ASSESSING THE FERTILISER VALUE OF CO-COMPOSTED BIOCHAR COMPOST MADE FROM BLACK SOLDIER FLY LARVAE FAECAL RESIDUE

by

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Submitted in partial fulfillment of the academic requirements

ofMaster of Science in Agriculture,

Crop Science

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Pietermaritzburg

South Africa

January 2021

The research contained in this thesis was completed by the candidate while based in the Discipline of Crop Science, School of Agricultural, Earth and Environmental Sciences, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg Campus, South Africa.

The contents of this work have not been submitted in any form to another university, and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

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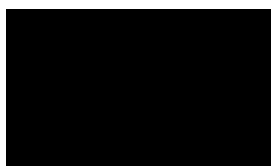
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DECLARATION 1: PLAGIARISM

I, Nqobile Nkomo, declare that:

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DECLARATION 2: PUBLICATIONS

The following publication forms part of the empirical research presented in this thesis.

Publication – Chapter 3 of this thesis

Nkomo N, Odindo A.O, Musazura W and Missengue R. 2021. Optimising pyrolysis conditions for high-quality biochar production using Black Soldier Fly Larvae faecal-derived residue as feedstock. *Heliyon, 7(5)* p.e07025 DOI: 10.1016/j.heliyon.2021.e07025

The data collection, analyses, and discussion of experimental results for the above-listed publication were conducted entirely by Nqobile Nkomo with technical advice from Prof. A. O Odindo, Dr. R. Missengue and Dr. W. Musazura. All figures, tables, and graphs were produced unless otherwise specified in the related publications.

DECLARATION 3: LIST OF CONFERENCE PRESENTATIONS

Nkomo N, Odindo A.O and Missengue R. Optimising pyrolysis conditions for high-quality biochar production using Black Soldier Fly Larvae faecal-derived residue as feedstock. "*Fourth Ukulinga Howard Davis Memorial Symposium.*" 20th -21st of August 2019. Ukulinga Research Farm, University of KwaZulu- Natal, Pietermaritzburg, South Africa.

GENERAL ABSTRACT

The Black Soldier Fly Larvae (BSFL) technology can treat faecal sludge emptied from full Urine Diverting Dry Toilets (UDDT's). A residue containing residual mineral elements such as nitrogen (N), phosphorus (P), potassium (K), and high organic matter, pathogens, and potentially heavy metals is left behind. Improper disposal of the residue can pose a challenge and lead to environmental pollution and health threats. However, there is potential for recycling BSFL Residue. The fertiliser value of BSFL Residue as an organic fertiliser has not been studied in South Africa. This study was carried out to evaluate the use of co-composted biochar compost made from BSFL Residue as a plant nutrient source for maize production. The residue was pyrolysed for 120, 90, and 60 minutes at 300 °C, for 60 and 45 minutes at 400 °C, and 60, 45, and 30 minutes at 500 °C. Biochar pyrolysed at 300,400, and 500 °C for 60, 45, and 30 minutes, respectively, was not burned and was analysed for physico-chemical, and biological characteristics. Biochar yield decreased significantly with increasing pyrolysis temperature. Surface area, pH, extractable phosphorus (P), exchangeable bases, trace metals significantly increased with pyrolysis temperature. Pathogens were destroyed with pyrolysis. Biochar pyrolysed at 500 °C for 30 minutes was chosen based on its characteristics as a bulking agent in the co-composting experiment. Co-composting of the residue was carried out, and chemical and physical characteristics of BSFL Residue composts (COMBI (compost with biochar) and (COMP (compost without biochar)) were compared to chicken manure (CM) and BSFL Heated Residue (HR). pH and exchangeable bases in BSFL Residue COMBI were higher than BSFL Residue Compost but less than BSFL HR and CM. Composting with biochar significantly increased trace elements, water holding capacity, total P, and total N in the BSFL Residue COMBI compared to BSFL Residue Compost. The BSFL Residue COMBI, BSFL Residue COMP, BSFL Residue, HR, CM, Chemical Commercial Fertiliser (CCF), and control were incubated in a sandy Cartref soil over a 112-day incubation period to determine phosphorus and nitrogen release patterns. Phosphorus decreased in all treatments during the first 21 days except for the control. Phosphorus release started after day 21, and the pattern for BSFL Residue COMBI and BSFL residue Compost were comparable, indicating the potential of using these amendments for crop production. Chicken manure had the least phosphorus released at the end of the incubation. Ammonium decreased with a concomitant increase in nitrates for all treatments. Nitrate release was lower for BSFL Residue COMBI compared to BSFL Residue Compost. An additional source of N is needed if BSFL Residue COMBI is to be used as a fertiliser based on P. The highest nitrate release was observed in CM. Amendments used in the incubation were used at the recommended and double recommended application rate to grow maize in a greenhouse pot

trial. Statistically similar yields were obtained in grain harvested from COMBI, COMP CCF, and CM. More research should be carried out on the residual effect of BSFL COMBI on subsequent maize growth to establish possible residual fertility on the second cycle of growth.

Keywords: biochar; biochar co-compost; black soldier fly larvae; faecal matter; fertiliser-value; nutrient recycling; phosphorus; pyrolysis; crop growth.

ACKNOWLEDGMENTS

"When something is meant for you, God will place it in your hands against all odds."

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ABBREVIATIONS

BSFL-   Black Soldier Fly Larvae

BSFL HR-   Black Soldier Fly Larvae Heated Residue

CM-   Chicken manure

CCF-   Chemical commercial fertiliser

COMBI-   Biochar compost

DEWATS-   Decentralised Wastewater Treatment

DWAF-   Department of Water Affairs and Forestry

HEDM-   Human Excreta Derived Material

NEMA-   National Environment Management Act

NUC-   Nitrified Urine Concentrate

SDG-   Sustainable Development Goal

SSP-   Single Super Phosphate
UDDT's-   Urine Diversion Dry Toilets

UNDP-   United Nations Development Programme

VIP-   Ventilated Improved Pit

WHO -   World Health Organisation (of the United Nations)

CHAPTER 1: GENERAL INTRODUCTION

1.1. Background

The rapid increase in population and urbanization has resulted in pressure on sanitation facilities and increased demand for food in cities. In addition, due to rural to urban migration, informal settlements that do not have access to formal facilities have emerged (Mlambo, 2018), leading to poor sanitation. Existing centralised waste-water treatment plants are operating beyond maximum capacity. It is expensive to construct new systems to cater to the increasing population due to high construction costs and inaccessibility of some areas because of rugged terrain and steep slopes.

In a bid to address this problem, the government of South Africa has provided several onsite innovation sanitation technologies. For example, by 2014, the eThekwini municipality (South Africa) undertook key initiatives to address the sanitation backlog in response to the White Paper on Sanitation, which aimed at ensuring that sufficient sanitation is available to all South Africans (DWAF, 2001). Provision of the Decentralised Wastewater Treatment System (DEWATS), Ventilated Improved Pit (VIP) latrines, and Urine Diverting Dry Toilets (UDDTs) have been made to cater for people without access to sanitation facilities. (Hudson, 2010; Kone, 2012).

The UDDTs are an improvement from the VIP, and they allow the separate collection of faecal matter and urine. The UDDT technology has been rolled out to Isipingo (South Africa), where the residue used in this study was taken. However, these systems face challenges of managing the collection of excreta, such as health risks and environmental pollution (DWAF, 2001; Seminar, 2011; Still et al., 2005). These technologies are of importance since, in recent years, waste management has focused on recycling rather than disposal, a way that protects the environment and benefits people (Murray and Buckley, 2009).

Although onsite sanitation technologies offer a solution to sanitation facilities, environmental and health challenges may arise. When the pits get full, there was no plan by the municipality to empty the pits. Full and overflowing pits pose health risks due to overflowing pits and vectors such as house flies which transmit diseases. However, there is a large potential source of human-derived Nitrogen (N), and Phosphorus (P), where the population is currently not served by improved sanitation. Human excreta-derived materials (HEDMs) have the potential for nutrient

recycling.

The use of HEDMs as fertilisers will help address interlinked Sustainable Developmental Goals (SDGs) according to SDG 2, which focusses explicitly on food by aiming to 'end hunger, achieve food security and improve nutrition and encourage sustainable farming'; SDG 6 on water and SDG 12 on sustainable production and consumption (Brooks, 2016). It also supports national policies such as the National Environmental Management Act (NEMA), which advocates for reducing pollution, degradation of the environment, and using non-renewable natural resources responsibly and fairly considering the consequences of depleting the resource. Implementing these strategies also provide basic sanitation without water use (Maleba et al., 2016).

Currently, the toilets in the Isipingo community have filled up, and the municipality does not have an emptying plan. The faecal matter is collected, and fed to BSFL (Banks, 2014; Mutsakatira et al., 2018). The larvae convert the faecal matter to body protein and fats, and it is used as chicken and fish feed. The residue that remains can be used as a nutrient source for crop production.

Faecal matter can be used as a source of P and organic matter (Rose et al., 2015). Hence the residue from the decomposition of faecal matter using BSFL can also be used in like manner. However, concerns arise due to health risks associated with the use of faecal matter. In addition. The raw residue has a high moisture content, which makes it challenging to handle and transport. Furthermore, the ammonium to nitrate ratio is high, which poses ammonium toxicity to plants. Hence, composting can eliminate threats posed to human health, improve safety and hygiene, and enhance the residue's nutritional quality. Some of the residue can be pyrolyzed to biochar and added to the residue before composting as a bulking agent to improve the compost quality.

Biochar is a solid carbonaceous material made from the pyrolysis of any organic material under limited or no oxygen conditions (Joseph and Lehmann, 2015). Research is needed on the use of BSFL Residue as a source of feedstock for co-composting with biochar made from BSFL Residue and the co-compost as a nutrient source for crop production. The research proposition in this study is that nutrient reuse and recycling from human excreta is an option. It can be put into practice, given the challenges of P depletion in a sustainable way to both soils and water bodies.

1.2. Problem statement

Poor sanitation experienced by both rural, urban, and peri-urban settlers is a major problem in South Africa. Several municipal authorities in South Africa have introduced innovative sanitation technologies such as UDDT's not connected to the centralised main sewer system. A key challenge UDDT users faces is that municipalities have not provided a clear plan for pit emptying. Unemptied full pits pose a risk of sanitation-related diseases. Improper disposal of faecal matter also result in contamination through pollution of groundwater bodies. There are initiatives where faecal matter is currently collected and fed to Black Soldier Fly Larvae. However, there are resultant challenges with the BSFL residue's disposal that remain after feeding: high moisture content, residual pathogens, low C: N ratio, and high ammonium: nitrate ratio. The residue still contains essential nutrients such as P, an important primary mineral nutrient for plant growth. Instead of disposing the residue, it can be used as a nutrient source since it contains essential nutrients such as P, an important primary mineral nutrient for plant growth. Research is needed on investigating the use of BSFL residue as a source of material for co-composting with biochar made from the BSFL residue and its use as an organic fertiliser source. Biochar is a carbon material made from pyrolysis of organic material. It is a good bulking agent for the composting process because it helps maintain moisture in the compost heap. It is porous; hence it is good for aeration. Biochar also helps to increase the C: N ratio needed to initiate the composting process. There is inadequate information on conditions that optimise the pyrolysis of BSFL Residue and the use of BSFL Residue biochar when co-composted with the BSFL Residue. There is also no information on the co-compost's effect on nutrient release and crop growth in P-depleted soils.

1.3. Justification

In the past, both biochar and compost have been added separately and used for crop growth (Bass et al., 2019). Few studies have dwelt on the use of biochar and composting material mixed before the composting process. In cases where this has been done, neutral, negative, and positive crop growth responses have been observed. However, this approach has not been done using feacal matter or any material of feacal origin. The use of BSFL faecal matter residue in the form of BSFL Residue COMBI (BSFL Residue Biochar + BSFL Residue after co-composting) addresses the waste management problem while potentially providing a pathway for closing the nutrient loop by providing P to nutrient-depleted soils. BSFL Residue COMBI adds organic matter and

nutrients to soils; thus, increasing crop yields and alleviating hunger while simultaneously tackling environmental and health hazards presented by improper and inadequate sanitation systems.

1.4. Study aims

The project aims to understand the conditions that influence high-quality biochar production using BSFL Residue obtained from the decomposition of faecal matter from Urine Diversion Dry Toilets (UDDT's) by Black Soldier Fly Larvae. It further investigates the potential of BSFL Residue co-composted with biochar as an organic fertiliser source and its effect on soils and crop growth.

1.5. Specific objectives

1.5.1 to optimise the pyrolysis conditions for high-quality biochar.

1.5.2 to co-compost Black Soldier Fly Larvae Residue with biochar and determine the physical, chemical, and biological properties of composts for its suitability for crop production

1.5.3 to investigate the nitrogen and phosphorus release patterns in a Cartref soil amended using co-compost made from Black Soldier Fly Larvae residue derived biochar mixed with the Black Soldier Fly Larvae residue.

1.5.4 to determine the optimum application rates for Black Soldier Fly Larvae Biochar COMBI on yield and nutrient uptake of maize planted on a sandy Cartref soil under controlled conditions.

1.6. Chapter Outline

This thesis consists of 7 chapters:

Chapter 1

This chapter gives a general introduction, summarises background information, problem statement, aims, objectives, and justification of the study.

Chapter 2

This chapter reviews sanitation problems faced by cities and how municipalities have and can respond to offer sanitation facilities. It also highlights technologies for treating human faecal matter, primarily focusing on Black Soldier Fly Larvae and the limitation to processing the resulting residue for use as a bio-fertiliser to provide nutrients.

Chapter 3

This chapter was a preliminary experiment to optimise pyrolysis conditions using Black Soldier Fly Larvae Residue from the decomposition of faecal matter from UDDT's as feedstock for biochar production.

Chapter 4

This chapter characterised the feedstock used for composting, i.e., Black Soldier Fly Larvae Residue, Black Soldier Fly Larvae Residue Biochar, and sawdust and how they relate to the final compost quality with regards to physicochemical and biological characteristics. It also assessed the compost quality through laboratory germination of radish seeds.

Chapter 5

This chapter was an incubation study investigating how Black Soldier Fly Larvae Residue COMBI, Black Soldier Fly Larvae Residue Compost, Black Soldier Fly Larvae Heated Residue affected N (NO_3^- and NH_4^+) and P (PO_4^{3-}) availability over time compared to chicken manure, chemical fertiliser and control.

Chapter 6

This chapter was a greenhouse experiment where maize was grown under controlled conditions using the Black Soldier Fly Larvae organic amendments to assess crop growth, soil nutrient responses, and grain nutritional quality.

Chapter 7

The chapter is a general conclusion on Black Soldier Fly Larvae Biochar Compost's agricultural potential when added to soils and the subsequent effect on crop growth. Critical issues leading to further questions were identified, and recommendations for further research were made.

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CHAPTER 2: CO-COMPOSTING: TOWARDS SUSTAINABLE FAECAL WASTE MANAGEMENT

2.1. Introduction

Lack of adequate provision of sanitation facilities in informal settlements is a major problem that causes health-related risks and pollution through improper disposal of human waste. Population increase is projected to reach nine billion by 2050 (UNDP, 2007), coupled with an increase in urban migration, poses challenges with the huge amounts of waste generated (Mara et al., 2010). Generally, most migrants settle in informal settlements with no access to sanitation facilities (Mannie and Bowers, 2014). Municipalities cannot provide these settlements with centralised waste treatment plants due to congestion. Those operating services are under stress as they have exceeded their maximum capacity (Mema, 2010). Coupled with this, centralised waste-water treatment plants' construction to balance the short fall is costly (Prihandrijanti et al., 2008). Hence, communities improperly dispose of human waste leading to pollution and water-borne diseases (Mara et al., 2010).

Municipalities have come up with technologies in response to these challenges, such as Ventilated Improved Pits (VIPs) and Urine-Diverting Dry Toilets (UDDT's), to cater for the sanitation needs of these communities (Goundenetal., 2006). Urine diverting dry toilets allow for separating urine and faeces (Rieck et al., 2012). Although adequate sanitation is an undesirable situation, it has presented opportunities for income generation steered by the formal resource management and co-operated with the informal sector (Diener, 2011; Fumasoli et al., 2016; Morales et al., 2013). Hence, the problem of faecal and urine disposal can be turned into a positive, valuable operation through valorisation.

