	56.4 ± 7.2	15.7 ± 2.0bc	18.2 ± 4.2cd	91.1 ± 11.4	57.0 ± 6.9
30N UREA	34.4 ± 14.8ab	57.3 ± 1.5a	85.6 ± 2.3b	54.4 ± 8.1	55.3 ± 11.8a	91.4 ± 15.2a	60.3 ± 9.5	67.4 ± 3.3
60N UREA	32.2 ± 6.0ab	27.7 ± 2.7ab	122.6 ± 35.0ab	56.8 ± 6.8	31.3 ± 0.6abc	60.3 ± 4.8abc	52.5 ± 3.8	51.8 ± 2.4
100N UREA	26.7 ± 0.9b	30.8 ± 5.7ab	95.8 ± 24.6ab	58.4 ± 1.9	22.4 ± 4.2bc	37.4 ± 9.0bcd	61.7 ± 4.9	60.6 ± 4.4
Control	na	na	na	55.2 ± 2.0	na	na	na	51.6 ± 5.9
p value	**	***	*	ns	***	***	ns	ns

Key: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$; ns = not significant ($p \geq 0.05$); AE_N = agronomic nitrogen efficiency; ANR_N = apparent nutrient recovery; APE_N = agro-physiological nitrogen efficiency; NHI = nitrogen harvest index; Control = unfertilized soil; 2.5BSF, 5BSF and 7.5BSF = application BSF frass fertilizer at rates of 2.5, 5 and 7.5 t ha^{-1}, respectively; 2.5SAFI, 5SAFI and 7.5SAFI = application SAFI organic fertilizer at rates of 2.5, 5 and 7.5 t ha^{-1}; 30N BSF, 60N BSF and 100N BSF = application of BSF frass fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; 30N SAFI, 60N SAFI and 100N SAFI = application of SAFI organic fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; 30N UREA, 60N UREA and 100N UREA = application of urea fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; BSF = Black soldier fly frass fertilizer; SAFI = commercial organic fertilizer; na = not applicable. In the same column, means (± standard error) followed by the same letters are not significantly different at $p \leq 0.05$.

Maize treated with 2.5 t ha^{-1} of BSF frass fertilizer had significantly ($p < 0.001$) higher ANR_N values than other treatments, except for plots where 5 t ha^{-1} of the same fertilizer were applied during the short rain season. The ANR_N of maize treated with 2.5 t ha^{-1} of BSF frass fertilizer was 1.9 and 4.1 times higher than that obtained using equivalent rates of SAFI organic fertilizer during the short and long rain season, respectively.

Maize grown in plots treated with 30 kg N ha^{-1} supplied as urea had significantly ($p < 0.001$) higher ANR_N values than other treatments in the short rain season, except for those treated with 30 and 60 kg N ha^{-1} supplied using SAFI organic fertilizer (Table 21). Similarly, the ANR_N of maize treated with 30 kg N ha^{-1} as urea, was significantly ($p < 0.001$) higher than those of other treatments during the long rain season, except for maize grown using 60 kg N ha^{-1} of urea, 30 and 100 kg N ha^{-1} of BSF frass fertilizer.

4.4.6.3 Agro-physiological nitrogen efficiency (APE_N)

The APE_N of maize under different sole organic ($p = 0.009$) and combined fertilizer treatments ($p = 0.013$) varied significantly during the short rain season only (Table 21). Maize treated with 5 t ha^{-1} applied as SAFI organic fertilizer had significantly ($p = 0.009$) higher APE_N values than other treatments. During the long rain season, the APE_N of maize treated with 5 t ha^{-1} of SAFI organic fertilizer was 16% higher ($p = 0.77$) than that obtained using an equivalent rate of BSF frass fertilizer.

On the other hand, the APE_N of maize grown in plots treated with 30 kg N ha^{-1} supplied as SAFI organic fertilizer was significantly ($p = 0.013$) higher than those attained by 100 kg N ha^{-1} of BSF frass fertilizer and 30 kg N ha^{-1} of urea fertilizer during the short rain season (Table 21). During the long rain season, APE_N values of maize treated with 30 kg N ha^{-1} of SAFI organic fertilizer was 63 and 23% ($p = 0.704$) higher than those obtained using equivalent rates of urea and BSF frass fertilizer, respectively.

4.4.6.4 Nitrogen harvest index (NHI)

The NHI of maize grown using different sole organic treatments varied significantly ($p = 0.006$) during the short rain season only (Table 21). Maize grown on plots treated with 5 t h^{-1} of SAFI organic fertilizer had significantly ($p = 0.006$) higher NHI values than those treated with 2.5 and 7.5 t ha^{-1} of the same fertilizer treatments. Also, the NHI of maize treated with 5 t ha^{-1} of SAFI organic fertilizer was 8% higher than that obtained using an equivalent rate of BSF frass fertilizer during the long rain season. On the other hand, the different combined fertilizer treatments did not influence the NHI of maize significantly (short rain season: $p = 0.894$, long rain season: $p = 0.312$) (Table 21). Maize treated with 100 kg N ha^{-1} of BSF frass fertilizer and 30 kg N ha^{-1} of urea fertilizer, had the highest NHI values during the short and long rain season, respectively.

Remarkably, higher apparent nitrogen recovery and agronomic nitrogen use efficiency values were observed in maize harvested from plots treated with BSF frass fertilizer compared to those from plots treated with commercial fertilizers (Table 21). The highest values of agronomic and agro-physiological nitrogen use efficiencies as well as nitrogen harvest indices observed at lower application rates of BSF frass fertilizer (2.5 t ha^{-1} and 30 kg N ha^{-1}) indicate sufficiency in nitrogen supply for maize growth. Interestingly, one of the major factors limiting the use of organic fertilizers is associated with the high rates of application required (≥ 5 t ha^{-1}) (Mucheru-Muna et al., 2014; Musyoka et al., 2017) arising from the low-quality of organic resources available on the market.

Our findings demonstrate that BSF frass fertilizer has potential to increase crop productivity in most developing countries where mineral fertilizer use is still limited by high prices yet less effective due to multiple soil degradation challenges. Findings from this study would encourage a shift in attitude towards embracing the use of organic fertilizers such as the BSF frass fertilizer. Nonetheless, further studies to determine the economically optimum rates of BSF frass fertilizers for maize production are warranted.

4.4.7 Maize grain and stover yields

4.4.7.1 Sole organic fertilizer treatments

The different sole organic fertilizer treatments during the short and long rain seasons had significant effects on maize grain yields (short rain season: $p < 0.001$, long rain season: $p < 0.001$) (Figure 28). Application of BSF frass fertilizer treatments increased grain yields by between 71 and 96% during the short rain season (Figure 28a) and 49 – 101% during long rains compared to the control (Figure 28b).

On the other hand, grain yields increased by 50 – 87% during the short rains and 32 – 77% during the long rains season due to SAFI organic fertilizer treatments. For SAFI organic fertilizer, significant ($p < 0.001$) increases in grain yield above the control were achieved at rates of 5 – 7.5 t ha^{-1} during the short rain season, and at 7.5 t ha^{-1} during the long rain season. Maize grain yields did not vary significantly at equivalent rates of SAFI and BSF frass fertilizers (Figure 28a and 28b).

Application of BSF frass fertilizer at 7.5 t ha^{-1} produced the highest maize grain yields of 5.4 and 7.2 t ha^{-1} during the short and long rain seasons, respectively, which were 5 and 14% higher than the highest grain yield obtained using SAFI organic fertilizer during the short and long rain seasons, respectively. Grain yield from plots treated with 7.5 t ha^{-1} of BSF frass fertilizer was significantly ($p < 0.001$) higher than that obtained from plots amended with 2.5 t ha^{-1} of SAFI during the short rain season. Similarly, grain yield from maize grown using 7.5 t ha^{-1} of BSF frass fertilizer was significantly ($p < 0.001$) higher than those of other treatments during the long rain season, except for maize treated with 5 t ha^{-1} BSF frass fertilizer and 7.5 t ha^{-1} for SAFI organic fertilizer (Figure 28).

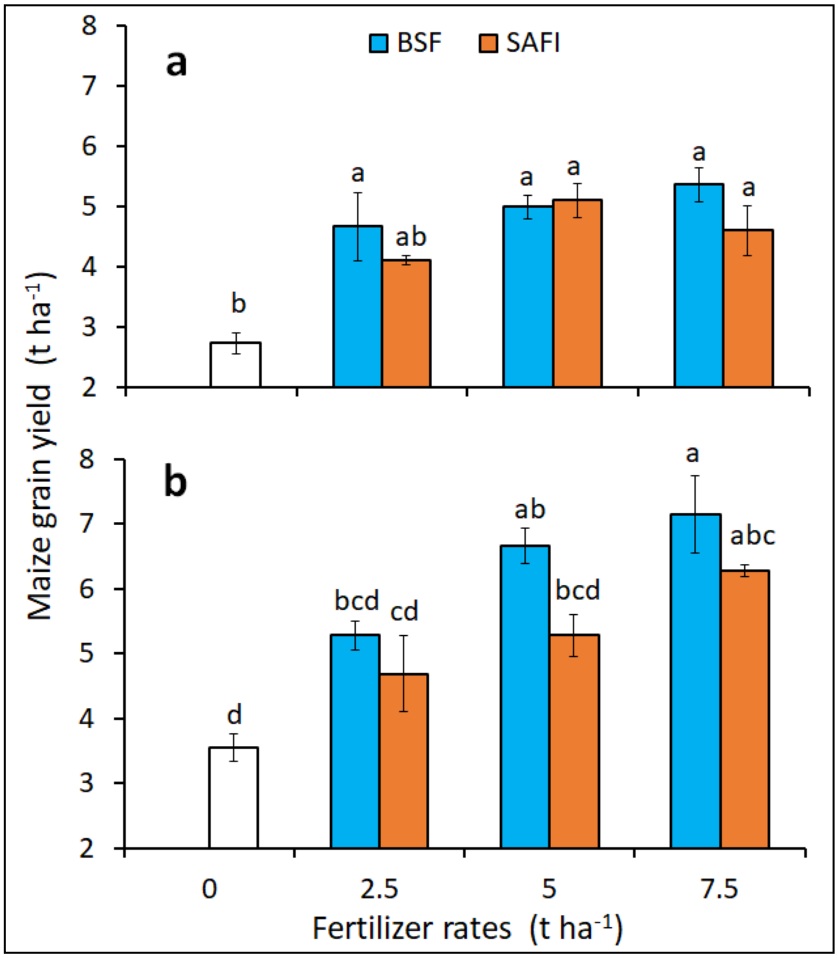

Figure 28: Effects of sole BSF frass and SAFI organic fertilizers on maize grain yields during the short (a) and long rain (b) season experiments.

Control = unfertilized soil; 2.5BSF, 5BSF and 7.5BSF = application BSF frass fertilizer at rates of 2.5, 5 and 7.5 t ha^{-1}, respectively; 2.5SAFI, 5SAFI and 7.5SAFI = application SAFI organic fertilizer at rates of 2.5, 5 and 7.5 t ha^{-1}; BSF = Black soldier fly frass fertilizer; SAFI = commercial organic fertilizer. In the same season, means (± stand error) followed by the same letters are not significantly different at $p \leq 0.05$

The different sole BSF frass and SAFI organic fertilizer treatments did not significantly influence maize stover yields during the experiments (short rain season: p = 0.118, long rain season: p = 0.259) (Table 22). However, plots treated with BSF frass fertilizer had higher stover yields (7.9 – 11.5 t ha^{-1}) than those treated with SAFI organic fertilizer (6.4 – 10.6 t ha^{-1}) during the long rain season.

Table 22: Effects of BSF frass fertilizer, commercial organic and mineral (urea) fertilizers on maize stover yield.

Fertilizer treatments	SEASON 2019A (short rains)	SEASON 2019B (long rains)
	Stover yield (t ha^{-1})	
Fertilizer treatments (t ha^{-1})		
Control	5.9 ± 0.78	5.8 ± 1.2
2.5 BSFF	8.4 ± 0.63	8.1 ± 0.6
5 BSFF	9.6 ± 0.76	9.8 ± 2.4
7. 5BSFF	7.9 ± 1.05	11.5 ± 2.3
2.5 SAFI	8.5 ± 0.70	7.2 ± 1.9
5 SAFI	6.4 ± 0.44	7.4 ± 0.6
7.5 SAFI	8.8 ± 1.49	10.6 ± 1.8
P value	ns	ns
Fertilizer treatments (kg N ha^{-1})		
30N BSFF	6.8 ± 1.78	7.7 ± 0.32abc
60N BSFF	8.3 ± 0.83	8.9 ± 0.26ab
100N BSFF	9.6 ± 1.41	10.3 ± 0.10a
30N SAFI	6.1 ± 0.60	5.0 ± 0.34c
60N SAFI	7.6 ± 0.97	7.3 ±0.27abc
100N SAFI	7.7 ± 0.71	8.0 ± 0.20abc
30N UREA	9.0 ± 1.23	7.1± 0.4babc
60N UREA	6.4 ± 0.89	9.1± 0.04ab
100N UREA	8.1 ± 0.54	7.7± 0.42abc
Control	5.9 ± 0.78	5.8 ± 1.2bc
p value	ns	**

Key: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$; ns = not significant ($p \geq 0.05$); 2.5BSF, 5BSF and 7.5BSF = application BSF frass fertilizer at rates of 2.5, 5 and 7.5 t ha^{-1}, respectively; 2.5SAFI, 5SAFI and 7.5SAFI = application SAFI organic fertilizer at rates of 2.5, 5 and 7.5 t ha^{-1}; 30N BSF, 60N BSF and 100N BSF = application of BSF frass fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; 30N SAFI, 60N SAFI and 100N SAFI = application of SAFI organic fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; 30N UREA, 60N UREA and 100N UREA = application of urea fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; BSF = Black soldier fly frass fertilizer; SAFI = commercial organic fertilizer. In the same column, means (± standard error) followed by the same letters are not significantly different at $p \leq 0.05$.