Faeces and urine are a source of nutrients (Table 2.1). Nutrients differ depending on diet, climate, and water intake (Del Porto and Steinfeld, 1999). When applied to the soil, human excreta can result in increased soil fertility and improved crop growth. However, the raw application of human waste to the soil poses risks of underground water contamination through nutrient leaching, especially water-soluble nutrients such as nitrates (NO_3^-). Pathogens such as total, faecal coliforms, *Salmonella spp*, *Helminths,* and *Ascaris* also pose a threat to humans as

they spread water-borne diseases (Jimenez-Cisnerosetal., 2001; Redlinger et al., 2001). Technologies that allow faecal matter and urine treatment result in their safe handling and usage as nutrient sources for crop production.

Researchers have focused on the processing of these wastes to produce waste-based fertilisers such as nitrified urine concentrate (NUC), a nitrogen (N) rich fertiliser, and struvite, a phosphorus (P) rich fertiliser (Udert et al., 2015). Currently, the technology of feeding faecal matter to Black Soldier Fly Larvae (BSFL) is becoming popular (Banks et al., 2014). This technology's end products are protein-rich feed, oil, and a residue that can be used as a soil amendment (Dortmans et al., 2017).

The BSFL technology could provide a feasible option for waste recycling, and the residue can potentially provide a new organic source of nutrients to be applied to nutrient-depleted soils. This technology has been used with organic feedstock such as food waste and mixtures of food and municipal solid waste. The residue proved to be a rich source of nitrogen (N), phosphorus (P) and, potassium (K) and is characterised by a decreased level of heavy metals (Sarpong et al., 2019). The BSFL technology is important because the residue can supply crops with N, P, and K. The high organic matter is essential for increasing poor sandy soils' organic matter and water holding capacity.

The residue's addition to the soil soon after feeding by BSFL is problematic due to high moisture content, possible residual pathogens, and low C: N ratio, making it challenging to handle and apply in the fields and may inhibit crop growth. These challenges hinder the use of residue in the field. Further treatment can be done to ensure the safe use of the residue and reduce moisture content and pathogen load. Post-treatment methods such as heating, pyrolysis to make biochar, and co-composting have been suggested.

The objective of this review is to highlight the sanitation and pollution problems faced by cities. The review seeks to explain strategies that have been put in place by municipalities to provide sanitation facilities; and discusses technologies for processing human faecal matter, focusing on using BSFL, the constraints faced to utilise the residue as a bio-fertiliser, and how it can be processed into a useable form. The review concludes by identifying knowledge gaps for future research and recommendations for further studies.

Table 2.1 Composition and characteristics of human faeces and urine (Chaggu, 2004).

Approximate Quantity	Faeces	Urine
Quantity (wet) per person per day (g)	70 – 520	100-1500
Quantity (dry solids) per person per day (g)	30 – 70	50 -70
Proximate composition (% of dry weight /matter)	88 – 97	65 -85
Moisture	66 – 85	93 -99.5
Organic matter	88 – 97	65 -85
Nitrogen	5.0 -7.0	15 -19
NK_j (gN)	1.4 -2.46	5.29
Phosphorus as (P_2O_5)	3.0 -5.4	2.5 -5.0
PO_4-P	-	0.20
Total Phosphorus (gP)	0.69 -2.5	1.08 -2.2
Total Potassium as (K_2O)	1.0 -2.5	3.0 -4.5
Potassium(K)	0.80 -2.1	2.5 -3.7
C	44 – 55	11.0 -17.0
Ca as (CaO)	4.5	4.5 -6.0
Ca (Ca)	2.9 -3.6	3.2 -4.3
C: N ratio	5.0 – 10	0.6 -1.1
COD $_{(total)}$(g/l)	46.23 -78.31	12.79
COD $_{(soluble)}$		11.33
COD $_{(particulate)}$	-	1.46
TS (%)	33	-
Ph	-	7.08 -9
Proteins (g)	4.0 -12.0	0.28
Total lipids (g)	4.0 -6.0	-
Polysaccharides (g)	4.0 -10.0	0.68

2.2. Sanitation problems

When improperly disposed, human excreta have been shown to cause many infectious diseases, including cholera, ascariasis, polio, hepatitis, cryptosporidiosis, typhoid, hepatitis, and schistosomiasis (Carr et al., 2001). It is estimated by the World Health Organization (WHO) that 2.2 million people die annually from diarrhoeal diseases. Furthermore, approximately 10% of the population in developing counties is affected severely by intestinal worms. All these diseases are related to improper waste and excreta management (Murray and Lopez, 1996; WHO, 1996). Most households in South Africa, especially informal settlements, do not have access to basic and proper sanitation facilities. Improper disposal of human excreta poses problems concerning sanitation health risks. Provision of basic sanitation facilities could help reduce morbidity due to *Helminth* infection, where approximately 1.8 million deaths occur yearly due to these diseases. The choice of sanitation technology should consider the permanence of the settlement,

the technical aspects, financial costs, design, and environmental considerations. Thus, new innovative technologies to address sanitation problems should seek to protect rather than harm the environment.

2.3. Innovative technologies

New sanitation technologies should be provided to cater to the populatin's needs who do not have access to adequate sanitation facilities. These technologies provide sanitation facilities and relieve pressure on the existing systems, thus providing adequate sanitation to the people using them (Zuma, 2016). On-site sanitation technologies such as the Ventilated Improved Toilet (VIP), UDDTs, and the low/pour-flush toilet are currently being used in South Africa (Mkhize et al., 2017; Tilley et al., 2008).

There is a need for alternative technologies that emphasize a re-conception of sanitation from the 'drop-flush-forget' model to 'drop and re-use' models that allow the protection of the environment at the source (Drangert et al., 2006). Such models allow recovery and reuse of nutrients. In 2002, the eThekwini municipality initiated the installation of UDDT's (Roma et al., 2013). The UDDT is characterised by two vaults, one for collecting urine and another for collecting faecal matter. Separate collection of faecal matter and urine is achieved by UDDT's. However,with time, the vaults fill up and require emptying. Both urine and faecal matter derived from these toilets can be processed into waste-based fertiliser products (Malkki, 1999; Udert et al.,2015).

2.4. Processing technologies

Processing technologies offer a solution to the disposal problems presented by the collected urine and faecal matter from UDDT and VIP toilets (Niwagaba, 2009). Both source-separated urine and faecal matter can be processed into plant useable fertilisers with the potential to supply crops with nutrients. Processing is important in affecting human waste's fertiliser value by concentrating nutrients and sanitisation so that the final product is safe for human handling (Harder et al., 2019).

2.4.1. Struvite and NUC

Source separated urine is a rich source of nutrients (N and P). Urine is processed into struvite and nitrified urine concentrate (NUC), rich P and N sources, respectively (Udert et al., 2015). Struvite, chemical formula $MgNH_4PO_4 \cdot 6H_2O$, is a solid precipitate formed after adding a

magnesium source such as $MgCl_2$, MgO, and $MgSO_4$ to urine (Udert et al., 2015). Phosphorus recovery is 93% P, and 97% of N remains in the supernatant (Etter et al., 2013; Grau et al., 2013). Hence, another process was developed for the complete recovery of nutrients by recovering N from the supernatant in the form of a concentrated solution called NUC (Udert and Wachter, 2012).

Nitrified urine concentrate is a nitrogen concentrated liquid fertiliser resulting from nitrification and distillation of source-separated urine. Water is evaporated by boiling during distillation, leaving behind a concentrated solution (Udert et al., 2003; Udert and Wachter, 2012). The nitrate (NO_3^-) concentration in NUC is more than ammonium (NH_4^+), and NUC has 21% total N. Nitrified urine concentrate has been tested as a fertiliser with success rates comparable to commercial chemical fertilisers (Bonvin, 2013; Bonvin et al., 2015).

2.4.2. Latrine Dehydration Pasteurization (LaDePa) pellets

Latrine Dehydration Pasteurization (LaDePa) pellets are processed from the output of VIP toilets. Latrine Dehydration and Pasteurization agricultural pellets are solid faecal matter pellets with the resemblance of spaghetti in structure. LaDePa pellet production involves drying faecal sludge using mid-infrared (MIR) radiation from lamps, usinga LaDePa machine (Nikiema et al., 2013; Septien et al., 2018). The resulting dry pasteurised pellets can be used as a soil conditioner due to high organic matter content or fertiliser value (Nikiema and Cofie, 2014; Waweru, 2017). According to a study carried out by Waweru (2017), the optimum conditions for pasteurisation were residence times higher than 17 minutes at temperatures above 87°C or 4 minutes at temperatures above 214°C. The nutrient concentration of LaDePa pellets increases with drying. However, there is no effect on the definite amount of P, K, Ca, and Mg. Pellets have reduced nitrates and ammonium; hence the amount of LaDePa applied should be ten times more than organic fertiliser sources to achieve a similar fertiliser value (Waweru, 2017).

2.4.3. Composting

Composting is a biological process involving microorganisms' aerobic decomposition of organic matter (Diaz et al., 2020; Haug, 2018). The resulting material is rich in humus, carbon (C), major plant nutrients, N, P, K, micronutrients, as well as a product free from pathogens, weed seeds, and phytotoxic organic leachates (Haug, 1993). Composting enhances the nutritional value of the composted material by making nutrients such as N and P available forplant uptake and

enriches the soil with organic matter (Niwagaba, 2009).

There are three types of composting, namely, aerobic, anaerobic, and vermicomposting. The different composting techniques affect the end-product quality due to different environmental conditions during organic waste degradation (Shah et al., 2015). Differences in end-product quality are attributed to N losses through nitrous oxide, ammonia emissions, and leaching.

Aerobic composting is the decomposition of organic matter by microorganisms that require oxygen. Mostly compost heaps are turned to allow air to pass through the compost. Aerobic composting yields better compost with regards to phytotoxicity. However, it also experiences a lot of nutrient losses due to gas escape during the turning process (Misra et al., 2003). During aerobic composting, easily degradable N compounds are broken down into stable N compounds during the early composting stages. When applied to the soil, such compost results in relatively lower decomposition and mineralisation (Shah et al., 2015). The addition of aerobically composted compost has been found to increase total N in the soil. Up to 60% of total N applied from aerobic composting has been reported to mineralise during the first year of application (Civeira, 2010; Martínez-Blanco et al., 2013).

Anaerobic composting involves the decomposition of organic material by microorganisms in the absence of oxygen. Methane is produced as a by-product (Shah et al., 2017). Anaerobic composting results in compost with greater N retention because fewer losses are incurred. Easily degradable organic compounds such as volatile fatty acids are produced during anaerobic composting resulting in higher microbial degradation and N mineralisation compared to aerobic composting. Both processes kill pathogens. However, in aerobic composting, the heat generated kills the pathogens, whereas, in anaerobic composting, acidic conditions kill the pathogens. Vermicomposting is the decomposition of organic material using earthworms such as white worms, red wigglers, and other worms (Chanu et al., 2018).

All the composting methods produce a stable nutrient-rich organic source for crops (Haug, 2018). The end compost product should be free from weed seeds and pathogens (Mangkoedihardjo, 2006). It should also be non-phytotoxic when assessed for germination using seeds such as cress or radish. Radish and cress seeds are preferred because they are rapidly growing, cheap, and easy to analyse (Mangkoedihardjo, 2006; Pavel et al., 2013).

2.4.4. Black Soldier Fly Larvae feeding technology.

Black soldier fly larvae can digest large volumes of organic waste such as animal and human cadavers, decomposing food scraps, animal manure, municipal organic waste, fresh faeces, and faecal matter from pit latrines (Banks, 2014; Diener, 2011; Tomberlin et al., 2005). The larvae's voracious appetite results in fast waste conversion; hence, they can act as an essential tool in faecal waste management. The short life cycle of the BSF also contributes to quickening the process of waste decomposition. Literature reports different waste reduction abilities of BSFL based on feedstock and larval density. There was a total reduction of 56% after feeding pig manure to BSFL. On the other hand, a 65-75% reduction was observed using sewage sludge (Diener, 2011; Newton et al., 1977). The quantity of the faecal matter influenced the reduction of waste.

The BSFL is characterised by a short life cycle (Figure 2.1). The adult flies are docile and depend on stored protein and fat; hence, they do not contact food and are not vectors of disease transmission (Diener et al., 2011). After mating, laid eggs are hatched near a nutrient-rich food source such as kitchen waste within 102 to 105 hours. After hatching, the larvae with translucent bodies are transferred to the waste, which they are supposed to feed on by spreading them over the waste's surface. The larvae digest the waste and convert it into body proteins and fatty acids. After digestion, the larvae pupate (pre-pupae) and self-harvest by crawling out from the feeding waste to a dry area and transforms to a darker colour in preparation for metamorphosis (Mutafela, 2015). It takes about 14 days for the pupae to emerge from the larvae (Diener et al., 2011).
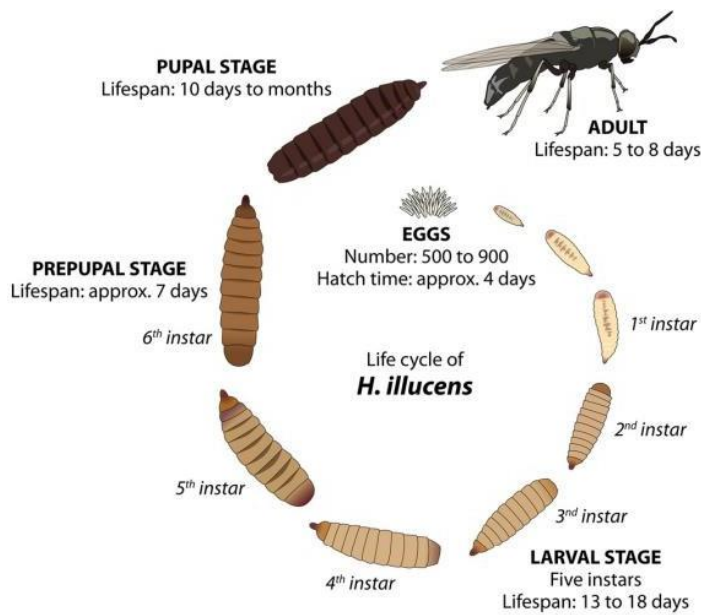
Figure 2.1 Life cycle of BSF indicating development stages and their corresponding duration (De Smet et al., 2018).

Harvesting of the pre-pupae is carried out by shaking on a screen. The nutritional composition of pre-pupae protein and fatty acids is 40% and 30%, respectively (Mutafela, 2015; St-Hilaire et al., 2007), but varies according to feedstock type (Wang and Shelomi, 2017). The pupae are harvested, and proteins are processed into a protein-rich meal used as animal feedstock (chickens and fish) and pet food. The lipids can be used for biodiesel production (Leong et al., 2016). The pupae's exoskeleton's cuticle is made of chitin embedded in a matrix of proteins and lipids. Chitin consisting of approximately 6.9% N, can be used as a chelating agent in the medical and cosmetic industry. However, the feasibility of using chitin from BSFL still needs research (Kumar, 2000).