4.4.7.2 Combined fertilizer treatments

The grain yields also varied significantly among the different fertilizer treatments during the short and long rain seasons (short rain season: $p < 0.001$, long rain season: $p < 0.001$) as indicated in Figures 29a and 29b. All plots treated with BSF frass fertilizer produced significantly ($p < 0.001$) higher grain yields than the control. Grain yields from plots treated with 100 kg N ha^{-1} of BSF frass fertilizer were significantly ($p < 0.001$) higher than those where 30 kg N ha^{-1} of urea and 60 kg N ha^{-1} of SAFI organic fertilizer were applied during the short rain season (Figure 29a). Similarly, plots treated with 60 and 100 kg N ha^{-1} of BSF frass fertilizer, and 100 kg N ha^{-1} of urea, produced significantly ($p < 0.001$) higher maize grain yields than where 30 and 60 kg N ha^{-1} of SAFI organic fertilizer were applied during the long rain season (Figure 29b).

Maize treated with 100 kg N ha^{-1} applied as BSF frass fertilizer produced the highest grain yield (5.7 t ha^{-1}) during the short rain season which was 6 and 27% higher than yields obtained using equivalent rates of urea and SAFI organic fertilizer, respectively (Figure 29a). Likewise, grain yield from maize treated with 100 kg N ha^{-1} applied as BSF frass fertilizer (6.2 t ha^{-1}) was 7 and 21% higher than those obtained using equivalent rates of urea and SAFI organic fertilizer, respectively, during the long rain season (Figure 29b).

The stover yields varied significantly ($p = 0.003$) during the long rain season only (Table 22). Plots treated with 100 kg N ha^{-1} supplied as BSF frass fertilizer produced significantly ($p = 0.003$) higher stover maize yields than the control treatment and where 30 kg N ha^{-1} supplied using SAFI organic fertilizer was applied.

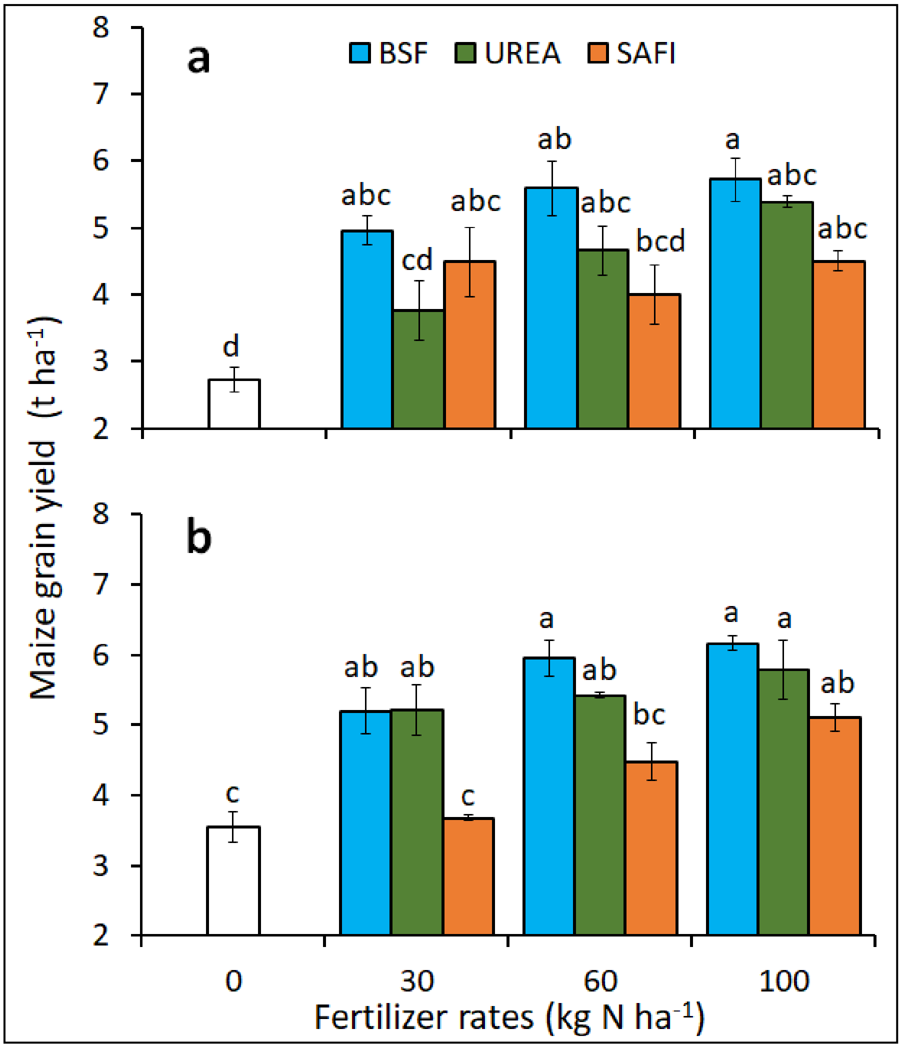

Figure 29: Effects of BSF frass, SAFI organic and urea fertilizers on maize grain yields during the short (a) and long rain (b) season experiments.

Control = unfertilized soil; 30N BSF, 60N BSF and 100N BSF = application of BSF frass fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; 30N SAFI, 60N SAFI and 100N SAFI = application of SAFI organic fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; 30N UREA, 60N UREA and 100N UREA = application of urea fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; BSF = Black soldier fly frass fertilizer; SAFI = commercial organic fertilizer. In the same season, means (± standard error) followed by the same letters are not significantly different at $p \leq 0.05$

The findings of this study revealed higher maize growth, nutrient uptake, and grain yields associated with all fertilizer treatments compared to the control (unamended soil) (Figures 28 and 29). From the results, it can be concluded that treating the soil with fertilizer significantly improved the maize yield and quality in terms of macronutrients (nitrogen, phosphorus, and potassium). This is consistent with other studies which clearly demonstrated that most parts of central Kenya have low soil fertility (Gachimbi et al., 2005; Muchena et al., 2005) and recommended regular fertilizer inputs for soil fertility improvement (Tully et al., 2015; Vanlauwe et al., 2015). Therefore, the increased maize plant height (Figure 22 and 23), chlorophyll concentration (Figure 24 and 25), and nitrogen (Figures 26 and 27) and phosphorus uptake (Table 20) observed in plots treated with BSF frass fertilizer compared to plots treated with the commercial organic and mineral fertilizers could be attributed to better supply and availability of nutrients from the newly introduced frass fertilizer. Furthermore, it is suggested that the high release of nutrients resulting from the high mineralisation rate of BSF frass fertilizer (Adin Yéton et al., 2019) and high availability of mineral nitrogen in the top 20 cm of soil depth (Tables 17 and 18) might have partly contributed to better synchrony of nutrients supply for maize growth, chlorophyll formation and high yields.

4.5 Soil moisture storage, nitrogen fertilizer equivalence value and synchrony of N mineralization from BSF frass and commercial organic fertilizer for maize production

4.5.1 Effect of BSF frass and SAFI organic fertilizers on soil moisture storage

The soil moisture content in the topsoil depth (0 – 20 cm) varied significantly during the short ($p < 0.001$) and long rain season ($p < 0.001$) experiments (Figure 30a and 30b). The soil moisture ranged between 14 and 27 g 100 g^{-1} at 35 days after planting (coinciding with early vegetative stage) after that it decreased significantly to minimum values at 91 and 70 days after planting during the short (10 g $100g^{-1}$) and long (12 g $100g^{-1}$) rain seasons, as shown in Figures 30a and 30b, respectively. The soil moisture content significantly ($p < 0.001$) increased at the 125th day after planting (corresponding to maturity stage) during long rain season.

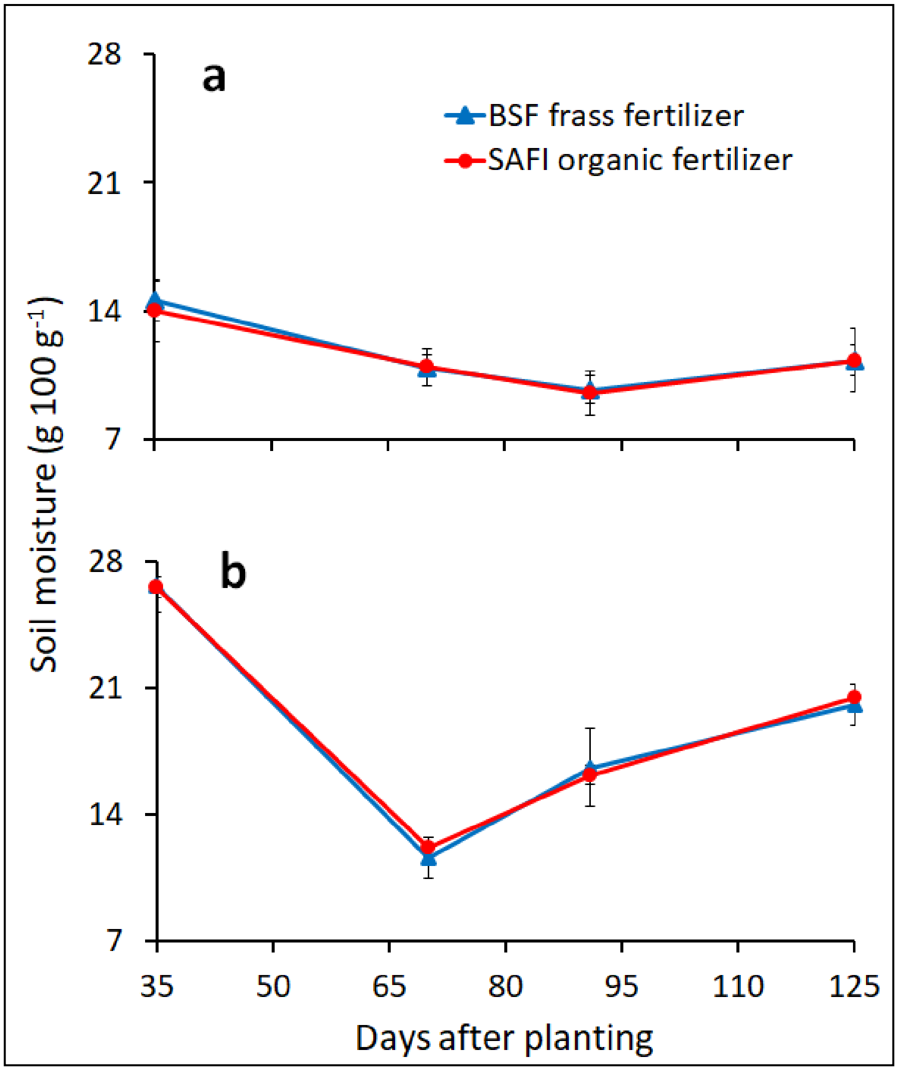

Figure 30: Soil moisture content in the topsoil (0 – 20 cm) depth of soil amended with BSF frass and SAFI organic

fertilizers during the short (a) and long rain (b) cropping seasons.

The soil moisture content in the subsoil depth (20 – 40 cm), also varied significantly during the maize growing period (short rain season: $p < 0.001$, long rain season: $p < 0.001$). However, after 70 days of planting, the soil moisture significantly ($p < 0.001$) decreased (corresponding to tasseling stage) and leveled off until maturity stage of the short rain season (Figure 31a and 31b).

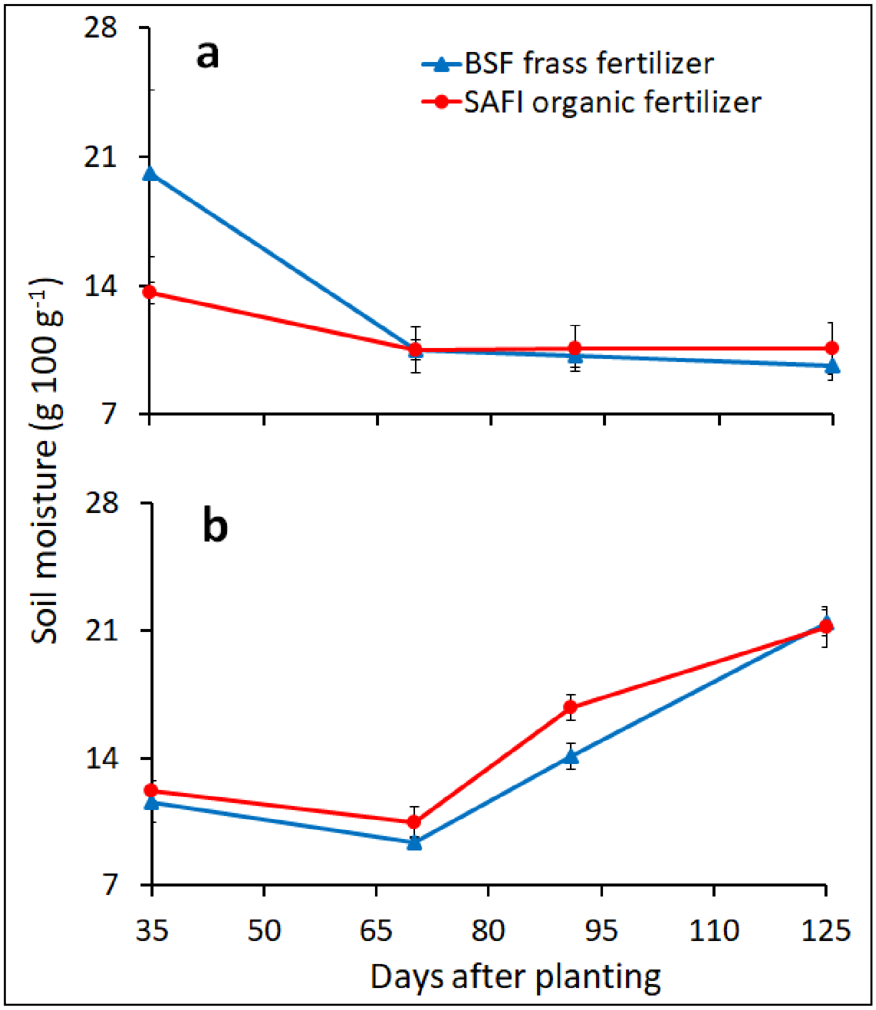

Figure 31: Soil moisture content in the subsoil (20 – 40 cm) depth of soil amended with BSF frass and SAFI organic fertilizers the short (a) and long rain (b) cropping seasons.