The remaining residue, referred to as frass, is receiving attention for its potential use as a soil conditioner (Zahn and Quilliam, 2017). Black soldier fly larvae residue is characterised by undecomposed, partially decomposed material, residual larvae, and excreta from the larvae (Alattar et al., 2016b; Zahn and Quilliam, 2017). Furthermore, frass also contains the outer skin after moulting, remains of larvae, pupae, chitin, metabolic substrates such as hormones, antibiotics, and organisms such as enzymes, bacteria, fungi, and protozoa (Vickerson et al., 2017). Figure 2.2 and Figure 2.3 summarises the process of BSFL decomposition and the resulting end-products.
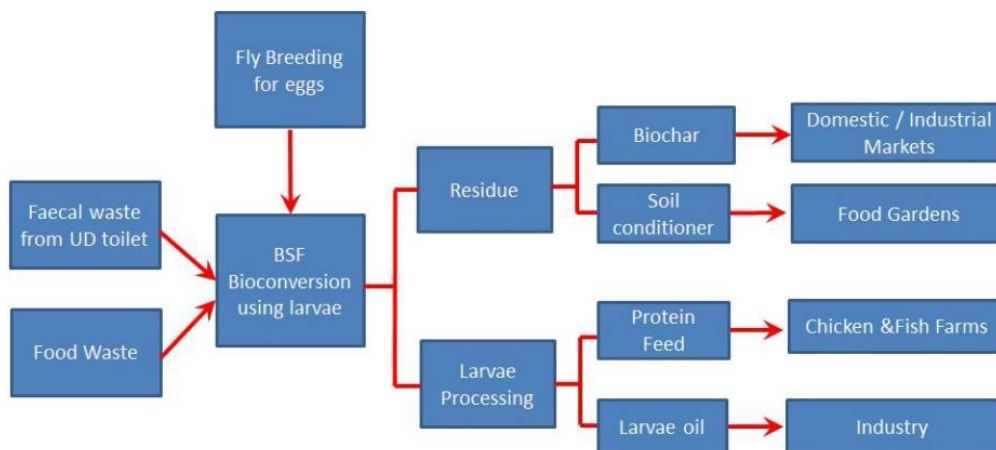
Figure 2.2 Concept of BSFL composting (Lalander et al., 2015).
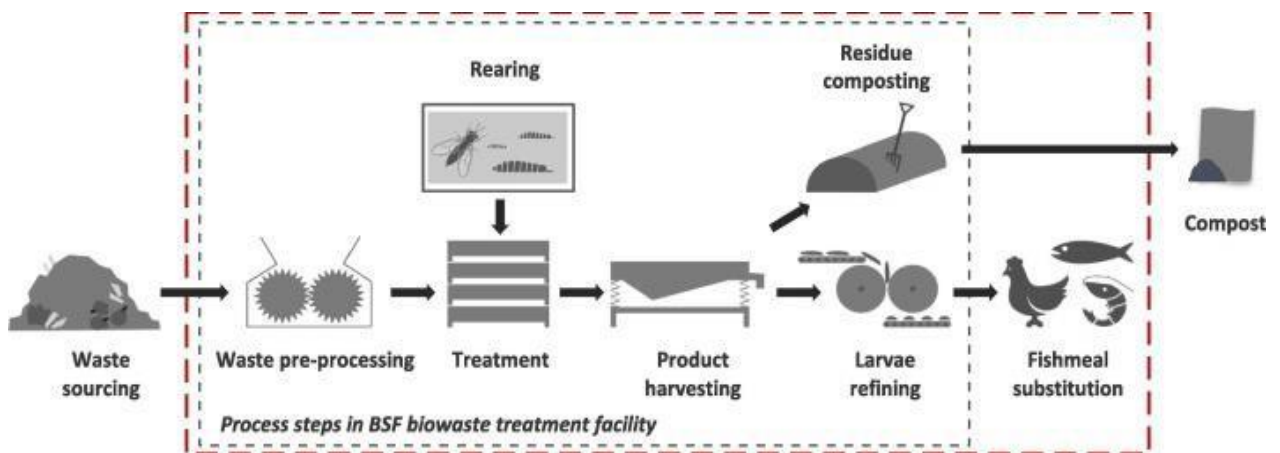
Figure 2.3 Steps in BSF treatment facility (Mertenat et al., 2019).

2.5. Raw BSFL residue as a soil amendment

Black soldier fly residual frass has been used in crop growth trials. Different results have been observed due to the differences in the quality of the frass because of different substrates and post-treatment of the residue following feeding (Vickerson et al., 2017; Choi et al., 2009). As observed, studies carried out revealed that the nutrient composition of frass could be used as a fertiliser with variations in crop responses ranging from positive to negative.

The raw residue from BSFL decomposition has the potential to be used as a nutrient source. An N, P, and K (5: 2: 2) nutritional value was observed when BSFL was fed a mixture of food waste

composed of approximately 70% bread, 20% grains, and 10% fish offals (Vickerson et al., 2017). The residual frass of the decomposition of food waste from the Agriprotein BSFL plant in Cape Town has a (4: 2: 2) N, P, K nutritional value (Table 2.2). Also, frass is characterised by other nutrients such as calcium (Ca), magnesium (Mg) as well as trace elements such as sulphur (S), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn) and boron (B) as indicated in Table 2.2

Table 2.2 Nutritional value of MagSoil from food waste processing by BSFL at Agriprotein Cape Town

(https://agriprotein.com/wp-content/uploads/2017/02/agriprotein-magsoil.pdf)

N (%)	P (%)	K (%)	Ca (mg/kg)	Mg (mg/kg)	
3.6	**1.6**	**1.4**	**1.7**	**0.3**	

pH	EC	H2O	Ash	C	C: N
6.9	**2.1**	**24.1**	**21**	**40**	**16:1**

			mg/kg		
S	Mn	Fe	Cu	Zn	B
355	**241.6**	**6286**	**11.3**	**78.2**	**12.2**

There is information on the use of BSF-derived compost (MagSoil) as a soil conditioner and its effect on root development and crop yields (Agriprotein, 2009). However, there is no data on the residue's use from the decomposition of faecal matter using BSFL for crop production on poor soils, application rates, nitrogen and phosphorus release patterns, and plant uptake availability. Research is needed on investigating the use of BSFL residue as a source of material for co-composting with biochar made from the BSFL residue and its use as an organic fertiliser source.

AgriProtein technologies compost the BSF larvae residue into a homogeneous organic fertiliser characterised by brown and loose crumbs remaining after the decomposition of food waste called MagSoil. The use of MagSoil, particularly in poor degraded soils, has two potential benefits. First, it can provide a potentially new fertiliser source to address poor soil fertility problems, limiting crop production among small-holder farmers in Sub-Saharan Africa. Second, it also

offers an opportunity for the sustainable management of waste by providing means of proper waste disposal in a manner beneficial to society while simultaneously protecting the environment, thus closing the nutrient loop by providing means of returning nutrients recovered from human waste back to the soil.

The residue from faecal matter faces resistance due to potentially harmful microorganisms such as faecal and total coliforms, *Ascaris,* and *Helminths,* which threaten human health. The residue is also characterised by high moisture content. Application of raw organic matter as a soil conditioner is associated with disadvantages such as phytotoxicity and immobilisation of nutrients hence the need for composting. Composting the residue alone faces challenges such as low C: N ratio, nitrate: ammonium ratio, and high moisture; hence it can be co-composted with biochar. Biochar is a solid carbonaceous material made from pyrolysis of organic material under limited or no oxygen conditions. The BSFL Residue can be used as a feedstock for the pyrolysis process and co-composted with the BSFL Residue.

Table 2.3 Nutrient value of BSFL food waste compost (A) and commercial chemical fertiliser (B) (Choi et al., 2009).

Compounds/ Group	Unit	A	B	P-value
pH		7.0	7.1	
EC	dS/m	0.5	0.3	0.05
OM	g/kg	33.2	31.3	
K2		0.1	0.1	-
Ca		8.8	8.7	
Mg	$cmol_c/kg$	1.7	1.7	
Na		0.4	0.2	0.05
P_2O_5		207.5	182.0	
NO3-N	mg/kg	8.7	5.7	
NH4-N		186.7	117.8	

The residue used in the experiment carried out by Choi et al. (2009) showed no significant differences in the growth and yield of Chinese cabbage compared to the commercial potting mix.

The nutritional composition in cabbage treated with BSFL residue did not differ significantly. However, the phosphates were less in the residue treated than in the commercial fertiliser treated plants. Basil plants grown with the same treatments showed maximum biomass at 5 and 10% w/w in clay and 10 and 20% w/w in the sand. Controls for both sand and clay showed stunted growth. However, the commercial potting mix performed the best. When the same residue was used on Sudan grass, 5% w/w yielded the best in clay and sandy soils (Newton et al., 2005). Growth responses were better in sandy soil in both cases.

Fruit residue was used as feedstock for BSFL feeding (Zhan and Quilliam, 2017)). Spring onions were grown in a tunnel with frass at application rates of 5, 10, 20 t ha^{-1} compared to compost and NPK fertiliser. Results showed a statistically similar increase in plant height with an increasing application rate. However, at 20 t ha^{-1}, there was stunted growth. Dry matter was also lowest at the highest application rate using frass (Zahn and Quilliam, 2017). Similar results were observed by Dieneret al. (2011), who observed a better response of basil at the lowest application rate of 5% compared to 10%. However, commercial chemical fertilisers outperformed all the residue treatments in both cases. Electric conductivity increased with increasing application rate for both frass and compost.

At high application rates, organic leachates produced during decomposition of food waste by BSFL contained high ammonia levels as high as six times more than material not fed to BSFL, which caused ammonium toxicity (Green et al., 2012). Crop growth is suppressed by high ammonium. Uric acid is the main N storage compound in frass. It is converted through allantoin and urea into NH_4^+. Allantoin is an intermediate chemical formed during the conversion of uric acid to ammonium. In the event of poor drainage systems, it can lead to NH_4^+ toxicity in plants. This study concluded that N-mineralisation is increased during BSFL feeding by elevating the concentration of NH_4^+ in the leachate fraction (Green et al., 2012).

In a field trial, testing BSFL residue's use from the decomposition of faecal sludge and market waste, the performance of crops was poor. The residue had a high moisture content of 82–86% and produced an odour. There is a need for post-treatment of the residue through composting if the residue needs to be used as a soil conditioner (Diener et al., 2011). The residue, when wet,can be composted with garden waste. Dry residue should be allowed to mature before applying it to the soil.

In another experiment carried out on the response of maize to BSFL residue as a proposed fertiliser, BSFL residue treated plants showed stunted growth (Alattar et al., 2016). The study compared the growth rate of maize treated with BSFL residue, microaerobic fermentation residue, and aerated compost at a rate of 1:2 residue to soil ratio (w/w). Three weeks after planting, microaerobic fermented and compost treated plants had grown almost three times taller than BSFL residue treated plants. At the end of 10 weeks, BSFL residue-treated plants had 19% less leaf growth and were 39% shorter than the control (Alattar et al., 2016). Poor crop growth response was linked to the low porosity of the residue. It was also suggested that the residue could have been phytotoxic to the plants due to a low C: N ratio of 14 when appliedat a 1: 2 residue: soil ratio. Furthermore, the harvested frass is suggested to be composted because of its high moisture content (Diener, 2011).

The residue's inhibitory effects can be eliminated by drying or composting the residue (Green and Popa, 2012). Further treatment such as composting is safer as it offers thermophilic temperatures to kill residual pathogens (Dortmans et al., 2017). However, due to the residue's low C and low porosity, a high C material can be added to increase the C: N ratio and allow the material to undergo further decomposition through composting.

Although nutrients characterise BSFL residue, potentially harmful organisms such as faecal coliforms, total coliforms, *Ascaris ova,* and Salmonella *ssp*. are most common in faecal material (Sidhu and Toze, 2009). Residue from the decomposition of faecal matter by BSFL may consist of residual pathogens. During feeding, pathogen load, especially *E. coli* and *Salmonella* was reduced (Erickson et al., 2004). A high reduction in Salmonella *spp*. concentration of $6_{log}10$ was observed after feeding sewage sludge faecal matter to BSFL in an eight-day small batch reactor larvae operating system compared to $<2_{log}10$ cfu/g in control (Lalander et al., 2013). However, *Ascaris ova Enterococcus spp* and bacteriophage ΦX174 remained in the residue from pit latrines. Therefore, further treatment is required to safely use BSFL residue from UDDT faecal matter decomposition in crop production (Banks, 2014).

2.5.1. Heating the residue

Heating is a pre-treatment method used to remove the moisture in sewage sludge (Panepinto and Genon, 2014; Septien et al., 2020). Heating sewage is a sanitary measure that allows safe handling before and during land application. The heated sludge is applied to the soil as a soil

conditioner or as a fertiliser. However, this method may have effects on the nutrient availability of sewage sludge. Phosphorus availability was negatively affected by heat drying of sewage sludge at 70, 130, 190, and 250 °C. Over a 190-day incubation study, water-extractable P release was reduced by 23% (Lemming et al., 2017). The same procedure can be tested on BSFL residue.

Heating the BSFL residue as a method to sanitise it and observing nutrient availability has not been done.

2.5.2. Pyrolysis to biochar

Biochar is a highly porous solid carbonaceous material formed from pyrolysis, a thermal process carried out at temperatures from 300 to 700 °C, under limited oxygen conditions (Lehmann and Joseph, 2015). The feedstock used during pyrolysis is any organic biomass, such as wood chips, plant residues, organic wastes, human waste, and poultry manure (Lehmann and Joseph, 2009; Sohi et al., 2010). The resulting material is a shiny black material structurally similar to the original material but shrunken in size (Wilson, 2014). It is characterised by a porous structure and various functional groups (Liu et al., 2015).

Biochar can be used as climate change mitigation, energy production, soil improvement and waste management tool, environmental remediation through the reclamation of contaminated sites, and suppression of foliar diseases (Lehmann and Joseph, 2009). Using biochar as a soil amendment is particularly important in adding organic matter to poor soils with low soil organic matter content (Zhang et al., 2016). High organic matter contributes to improved soil fertility and hence crop productivity, especially in sandy soils. The use of faecal matter as a feedstock for pyrolysis has been studied (Koetlisi and Muchaonyerwa, 2017; Ward et al., 2014).

Several studies have shown that pyrolysis of faecal sludge increases the P content of biochar, which can be used as a nutrient source for crop production. The addition of faecal biochar can be used as an amendment to replenish P in P-depleted soils (Dai et al., 2016; Woldetsadik et al., 2017). However, there are no studies on residue pyrolysis after decomposition of faecal matter from UDDT's by BSFL.

2.5.2.1. Biochar characteristics

Biochar is mostly composed of two carbon (C) forms, namely recalcitrant and labile C. Recalcitrant form contributes to C's major component, is humus-like, and is resistant to decay. In contrast, the labile form contributes a minor part and is easily accessible in a bioavailable form

for degradation by microorganisms (Ok et al., 2015). Carbon is arranged in hexagonal ring structures where atoms of hydrogen (H) and oxygen (O) are attached. The rings are fused and referred to as aromatic C (Keiluweit et al., 2010). Main elements (N, P, K, Ca, Mg, Na, Fe,Si, and S) and trace metals (Pb, Cd, Cu, Ni, Hg, Zn, Cr, B, Mn, and As) are found in biochar especially when the biochar is of sewage sludge origin (Liu et al., 2015).

During pyrolysis, C is left intact, while N is lost through volatilisation. This results in a high C:N ratio. The C: N ratio is affected by temperature (Figueiredo et al., 2018). The degree of N loss also depends on the feedstock used since different faecal materials possess different characteristics. Biochar has a higher C: N ratio than the original feedstock because of the higher proportion of recalcitrant C and lower N resulting from high heat.

A wide range of surface functional groups exist on the biochar graphene sheets' surface and within the biochar pores. As chemical bonds are created and rearranged during biochar making, these groups are formed (e.g., hydroxyl, $-OH$; amino, $-NH_2$; ketone, $-OR$; ester, $-(C = O)OR$; nitro, $-NO_2$; aldehyde, $-(C = O)H$; carboxyl, $-(C = O)OH$ and phenolic groups) (Van Zwieten et al., 2009).

Another interesting property of biochar is the co-existence of negative and positive charges resulting from the surface organic functional groups. Anion exchange capacity mechanism is explained by the presence of oxonium groups, which are positively charged (Lawrinenko and Laird, 2015). Negative charges arise from the deprotonation of carboxylic and phenolic groups resulting in cation exchange capacity. Cation exchange capacity (CEC) is also attributed to the deprotonation of proton-containing surface functional groups (Ok etal., 2015).

Biochar is characterised by high porosity, which develops during heating (Mukherjee et al., 2011). Three pore categories characterise biochar: namely micropores (less than 2 nm in diameter), mesopores (between 2 nm and 50 nm), and macropores (more than 50 nm). (Ok et al., 2015). Macro pores are responsible for aeration and offering a habitat for microorganisms (Downie et al., 2009). Micro-pores contribute to the surface area and mesopores for holding water.

2.5.2.2.   *Factors affecting biochar production.*
Four main factors that affect biochar characteristics are feedstock type, pyrolysis temperature,

heating time, and heating rate (Gai et al., 2014). Feedstock from animal origin, such as animal manure, including human waste, generally has a low C content and high N content than woody and lignocellulose material. Hence the C: N ratio of human waste is low. Increasing pyrolysis temperature reduces biochar yield due to volatilisation as well as evaporation of moisture. Increasing temperature also increases pore size. However, high temperatures of 700 °C and above result in the collapse of pores. An increase in the surface has been observed due to increased porosity directly proportional to the surface area. High temperatures burn the organic fraction leaving behind the basic minerals (Mg, Ca, and K), contributing to pH.

2.5.2.3. *Biochar as a soil amendment and effect on crop growth*

Biochar can change the soil's physical and chemical properties when added to the soil; hence it has been used as a soil amendment (Aslam et al., 2014). The high porosity of biochar allows it to retain water, increasing the water-holding capacity of the soil. Water-soluble nutrients such as nitrates are simultaneously held by biochar and prevented from leaching (Jeffery et al.,2011). According to a study carried out by Peake et al. (2014), biochar increased the amount of available water by 22%. Biochar addition to poorly structured sandy soils improved soil structure by forming soil aggregates and improving its hydraulic conductivity (Ouyang et al., 2013).