Soil amended with SAFI maintained higher moisture contents than that amended with BSF frass fertilizer from the 70th to 125th day after planting (Figure 31a and 31b). The higher soil moisture content obtained during the long rain season could be largely attributed to higher rainfall and lower temperatures experienced than during the short rain season (Figures 2a – c). The findings of this study are consistent with findings of Rusinamhodzi et al. (2013) who reported high water infiltration and moisture storage in soil amended with organic fertilizers. Furthermore, Vanlauwe et al. (2015) highlighted the positive roles of manure application in improving soil moisture conservation, fertilizer use efficiency and crop productivity. The increased soil moisture storage achieved using organic fertilizers is a positive step towards climate change adaptation and could act as a strategy for maintaining crop production in rainfed production systems which characterise most regions of SSA.

4.5.2 Effects of BSF frass and SAFI fertilizers on N mineralization

Application of BSF frass and SAFI organic fertilizers caused significant differences on N mineralization during the two cropping seasons (short rains season: $p < 0.05$, long rain season: $p < 0.01$) as indicated in Figures 32a and 32b. Mineral N released from soil amended with BSF frass fertilizer reached peak levels at 70 days of incubation which coincided with the tasseling stage of maize crop in both seasons, after which it decreased until the 125th day of incubation (coinciding with the maturity stage). For SAFI organic fertilizer, the peak levels in mineral N release were attained after 91 days of incubation which coincided with silking stage of maize crop during the short rain season (Figure 32a) while in the long rain season, the N release kept on a decreasing trend throughout the season (Figure 32b).

The soils amended with SAFI organic fertilizer released significantly ($p < 0.05$) higher mineral N at tasseling and at maturity stages than BSF frass fertilizer amended soils during the short rain season (Figure 32a). However, the mineral N released from soil amended with BSF frass fertilizer was significantly ($p < 0.01$) higher than that released from soil amended with SAFI organic fertilizer at the early vegetative and tasseling stages during the long rain season (Figure 32b).

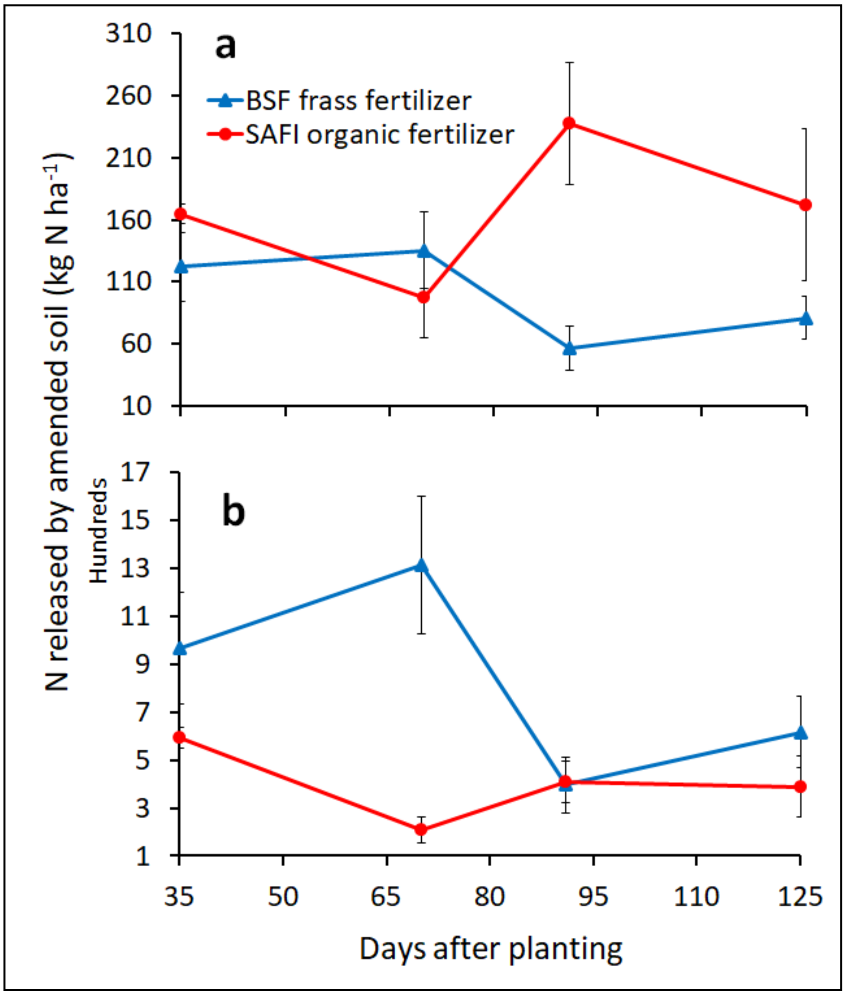

Figure 32: Mineral nitrogen released by soil amended with BSF frass and SAFI fertilizers during the short (a) and long rain (b) cropping seasons.

The rates of mineral N release from soils amended with organic inputs also varied significantly during the short ($p < 0.001$) (Figure 33a) and long ($p < 0.001$) rain seasons (Figure 33b). There was N immobilization in soil amended with BSF frass fertilizer in the first 91 days of incubation, with highest rates observed after 35 days for short rain season (Figure 33a) and 91 days for long rain season (Figure 33b), coinciding with early vegetative and silking stages, respectively.

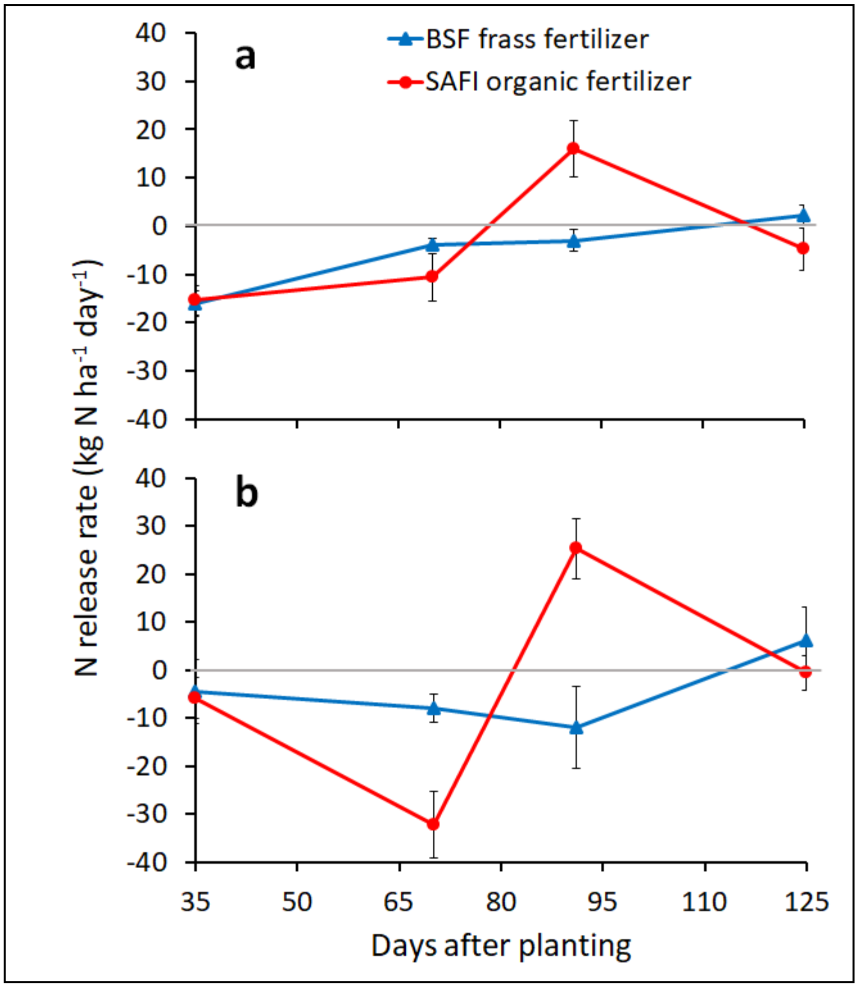

Figure 33: Mineral nitrogen release rate by soil amended with BSF frass and SAFI fertilizers during the short (a) and long rain (b) cropping seasons.

On the other hand, net N mineralization of up to 6 kg N ha^{-1} day^{-1} at 125 days of incubation was recorded in soil BSF frass fertilizer which coincided with the maturity stages. Soil amended with SAFI organic fertilizer immobilized significantly ($p < 0.001$) higher N (-32 kg N ha^{-1} day^{-1}) at the tasseling stage of the long rain season than soil amended with BSF frass fertilizer (Figure 33b). However, soil amended with SAFI organic fertilizer achieved higher and earlier net N mineralization (up to 25 kg N ha^{-1} day^{-1} after 91 days of incubation) compared to soil amended with BSF frass fertilizer (Figure 33).

The net N immobilization observed at the active stages of maize growth (Figure 33a and 33b) has been previously reported (Johnson et al., 2012; Loecke et al., 2012; Musyoka et al., 2019a) and could be largely attributed to the quality of organic inputs applied (Table 5). Fertilizers generated from insect frass contain high levels of labile organic carbon and organically bound ammonium nitrogen (Adin Yéton et al., 2019; Houben et al., 2020; Kagata & Ohgushi, 2012; Lalander et al., 2015), which requires time to be converted into a plant available form (NO_3^-) through the nitrification process. At the same time, ammonium nitrogen is a highly preferred by soil microorganisms making it prone to immobilization and denitrification pathways (Fornara et al., 2011).

Results from the current study are consistent with those of Li & Li (2014) who established that ammonium nitrogen is one of the major N fractions that influences the quantity of N released from organic fertilizers. Due to the high demand for available N by microorganisms, there is a high possibility that most of the ammonium N from BSF frass fertilizer was rapidly immobilized by microbes immediately after application (Fornara et al., 2011; Loecke et al., 2012), thereby, reducing N supply to the plant at the early growth stages.

Furthermore, some ammonium could have also been lost through denitrification (via nitrification) and volatilization especially in soils with pH values (>7.5) and high temperature (Bernal et al., 2009), thus reducing the quantity of available N for plant uptake at the early stages of maize growth. On the other hand, biochar consists of recalcitrant organic carbon that is resistant to microbial decomposition and, consequently, causes delays in N release (Kleber, 2010; Rovira & Vallejo, 2002). Biochar contains compounds such as lignin and polyphenols (Sanchez-Monedero et al., 2018) which have been reported to cause N immobilization (Kimetu et al., 2004; Musyoka et al., 2019a). Low N content, such as that observed in SAFI amended soils, has also been reported to stimulate N immobilization (Gómez-Muñoz et al., 2015), thereby, reducing the quantity of available N for plant use.

Furthermore, the low ammonium/nitrate ratio (0.43) and high nitrate concentration associated with SAFI organic fertilizer (Table 5) could have led to N leaching, especially during the long rain season, when moisture content was high (Loecke et al., 2012). High nitrate leaching (80% of total N), up to 1 m down the soil profile, has been reported in maize cropping systems in Kenya (Musyoka et al., 2019b) especially in soils with high sand and low organic matter levels comparable to the soils of this study (Table 1).

4.5.3 Effects of BSF frass and SAFI fertilizers on mineral N distribution in soil

The mineral N content in the topsoil depth (0 – 20 cm) varied significantly at different stages of maize growth during the short rain season ($p < 0.05$) (Figure 34a and 34b) with mineral N content ranging between 3 and 29 kg N ha^{-1} at early vegetative stage after which the amount decreased until the end of the season. Soil amended with BSF frass fertilizer maintained significantly ($p < 0.05$) higher mineral N content during the short rain season than that amended with SAFI organic fertilizer (Figure 34a) while in the long rain season, the mineral N amount increased to peak levels at the tasseling stage, with higher values recorded in BSF frass fertilizer treated soils than those treated with SAFI (Figure 34b).

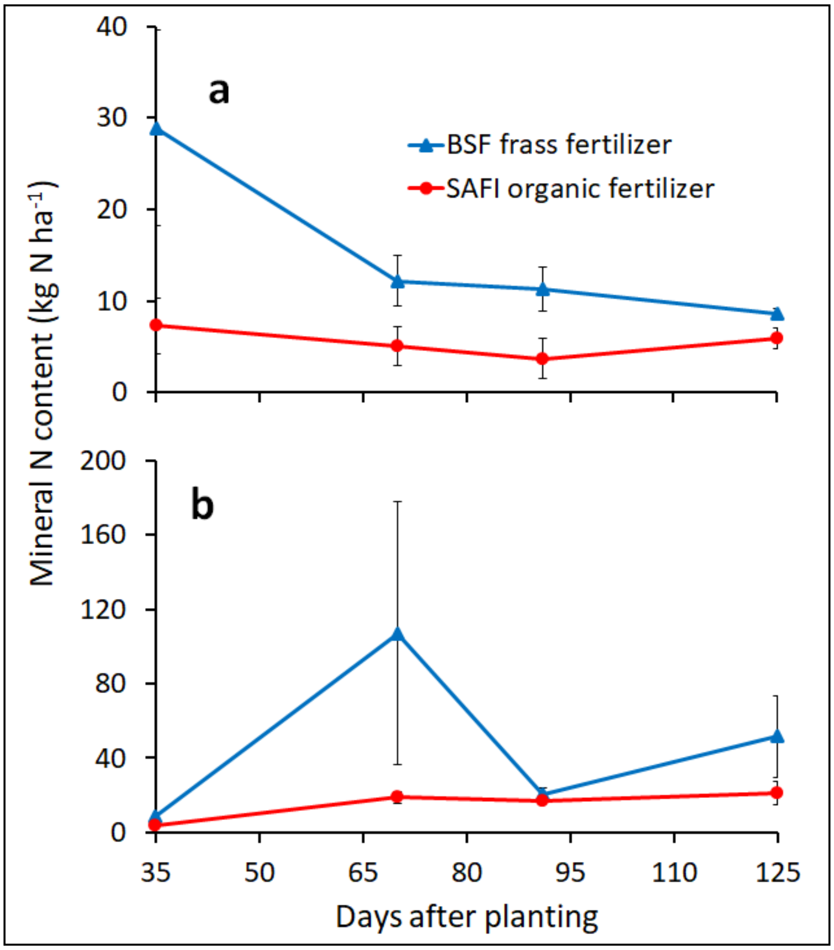

Figure 34: Soil mineral nitrogen content in the topsoil depth (0 – 20 cm) following amendment with BSF frass and SAFI fertilizers during the short (a) and long rain (b) cropping seasons.