The high pH of biochar, attributed to basic cations, K, Mg, and Ca, offered a liming effect when added to acidic soils (Chintala et al., 2014). Chintala et al. (2014) observed that the release of these basic cations in an incubation study increased the soil pH and lowered the (Aluminium) Al saturation of acid soil. Soil pH was raised from 4.77 to 5.39 after adding rice husk biochar (Carter et al., 2013). Jien and Wang (2013) observed an increase in pH from 3.9 to 5.1 after amending a highly weathered acidic Ultisol at the end of a 105-day incubation study. Biochar is rich in exchangeable cations Mg, Ca, and K; hence its addition to the soil increases the amount of exchangeable base cations (Carter et al., 2013).

Cation exchange capacity, defined as a soil's ability to retain cations, increases with biochar addition. The addition of biochar derived from wood waste of white lead trees to soil increased CEC from 7.41 to 10.8 $cmol_c\ kg^{-1}$ (Jien and Wang, 2013). Retention of cations on biochar surfaces prevents leaching, making biochar addition an important amendment for crop productivity. Biochar added increased base cation percentage from 6.40 to 26.0% regarding cations K, Ca, and Mg (Jien and Wang, 2013). An increase in exchangeable base cations was also observed by Martinsen et al. (2015).

Amending soils with biochar reduces the leaching of nitrates and phosphates, which could be attributed to biochar's high anion exchange capacity. The mechanism is explained by the presence of oxonium groups, which are positively charged (Lawrinenko and Laird, 2015). The addition of coffee husk biochar to clay loam soil resulted in a 76.7% increase in available P in a three-month incubation study (Bayu et al., 2016).

Owing to the high C, the addition of biochar to the soil results in an increase in organic matter. The organic matter in biochar acts as binding agents to hold soil particles together (Liang et al., 2006). The addition of biochar decreases the bulk density of soil. These modifications of the soil's physical properties contribute to soil fertility. When biochar is added to sandy soil, it improves the soil structure by binding soil particles together, which increases soil nutrient and water retention. Amendment of soil with cotton trash biochar increased soil organic matter (Zhang et al., 2016).

Biochar, especially from woody and crop residue material, is not a source of nutrients. However, it has been observed to increase plant growth and improve soil properties when used in combination with commercial chemical fertilisers and organic fertiliser sources (Banomani et al., 2017). A meta-analysis carried out by Jeffery et al. (2011), and Biederman and Harpole (2013) recorded the effects of biochar on crop productivity. An average of 10% increase in crop productivity was observed when biochar was applied to soils amended with fertilisers. In their meta-data analysis, Biederman and Harpole (2013) observed that the average effect of biochar addition was neutral to positive despite variation in soil type, climate, and production methods. Positive responses were attributed to biochar's ability to make P available.

Biochar-induced alkalinity affects P availability by preventing P from being adsorbed onto iron oxides, making it available to plants. Liming agents reduce Aluminium (Al) and iron (Fe), allowing Al and Fe bound P to be released in the soil solution (Cui et al., 2011). Biochar can also improve soil physical properties such as water-holding capacity, soil aggregation, and nutrient leaching prevention. Some studies showed negative crop growth responses when biochar was used alone (Calderon et al., 2015; Wisnubroto et al., 2011). When biochar is used for agronomic purposes, it is usually used in combination with a fertiliser source since it is not treated as a fertiliser. However, biochar derived from faecal matter showed positive effects compared to the control due to the presence of P, mostly attributed to the original feedstock's nutrient content.

2.5.3. Co-composting

Co-composting is like composting, except that additional materials known as bulking agents and additional feedstock of different origins are added to the composted material before aerobic composting. Bulking agents help to increase porosity, increase C: N ratio, and reduce moisture. Numerous articles have reported co-composting solid waste such as chicken manure, pig manure, and faecal sludge with organic solids such as sawdust, straw, maize stalks, and biochar (Cofie et al., 2016; Nartey et al., 2017).The addition of biochar during composting has the advantage of reducing gases such as ammonia, nitrous oxide, and carbon dioxide (Steiner et al., 2011). Higher N retention was achieved when organic solid waste was composted with biochar due to reduced N losses and decreased volatilisation of gases such as ammonia (Xiao et al., 2017). Water-soluble nutrients such as nitrates are held in the pores of biochar, preventing them from being lost (Malinowski et al., 2019).

The residue from the decomposition of faecal matter using BSFL can be composted to produce organic fertiliser. In most studies where raw residue was used, negative crop responses were observed due to low initial C: N ratio, phytotoxicity, and high moisture content making it unattractive for use without further processing (Green et al., 2012). Suggestions have been made to compost the residue and assess the impact of the compost on crop production. Residue from the decomposition of organic food wastes (cooked rice, uncooked yam, and vegetables) by BSFL was mixed with chicken faecal matter, and sawdust had NPK values ranging from 0.95 to 1.68%, 0.27 to 1.13%, and 0.27 to 2.11% (Attiogbe et al., 2019).

2.5.3.1.   *The implication of compost on soils*

Adding compost to soils has two significant effects of increasing soil nutrients and improving soil organic matter. Soil organic matter positively affects the soil's biological, physical, and chemical properties (Głąb et al., 2018). Although composts of faecal origin are nutrient-rich and offer the benefits mentioned above, they are limited by the presence of trace metals. Trace element concentration must fall below the recommendations for biosolid application to soils for crop production.

2.5.3.2.   *Implications for health*

Most pathogens are killed due to the thermophilic temperatures reached during the composting process (Sunar et al., 2014). Total heterotrophic bacteria and total coliforms decreased from

1.17×10^6 and 4.7×10^4 to 4.3×10^4 to 7.4×10^2 CFU g^{-1}. *Salmonella* sp., and *Shigella* sp. were 7.3×10^4 CFU g^{-1}, yeasts, and moulds in the initial compost were 9.0×10^4 CFU g^{-1} reduced to non-detectable levels in the final compost after a 40-day in-vessel composting trial (Fatunla et al., 2017). Sewage sludge composting using sawdust as a bulking agent reduced the pathogen concentration. However, *Ascaris* is the major problem when dealing with a compost of faecal origin. Some composting methods reduce the concentration of *Ascaris*. A minimum reduction of 82% and 84% was observed for *Ascaris* and *Trichuris,* respectively, when sewage sludge was composted with a mixture of shredded maize cobs using a rotary drum composter (Appiah-Effah et al., 2018).

2.5.3.3.   Implications for crop production

Throughout history, the use of composted sewage sludge has been used for crop production. The use of composted residue from BSFL can be used similarly to provide crops with nutrients. Composting sanitises the waste material making it a safe organic fertiliser for crop production. Composting also reduces the finished compost's moisture content, makes plant nutrients available, and provides organic matter. It is hypothesized that crops will respond to BSFL co-compost as a slow-release fertiliser like other organic fertilisers.

2.6 Summary and conclusions

According to this literature review, it is evident that human excreta generated by informal settlements can be managed and recycled into valuable sources of nutrients. One method used for reducing waste is the BSFL technology to feed on the faecal matter from UDDTs. There is evidence that the residue left behind has fertilizer value to supply crops with nutrients. However, the use of the residue alone has several negative effects on crop growth. Post-processing methods such as co-composting the residue have not been investigated on the release of nutrients and response of crops (Banks et al., 2014).

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CHAPTER 3: OPTIMISING PYROLYSIS CONDITIONS FOR HIGH-QUALITY BIOCHAR PRODUCTION USINGBLACK SOLDIER FLY LARVAE FAECAL-DERIVED RESIDUE AS FEEDSTOCK

Residue from the decomposition of faecal matter from Urine Diversion Dry Toilets (UDDT's) using Black Soldier Fly Larvae (BSFL) is considered a waste. Discarding this waste can cause pollution, and dumping wasteland is limited. The BSFL residue can be pyrolysed to produce biochar. Faecal matter biochar offers a guaranteed 100% pathogen elimination and reductions in weight and volume during transport and storage. A preliminary study was conducted to (i) optimise pyrolysis conditions (temperature and residence time) for biochar production using BSFL faecal residue; and (ii) determine the physicochemical and morphological characteristics of BSFL Residue biochar produced. The BSFL residue was pyrolysed at 300°C for 120, 90, and 60 minutes, 400°C for 60 and 45 minutes, and 500°C for 60, 45, and 30 minutes using a muffle furnace. Biochar from each temperature that completely pyrolysed was characterised. Chemical characteristics analysed were total carbon and nitrogen, exchangeable bases, $pH_{(KCl)}$, total phosphorus, and ammonium and nitrates. Morphological characteristics were determined by scanning electron microscopy and BET surface area analysis. Biochar pyrolysis was optimum at 300, 400, and 500°C, at residence times of 60, 45, and 30 minutes, respectively. Carbon and nitrogen decreased with increasing temperature. Surface area ($6.61 \text{ m}^2 \text{ g}^{-1}$), pore size, and C: N (9.28) ratio increased at 500 °C. Exchangeable bases and pH significantly increased with increasing pyrolysis temperature, with biochar pyrolysed at 500 °C having the highest values. Increasing pyrolysis temperature increased P to $3\,148 \text{ mg kg}^{-1}$ at 500°C. Ammonium decreased with increasing pyrolysis temperature. Biochar produced at 500°C for 30 minutes had desirable characteristics; total P, exchangeable bases, and pH and was chosen for the composting experiment. Biochar can be used as a phosphorus source with potential for crop production. Pathogens were eliminated, making BSFL Residue biochar safe for handling.

Keywords: biochar; black soldier fly larvae; faecal matter; phosphorus; pyrolysis

3.1. Introduction

Biochar is a stable carbon (C) rich material made through pyrolysis — the heating of any organic material (feedstock) at high temperatures ranging from 300 to 700 °C under oxygen-limited conditions (Lehmann and Joseph, 2015). The quality of biochar depends on the type of feedstock, pyrolysis temperature, and the residence time (the amount of time the feedstock is held at a particular temperature) (Tomczyk et al., 2020). A wide range of organic materials such as crop residues (wheat straw, maize straw), woody material with a recent focus on invasive plants, algae, and fruit peels can be used as feedstock for pyrolysis (Zhao et al., 2019). In addition, animal manure, wastewater sludges have been used as pyrolysis feedstock (Callegari and Capodaglio, 2018; Ro et al., 2010). An interest in using faecal matter from Urine Diverting Dry toilets (UDDTs) as feedstock for pyrolysis is increasing (Zhao et al., 2019).

Faecal matter from UDDTs is fed to Black Soldier Fly Larvae (BSFL) to produce protein-rich larvae used as animal feed (Banks et al., 2014; Maleba et al., 2016). The process also generates residual waste, comprising partly digested material, residual larvae, and larvae droppings, which can be processed to produce biochar as a nutrient-rich waste-based fertilizer due to the inherent high phosphorus (P) property of faecal matter. The high-value biochar can be used as a plant nutrient source to supply P, increase soil organic matter, and lime acidic soils due to the high pH of biochar (Sun et al., 2018).

Although there is potential for the BSFL technology to be applied in faecal sludge management, there is little information on BSFL residue's pyrolysis derived from faecal matter collected from UDDTs to produce biochar (Mutsakatira et al., 2018). Research is needed to optimise pyrolysis conditions (pyrolysis temperature and residence time effect) on biochar's physical and chemical properties derived from faecal-derived BSFL residue and assess the product's safety by undertaking a microbial study analysis.

No studies have been done using BSFL Residue from UDDT faecal matter decomposition as feedstock for pyrolysis. Hence the objectives of the study were to (i) determine the optimal temperature and residence time for high-quality biochar production using BSFL residue as feedstock and (ii) characterise the biochar produced with respect to the physical, chemical properties, morphological, and microbial analysis.

3.2. Materials and methods

Black soldier fly larvae (BSFL) residue used during the study was collected and stored in airtight jars at 4 °C until pyrolysis was done.

3.2.1. Biochar production

The BSFL Residue was first air-dried before pyrolysis. Pyrolysis was carried out in metal trays using a temperature-controlled muffle furnace at the Soil Science Laboratory (University of Kwa-Zulu Natal Pietermaritzburg, South Africa). Three different temperatures (300, 400, and 500°C) were used and timed to determine optimum pyrolysis conditions for complete charring. The pyrolysis process can be done from a temperature range between 300 and 950°C (Aller etal., 2017). However, in this study, the basis for selecting lower temperatures of 300, 400, and 500°C was because the biochar produced is intended for agricultural use. Pyrolysis was carried out for 120, 90, and 60 minutes at 300 °C, 60 and 45 minutes at 400 °C, 60, 45, and 30 minutes at 500 °C.

Biochar samples were crushed and passed through a 2 mm sieve and stored in airtight Mason jars. The biochar samples were analysed for moisture content, total solids, volatile matter, organic matter, and fixed carbon following proximate methods Aller et al. (2017) described.

Biochar yield was calculated as a percentage of char mass generated after pyrolysis (Equation 1).

$$\text{Biochar yield (\%)} = \frac{\text{Initial mass (g) – Final mass (g)}}{\text{Final Mass (g)}} * 100$$

3.2.2. Proximate analysis

Approximately 10 g of the biochar sample was added to glass beakers in triplicate. The moisture content and total solids were determined by oven heating at 105 °C for 24 hours (Zhang et al., 2017). The ash content was determined by adding 5 g samples of biochar from the moisture-determined samples used for ash content in a muffle furnace at 550 °C for 6 hours(Mierzwa-Hersztek et al., 2019; Singh et al., 2017). Volatile matter was determined on the moisture determined samples by combusting 1 g of biochar at 950 °C for 7 minutes in crucibles with the lid on, according to the American Society of Testing Materials (ASTM D 271-48, 1954). Moisture,

ash and fixed C were calculated according to ASTM D1762–84 (2007).

3.2.3. Chemical analyses

A 1:20 weight: volume mixing ratio (1 g biochar in 20 mL water) was used for pH determination. Samples were stirred for 30 minutes using a magnetic stirrer, and pH was read using a calibrated H198129 pH meter (HANNA Instruments, Romania). The same sample solution used for pH was used for measuring electrical conductivity (EC) using a conductivity meter (HANNA Instruments, Romania). The pH in KCl was determined using 20 mL of 1M KCl. The ammonium acetate method, buffered at pH 7, was used to assess cation exchange capacity (Munera-Echeverri et al., 2018; Song and Guo, 2012). Ammonium ions were analysed using the Thermo-Scientific Discreet Gallery (Scientific Therm Fisher, Waltham, Massachusetts, USA, 2014). The leachate's exchangeable cations were determined by an atomic absorption spectrophotometer (AAS) (Varian, AA280FS, California, USA). Cation exchange capacity was calculated following Equation 2 below (Munera-Echeverri et al., 2018).

$$\text{CEC (cmolc kg}^{-1}) = \frac{\text{NH4}-\text{N(mgL}-1)(0.25\text{L})*100}{\text{Mass of biochar (g)(MeqNH4}-\text{N per 14mgNH4}-\text{N}}$$

Mineral elements from biochar were analysed from a 0.5 g sample of biochar. Ashing was done for 6 hours at 550 °C in a muffle furnace. The ash was solubilised on porcelain crucibles using 10 ml aqua regia (HNO_3 and HCl mixed in ratio 1:3) and digested using microwave-assisted digestion (von Gunten et al., 2017). Calcium, Mg, K, Zn, Cu, Fe, and Mn concentrations were determined using Flame Atomic Absorption Spectroscopy (FAAS) (Varian AA280FS, California, USA). Potassium and sodium were analysed using Flame Atomic Emission Spectroscopy (FAES) with a fast-sequential absorption spectrometer (Varian AA280FS, California, USA). A mass of 0.2 g of the air-dried biochar sample in crucibles was heated at 1450 °C for 6 minutes under furnace conditions to analyse carbon, nitrogen, and sulfur (CNS) using the Leco-TruMac CNS Autoanalyser (Leco-cooperation, LECO CNS-2000, St Joseph MI, USA, 2012).