Similarly, in the subsoil depth (20 – 40 cm), the organic fertilizers also showed significant differences in mineral N content at different maize growth stages during the short ($p < 0.001$) and long ($p < 0.001$) rain seasons (Figure 35a and 35b). At early vegetative stage (35 DAP), the mineral N amount lay between 3 – 42 kg N ha^{-1} but increased to peak levels (9 – 32 kg N ha^{-1}) at silking stage, from thereafter, the amount decreased up to the end of the experiments. However, the mineral N content of soil amended with BSF frass fertilizer decreased throughout the long rain season (Figure 35b).

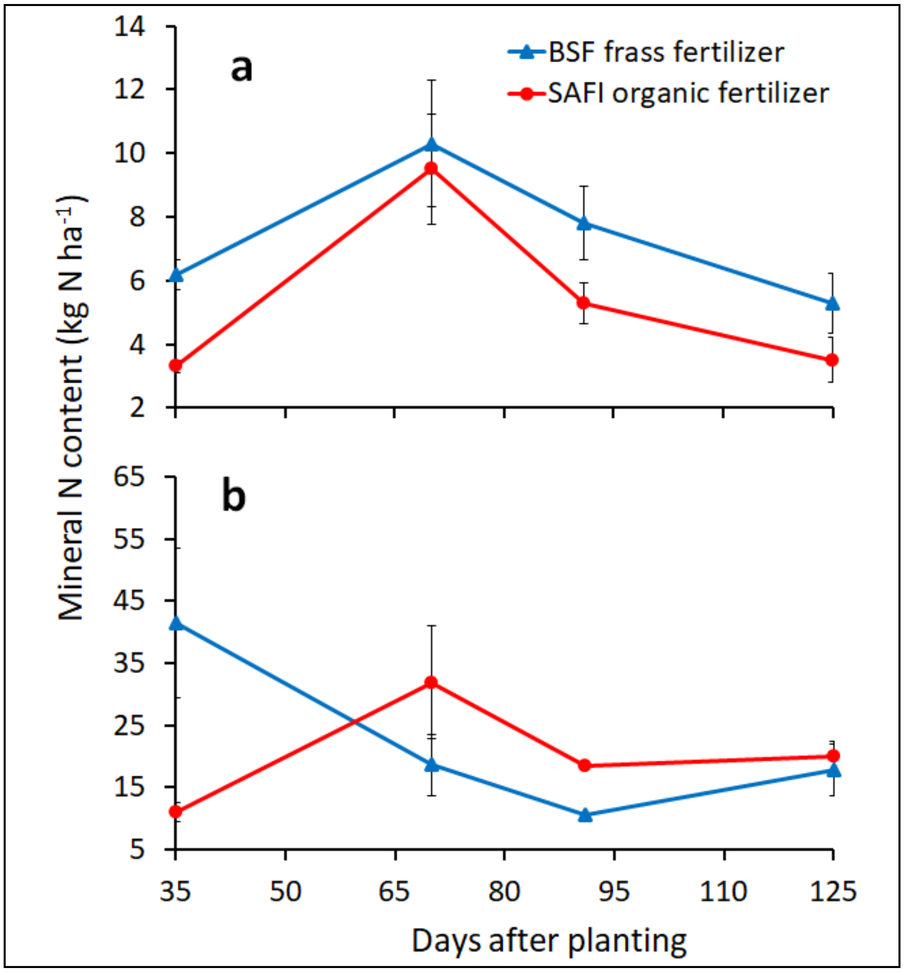

Figure 35: Soil mineral nitrogen content in the subsoil depth (20 – 40 cm) following amendment with BSF frass and SAFI fertilizers during the short (a) and long rain (b) cropping seasons.

Soil amended with BSF frass fertilizer attained significantly ($p < 0.001$) higher mineral N content than that treated with SAFI organic fertilizer at early vegetative stage during both seasons, and at the silking and harvesting stages during the short rain season. At the same time, the mineral N content in soil amended with SAFI organic fertilizer was significantly ($p < 0.001$) higher than that of soil amended with BSF frass fertilizer from tasseling stage to silking stage (Figure 35b) during the long rain season.

4.5.4 Effects of BSF frass and SAFI fertilizer on maize N uptake

There were significant differences in N uptake by maize grown in soil amended with organic fertilizers during the short ($p < 0.001$) and long rain ($p < 0.001$) seasons (Figures 36a and 36b). The N uptake increased significantly to peak levels (82 – 139 kg N ha^{-1}) at tasseling and silking stages, after which they decreased up to harvesting stage. Maize grown in soil amended with BSF frass fertilizer, accumulated significantly ($p < 0.001$) higher N levels at the silking stage in both seasons, but only at the harvesting stage during the short rain season (Figure 36a).

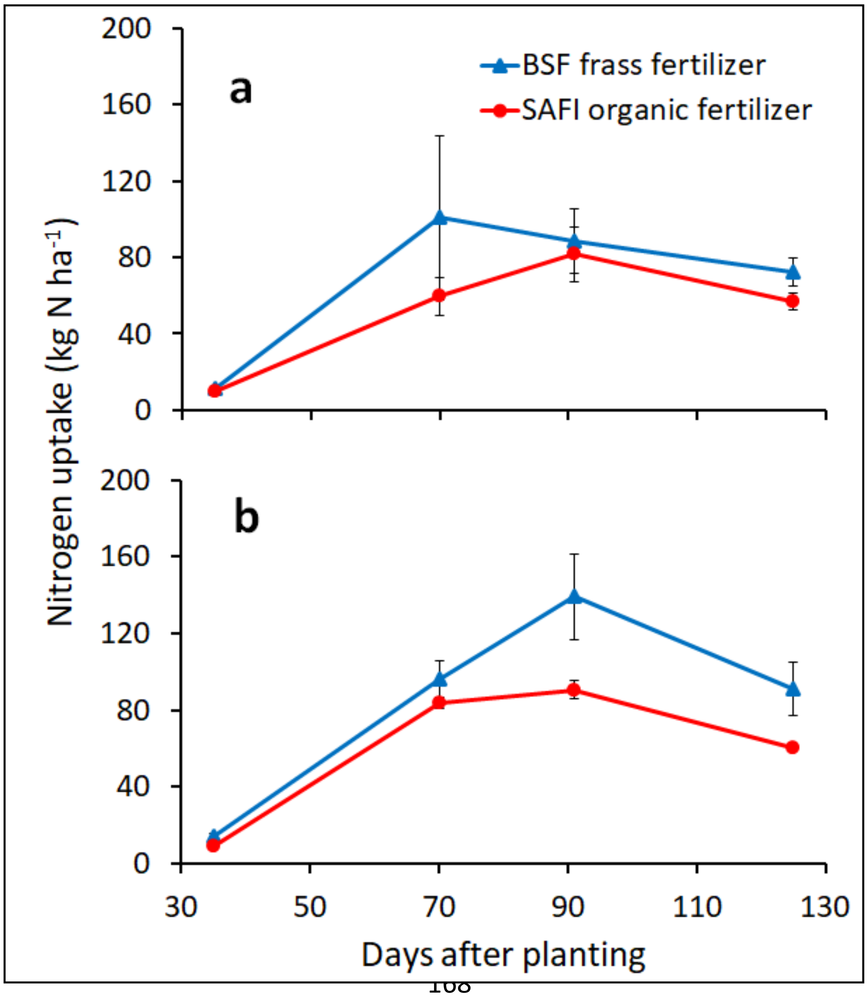

Figure 36: Nitrogen uptake by maize grown using BSF frass and SAFI fertilizers during the short rain (a) and long rain (b) cropping seasons.

The N uptake rates also varied significantly ($p < 0.01$) at different growth stages during the long rain season only (Figures 37a and 37b). The trends in N uptake rates followed those of N uptake, with peak values of between 0.4 and 1. kg N ha^{-1} day^{-1}. The N uptake rate of maize grown in soil amended with BSF frass fertilizer decreased to negative values (-1.2 kg N ha^{-1} day^{-1}) at the silking stage of the short rain season and at the harvesting stages during both seasons. On the other hand, maize grown in soil amended with SAFI organic fertilizer had positive N uptake rate at all stages of growth except at the harvesting stage during the short rain season where uptake rates of -0.4 kg N ha^{-1} day^{-1} were recorded.

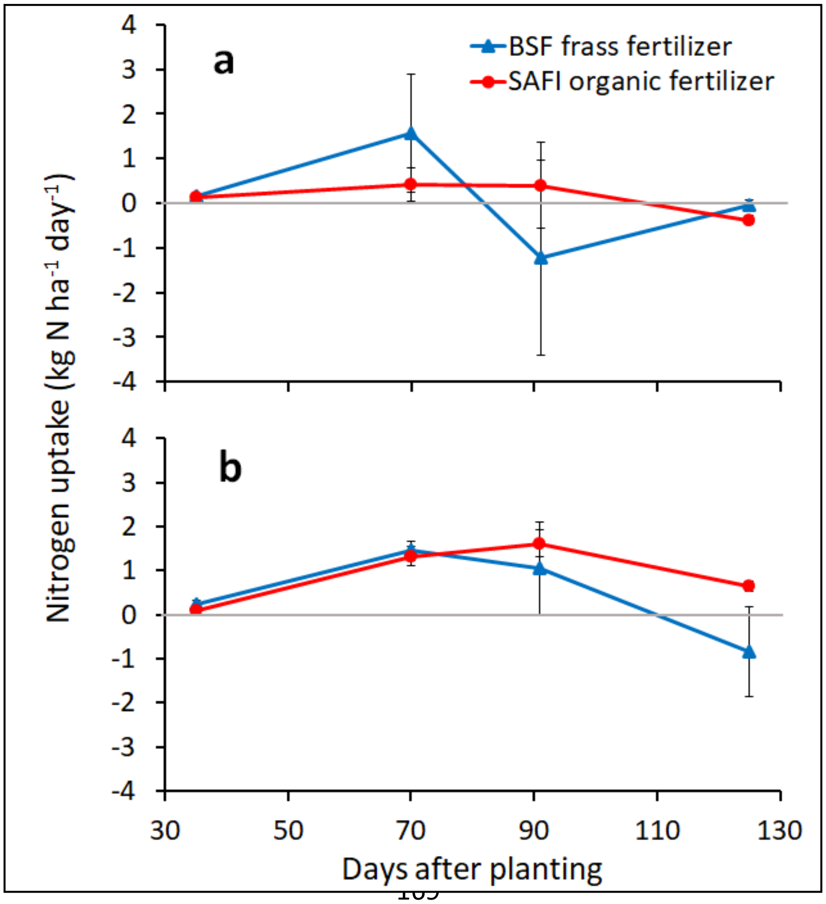

Figure 37: Nitrogen uptake rate by maize grown using BSF frass and SAFI fertilizers during the short rain (a) and long rain (b) cropping seasons.

4.5.5 Effect of BSF frass and SAFI fertilizers on degree of N synchrony

The different organic fertilizers showed significant variations in N flux during the short ($p < 0.01$) and long rain ($p < 0.001$) seasons (Figure 38a and 38b). Nitrogen fluxes for maize grown on soil amended with BSF frass fertilizer ranged between -16 and 7 kg N ha^{-1} day^{-1} while those of SAFI organic fertilizer treatments ranged between -34 and 24 kg N ha^{-1} day^{-1}. The N fluxes for BSF frass fertilizer amended treatments increased from early vegetative stage during the short rain season towards positive values (Figure 38a), while the N flux during the long rain season decreased with higher negative values up to the silking stage (Figure 38b).

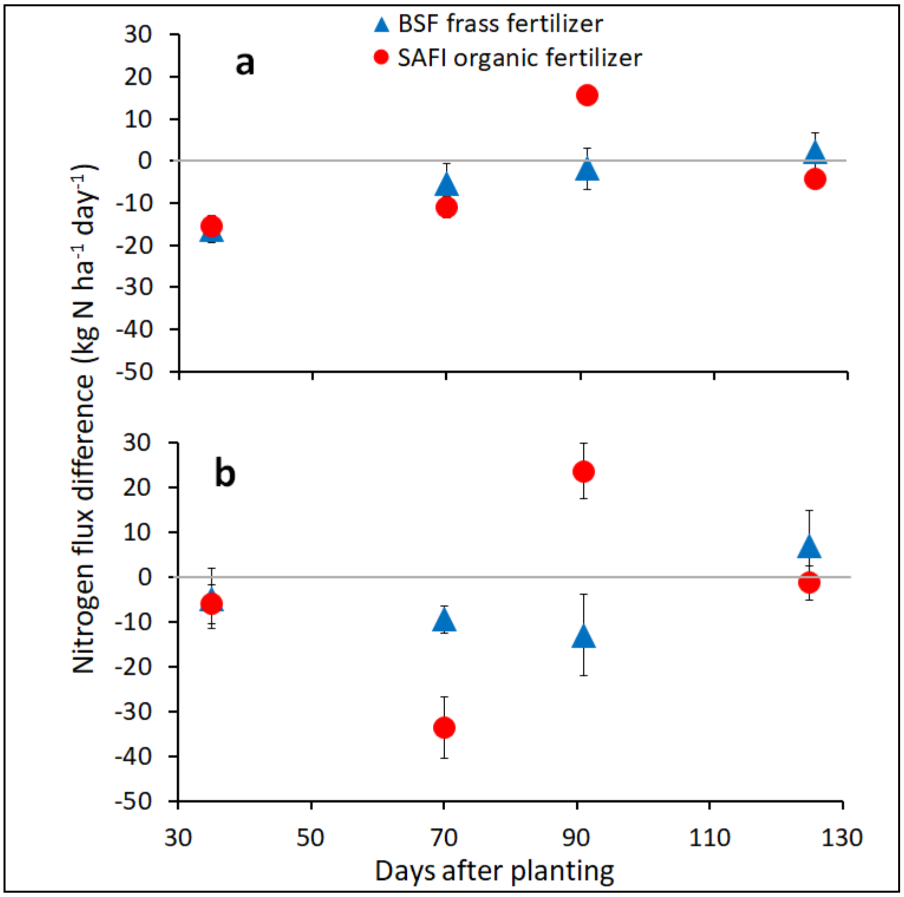

Figure 38: Nitrogen flux differences between N released by soil amended using BSF frass and SAFI fertilizers and N uptake by maize during the short (a) and long rain (b) cropping seasons.

Positive N flux differences for soil amended with BSF frass fertilizer were observed at the harvesting stages of both seasons. In comparison however, positive N flux differences for soil amended with SAFI organic fertilizer were attained at silking stage in both seasons (Figure 38a and 38b). Higher positive N flux differences were recorded during the long rain season compared to the short rain season. Soil amended with SAFI organic fertilizer produced significantly ($p < 0.001$) higher positive N flux differences than that amended with BSF frass fertilizer in both seasons.

It was noted that BSF frass fertilizer treatments had less negative N fluxes at the tasseling stage, whereas soil amended with SAFI organic fertilizer achieved positive N flux differences earlier (silking stage) than where BSF frass fertilizer was applied (maturity stage) (Figure 38a and 38b). The negative N fluxes observed during the active growth stages could be attributed to N immobilization and leaching, as earlier explained in section 4.5.2.

Previous research efforts have suggested supplementation with mineral N (Johnson et al., 2012; Cavalli et al., 2016; Musyoka et al., 2019a) or application of organic fertilizers before planting (Li & Li, 2014) to compensate for N immobilization during the early growth stages and periods of peak N demand. On the one hand, the present study established that an application of 2 – 16 kg ha^{-1} of mineral N at periods between the early vegetative and silking stages (35 – 91 days after planting) was necessary to compensate for the deficits in N release observed, while using the BSF frass fertilizer for maize production. On the other hand, 6 – 36 kg N ha^{-1} of mineral N fertilizer would be required between the early vegetative and tasseling stages (35 – 70 days after planting) to cause N synchrony for maize growth, while using SAFI organic fertilizer.

However, it is expected that with continued organic fertilizer application, mineral N release could increase gradually and consequently reduce the period of N immobilization. Conversely, the positive N flux difference observed at the silking stage (91 DAP) (Figure 38a and 38b) associated with SAFI organic fertilizer amendment could be explained by the increase in mineral N release (Figure 32 and 33) at 91 days of incubation. Such excesses in N could have resulted into N leaching to deeper subsoil layers (Musyoka et al., 2019b). However, the higher N uptake achieved by maize grown on soil amended with BSF frass fertilizer as compared with SAFI organic fertilizer (Figure 36 and 37) could be partly attributed to the higher mineral N content in the topsoil (Figure 34a and 34b).

It was also noted that peak N uptake by maize grown on soil amended with BSF frass fertilizer did not coincide with periods of positive N fluxes, indicating asynchrony (Figure 37). This mismatch has been previously attributed to N uptake from deeper soil layers or acquisition from N rich microsites in soil (Loecke et al., 2012; Musyoka et al., 2019a). Deeper plant root growth and spread due to the presence of plant growth promoting organisms and growth hormones, such as auxin and gibberellins, have been reported, while using insect frass as a biofertilizer (Poveda et al., 2019). Such behavior of root growth could have been crucial in nutrient acquisition by maize grown using BSF frass fertilizer. In addition, the higher N release and uptake in the second season (long rain season) could be attributed to residual effect of inputs applied in the first season and higher rainfall during the long rain season (Rovira & Vallejo, 2002; Musyoka et al., 2019a).

4.5.6 Nitrogen fertilizer equivalence values of BSF frass and SAFI fertilizers

The N fertilizer equivalence values (NFE) of organic fertilizers applied at different N rates varied significantly during the short ($p < 0.05$) and long rain ($p < 0.01$) seasons (Table 23). At equivalent N rates, BSF frass fertilizer had higher NFE values than SAFI organic fertilizer. During the short rain season, the NFE of BSF frass fertilizer applied at 30 kg N ha^{-1} was 3.8 and 4.4 times higher ($p < 0.05$) than that of SAFI organic fertilizer applied at 60 and 100 kg N ha^{-1}, respectively. Likewise, the NFE of BSF frass fertilizer applied at 60 kg N ha^{-1} was significantly ($p < 0.01$) higher than those of all SAFI organic fertilizer treatments which was even 5 times higher than the NFE of SAFI fertilizer applied at 60 kg N ha^{-1}.

Table 23: Nitrogen fertilizer equivalence values of BSF frass and SAFI fertilizers.

Fertilizer	Rate (kg N ha^{-1})	N fertilizer equivalence value (%)	
		Season 2019A (Short rains)	Season 2019B (Long rains)
BSF frass fertilizer	30	229.4 ± 28.4a	131.8 ± 34.3ab
	60	165.9 ± 32.7ab	151.0 ± 37.5a
	100	105.3 ± 17.7ab	110.6 ± 8.6ab
SAFI organic fertilizer	30	178.6 ± 59.7ab	3.5 ± 1.3c
	60	61.2 ± 21.9b	30.4 ± 10.9bc
	100	52.1 ± 5.3b	35.6 ± 6.5bc
	p value	*	**

Key: ** $p < 0.01$, * $p < 0.05$; 30, 60 and 100 = fertilizer application rates equivalent to 30, 60 and 100 kg N ha^{-1}. In the same column, means (± standard error) followed by the same letters are not significantly different at $p \leq 0.05$.

The NFE of BSF frass fertilizer treatments decreased with increase in N application rates, with highest values observed at the rate of 30 kg N ha^{-1} (Table 23). Increasing N rate of BSF frass fertilizer to 100 kg N ha^{-1} decreased the NFE by 2.2 and 1.2 times during the short and long rain seasons, respectively. However, NFE of SAFI organic fertilizer did not follow a consistent trend, whereby it decreased with increase in N rates during the short rain season but varied proportionally with N rates during the long rain season.

The higher nitrogen fertilizer equivalence values associated with the BSF frass fertilizer (Table 23) at all N rates than SAFI organic fertilizer are an indication of the high quality of this fertilizer as compared with the commercial organic fertilizer, SAFI. Our results are supported by previous studies which have reported improved drought tolerance, disease suppression, and higher crop growth yield, while using insect frass fertilizer (Houben et al., 2020; Kagata & Ohgushi, 2012; Poveda et al., 2019). Furthermore, the chitin contained in BSF frass fertilizers has been reported to improve plant health by stimulating disease resistance in crops (Quilliam et al., 2020). Such additional benefits provided by BSF frass fertilizer could have been responsible for the higher grain yields achieved from maize grown in soil amended with this fertilizer (BSF frass). The SAFI organic fertilizer is made of biochar and organic inputs with high recalcitrant carbon which are associated with N immobilization (Musyoka et al., 2019a). Furthermore, the SAFI organic fertilizer has a high nitrate concentration, that could have been lost through leaching, especially during the long rain season (Musyoka et al., 2019b).

The nitrogen fertilizer equivalence values (105 – 229%) of BSF frass fertilizer obtained in this study are comparable to those obtained by Kimetu et al. (2004), who used tithonia as organic fertilizer in the same study. Furthermore, the nitrogen fertilizer equivalence values of BSF frass fertilizer reported in the present study are higher than those previously reported for calliandra and senna (Kimetu et al., 2004), lucerne (De Notaris et al., 2018), poultry litter and yard waste compost (Bowden et al., 2007), and slurry from cattle (Cavalli et al., 2016; De Notaris et al., 2018; Lalor et al., 2011), and pigs (van Middelkoop & Holshof, 2017). On the contrary, the low and inconsistent nitrogen fertilizer equivalence recorded from SAFI organic fertilizer could be improved by combining it with a mineral N fertilizer to increase the N supply (Musyoka, et al., 2019a).

Whereas previous studies where nitrogen fertilizer equivalence values of different organic amendments were found to increase with an increase in N rates (Hijbeek et al., 2018), this study established that the nitrogen fertilizer equivalence values of BSF frass fertilizer decreased with an increase in N application rates (Table 23). The high value of nitrogen fertilizer equivalence obtained at low N rates implies that even at low application rates, the BSF frass fertilizer can perform equally good or even better than the mineral N fertilizer, and indication of its high quality as an organic fertilizer input (Quilliam et al., 2020). Findings from the present study are crucial in changing the negative attitude towards use of organic fertilizers, with the advantage of less bulkiness and high nutrient quality associated with the BSF frass fertilizer.

Subsequently, the heavy reliance on the highly expensive mineral N fertilizers could be lessened by adopting high-quality organic fertilizers such as BSF frass fertilizer.

4.6 Economic value of frass fertilizer production alongside BSF rearing and profitability of crop production using BSF frass fertilizer

4.6.1 Larval and frass fertilizer yields, gross and net income from BSF farming

The yields of BSF larva, BSF frass and economic output from the two BSF farming scenarios using sawdust, biochar and gypsum amended substrates are presented in Tables 24 and 25, respectively. Amending the rearing substrate with sawdust and > 5% gypsum decreased the gross income from BSF larvae by 4 – 29% (Table 24) and 8 – 24% (Table 25), respectively while substrate amendment with biochar increased the gross income generated from BSF larvae by 20 – 88% (Table 25). On the positive side, generating frass fertilizer from substrates amended with sawdust, gypsum and biochar increased the gross income generated from BSF farming by 4 – 6, 8 – 9 and 7 – 10 times, respectively, compared to if BSF are reared for larvae production alone. It was established that even without sawdust, biochar, or gypsum amendment of the substrates, the frass would raise the gross income by 4.5 – 6.6 times if converted into organic fertilizer (Tables 24 and 25). This implies that frass fertilizer is an important component of BSF farming.

For a farmer rearing BSF for both larvae and organic fertilizer production, the revenue from frass fertilizer accounted for 78 – 86% (Table 24) and 80 – 92% (Table 25) of the gross income generated from insect farming, with significantly ($p < 0.001$) higher values obtained from frass fertilizer generated from substrates amended with 20% sawdust, 15% gypsum or 20% biochar. This could be attributed to the significantly

($p < 0.001$) higher yields of frass fertilizer generated from substrates amended with 20% sawdust (Table 24), 15% gypsum and 20% biochar (Table 25).

Table 24: Economic returns from BSF production using rearing substrates amended with sawdust.

Rearing substrates	Cost of rearing substrate as % of production costs	Larval yield per tonne of dry substrate (kg)	Frass fertilizer yield (kg)	Income from BSF larvae (USD)	Income from BSF frass fertilizer (USD)	Gross income (USD)	Income from frass fertilizer as % of gross income %)	Net income (USD)		
								Substrate purchased & transported	Substrate transported only	Zero cost on substrate
					BSF farming for larvae production only					
BSG	89.3±0.0a	45.3±0.80a	na	40.7±0.7a	na	40.7±0.7c	na	-214.1±0.7c	-36.6±0.7c	13.4±7.1c
				BSF farming for larvae and frass fertilizer production						
BSG	86.7±0.0b	45.3±0.8a	472±22.8b	40.7±0.7a	141.7±6.8b	182.5±6.2b	77.6±1.1b	-79.9±6.2b	97.6±6.2b	147.6±6.2b
BSG+9SD	85.7±0.0c	43.2±1.2ab	451±26.6b	38.9±1.1ab	135.4±8.0b	174.3±8.1b	77.6±1.1b	-68.8±8.1b	84.0±8.1b	134.0±6.2b
BSG+20SD	84.3±0.0d	37.4±0.8bc	696±36.8a	33.6±0.7bc	208.8±1.1a	242.4±11.6a	86.1±0.5a	22.4±11.6a	145.9±11.6a	195.9±11.6a
BSG+30SD	82.6±0.0e	32.1±3.4c	555±23.3b	28.9±0.3c	166.5±7.0b	195.4±6.8b	85.2±1.6a	-50.7±6.8a	93.3±6.8b	143.3±6.8b
BSG+44SD	81.0±0.0f	34.2±0.4c	580± 9.2ab	30.9±0.4c	174±12ab	204.8±12ab	84.8±0.9a	21.1±11.8a	97.2±11.8b	147.2±11.8b
p value		***	***	***	***	***	***	***	***	***

Key: *** p < 0.001. BSG = brewery spent grains; SD = sawdust; BSG+9SD, BSG+20SD, BSG+32SD and BSG+44SD = amendment of brewery spent grain with sawdust at inclusion levels of 9, 20, 32 and 44% (weight/weight), respectively; na = not applicable. The amendments with sawdust at 9, 20, 32 and 44% corresponded to C/N ratios of 15, 20, 25 and 30, respectively. In the same column, means (± standard error) followed by the same letters are not significantly different at p ≤ 0.05.

Table 25: Economic returns from BSF production using rearing substrates amended with biochar and gypsum.

Rearing substrates	Cost of rearing substrate as % of production costs	Larval yield per tonne of dry substrate (kg)	Frass fertilizer yield (kg)	Income from BSF larvae (USD)	Income from BSF frass fertilizer (USD)	Gross income (USD)	Income from frass fertilizer as % of gross income (%)	Net income (USD)		
								Substrate purchased & transported	Substrate transported only	Zero cost on substrate
BSF farming for larvae production only										
BSG	89.6±0.0c	34.6±1.7cd	na	31.1±1.5cd	na	31.1±1.5f	na	-232.6±1.5c	-46.2±1.5c	37.9±1.5c
BSF farming for larvae and frass fertilizer production										
BSG	87.2±0.0h	34.6±1.7cd	585±17.1c	31.1±1.5cd	175.4±5.1c	206.5±4.9e	84.9±0.8abc	-64.7±4.9b	121.7±4.9b	171.7±4.9b
BSG+5GY	88.2±0.0f	36.3±1.2cd	696±30.5bc	32.6±1.1cd	208.9±9.2bc	241.6±8.5cd	86.4±0.9abc	-53.9±8.5b	132.5±8.5b	182.5±8.5b
BSG+10GY	89.1±0.0d	31.7±3.5cd	802±25.4ab	28.6±3.1cd	240.6±7.6ab	269.2±4.5bc	89.3±1.3ab	-50.6±4.5b	135.9±4.5b	185.6±4.5b
BSG+15GY	89.9±0.0a	26.1±3.8d	878±15.3a	23.5±3.4d	263.4±4.6a	286.9±2.7b	91.8±1.2a	-56.8±2.7b	129.6±2.7b	179.6±2.7b
BSG+5BC	87.9±0.0g	41.4±4.0bcd	633±34.3c	37.3±3.6bcd	190.0±10.3c	227.2±10.1de	83.6±1.6bc	-60.5±10.1b	125.9±10.1b	175.9±10.1b
BSG+10BC	88.5±0.0e	53.6±4.8abc	696±20.7bc	48.3±4.3abc	208.9±6.2bc	257.2±2.3bcd	81.2±1.8c	-47.1±2.3b	139.3±2.3b	189.3±2.3b
BSG+15BC	89.1±0.0d	63.4±6.3ab	759±8.6b	57.1±5.7ab	227.7±2.6b	284.8±4.8b	80.0±1.7c	-36.0±4.8ab	150.4±4.8ab	200.4±4.8ab
BSG+20BC	89.7±0.0b	64.9±8.4a	876±33.9a	58.4±7.6a	262.8±10.2a	321.1±9.4a	81.8±2.2c	-16.1±9.4a	170.3±9.4a	220.3±9.4a
p value	***	***	***	***	***	***	***	***	***	***

Key: *** $p < 0.001$. BSG = brewery spent grains; GY = Gypsum; BC = Biochar; BSG+5GY, BSG+10GY and BSG+15GY = amendment of brewery spent grains with gypsum powder at inclusion rates of 5, 10 and 15% (weight/weight), respectively; BSG+5BC, BSG+10BC, BSG+15BC and BSG+20BC = amendment of brewery spent grain with biochar at inclusion rates of 5, 10, 15 and 20%(weight/weight), respectively; na = not applicable. In the same column, means (± standard error) followed by the same letters are not significantly different at $p \leq 0.05$.