Mineral nitrogen (NH_4^+-N and NO_3-N) was extracted with 2M KCl solution using 1:10 biochar: extracting solution volume ratio. From each sample, 2 g of sample was added to 50 mL plastic tubes with 20 mL of the extracting solution (Maynard et al., 1993). An overhead shaker was used to shake the mixture for 30 minutes. Whatman 1 Filter paper was used to filter the mixture, and the filtrate was transferred to storage bottles. The NH_4^+-N and NO_3-N were analysed using the Thermo-Scientific Discreet Gallery (Scientific Therm Fisher, Waltham, Massachusetts, USA, 2014).

Phosphorus was extracted following the AMBIC method (Van der Merwe et al., 1984). The extracted solution was mixed with a colour reagent and changed to a blue colour. The extractable P was thus determined after reading the blue liquid's absorbance at 670 nm using an ultraviolet spectrophotometer (Varian Alpha UV-VIS, Spectronic Unicam, Berlin, Germany).

3.2.4. Physical properties analyses

The surface morphology of BSFL biochars was examined using a Scanning Electron Microscope (SEM) (EVO LS15, Carl Zeiss Microscopy, New York, USA). The biochar samples were sputtered with gold coating before viewing using a gold sputtering machine (Quorum Q150R ES, Quorum Technologies, East Sussex, UK). The diameter of pores was measured using Image J software (Soft Imaging System, Münster, Germany). The surface area was analysed in liquid nitrogen at a temperature of 25 °C using the Brunauer–Emmett–Teller (BET) surface area analyser (NOVA 2200e analyser Quantachrome Instruments, Boynton Beach, FL, USA) (Brunauer et al., 1938). Physisorption isotherms were plotted based on nitrogen adsorption at

increasing pressure using the Barret, Joyner, and Balenda (BJH) method. This method was used for the determination of mesopore size distribution (Barrett et al., 1951).

3.2.5. Microbiological analyses

Both BSFL residue and BSFL residue biochar samples were analysed for total coliforms, faecal coliforms, *Escherichia coli (E. coli)*, *Ascaris,* and helminths at Talbot & Talbot (Pty) Ltd, Pietermaritzburg, South Africa (29°37'55.6"S 30°22'41.4"E). The pour plate membrane filtration method was used to analyse total coliforms and faecal coliforms, while the membrane filtration coupled with 4-methylumbelliferyl-beta-D-glucuronide (MUG) fluorescence was used for *E. coli*. A water sample was filtered through a membrane filter upon which the bacteria were entrapped. The filter was placed on a selective growth medium and incubated at 35°C for 24 hours, after which all characteristic colonies were counted. A dilution of 1:10 of the sample was made using sterile deionised water. Several sequential dilutions were made from this and filtered.

The *E. coli* was determined after a positive count was made on colonies that developed a blue fluorescence around the colony's margin when grown in an NA-MUG (Nutrient Agar + MUG) medium at 35 ℃ ±0.5 ℃ for 4 hours. Total coliforms were determined from counts with red bacterial colonies with a (golden-green) metallic sheen within 18-24 hours when incubated at 35 °C on m-Endo type medium containing lactose. To determine faecal coliforms, counts were made on bacteria that produced typical blue colonies on m-FC medium without Rosolic acid within 18-24 hours when incubated at 45 °C.

Ascaris and helminths were analysed using "tween 80" as a surfactant. A 10 g dried sample was weighed and soaked in "tween 80 solution". This solution was filtered using a 150 µm sieve followed by centrifugation. The filtrate that passed through the sieve was collected and transferred to a centrifuge tube, and centrifuged. The pellet was collected and re-suspended using zinc sulphate, allowing helminth eggs to float in a solution. The supernatant was filtered using a 20 µm sieve. The particles/residue that remained on the sieve was then collected and centrifuged. After centrifuging, the supernatant was collected and transferred to a slide for observation under a microscope. Helminth eggs observed were counted and calculated per g of dry biochar or residue (APHA, 2005).

3.2.6. Data analysis

All data sets were subjected to a general Analysis of Variance (ANOVA) using GenStat 18th Edition Statistical Package VSN International, Hemel Hempstead, UK. Means were separated using Fisher's Least Significant Difference at a 5% level of significance.

3.3. Results

3.3.1. Biochar yield and proximate results

Figure 3.1 shows images of BSFL residue pyrolysed at different temperate pyrolysis temperatures and residence times. Increasing residence time and temperature resulted in the burning of residue. Optimum pyrolysis was achieved at the highest pyrolysis temperature of 500 °C and the shortest residence time of 30 minutes. Optimum pyrolysis was also achieved at lower temperatures of 300 and 400 °C when residence time increased to 60 and 45 minutes, respectively (Figure 3.1)
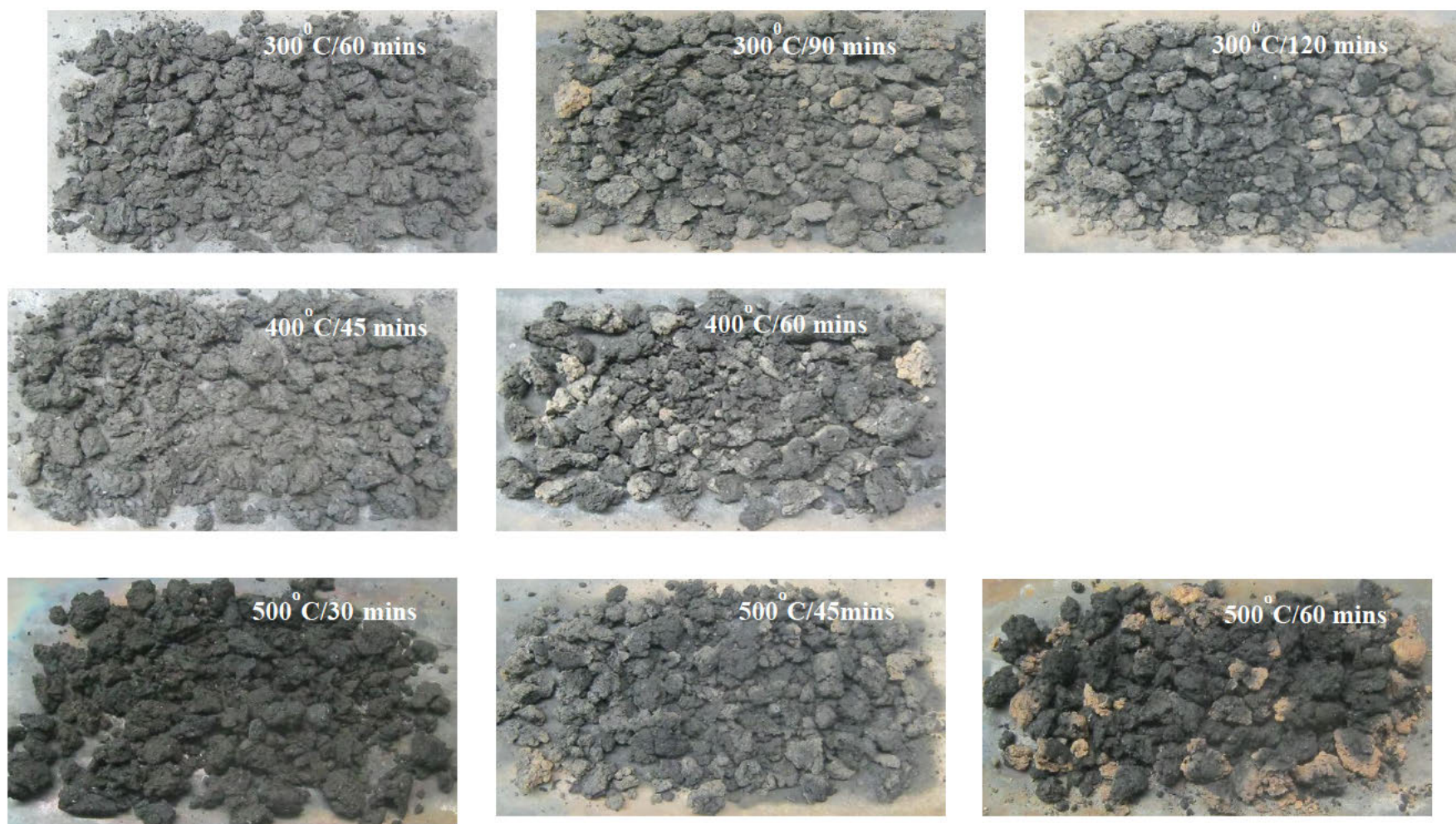
Figure 3.1 Black soldier fly larvae residue biochar at different pyrolysis temperatures and different heating times.

Figure 3.2 shows the yield of biochar pyrolysed at 300, 400, and 500 °C. Black Soldier Fly Larvae Residue subjected to the highest pyrolysis temperature (500 °C) and the shortest residence time (30 minutes) produced the lowest yield compared to biochar pyrolysed at 400 °C and 300 °C for 45 and 60 minutes, respectively (Figure 3.2). Biochar yields decreased significantly ($p<0.001$) with increasing pyrolysis temperature from 51.5 to 56.9% at 300 and 500 °C, respectively.
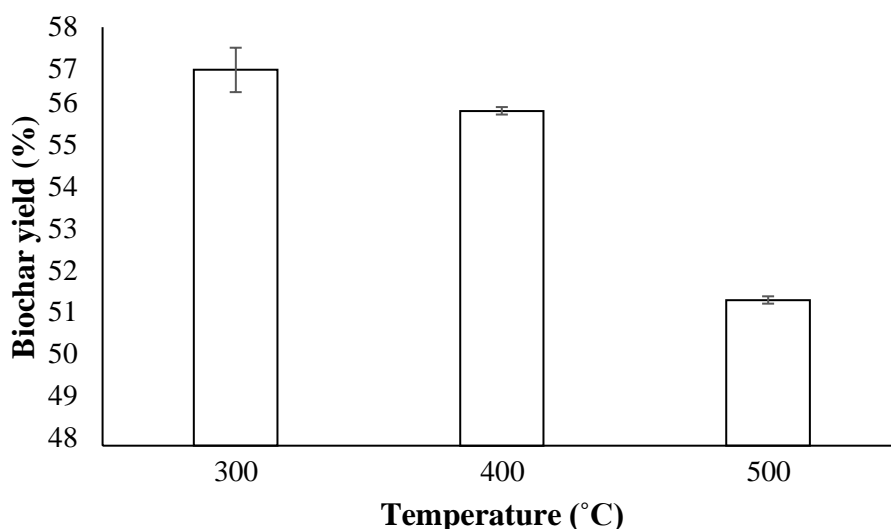
Figure 3.2 Biochar yield at different pyrolysis temperatures and residence times.

Table 3.1 shows the proximate analysis results of biochar pyrolysed at different pyrolysis temperatures and different residence times. The residue had the lowest ash content of 12.40%, which increased with increasing pyrolysis temperature. Ash content increased to 15.42% at 300 °C, 18.57% at 400 °C and 24.18% at 500°C (Table 3.1). Volatile matter (VM) decreased with increasing pyrolysis temperature, with the residue having the highest value (76%). Further increase in pyrolysis temperature reduced VM to 63, 55, and 33% at 300, 400, and 500 °C, respectively. Organic matter decomposition by loss on ignition was highest in biochar pyrolysed at 500 °C. The residue had the lowest fixed C of 12%, which increased with increasing pyrolysis temperature. The highest fixed C was 43% at 500 °C. Pyrolysis resulted in a significant decrease ($p<0.001$) in moisture from the residue (51.7%) to 0.54% (300 °C), 0.24% (400°C) and 0.18% (500 °C) (Table 3.1).

Table 3.1 Proximate values (percentage of dry matter) of biochar produced under different pyrolysis conditions.

Treatment	Ash (%)	Volatile (%)	Fixed C (%)	Moisture (%)	TS (%)	OM (%)
Residue	12.40[a]	75.83[a]	11.78[a]	51.07[b]	48.93[a]	87.60[a]
B300	15.42[b]	62.80[b]	21.78[b]	0.54[a]	99.46[b]	84.58[b]
B400	18.57[c]	55.01[c]	26.42[c]	0.24[a]	99.76[b]	81.43[c]
B500	24.18[d]	32.77[d]	43.06[d]	0.18[a]	99.82[b]	75.82[d]
s.e.d	0.1002	0.415	0.459	0.4080	0.4080	0.1002
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
c.v(%)	1.1	0.9	2.2	1.9	0.3	0.2

Superscripts represent mean differences ($P<0.05$) within each column according to Fisher's test

B300, B400, and B500 indicate biochar pyrolysed at 300 C, 400 C, and 500 C for 60, 45, and 30 minutes respectivelyTS is the total solids; OM is the organic matter; Fixed C is the fixed carbon

s.e.d. is the standard error of deviation

l.s.d. is the least significant difference at the 5% level

c.v. is the coefficient of variation

3.3.2. Chemical characteristics

Table 3.2 shows the chemical characteristics of biochar pyrolysed at different pyrolysis temperatures and residence times. The C: N ratio increased with increasing pyrolysis temperature, with the highest C: N ratio of 9.28 at 500°C and the lowest ratio of 8.33 at 300°C (Table 3.2). The residue's pH value was 6.79 and increased with increasing temperatures (8.8, 9.3, and 9.82 at 300, 400, and 500°C, respectively) (Table 3.2). The residue had the highest EC, which decreased at 300°C and increased at 400 and 500°C (Table 3.2). Table 3.2 shows that cation exchange capacity increased with increasing pyrolysis temperature. At 300, the CEC was 29.07 cmol$_c$ kg^{-1} and increased at 400 and 500°C, from 70.01 to 70.06 cmol$_c$ kg-^{1}, respectively. Exchangeable bases and trace elements increased with increasing pyrolysis temperature, and the trace element concentrations were above the limits for disposal for sewage sludge

Table 3.2 Chemical (C: N, pH, EC, CEC, exchangeable cations, and trace elements concentrations) properties of biochar produced under different pyrolysis conditions.

	C: N	pH	EC mS cm^{-1}	CEC (cmolc kg^{-1})	Exchangeable bases (cmolc kg^{-1})				Trace elements (mg kg^{-1})						
					Ca	K	Mg	Na	Cr	Cd	Cu	Fe	Mn	Ni	Zn
Residue	8.07[ab]	6.70[a]	2.11[a]	15.11a	0.39[a]	0.07[a]	0.20[a]	0.17[a]	17.8[a]	1.0[a]	89.7[a]	3407[a]	336.6[a]	22.1[a]	347.1[a]
B300	8.33[b]	8.80[b]	2.22[b]	29.07[b]	0.99[b]	0.19[b]	0.59[b]	0.30[b]	153.7[b]	3.0[c]	135.9[b]	6224[b]	458.5[b]	34.7[a]	649.8[b]
B400	7.94[a]	9.32[c]	2.47[c]	70.01[c]	1.07[c]	0.18[b]	0.63[c]	0.32[b]	176.8[c]	2.7[b]	148.0[c]	8929[c]	448.3[b]	54.8[b]	683.5[b]
B500	9.28[c]	9.82[d]	2.51[c]	70.06[c]	1.12[c]	0.20[b]	0.65[c]	0.33[b]	210.6[c]	4.0[d]	221.6[d]	14737[d]	504.0[c]	64.7[b]	805.2[c]
s.e.d	0.094	0.018	0.013	0.352	0.022	0.004	0.008	0.005	8.370	0.104	1.790	30.0	11.54	4.15	11.51
p value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
cv (%)	1.4	0.2	0.7	0.9	3.0	3.7	1.8	2.0	7.3	4.8	1.5	0.4	3.2	11.5	2.3

* Superscripts represents mean differences (P<0.05) within each
column according to Fisher's test CEC is the cation exchange capacity
s.e.d. is the standard error of deviation
l.s.d. is the least significant difference at a5 % level of significance
c.v. is the coefficient of variation
B300, B400, and B500 indicate biochar pyrolysed at 300 C, 400 C, and 500 C for 60, 45, and 30 minutes respectively