This study established that for every kg of BSF larvae, 10 – 17, 10 – 19, 19 – 34 and 12 – 15 kg of frass fertilizer were produced while using the unamended substrate, and substrates amended with sawdust, gypsum, and biochar, respectively. The cost of rearing substrate accounted for most expenses of BSF farming (81 – 90%) (Tables 24 and 25), whereby expenses incurred on substrates amended by sawdust were significantly ($p < 0.001$) lower than using unamended substrates. On the contrary, substrate amendment using biochar or gypsum significantly increased the cost of rearing substrate (Table 25). It was established that cost of purchasing and transporting the BSF rearing substrates, would lead to loss as indicated by the negative net income values (Tables 24 and 25). Results on net income show that BSF farming would still not be profitable even if the farmer only incurred the costs of transporting substrate but does not utilize the frass for organic fertilizer production. Contrarily, amending the substrate with 20% sawdust and generating organic fertilizer from the frass, can deliver little profits even if the BSF farmer incurs expenses of purchasing and transporting rearing substrate.

The net income generated from BSF farming increased positively in values when the cost of purchasing the rearing substrate was removed (Tables 24 and Table 25). However, this was only achieved in systems designed for larvae and frass fertilizer production, indicating that frass fertilizer is crucial for improving the cost-effectiveness of BSF rearing. Considering a scenario where the rearing substrate is available on the farm, i.e. no incurring expense on rearing substrate, the estimates indicate that generating frass fertilizer would increase the net income from BSF farming by 11 – 15, 4.8 – 4.9, and 5 – 6 times for substrates amended with sawdust (Table 24), gypsum and biochar (Table 25), respectively, compared to rearing BSF for larvae production alone.

The significantly ($p < 0.001$) higher net income achieved from substrates amended with 20% biochar could be attributed to the positive impact of biochar on both larval and frass fertilizer yields (Table 25). Biochar is a bulking agent and its amendment improves nutrients, moisture retention, and porosity of organic substrates, which are favourable for BSF larval growth and yield and frass fertilizer production (Sanchez-Monedero et al., 2018).

4.6.2 Gross margin, benefit-cost ratio and return on investment from BSF farming

When the costs on purchasing and transporting of BSF rearing substrate are incurred, this resulted into loss as indicated by negative values of gross margin (Figures 39a and 40a), benefit cost ratio (Figures 39b and 40b) and return on investment (Figures 39c and 40c). However, amending the rearing substrates with 20% sawdust and generating organic fertilizer from the frass could deliver little profits even if the farmer had to incurred expenses of purchasing and transporting rearing substrates.

Generating frass fertilizer as a second product would significantly ($p < 0.001$) increase gross margin, BSR and ROI compared to larval production alone (Figures 39 and 40). It was established that without the cost of rearing substrate i.e., incurring transportation costs only, there is potential to increase gross margin, benefit-cost ratio (BCR) and return on investment (ROI) if the frass is converted into an organic fertilizer (Figures 39 and 40). Nevertheless, the highest gross margin of 81 and 83%, BCR of 4.2 and 4.9 and ROI of 424 and 493% were attained using unamended substrate. Among the sawdust amended substrates, the substrates amended with 20% sawdust generated the highest gross margins (81%), BCR (4.2) and ROI (421%) (Figure 39a and 39c).

In rearing systems where the farmer incurs no cost or cost of transport alone, substrates amended with 20% sawdust generated significantly ($p < 0.001$) higher gross margins than substrates with 32 and 44% sawdust. Also, the BCR and ROI achieved using substrates amended with 20% sawdust were significantly higher than those of other amended substrates. On the other hand, substrates amended with 5% gypsum produced the highest gross margin (76%), BCR (3.1) and ROI (309%) among gypsum amended substrates, while of substrates amended with 5% biochar generated the highest gross margins of (77%), BCR (3.4) and ROI (343%) among the biochar amended substrates (Figure 40a – c).

The gross margins were not significantly different when the cost of transport was considered while using biochar and gypsum amendments to produce both larval and frass fertilizer (Figure 40). Furthermore, the unamended substrate generated significantly higher gross margin than substrates amended with 10 – 15% gypsum, and 10 – 20% biochar when the costs of substrate purchase and transportation were removed. While considering the cost of substrate transportation, unamended substrate generated significantly higher BCR and ROI than substrates amended with 10 – 15% gypsum, and 15 – 20 % biochar (Figure 40). When the costs of substrate purchase and transportation were removed, unamended substrate produced significantly higher BCR and ROI than other substrates. It was noted that increasing biochar and gypsum amendment rates caused significant decreases in BCR and ROI (Figure 40).

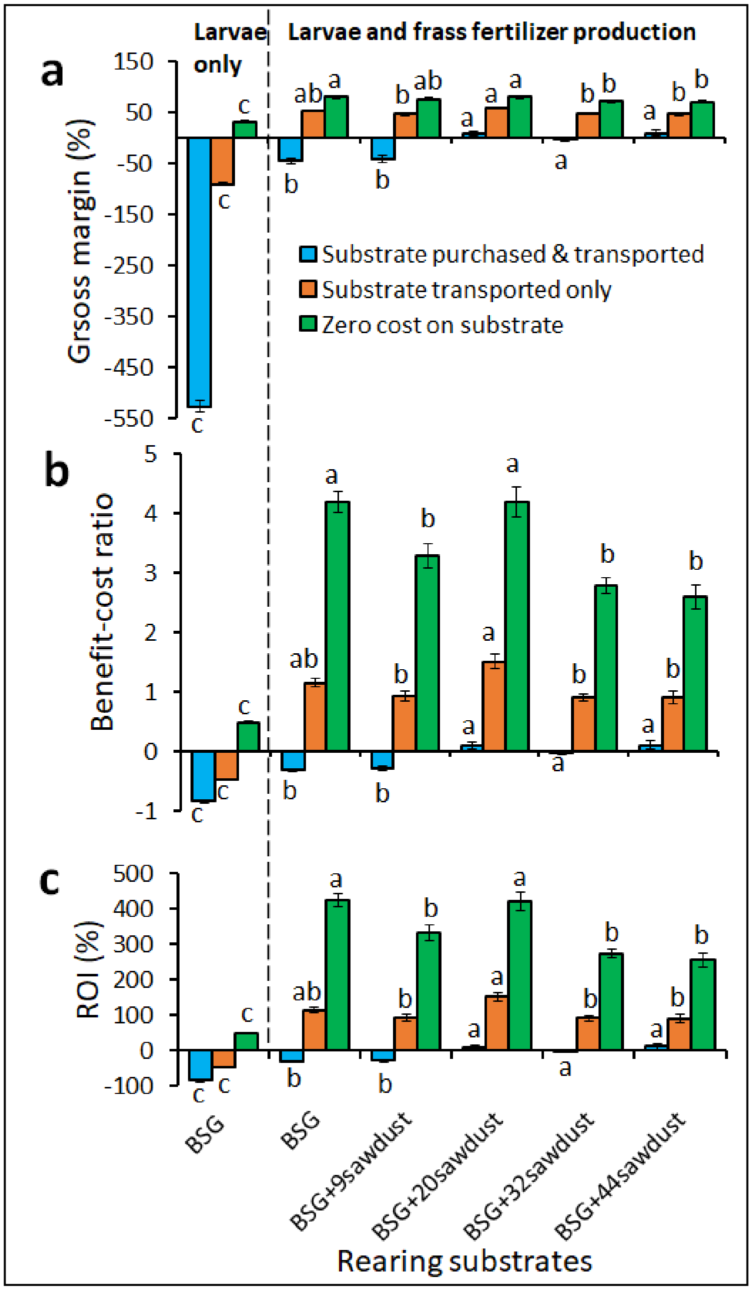

Figure 39: Gross margins (a), benefit-cost ratio (b) and return on investment (ROI) (c) on two BSF farming scenarios using rearing substrates amended with sawdust.

Key: BSG = brewery spent grains; 9sawdsut, 20sawdust, 32sawdust and 44sawdust = amendment of brewery spent grain with sawdust at inclusion levels of 9, 20, 32 and 44% (weight/weight), respectively. The amendments with sawdust at 9, 20, 32 and 44% corresponded to C/N ratios of 15, 20, 25 and 30, respectively. 5gypsum, 10gypsum and 15gypsum = amendment of brewery spent grains with gypsum powder at inclusion rates of 5, 10 and 15% (weight/weight), respectively; 5biochar, 10biochar, 15biochar and 20biochar = amendment of brewery spent grain with inclusion rates of 5, 10, 15 and 20% (weight/weight) gypsum, respectively. Per parameter and per panel, means (±standard error) followed by the same letters are not significantly different at $p \leq 0.05$.

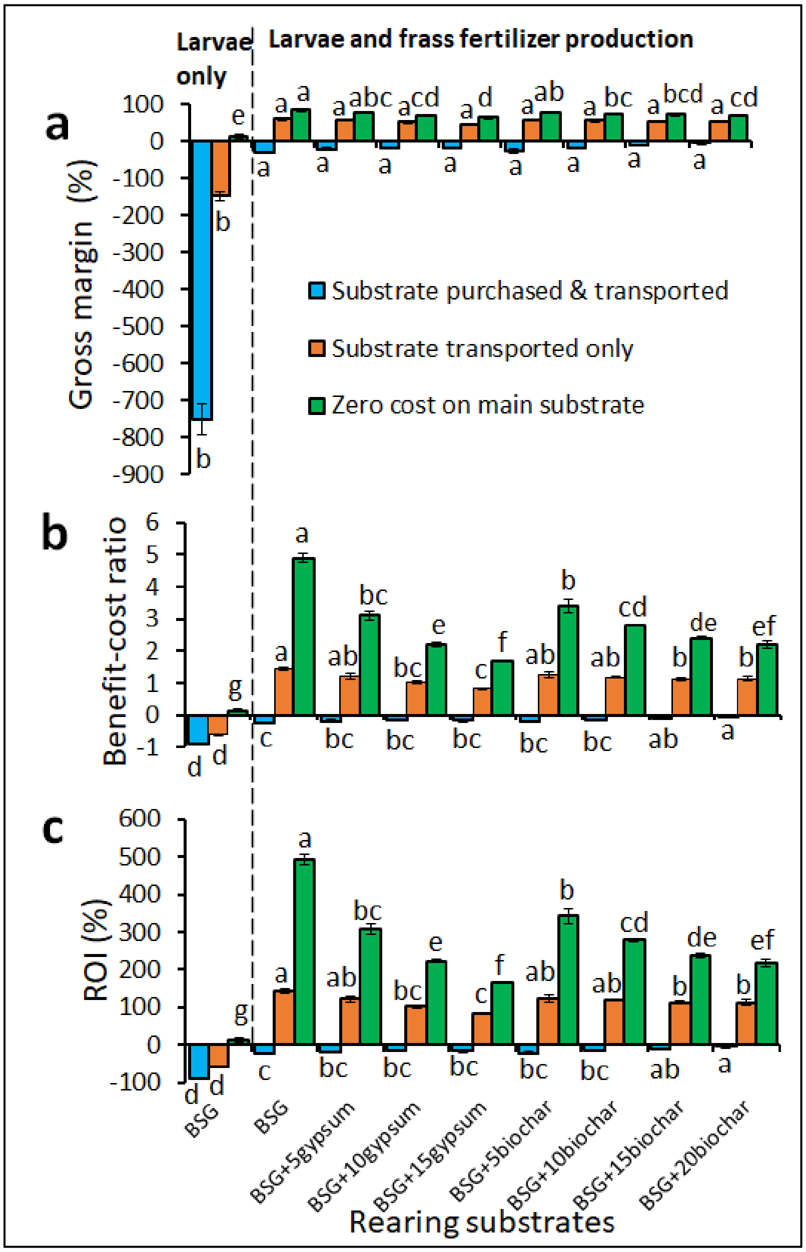

Figure 40: Gross margins (a), benefit-cost ratio (b) and return on investment (ROI) (c) on two BSF farming scenarios using rearing substrates amended with gypsum and biochar.

Key: BSG = brewery spent grains; 9sawdsut, 20sawdust, 32sawdust and 44sawdust = amendment of brewery spent grain with sawdust at inclusion levels of 9, 20, 32 and 44% (weight/weight), respectively. The amendments with sawdust at 9, 20, 32 and 44% corresponded to C/N ratios of 15, 20, 25 and 30, respectively. 5gypsum, 10gypsum and 15gypsum = amendment of brewery spent grains with gypsum powder at inclusion rates of 5, 10 and 15% (weight/weight), respectively; 5biochar, 10biochar, 15biochar and 20biochar = amendment of brewery spent grain with inclusion rates of 5, 10, 15 and 20% (weight/weight) gypsum, respectively. Per parameter and per panel, means (±standard error) followed by the same letters are not significantly different at $p \leq 0.05$.

The higher values of gross margins (53 – 83%) and returns on investment of > 1 obtained when using unamended substrate, and substrates amended with 9 – 20% sawdust (Figure 39c), 5 – 10% gypsum, and 5 – 20% biochar (Figure 40c) indicate that with or without substrate amendment, generating organic fertilizer as a second product from BSF farming has high potential for increasing cost-effectiveness of insect rearing systems (Makkar et al., 2014; Chia et al., 2019a; Singh & Kumari, 2019).