Figure 3.3 shows ammonium and orthophosphate content of biochar pyrolysed at different pyrolysis temperatures and residence time. Ammonium was highest at the lowest temperature of 300 (2.07 mg kg^{-1}) and decreased with increasing pyrolysis temperature to 1.22 and 0.90 mg kg^{-1} at 400 and 500°C, respectively. Increasing pyrolysis temperature increased the P content of biochar at 500°C by 25.6%.
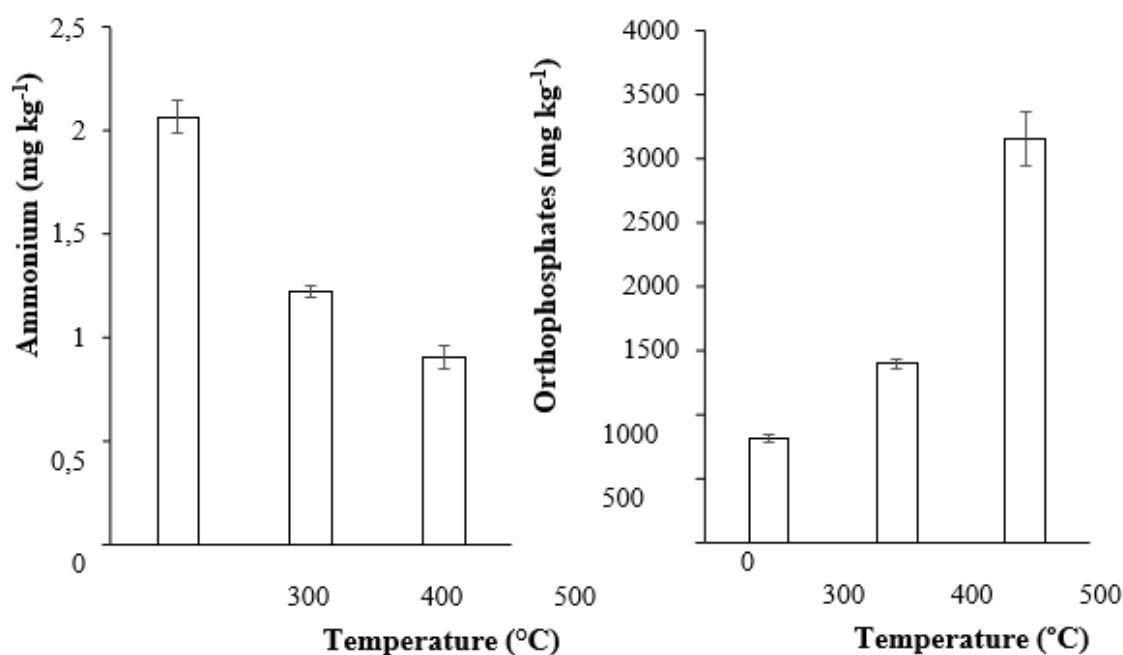
Figure 3.3 Extractable ammonium and inorganic P (orthophosphate) concentrations (mean ± standard error of means; n=3) in biochar exposed to three contrasting pyrolysis temperatures

3.3.3. Physical characteristics

Figure 3.4 shows isotherms for biochar produced at 300 °C (A), 400 °C (B), and 500 °C (C) with their respective Scanning Electron Microscopy (SEM) images. All biochars had similar type IV isotherms (Figure 3.4). As the pressure increased in all isotherms, the amount of nitrogen gas adsorbed onto the biochar's surface increased, forming a monolayer indicated by the knee on the graph. At 300 °C, the pores were smaller and increased in size with increasing pyrolysis temperature. As the temperature increased, the pores merge to form bigger pores. The corresponding SEM images of the biochars are shown on the right (Figure 3.4). Increasing the pyrolysis temperature changed the surface morphology of biochar. Biochar pyrolysed at 300 °C had a smooth surface structure. At low pyrolysis temperatures, smaller pores were observed.

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Pore quantity and size increased with increasing pyrolysis temperatures, as is evident in the electron microscope images. As the pyrolysis temperature increase, the surface of the biochar became more rugged.
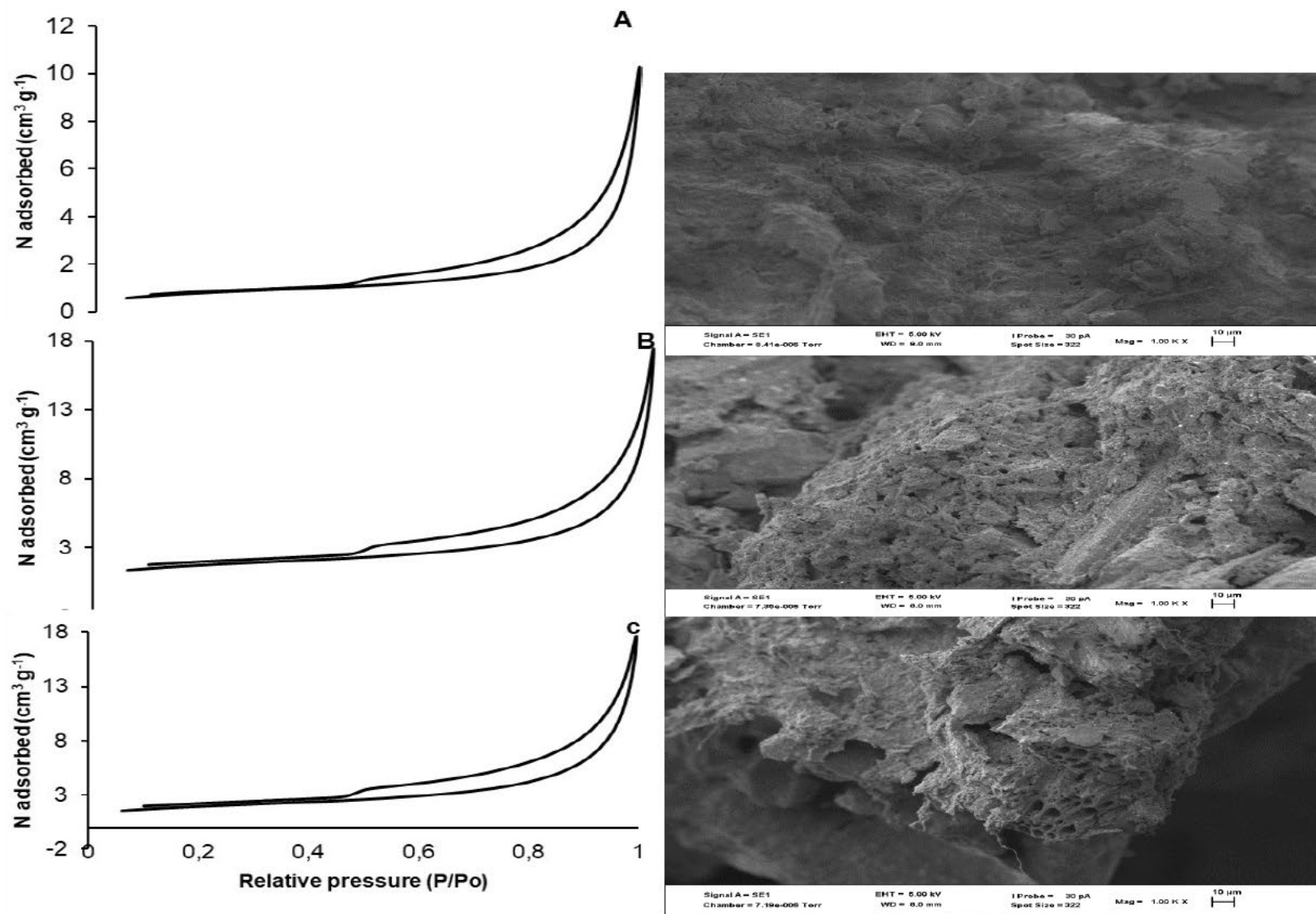
Figure 3.4 Isotherms for biochar produced at 300 °C (A), 400 °C (B), and 500 °C (C) with their respective SEM images.

Figure 3.5 shows the Brunauer–Emmett–Teller (BET) surface area and pore volume of biochar produced at different pyrolysis temperatures and residence times. Pore diameter, pore area, and surface area increased significantly ($p<0.001$) with increasing pyrolysis temperature. The surface area was lowest at 300 °C (2.84 $m^2 g^{-1}$), followed by 6.14 $m^2 g^{-1}$ at 400 °C and the highest at 500 °C (6.61 $m^2 g^{-1}$), which did not differ significantly. The BET surface area increased with increasing pyrolysis temperature (Figure 3.5). Pore volume increased with increasing pyrolysis temperatures ranging from 0.017 to 0.028 at 300 °C, and 400, and 500 °C, respectively.
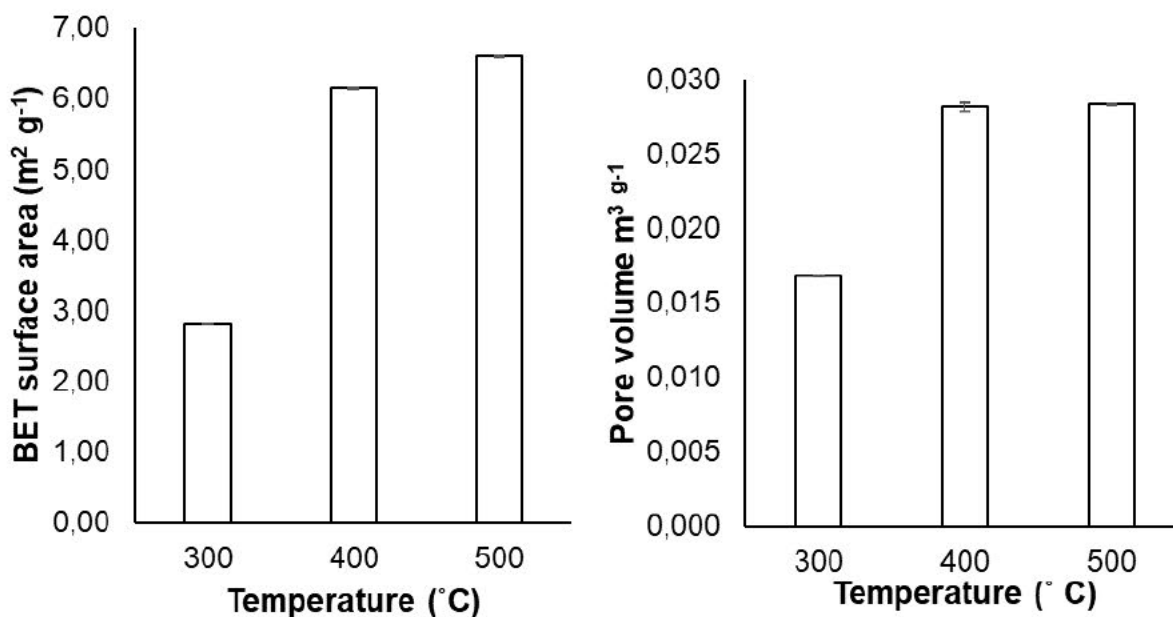
Figure 3.5 Relationship between BET surface area and pore volumes of biochar subjected to three various temperatures.

Table 3.3 shows the distribution of micropores, mesopores, and macropores in biochar pyrolysed at 300, 400, and 500 °C. Increasing pyrolysis temperature from 300 to 500 °C increased the area contributed by micropores, mesopores, and macropores.

Table 3.3 Pore area distribution, micropores, mesopores, and macropores for biochar pyrolysed at different pyrolysis temperatures.

Temperature (˚C)	Micropores	Mesopores	Macropores
300	0.12[a]	0.40[a]	1.86[b]
400	0.14[a]	4.41[c]	0.38[a]
500	0.22[b]	1.09[b]	3.21[c]
s.e.d	0.01	0.02	0.06
l.s.d	0.02	0.05	0.02
P value	<0.001	<0.001	<0.001
c.v (%)	5.4	1.2	0.4

Superscripts represent mean differences ($P<0.05$) within each column according to Fisher's test

B300, B400, and B500 indicate biochar pyrolysed at 300 C, 400 C, and 500 C for 60, 45, and 30 minutes respectively is

the total solids; OM is the organic matter; Fixed C is the fixed carbon

s.e.d. is the standard error of deviation

l.s.d. is the least significant difference at the 5% level

c.v. is the coefficient of variation

3.3.4. Microbiological characteristics

The residue was characterised in terms of *Ascaris*, viable helminths, *Escherichia coli*, total coliforms, and faecal coliforms (Table 3.4). Pyrolysis killed all the pathogens, making safe biochar amendments that do not pose a health risk to users.

Table 3.4 Biological characteristics of residue used as feedstock for pyrolysis.

	Ascaris (g^{-1} dry mass)	Viable helminth (g^{-1} dry mass)	*E. coli* ($cfu\ ml^{-1}$)	Faecal coliforms ($cfu\ ml^{-1}$)	Total coliforms ($cfu\ ml^{-1}$)
Residue	1	2	4×10^{-1}	7×10^{-1}	1.1×10^{-1}
Biochar (300 °C)	0	0	0	0	0
Biochar (400 °C)	0	0	0	0	0
Biochar (500 °C	0	0	0	0	0

3.4. Discussion

The study sought to determine the optimal temperature and residence time for high-quality biochar production using BSFL residue as feedstock. The study also characterised the biochar produced with respect to physical, chemical, and microbial properties. Exposure of BSFL faecal residue for longer durations during the pyrolysis does not yield high-quality biochar. These results are comparable with studies carried out at Makerere University using temperatures of 350, 450, and 600 °C and residence times of 10, 20, and 40 minutes using faecal matter as feedstock (Gold et al., 2018). The burning at high temperatures and longer residence times in the BSFL residue could be attributed to residual larvae containing proteins and fatty acids that quickly heat up.

The observed decrease in biochar yield could probably be attributed to thermal dehydration of hydroxyl groups as moisture is lost through evaporation and the volatilisation of organic fractions (Koetlisi and Muchaonyerwa, 2017; Liu et al., 2014). Organic C is lost during pyrolysis due to the thermal degradation of lignocellulose material in the human excreta (Liu et al.,2014). At 300 °C, torrefaction (the slow pyrolysis process where organic material starts to pyrolyse at temperatures between 200 and 320 °C) results in a more significant mass loss. These results are consistent with Sun et al. (2016), who observed an initial rapid mass loss at 300 °C during the pyrolysis of sewage sludge; and reported low biochar yields with increasing residence time at a low temperature of 300 °C, using different agricultural wastes (from low ash to high ash). Volatile matter findings are consistent with familiar progressions where more VMs have been removed with rising temperatures leading to a stable, fixed carbon (Antal and Grønli, 2003; Titiladunayo et al., 2012). Liu et al. (2014) established a positive relationship between VM organic matter and fixed C. As pyrolysis temperature increased, more volatiles were driven from the carbon matrix resulting in biochar with low VM and increasing the fixed C (Leng et al., 2019). Low VM is also indicative of low organic matter. At higher temperatures, as more volatiles are driven off, more organic origin material is burnt.

A decrease in the C: N ratio could be attributed to the decline in both total C and total N; however, total N decreased faster than C with increasing pyrolysis temperature (Table 3.2). Increasing temperature results in the conversion of organic C from the solid phase to the condensable liquid phase products of the pyrolysis process, such as tar. A comparable trend of decreasing C with increasing pyrolysis temperature was observed when poultry litter was pyrolysed at 300, 400, and 500 °C (Song and Guo, 2012). The decrease in total N is attributed to the loss of NH_4^+-N

and NO_3^--N groups at lower temperatures (300 and 400 °C). Volatile matter containing N groups are also lost at 200 °C (Bagreev et al., 2001). Further increase in temperature could have resulted in a different nitrogen group (pyridine), resulting in a further decrease in total N (Khanmohammadi et al., 2015).

Increasing pyrolysis temperature produced more alkaline biochar. An increase in pH is linked to an increase in basic cations such as Ca, K, and Mg. Also, higher amounts of inorganic compounds such as K, P, Mg, and Ca, compared to the residue which remained in the biomass after degradation of organic material and volatilisation of C, H, O, and volatile solids result in high ash content (Domingues et al., 2017). Alkali metals become separated from the organic matrix at increasing temperature during pyrolysis, resulting in biochar enriched with alkali metal salts due to loss of mass (Cao and Harris, 2010; Gasco et al., 2005; Gaskin et al., 2008; Singh et al., 2010; Yuan et al., 2011). Hence the relative increase in pH with increasing pyrolysis temperature canbe attributed to increased ash content. During pyrolysis, carboxyl groups in the biochar are reduced, and acidic groups are deprotonated to conjugate bases resulting in alkalinity. Furthermore, pyrolysis has been associated with an increase in basic functional groups and a decrease in carboxylic functional groups, increasing pH, as observed by Ronsse et al. (2013). The high pH characteristic of biochar is beneficial when applied to acidic soils (Berek et al., 2011).

Biochars produced at low temperatures up to 400 °C have been found to have lower electrical conductivities than the residue (Figueiredo et al., 2018; Singh et al., 2010). However, as pyrolysis temperature increases, EC increased. The EC is an indicator of total salts, and it canbe used to estimate the quantity of total dissolved salts in a sample. Electrical conductivity is important when considering biochar use for crop production because materials with high salinity can have toxic effects on plants (Song and Guo, 2012). The minimum range for EC is 2 to 3.5 mS cm^{-1}; hence all the three biochars can be used without risk of toxic salinity effects to plants (Abou-Hadid et al., 1996). Cation exchange capacity has been found to increase in biochar because of an increase in surface area. There is a relationship between increased CEC and phenolic functional groups' formation with increasing pyrolysis temperature (Mukherjee et al., 2011).

Exchangeable cations (K, Ca, and Mg) and trace elements such as Cd, Cu, Cr, Mn, Ni, and Zn increased after pyrolysis since they are not volatilised (Gondek and Mierzwa-Hersztek, 2017; Sadaka et al., 2014). Gezahegn et al. (2019) discovered an increase in the concentration of main

elements Ca, Mg, and K with increasing pyrolysis temperature using wood feedstocks. Calcium, Mg, and K are essential macronutrients; hence, their biochar availability benefits crop production. Human beings do not excrete heavy metals. Trace amounts are only found when the heavy metals originate from dietary sources, that is, if human beings ingest heavy metal contaminated crops (Tervahauta et al., 2014). Also, there is possible contamination from external sources such as foreign objects such as batteries thrown into pits.

There is a need for education on the proper use of UDDTs to prevent the accumulation of heavy metals in pits. There is variability in the guidelines for acceptable trace elements' limit levels (IBI, 2012; Meyer et al., 2017; Schmidt et al., 2016). However, the biochar produced in this study did not fall within limits. After pyrolysis, the concentration of trace metals increases due to a decrease in the biochar volume, while heavy metals do not change. Trace metals are of concern when the biochar is handled for crop production.

The decrease in inorganic N in the form of nitrates and ammonium is due to the loss of NH groups. Low detection values of less than 0.2 mg kg^{-1} of nitrates were observed at 300 and 400°C. However, these results are contrary to reports by Hossain et al. (2011), who observed that increasing pyrolysis temperature resulted in an increase in nitrates to 0.24 and 0.32 mg kg^{-1} at 500 and 700°C, respectively. When sewage sludge was used, there was an increase of 43% in nitrates at 700°C (Hossain et al., 2011). In the same study, ammonium decreased from 1175 mg kg^{-1} to 25 mg kg^{-1} at 500°C. Manure-based biochars are popular for their richness in P (Cantrell et al., 2012). In this study, orthophosphates increased four-fold at the highest pyrolysis temperature of 500 °C (Figure 3.3). Phosphorus is related to the residue's inorganic fraction (Capodaglio et al., 2017; Khanmohammadi et al., 2015). The increased P content suggests that faecal matter pyrolysis promises to be a potential alternative P source. Phosphorus also increased in an experiment when sewage sludge was pyrolysed at 450 °C, which resulted in a 2-3-fold increase in P (Fristak et al., 2018).

The type IV isotherms generated from the BET surface area analysis are indicative of a mesoporous pore structure. Pores were clogged by volatiles, which could not escape at 300 °C. Increasing pyrolysis temperature increased the amount of volatiles driven off the feedstock resulting in more pores and more voids within the biochar matrix (Ma et al., 2016; Zhang et al., 2017). However, this increase in temperature did not result in considerable removal of volatile to form bigger pores.

The BET surface area findings are consistent with those of Gao et al. (2014), who observed that as temperatures rise, pores are created, and the structural sheets of the biochar crack resulting in increased surface area. However, the low surface area is a characteristic of biochar derived from feedstock of faecal origin. Koetlisi and Muchaonyerwa, (2017) observed a similar low surface area after pyrolyzing sewage sludge and pit latrine waste. Sewage sludge biochar had surface areas of 4.17, 2.31, and 2.40, and pit latrine biochars had surface areas of 5.09, 4.91, and 5.65 cmolc kg-1 at pyrolysis temperatures of 350, 550, and 650 °C, respectively. Pores are created due to the devolatilisation of organic matter and the removal of carbon mass as volatile matter, creating pores (Song and Guo, 2012) from 300 to 400 °C. However, in this study, further increasing the pyrolysis temperature to 500 °C did not result in a significant difference in SA from 400°C.

Pyrolysis at all the optimum temperatures and their respective residence times resulted in 100% pathogen elimination. *Ascaris*, viable helminth, *E. coli*, faecal coliforms, and total coliforms were not detected in all the three biochar samples pyrolysed at 300, 400, and 500 °C for 60, 45, and 30 minutes respectively. Pyrolysis proved to be a way to sanitise the BSFL faecal-derived residue making it safe for handling without posing health hazards.

3.5. Conclusions

Pyrolysis temperature significantly affects biochar's physical and chemical characteristics produced from the residue after decomposition of faecal matter from UDDTs by BSFL. The characterisation is important in determining the pyrolysis temperature suitable for producing biochar used in agriculture. This study confirmed that biochar yield decreased with increasing pyrolysis temperature. Biochar pyrolysed at 500 °C for 30 minutes showed excellent characteristics regarding C: N ratio, porosity, surface area, major elements, CEC, pH, and fixed C. The highest pyrolysis temperature of 500 °C resulted in the lowest biochar yield when pyrolysed for 30 minutes. All the biochars had low inorganic N (nitrates and ammonium), indicating that biochar is not a source of N. Phosphorus (orthophosphates) was high at 500 °C. The isotherms for biochar produced at 500°C showed a mesoporous material meaning it has good water and nutrient holding capacity. The high temperatures killed pathogens during pyrolysis making it safe for agricultural use. Hence biochar pyrolysed at 500 °C for 30 minuteswas chosen as a bulking agent for the co-composting experiment.

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CHAPTER FOUR: ASSESSING THE PHYSICAL, CHEMICAL AND PHYTOTOXIC CHARACTERISTICSOF COMPOST MADE FROM BLACK SOLDIER FLY LARVAE RESIDUE AND BIOCHAR

Residue from the decomposition of faecal matter from urine-diverting dry toilets using Black Soldier Fly Larvae (BSFL) residue presents challenges such as high moisture, ammonium, residual pathogens, and low C: N when used raw for crop production. Processing of the residue, for example, composting can address these problems to improve BSFL Residue nutrient quality. In this study, BSFL residue was co-composted with biochar (BSFL Residue COMBI) and without biochar (BSFL Residue Compost). The composts were characterised for physical, chemical, and phytotoxic properties compared to chicken manure (CM), BSFL Heated Residue (BSFL HR), and the BSFL Residue. During the composting process, temperature and pH were measured. At the end of the composting period, water holding capacity exchangeable bases cation exchange capacity, total phosphorus, total carbon and nitrogen, trace metals, and phytotoxic tests were carried out. BSFL COMBI reached a higher thermophilic temperature (59.9 °C) compared to BSFL Residue Compost (55.2 °C). Composting increased pH from 6.48 (residue) to 7.53 and 7.47 in the BSFL Residue COMBI and BSFL Residue Compost, respectively. BSFL Residue COMBI had a higher electrical conductivity (4.72 mS cm^{-1}), water holding capacity 49.6%, exchangeable bases, cation exchange capacity (18.15 cmol$_c$ kg^{-1}), total P (5175.96 mg kg^{-1}), and C: N ratio (11.3: 1). Trace metal concentrations were higher in both BSFL Residue COMBI and BSFL Residue Compost and were higher than the limits for agricultural use. Both BSFL Residue COMBI and BSFL Residue Compost passed the phytotoxic test with germination for 80 and 88 % radish seeds. Composting with BSFL Residue biochar improved compost physical and chemical qualities, especially P, which makes it a potential source that can be used to amend P deficient soils.

4.1 Introduction

Black soldier fly larvae (BSFL) residue is the remaining material, also known as frass after organic matter has been fed to BSFL (Kawasaki et al., 2020). The residue is characterised by partly digested material, larvae remain, and larvae droppings. Organic materials such as kitchen, animal, and human waste can be fed to BSFL to reduce waste volume (da Silva and Hesselberg, 2020). The larvae are harvested and used as a protein source. Oil can also be extracted from the larvae. There has been an interest in using the BSFL technology to treat faecal waste from Urine Diversion Dry Toilets (UDDTs) (Maleba et al., 2016). The residue that remains is a waste product. There are challenges with the disposal of the residue. However, the residue has residual nutrients and can be composted to produce compost with the potential to provide crops with nutrients (Sarpong et al., 2019).

Composting is a technology that transforms organic matter into a stable nutrient-rich product that can benefit soil fertility and, ultimately, agricultural productivity (Araceli and Dios, 2015; Haug, 2018). The addition of compost increases the soil organic matter (SOM), improves the formation of soil aggregates in sandy soils, thus preventing rapid leaching of nutrients and increasing the water holding capacity of the soil and the availability of soil nutrients. The microbial biomass population involved in nutrient mineralisation is also improved. Compost performance has been recorded to be as effective as synthetic mineral fertilisers (Moya et al., 2019). It is also a cost-effective and environmentally friendly method of adding nutrients to the soil. Compost can improve the structure of sandy soils and, subsequently, nutrient retention in such soils.

Furthermore, composting decreases the volume of waste and removes weed seeds and pathogenic bacteria (Cofie et al., 2009). Animal manure such as chicken, pig, goat, cattle can be used for the composting process due to the presence of nutrients such as (N), phosphorus (P), and potassium (K). Human excreta also contain all the macronutrients essential for crop growth and can be used as a feedstock for the composting process. Although there are many ways of treating human excreta, composting is often considered the best option because useful humus is the outcome of this procedure (Nagy et al., 2019). In Tahiti and Kenya, faecal matter is composted and used as a nutrient source for crop growth (Krounbi et al., 2019; Moya et al., 2019).

The residue from the decomposition of faecal matter by BSFL can be used as a feedstock for

composting since it contains residual nutrients. Converting the residue into a useable form closes the nutrient loop by returning the much-needed nutrients to the soil for crop production(Moya et al., 2019). However, when feedstock of faecal origin is used, there is a risk of residual heavy metals and pathogens that present health risks to human beings. Generally, the residue is also characterised by high moisture and high ammonium: nitrogen ratio. Depending on the organic material used for feeding the BSFL, the residue can be directly used for crop production or needs further processing. When added directly to the soil, the high ammoniumcan cause ammonium toxicity to plants (Alattar et al., 2016b). The BSFL Residue can be composted to overcome this problem. In a study using kitchen waste as feedstock,the BSFL residue used on maize plants' growth showed stunted growth. These effects were attributed to too high ammonium concentrations of BSFL residues, resulting in phytotoxicity and low residue porosity (Alattar et al., 2016b).

Further research on methods to post-processing the BSFL residue, such as composting, is needed before this material can be used as a fertilizer (Alattar et al., 2016; Beesigamukama etal., 2020). BSFL residue was composted for five weeks after harvesting larvae at two weeks using the heap method to obtain a mature and stable frass product used in experiments as BSFfrass fertilizer (Beesigamukama et al., 2020). In this study, the residue compost yielded higher than commercial chemical fertiliser.

In chapter three, it was established that BSFL residue could be pyrolysed to make biochar. It is hypothesized that mixing biochar with the residue and composting the mixture results in a waste-based amendment with improved physical, chemical, and biological properties for improving poor soils (Agegnehu et al., 2016; Prost et al., 2013; Vandecasteele et al., 2016; Zhang and Sun, 2014). The use of biochar as a compost amendment has recently been proposed (Camps and Tomlinson, 2015; Sanchez-Monedero et al., 2018). The whole composting process can be done without biochar. However, biochar's addition before composting impacts compost processes by reducing the time needed to finish composting and attaining higher thermophilic temperatures. Biochar also improves the compost qualities such as nutrient and humus content and enhances environmental conditions for microbial growth in composting piles (Sanchez-Monedero et al., 2018). Biochar improves the composting process by increasing the water holding capacity, increasing aeration, and reducing gas emissions (López-Cano et al.,2016).

The objective of the study was to co-compost BSFL residue with BSFL Residue biochar and assessed the physical (water holding capacity), chemical (mineral element composition, pH, and heavy metal content), and phototoxicity compared to other organic amendments (chicken manure and BSFL HR).

4.2 Materials and methods
4.2.1 Experimental material
The experimental material comprised of biochar + BSFL residue compost (COMBI), BSFL residue Compost, BSFL Heated Residue, chicken manure sourced from Ukulinga farm, and the BSFL residue. Biochar pyrolysed at 500 ˚C for 30 minutes, characterised in chapter three, was used as a bulking agent for the composting process. Two composts were made from BSFL residue. One was made from BSFL Residue and sawdust (BSFL Residue Compost). The other compost was made from BSFL residue, BSFL Residue biochar, and sawdust hereafter referred to as BSFL Residue COMBI. The BSFL heated residue (BSFL HR) was made by blowing hot air using an inlet at 200 °C into a rotary drier with BSFL Residue. The BSFL HR moisture was reduced to 5%.

4.2.2 Composting process
Two compost bins (Compost Figure 4.1A and COMBI Figure 4.1B) were set up in a greenhouse for 108 days. A C: N ratio of 26:1 in both composts was achieved by mixing ratiosfor BSFL Residue COMBI and BSFL Residue Compost, respectively as follows:

$$\text{Total C: N} = \frac{x\% \; x C{:}N \; (A) + y\% \; x \; C{:}N \; (B) + z(\%) \; x \; C{:}N \; (C)}{x+y+z}$$

where A = C: N ratio of residue

B = C: N ratio of sawdust

C = C: N ratio of biochar

$$\text{Total C: N} = \frac{x\% \; x C{:}N \; (A) + y\% \; x \; C{:}N \; (B)}{x+y}$$

Biochar was added at approximately 4% w/w (Głąb et al., 2018). Only sawdust was added as a bulking agent at the same ratio in the compost where BSFL Residue biochar was not added. Table 4.1 shows the calculated BSFL Residue, BSFL Residue biochar, sawdust quantities used to

make BSFL Residue COMBI and BSFL Residue, and sawdust quantities used to make BSFL Residue Compost. The C: N ratio of sawdust used was 497:1.

Table 4.1 Mixing quantities of residue and biochar for BSFL Residue COMBI feedstock and BSFL Residue and sawdust for BSFL Residue Compost feedstock.

Compost name	BSFL Residue (kg wet mass)	BSFL Residue Biochar (kg dry mass)	Sawdust (kg dry mass)	Total feedstock mass (kg)	Pre-compost mix C: N
BSFL Residue COMBI	55.56	2.22	2.22	60	26.2
BSFL Residue Compost	57.78	-	2.22	60	26.2

The bins were turned twice a week for the first month by rolling on the side down a gradient to allow aeration, then once every week after that. Temperature measurements were taken using a digital thermocouple (MT645, Major Tech) from the composting bin's centre twice a week for the first three weeks and once a week after that. After three months, the composts were left to cure for a month. Five random samples from the compost were taken and composited, crushed to pass through a 2 mm sieve for characterisation. During the composting process, moisture content was maintained by adding water to the piles once a week using the squeeze method (Vpa et al., 2017). Representative samples of approximately 200 g (dry weight) from each of the two piles were air-dried, milled to pass through a 2 mm sieve, and used for analysis.

Figure 4.1 Composting set up in glasshouse, BSFL Residue Compost (A) and BSFL Residue COMBI (B).

4.2.3 Co-compost characterisation

The BSFL Residue COMBI and BSFL Residue Compost were characterised for pH, EC, exchangeable cations, mineral element, N, P, K, and water holding capacity compared to other organic amendments CM, BSFL HR, and the BSFL Residue without treatment. A phytotoxicity test was also carried out using radish seeds.

4.2.3.1 Physical characteristics

4.2.3.1.1 Organic matter determination

For each material, 5 g samples were weighed from moisture pre-determined samples into crucibles and combusted in a muffle furnace at 550 °C for 6 hours (Singh et al., 2017). The difference between initial mass (5 g) and the combusted ash was used to calculate the organic matter content using the loss on ignition principle (Nelson et al., 1983).