Since the net income generated while using substrate amended with either 20% sawdust (Table 24), 10% gypsum or 20% biochar (Table 25) was greater than that obtained while using unamended substrate, substrate amendment is recommended for higher profit margins. Such innovations in BSF rearing have potential for maximizing profits from BSF farming and improving the sustainability of insect farming as a business (van Huis, 2013).

4.6.3 Economic value of BSF frass fertilizer on maize production

Maize grown using 100 kg N ha^{-1} of frass fertilizer generated higher gross income than where the commercial organic (24 – 48%) and mineral fertilizer (urea) (8 – 32%) were applied (Table 26). However, maize grown using 100 kg N ha^{-1} of urea generated significantly ($p < 0.001$ higher net income than that grown using the BSF frass and SAFI organic fertilizers. Also, maize grown using urea fertilizer treatments produced significantly ($p < 0.001$) higher BCR and ROI than those grown using where SAFI and BSF frass fertilizers. Maize grown using 30 kg N ha^{-1} of BSF frass fertilizer achieved significantly higher BCR and ROI than where 100 kg N ha^{-1} of SAFI was applied. The gross margin achieved using urea fertilizers was significantly ($p < 0.001$) higher than those of other treatments, except where 30 kg N ha^{-1} of BSF frass fertilizer.

In comparison however, the gross margin achieved using 30 kg N ha^{-1} of BSF frass fertilizer was significantly ($p < 0.001$) higher compared to other organic fertilizer treatments, except where 30 kg N ha^{-1} of SAFI was applied. The findings on net income are consistent with previous studies which reported higher net income from maize treated with mineral fertilizer than where organic fertilizers were applied (Mucheru-Muna et al., 2014). It was noted that maize grown using 30 kg N ha^{-1} applied as BSF frass fertilizer produced significantly ($p < 0.001$) higher net income than all the SAFI treatments. The values of net income obtained in the current study are higher than those reported by Mucheru-Muna et al. (2007) who used organic fertilizers generated from agroforestry trees for maize production in central Kenya. Results of BCR indicated that it is profitable to apply frass fertilizer at rates equivalent to 30 kg N ha^{-1} (Table 26).

Frass fertilizer applied at a rate of 2.5 t ha^{-1} generated the highest net income, that was significantly ($p < 0.001$) higher than the value achieved using 5 t ha^{-1} of SAFI by 4.5 folds. Also, the net income generated using 2.5 t ha^{-1} frass fertilizer was 33% higher than that obtained using equivalent rate of the commercial organic fertilizer (Table 27). Maize grown using 2.5 t ha^{-1} of frass fertilizer generated significantly higher BSR and Roi than SAFI treatments, except where 2.5 tha^{-1} of SAFI was applied. Also, the profit margin achieved using 2.5 t ha^{-1} of frass fertilizer was significantly ($p < 0.001$) higher compared to other sole organic fertilizer treatments, except where 5 t ha^{-1} of frass fertilizer and 2.5 t ha^{-1} of SAFI were applied. The decrease in net income, BCR (< 1), ROI and profit margins at rates beyond 2.5 t ha^{-1} or 30 kg N ha^{-1} suggest that it may be less profitable to apply purchased BSF frass fertilizer at higher rates.

Table 26: Economic returns from maize production if BSF frass fertilizer is bought at same price as the commercial organic fertilizer (SAFI) and applied in terms of nitrogen rates.

Treatment	Grain yield (kg ha^{-1})	Gross income (USD)	Net income (USD)	Benefit-cost ratio	Return on investment (%)	Gross margin (%)
30N BSF	5,077 ± 180.9 abc	2,038 ± 64.5abcd	1,233 ± 65.5bcd	1.5 ± 0.08b	153.3 ± 8.0b	60.3 ± 1.3ab
60N BSF	5,768 ± 227.4a	2,324 ± 95.9ab	1,091 ± 95.9cde	0.9 ± 0.08bc	88.4 ± 7.8bc	46.4 ± 2.4c
100N BSF	5,937 ± 180.8a	2,417 ± 74.6a	548 ± 74.6f	0.3 ± 0.04c	29.3 ± 4.0c	22.3 ± 2.5d
30N SAFI	4,048 ± 296.2c	1,635 ± 111.8d	959 ± 111.8def	1.4 ± 0.17b	141.8 ± 16.5b	57.8 2.7±b
60N SAFI	4,242 ± 255.7c	1,733 ± 93.5d	757 ± 93.5ef	0.8 ± 0.10bc	77.5 ± 9.6bc	42.8 ± 3.5c
100N SAFI	4,809 ± 177.0abc	1,952 ± 69.4bcd	756 ± 69.4f	0.4 ± 0.05c	41.9 ± 5.0c	29.1 ± 2.5d
30N UREA	4,481 ± 411.4bc	1,836 ± 141.0cd	1,425 ± 141.0abc	3.5 ± 0.34a	346.4 ± 34.3a	76.9 ± 1.94a
60N UREA	5,039 ± 235.2abc	2,035 ± 93.6abcd	1,589 ± 93.6ab	3.5 ± 0.21a	355.9 ± 21.0a	77.8 ± 1.10a
100N UREA	5,588 ± 210.4ab	2,242 ± 77.3abc	1,748 ± 77.3a	3.6 ± 0.16a	354.3 ± 15.7a	77.8 ± 0.76a
p value	***	***	***	***	***	***

Key: *** $p < 0.001$. 30N BSF, 60N BSF and 100N BSF = application of BSF frass fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; 30N SAFI, 60N SAFI and 100N SAFI = application of SAFI organic fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; 30N urea, 60N urea and 100N urea = application of urea fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; BSF = Black soldier fly frass fertilizer; SAFI = commercial organic fertilizer. In the same column, means (± standard error) followed by the same letters are not significantly different at $p \leq 0.05$.

Table 27: Economic returns from maize production if BSF frass fertilizer is bought at same price as the commercial organic fertilizer (SAFI) and applied in terms of tonnage.

Treatment	Grain yield (kg ha^{-1})	Gross income (USD)	Net income (USD)	Benet-cost ratio	Return on investment (%)	Gross margin (%)
2.5 BSF	4,972 ± 188.5ab	2,022 ± 68.6ab	896.1 ± 75.1a	0.80 ± 0.07a	79.6 ± 6.7a	43.9 ± 2.1ab
5 BSF	5,826 ± 467.3ab	2,370 ± 158.9ab	493.5 ± 174.1abc	0.30 ± 0.09bc	26.3 ± 9.3bc	18.2 ± 7.3bc
7.5 BSF	6,252 ± 497.6a	2,527 ± 194.1a	-98.6 ± 212.7cd	-0.04 ± 0.08c	-3.8 ± 8.1c	-7.5 ± 8.6cd
2.5 SAFI	4,396 ± 295.6b	1,800 ± 113.2b	673.9 ± 124.0ab	0.60 ± 0.11ab	59.9 ± 11ab	36.2 ± 3.8b
5 SAFI	5,190 ± a195.5b	2,075 ± 67.8ab	198.6 ± 74.3bcd	0.10 ± 0.04bc	10.6 ± 4.0bc	9.0 ± 3.2c
7.5 SAFI	5,436 ± 421.3ab	2,226 ± 153.2ab	-400.1 ± 167.8d	-0.20 ± 0.06c	-15.2 ± 6.4c	-21.8 ± 10.1d
p value	***	***	***	***	***	***

Key: *** $p < 0.001$. 2.5 BSF, 5 BSF and 7.5 BSF = application of BSF frass fertilizer at rates of 2.5, 5 and 7.5 t ha^{-1}, respectively; 2.5 SAFI, 5 SAFI and 7.5 SAFI = application of BSF frass fertilizer at rates of 2.5, 5 and 7.5 t ha^{-1}, respectively; BSF = Black soldier fly frass fertilizer; SAFI = commercial organic fertilizer. In the same column, mean (± standard error) followed by the same letters are not significantly different at $p \leq 0.05$.

Considering, a farmer who produces own frass fertilizer (homemade) and uses it for crop production (i.e., incurs labour costs for making the frass fertilizer at 45 USD per tonne), the net income would increase by 30, 67 and 232% for maize grown using 30, 60 and 100 kg N ha^{-1}, respectively compared to purchasing the same frass fertilizer (Table 28). Estimates indicated that this approach of using homemade BSF frass fertilizer could generate higher net income from maize production compared to using urea fertilizer. Likewise, using homemade frass fertilizer would increase the profitability of maize production by 71 and 259% increase in net income from maize grown using 2.5 and 5 t ha^{-1} of homemade frass fertilizer, respectively (Table 28).

Table 28: Economic returns from maize production if BSF frass fertilizer is made from the farm.

BSF frass fertilizer treatments	Fertilizer rates (kg N ha^{-1})			
	Net income (USD)	Benefit-cost ratio	Return on investment (%)	Gross margin (%)
30	1,598 ± 64.5a	3.63 ± 0.15a	363 ± 14.7a	78.3 ± 0.69a
60	1,819 ± 95.9a	3.61 ± 0.19a	361 ± 19.0a	78.1 ± 0.97ab
100	1,817 ± 74.6a	3.03 ± 0.12b	303 ± 12.4b	75.0 ± 0.80b
p value	ns	*	*	*
	Fertilizer rates (t ha^{-1})			
2.5	1,534 ± 75.1a	3.1 ± 0.15a	314 ± 15.4a	75.7 ± 0.92a
5	1,769 ± 174.1a	2.9 ± 0.29a	294 ± 29.0a	73.8 ± 2.33a
7.5	1,814 ± 212.7a	2.5 ± 0.30a	254 ± 29.8a	70.8 ± 2.33a
p value	ns	ns	ns	ns

Key: * $p < 0.05$, ns = nonsignificant ($p \geq 0.05$). 30, 60 and 100 = application of BSF fertilizer at rates equivalent to 30, 60 & 100 kg N ha^{-1}, respectively; 2.5, 5 and 7.5 = application rates of BSF fertilizer at 2.5, 5 and 7.5 t ha^{-1}, respectively; BSF = Black soldier fly frass fertilizer. In the same column, mean (± standard error) followed by the same letters are not significantly different at $p \leq 0.05$.

The net income generated at different N application rates of BSF frass fertilizer did not vary significantly (Table 28). Furthermore, the net income, BCR, ROI and gross margin did not vary significantly when BSF frass fertilizer was applied at sole rates. It was noted that application of BSF fertilizer at 30 and 60 kg N ha^{-1} would generate significantly higher values of benefit-cost ratio ($p = 0.024$) and ROI ($p = 0.025$) than using 100 kg N ha^{-1} of the same fertilizer. Also, the gross margin achieved using 30 kg N ha^{-1} of BSF frass fertilizer was significantly ($p = 0.024$) higher than the value obtained using 100 kg N ha^{-1}.

The higher BCR (2.5 – 3.1), ROI and gross margins (71 – 76%) (Table 28) imply that application of frass fertilizer alone could increase economic gains if the farmer makes own organic fertilizer. Results from the current study are consistent with other researchers who established that proper utilization of organic resources can boost crop productivity (Rusinamhodzi et al., 2013, 2016). The approach of using homemade frass fertilizer would contribute to the concept of circular economy, whereby the frass from BSF farming is converted into organic fertilizer for use in crop fields. Such innovative approaches have potential to improve farm productivity through sustainable use of available resources (Vanlauwe et al., 2014).

4.6.4 Economically optimum frass fertilizer rates and stochastic dominance analysis

The BSF frass fertilizer had a lower optimum N rate (79 kg N ha^{-1}) than urea (100 kg N ha^{-1}) and SAFI fertilizers (120 kg N ha^{-1}) (Figures 4a – c). On the other hand, the economic optimum rate of sole BSF frass fertilizer (7.4 t ha^{-1}) was comparable to that of sole SAFI organic fertilizer (7.5 t ha^{-1}) (Figures 4c and d).

The lower economically optimum N rate achieved using of frass fertilizer (Figures 4a – c) implies that one would incur less fertilizer expenses while using frass fertilizer for maize production compared to using urea and SAFI where higher quantities of fertilizers required. Although the economic optimum rate of sole frass fertilizer (7.4 t ha^{-1}) was comparable to that of sole commercial organic fertilizer (7.5 t ha^{-1}) (Figure 4c and 4d), it was found that using frass fertilizer would increase grain yield by 15% compared to commercial organic fertilizer (Table 27). The results of economic optimum rates are higher than the rates which produced the highest net profits, returns on investment and gross margins because the model used to determine optimum yields only considers the cost of fertilizer (Naher et al., 2011; Bachmaier & Gandorfer, 2012) and does not take into account the other costs of maize production such as labour.

To determine if the BSF frass fertilizers would be preferred to commercial organic fertilizers, first order stochastic dominance analysis was performed on the grain yield obtained from each fertilizer treatment. The results of the stochastic dominance analysis (Figure 41) indicate that for all fertilizers, grain yield cumulative distribution with frass fertilizer is to the right of yield distributions of SAFI organic and urea fertilizers, implying that yield with frass fertilizer absolutely holds first-order stochastic dominance over those from commercial organic and urea treated plots. This, therefore, indicates that when compared to the SAFI commercial organic fertilizer and urea, application of frass fertilizer produced higher maize grain yields.

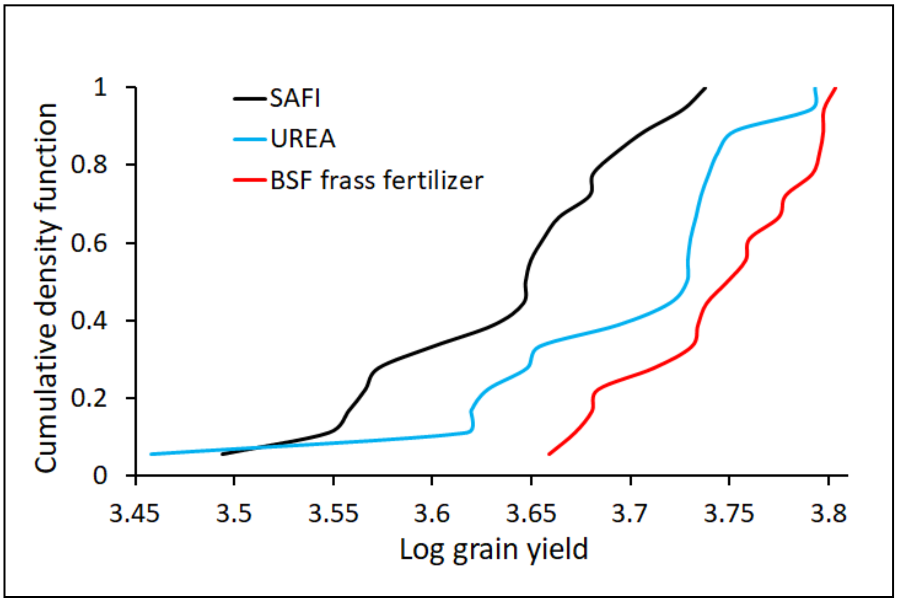

Figure 41: Stochastic dominance analysis of impact of BSF frass fertilizer on maize production.

The yield distribution from plots amended with urea fertilizer dominated yield distributions of commercial organic fertilizer, an indication that use of urea fertilizer produces significantly higher grain yields than where commercial organic fertilizer is applied, as indicated by results of the two-sample Kolmogorov- Smirnov test (Table 29). Grain yield from plots treated with BSF frass fertilizer and urea fertilizer did not vary significantly, indicating that BSF frass fertilizer can perform equally good as urea. Therefore, frass fertilizer could be a complete or partial substitute to the scarce yet low-quality organic resources used in most Sub-Saharan African countries (Ndambi et al., 2019).

Table 29: Two sample Kolmogorov-Smirnov statistical test for first-order stochastic dominance.

Fertilizer treatments	Difference	p value
BSF frass fertilizer vs SAFI organic	0.67	0.001
Urea vs SAFI organic	0.50	0.022
BSF frass fertilizer vs urea	0.39	0.131

CHAPTER FIVE: SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary of the key findings

i. It was established that the critical carbon to nitrogen ratio for optimal black soldier fly (BSF) growth and quality organic fertilizer production is 15 as depicted by the highest concentrations of nitrogen and phosphorus in frass compost, and minimal reduction in the yields of BSF larvae reared on substrates with C/N ratio of 15.

ii. Amending the BSF rearing substrate with biochar and gypsum improved nutrient retention in frass compost compared to the unamended substrate. Whereas substrate amendment with biochar increased BSF larval yields (13 – 88%), amendment with gypsum decreased larval yields by 0.6 – 30%.

iii. Using the BSF larvae to recycle organic substrates shortened the compost maturity time to 5 weeks compared to 8 – 24 weeks in conventional composting methods.

iv. Soil amended with BSF frass fertilizer had more mineral N in topsoil which resulted in higher maize growth, chlorophyll concentration and N uptake than the commercial organic and mineral fertilizer treatments. However, the N release from both organic fertilizers during the early crop growth stages was slow, which led to insufficient N synchrony at periods of peak N demands.

v. Application of sole BSF frass fertilizer at 7.5 t ha^{-1} produced the highest maize grain yield (7.2 t ha^{-1}), which was 17% higher than the yield achieved when using equivalent rate of the commercial organic fertilizer (SAFI).

vi. Furthermore, maize grain yield of 6.2 t ha^{-1} from plots treated with 100 kg N ha^{-1} of BSF frass fertilizer was 27 and 7% higher than the highest yields obtained using equivalent rates of commercial organic fertilizer and urea, respectively.

vii. The agronomic nitrogen use efficiency (AE_N) of maize treated with 30 kg N ha^{-1} of BSF frass fertilizer was 27 and 116% higher than those attained using equivalent rates of the commercial organic fertilizer and urea, respectively. Likewise, the AE_N of maize treated with 2.5 t ha^{-1} of BSF frass fertilizer was 2.4 times higher than that achieved using a similar rate of the commercial organic fertilizer.

viii. The nitrogen fertilizer equivalence of BSF frass fertilizer was 1.3 – 37 times higher than that of the commercial organic fertilizer applied at equivalent rates.

ix. Generating frass fertilizer as a second product from BSF farming would increase the profitability of insect farming between 5 and 15 folds. It was estimated that the production of 1 tonne of dry BSF larvae would generate an income of US$ 900. An estimated 10 – 34 tonnes of frass fertilizer would be produced from the frass obtained above with an additional cost of US$ 100 – 330. The income from the 10 – 34 tonnes of frass fertilizer would be worth US$ 3,000 – 10,200.

x. Maize grown on plots treated with frass fertilizer yielded between 29 and 44% higher net income compared to plots with commercial organic fertilizer. The direct use of frass fertilizer by smallholder insect farmers for maize production would generate 30 – 259% higher net income compared to farmers purchasing similar frass fertilizer.

5.2 Conclusions

1. This study has demonstrated that production of organic fertilizer using the BSF larvae is a promising technology worth exploring in the low-income countries of Sub-Saharan Africa where mineral fertilizer use is still limited due to high prices, low availability, and less effective if applied alone.

2. The ability of BSF larvae to develop on substrates with different C/N ratios, and various amendment rates of biochar and gypsum implies that with properly formulated feedstocks, the BSF can be successfully utilized for both larvae and organic fertilizer production without significantly affecting larval yields.

3. The ability of BSF larvae to recycle organic substrates into high-quality fertilizer within 5 weeks indicates the efficiency of this technology, and potential to benefit many farmers within a short period.

4. The higher maize growth performance, grain yield, N uptake, and N use efficiency associated with BSF frass fertilizer indicate superior agronomic performance compared to commercial fertilizers assessed during the study.

5. The high nitrogen fertilizer equivalence values of BSF frass fertilizer imply that it can be a complete or partial substitute to existing commercial fertilizers used in most farming practices. In a nutshell, the advantages of BSF frass fertilizer such as shorter production time and less purchase and/or production costs, higher N mineralization rate, increased nutrient availability for plant uptake, improved soil moisture storage and presence of plant growth promoting organisms, make it a better alternative or potential complement to the conventional fertilizers.

vi. The higher economic returns achieved in BSF farming systems designed for both larval and organic fertilizer production confirm that frass fertilizer is vital for improving the cost-effectiveness of BSF farming.

vii. The higher economic gains from maize production achieved while using BSF frass fertilizer indicate ability to improve profitability of fertilizer use when BSF larvae are used to produce frass fertilizer compared to commercially available organic and inorganic fertilizers. The additional use of frass fertilizer for crop production would generate much higher profits and close the nutrient cycles on the farm. Adoption of such production technologies would sustainably increase farm productivity.

5.3 Recommendations

1. For a farmer targeting to generate frass compost as a second product from black soldier fly rearing without significantly reducing larval yields, a substrate C/N ratio of 15 is recommended for generation of nutrient rich compost. A substrate with C/N ratio of 20 is recommended for farmers majorly targeting organic fertilizer production using the black soldier fly larvae.

2. Amendment of black soldier fly rearing substrates with 20% biochar is recommended for increased yields of BSF larvae and frass compost as well as higher nutrient retention in frass compost and economic returns. Gypsum amendment (10 – 15%) is suggested for farmers targeting frass compost production only.

3. A composting period of four weeks after larval harvesting is recommended to convert black soldier fly frass into mature and stable compost for field application.

4. To achieve high maize grain yield and net income while using purchased black soldier fly frass fertilizer, rates of 2.5 t ha^{-1} and 30 kg N ha^{-1} are recommended for sole and combined application, respectively. Higher grain yields and net incomes could be obtained at 7.5 t ha^{-1} and 100 kg N ha^{-1} but this is only economically viable for farmers who make frass fertilizer and use it directly on the farm.

5. Inorganic N supplementation could be necessary to compensate for N immobilization observed at the earlier growth stages of maize treated BSF frass and SAFI organic fertilizers.

5.4 Areas for future research

1. The current studies only determined the amount of ammonium retained in the frass but did not measure gaseous N losses in form of ammonia gas emissions through volatilization process and other gaseous forms of nitrogen loss (N_2 and NOx gases) through denitrification process plus carbon emissions. Future studies should explore this to assess effect the of BSF-mediated composting on the environment. Use of tagged N forms will be ideal in this case.

2. The population and species of bacteria and fungi associated with the BSF larvae during the composting processes should be investigated to develop microbe-

based strategies for optimization of the black soldier fly-assisted composting process.

3. Field experiments should be carried out in other agro-ecological zones for longer periods and on other crop species to further validate the results obtained in this study and determine the effects of BSF frass fertilizer on soil physical, chemical, and biological properties.
4. Comparative analysis of performance of BSF frass fertilizer and other organic resources such as calliandra, senna, and lucerne should be investigated.
5. Future studies will be necessary to determine the immobilized N both by the root and microbial biomasses to accurately estimate N fluxes in soils amended with BSF frass fertilizer.

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APPENDICES

Appendix I: Papers emanating from the thesis

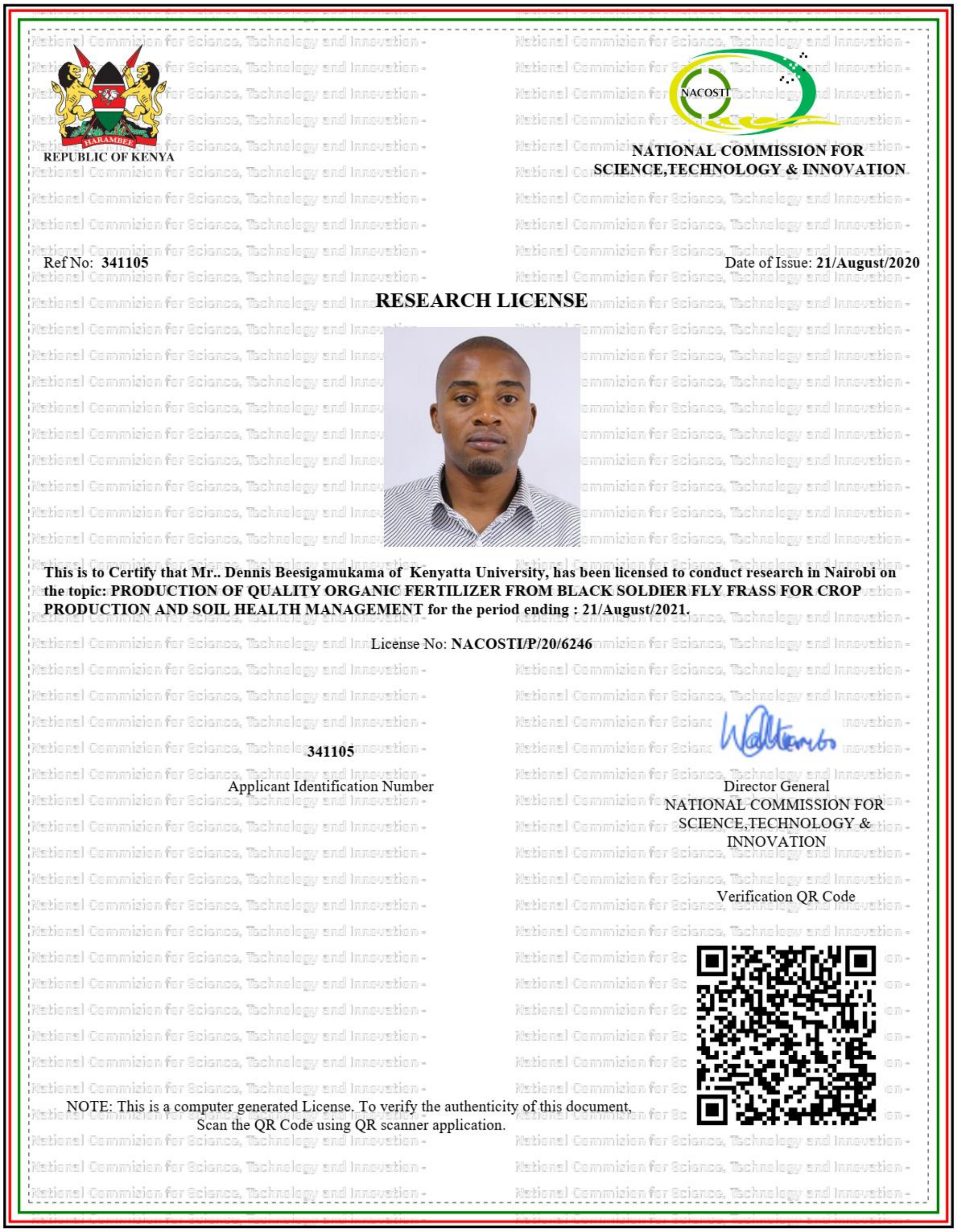
1. Beesigamukama, D., Mochoge, B., Korir, N. K., Fiaboe, K. K. M., Nakimbugwe, D., Khamis, F. M., Dubois, T., Subramanian, S., Wangu, M. M., Ekesi, S., & Tanga, C. M. (2020). Biochar and gypsum amendment of agro-industrial waste for enhanced black soldier fly larval biomass and quality frass fertilizer. PloS One, 15(8), e0238154. https://doi.org/10.1371/journal.pone.0238154

2. Beesigamukama, D., Mochoge, B., Korir, N. K., Fiaboe, K. K. M., Nakimbugwe, D., Khamis, F. M., Subramanian, S., Dubois, T., Ekesi, S., Kelemu, S., & Tanga, C. M. (2020). Exploring Black Soldier Fly Frass as Novel Fertilizer for Improved Growth, Yield, and Nitrogen Use Efficiency of Maize Under Field Conditions. Frontiers in Plant Science, 11(September), 1-17. https://doi.org/10.3389/fpls.2020.574592

3. Beesigamukama, D., Mochoge, B., Korir, N., Musyoka, M. W., Fiaboe, K. K. M., Nakimbugwe, D., Khamis, F. M., Subramanian, S., Dubois, T., Ekesi, S., & Tanga, C. M. (2020). Nitrogen Fertilizer Equivalence of Black Soldier Fly Frass Fertilizer and Synchrony of Nitrogen Mineralization for Maize Production. Agronomy, 10(1395), 1–9.

4. Beesigamukama, D., Mochoge, B., Korir, N. K., K.M. Fiaboe, K., Nakimbugwe, D., Khamis, F. M., Subramanian, S., Wangu, M. M., Dubois, T., Ekesi, S., & Tanga, C. M. (2021). Low-cost technology for recycling agro-industrial waste into nutrient-rich organic fertilizer using black soldier fly. Waste Management, 119, 183–194. https://doi.org/10.1016/j.wasman.2020.09.043

Appendix II: NACOSTI research permit



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