For water holding capacity, the percolation method was used. A 25 g dry sample was weighed into a funnel lined with filter paper for draining. The funnel was positioned on top of a measuring cylinder. A volume of 100 ml distilled water was poured onto the sample and allowed to drain for 24 hours. The amount of water retained by the dry sample was calculated as the water holding capacity (WHC) (Brischke and Wegener, 2019). The WHC (%) was calculated as follows:

WHC (%) = (Wi - Ws) x100
$$\overline{\qquad\qquad}$$
                    Wi

Where:

Wi = the initial water volume

(mL)Ws = the final water volume

(mL)

4.2.3.1.2 Moisture content, determination

Approximately 10 g of the sample was added to glass beakers in triplicate. Crucibles were placed in an oven at 105 °C for 24 hours (Agnew and Leonard, 2003). The mass of soil after drying was weighed. The following formula was used to calculate moisture content:

Moisture (%) = (A – B) x 100
$$\overline{\qquad\qquad}$$
                  A

Where: A = grams of air-dry sample and,

   B = grams of the sample after drying at 105 °C (24 hours until constant moisture)

4.2.3.2 Chemical characterisation

4.2.3.2.1 pH determination

pH was determined by mixing a 1:10 (w/v) sample: water mixing ratio (1 g sample in 10 ml water). The solution pH was read using a calibrated H198129 pH meter (HANNA Instruments, Romania). The same sample solution used for pH was used for measuring EC using a conductivity meter (HANNA Instruments, Romania). The same procedure was done for pH in KCl using 1M KCl (Agnew and Leonard, 2003). The paste was mixed with a stirring rod, and pH was measured using a calibrated H198129 pH meter (HAN NA Instruments, Romania).

4.2.3.2.2 Cation exchange capacity (CEC)

Cation exchange capacity was determined by the ammonium acetate method buffered at pH 7 (Munera-Echeverri et al., 2018; Song and Guo, 2012). Briefly, a 5 g dry sample was weighed and added to a centrifuge tube. Forty milliliters of 1 M ammonium acetate were added and stoppered. The mixture was shaken for 5 minutes and left overnight to stand. The mixture was shaken for 15 minutes the next day and vacuum filtered using Whatman 42 filter papers. The ammonium ions displace the cations on the exchange site. Four washings with 30 mL of 1 M ammonium acetate were carried out. The filtrate was poured into a 200 mL volumetric flask and

filled to volume with ammonium acetate for exchangeable bases quantification using the atomic absorption spectrophotometer (AAS) (Varian, AA280FS, Shimadzu). Cation exchange capacity was determined as described in Chapter 3.

A mass of 0.5 g from the ashed sample was solubilised on porcelain crucibles using 10 ml aqua-regia (HNO_3 and HCl mixed in ratio 1:3) (von Gunten et al., 2017) and digested using microwave-assisted digestion for elemental analysis. The elements Ca, Mg, K, Zn, Cu, Fe, and Mn concentrations were determined using Flame Atomic Absorption Spectroscopy (AAS) (Varian AA280FS, California, USA). Total K was determined using Flame Atomic Emission Spectroscopy (FAES) with a fast-sequential absorption spectrometer (Varian AA280FS, Shimadzu, California, USA).

4.2.3.2.3 Total phosphorus

Total P was extracted using sulfuric acid, hydrofluoric acid, and hydrogen peroxide on a hot plate. Briefly, a 0.25 g well-mixed sample was weighed into a 100 mL Teflon beaker. A volume of 5 mL concentrated sulphuric acid was added and swirled gently. Three milliliters of 30% hydrogen peroxide were added in 0.5 mL portions and swirled vigorously to avoid foam overflow. When the reaction with hydrogen peroxide had subsided, 1 mL of concentrated hydrofluoric acid was added in 0.5 mL portions and swirled gently. The beaker was placed on a hot plate at 150 °C for 20 minutes to eliminate excess hydrogen peroxide. After slight cooling, the beaker was washed down the sides with approximately 15 mL of distilled water. The mixture was mixed and allowed to cool to room temperature. The beaker contents were quantitatively transferred to a 50 mL volumetric flask, passing it through a Whatmann 1 filterpaper. Two additional washings of the beaker were made with 10 mL of distilled water, filtered, and made up to volume (Margesin and Schinner, 2005). The molybdenum blue method was used on the extract. The blue liquid was read on an ultraviolet spectrophotometer (Varian Alpha UV-VIS, Spectronic Unicam, Berlin, Germany) at a wavelength of 670 nm. The total concentration of P (wPT; mg/kg oven-dried soil) was calculated using the following equation:

$$w\text{PT} = P\text{p} \; \text{x} \; \frac{50}{\text{m}} \; \text{x} \; \frac{(100 + \text{Ww})}{100}$$

*P*p - concentration of P (mg/l) measured according to the molybdenum blue method

m - mass of air-dried sample (g)

Ww - percentage of water content (mass fraction) based on an oven-dried sample.

4.2.3.2.4   Total carbon, nitrogen, nitrates, and ammonium

Total C and N were determined using the Leco-TruMac CNS Autoanalyser (Leco-cooperation, LECO CNS-2000, St Joseph MI, USA, 2012). A mass of 0.2 g of the air-dried sample was exposed to high temperatures of 1450 °C for six minutes under furnace conditions.   For ammonium and nitrates, a mass of 2 g of 2 mm sieved sample was extracted using 20 mL of 2M KCl to analyse nitrates and ammonium. The mixture was shaken with an overhead shaker for one hour (Kammann et al., 2015). The filtrate collected was transferred to storage bottles. Ammonium-N and nitrates-N were determined using the Thermo-Scientific Discreet Gallery (Scientific Therm Fisher, Waltham, Massachusetts, USA, 2014).

4.2.3.2.5   Stability and maturity

The stability of the compost was measured by determining the C: N ratio. The Leco-TruMac CNS Autoanalyser (Leco-cooperation, LECO CNS-2000, St Joseph MI, USA, 2012) was used to quantify total N, and C. Mature compost must have a C: N ratio lower than 20. The biological assay was used to determine the compost's phytotoxicity using radish (*Raphanus sativa*) seeds. A 1:10 (w/v) compost: distilled water extract was made by combining the compost with water and stirring for one hour with a magnetic stirrer, centrifuging for one hour at 8000 rpm, and filtering. The same was done for the residue, heated residue, and chicken manure. Ten seeds were germinated in petri dishes using a 3 mL aliquot of the extract replicated three times. The control was set using distilled water. A standard bench germination test was carried out. Germination was considered successful if the radicle length was at least 2 mm. The experiment was terminated on day 6 when a constant curve was obtained (Warman, 1999). Germination percentage and relative germination were calculated using the following formulae:

$$\text{Germination \%} = \frac{\text{Number of germinated seeds x 100}}{\text{Total number of seed}}$$

4.3 Results

4.3.1   Physical and chemical characteristics

4.3.1.1 *Temperature and pH during composting*

Figure 4.2 shows the BSFL Residue COMBI and BSFL Residue Compost heap temperatures over the 108-day composting period. There was a rapid initial increase in temperature in both BSFL Residue COMBI and BSFL Residue Compost, reaching a peak around day 9. BSFL Residue COMBI reached the highest thermophilic temperature of 59.9 °C, whereas the BSFL Residue Compost reached a lower thermophilic temperature of 55.2 °C. The temperature started to decrease slowly over time for both the BSFL Residue COMBI and BSFL Residue Compost. However, the rate of decrease was higher for BSFL Residue Compost than BSFL Residue COMBI. Eventually, both BSFL Residue COMBI and BSFL Residue Compost reached room temperature at the end of the 108-day composting period.
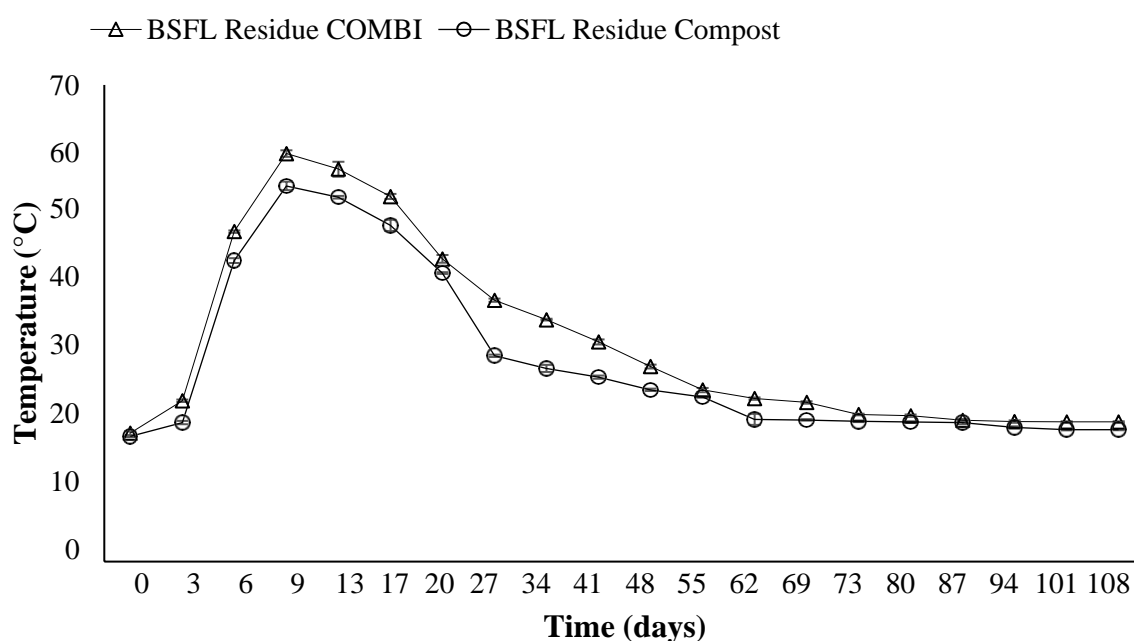
Figure 4.2 Changes in temperature during composting of BSFL Residue COMBI and BSFL Residue Compost over a 108-day composting period.

Figure 4.3 shows the BSFL Residue COMBI and BSFL Residue Compost heap's pH over the 108-day composting period. pH initially dropped during the composting period to day six. After day six, there was an increase in pH to around pH nine on day 17. pH slowly decreased until the end of the composting period. BSFL Residue COMBI had a higher pH of 7.53 compared to BSFL Residue Compost (7.47).

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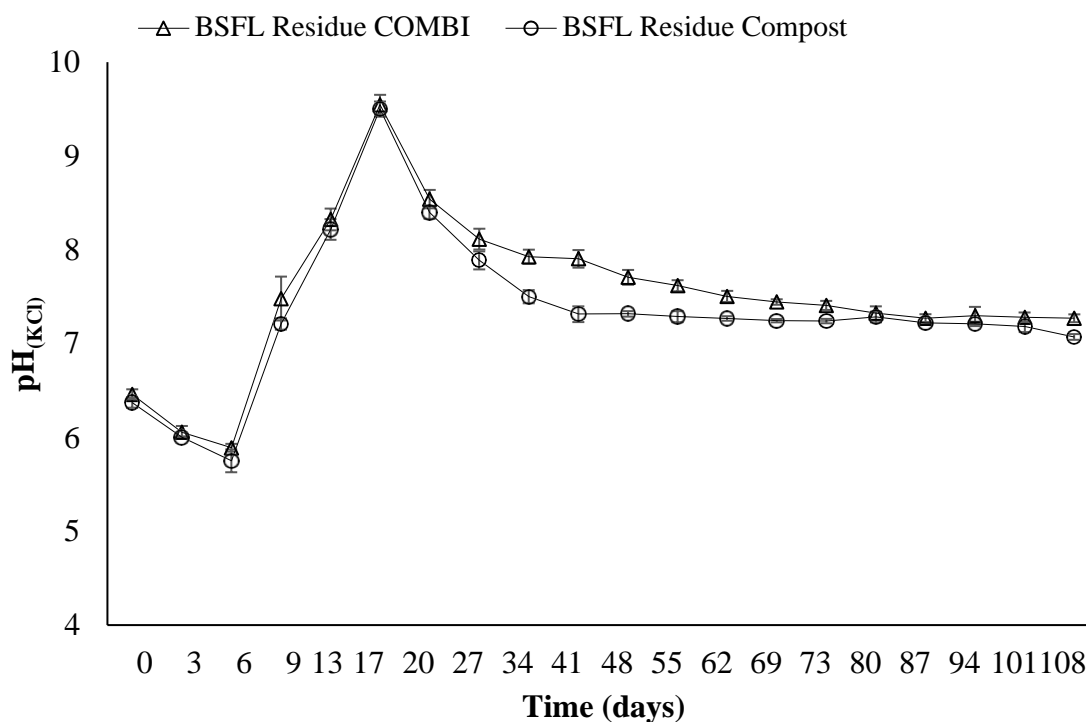
Figure 4.3 Changes in pH during composting of BSFL Residue COMBI and BSFL Residue Compost over a 108-day composting period.

4.3.1.2 *pH, electrical conductivity, water holding capacity, moisture, and organic matter of organic amendments.*

Table 4.2 shows the pH, EC, water holding capacity, moisture, organic matter, and ash content of BSFL Residue COMBI and BSFL Residue Compost made from BSFL residue compared to BSFL HR and CM. All these characteristics differed significantly among amendments (p<0.001) (Table 4.2). The pH ranged from 6.48 to 8.05. Black Soldier Fly Larvae Residue had the lowest pH, while BSFL HR had the highest pH. The pH values in water were higher than in KCl except for CM but followed a similar trend. All the organic amendments had a pH above 7. Electrical conductivity ranged from 3.09 mS cm^{-1} (residue) to 6.02 mS cm^{-1} in BSFLR Residue and BSFL Residue COMBI, respectively). Water holding capacity ranged from 54.9% for BSFL HR to 31.67% for the BSFL Residue. Organic matter ranged from 30.9% in BSFL Residue COMBI to 87.6 % in the BSFL Residue. Ash content ranged from 12.4 to 69.08% in the BSFL Residue and BSFL Residue COMBI, respectively.

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Table 4.2 pH, electrical conductivity, water holding capacity, moisture, and organic matter of BSFL Residue, BSFL Residue COMBI, BSFL Residue Compost, BSFL HR, and CM.

Sample ID	$pH_{(KCl)}$	$pH_{(H2O)}$	EC mS cm^{-1}	Water holding capacity (%)	OM (%)	Ash (%)
CM	7.81^d	7.70^b	5.93^c	34.67^b	80.75^d	19.25b
BSFL Residue	6.48^a	6.79^a	3.09^a	31.67^a	87.60^e	12.40^a
BSFL HR	8.05e	8.32c	5.95c	54.90e	32.32c	67.68c
BSFL Residue Compost	7.47^b	7.66^b	4.72^b	38.33^c	30.92^a	68.52^d
BSFL Residue COMBI	7.53^c	7.71^b	6.02^c	49.63^d	31.48^b	69.08^e
s.e.d	0.018	0.024	0.260	1.116	0.134	0.130
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
c.v (%)	0.3	0.4	6.2	3.3	0.3	0.3

According to Tukey's test, averages followed by the same letter in the same column do not differ significantly (p<0.05).
CM = Chicken Manure; BSFL Residue = Black Soldier Fly Larvae Residue; BSFL HR = Black Soldier Fly Larvae Heated Residue; BSFL Residue Compost = Black Soldier Fly Larvae Residue Compost; BSFLR Residue COMBI = Black Soldier Fly Larvae Residue Biochar Co-compost.

4.3.1.3 Total N, P, K, C, and C: N ratio of amendments

Table 4.3 shows total N, P, K, C, and C: N ratios of the BSFL Residue, subsequent composts (BSFL Residue COMBI and BSFL Residue Compost), compared to BSFL HR and CM. All these characteristics differed between the two composts (Table 4.3). Total N ranged from 1.17 to 2.6 % in BSFL Residue Compost and CM, respectively. Total carbon was highest in CM (34.9%) and lowest in BSFL Residue Compost (12.5%). Total P ranged from 4582.04 mg kg^{-1} to 5785.15 mg kg^{-1} in the BSFL Residue and BSFL HR, respectively. Composting using BSFL Residue biochar increased P to 5429.39 mg kg^{-1} in the BSFL Residue COMBI, comparable to CM (5327.98 mg kg^{-1}). Composting BSFL Residue using BSFL Residue biochar increased the K content of BSFL Residue COMBI (8427.57 mg kg^{-1}) compared to BSFL Residue Compost (8125.90 mg kg^{-1}). Black Soldier Fly Larvae HR and CM had higher K compared to both BSFL

Residue COMBI and BSFL Residue Compost. The C: N ratios ranged from 8:1 (BSFL Residue) to 13:1 (CM). Composting with BSFL Residue biochar resulted in a similar C: N ratio (11:1) in BSFL Residue COMBI and BSFL Residue Compost.

Table 4.3 Total N, P, K, C, and C: N ratio of BSFL Residue, BSFL Residue COMBI, BSFL Residue Compost, BSFL HR, and CM.

Sample ID	Total N (%)	Total C (%)	Total P (mg kg^{-1})	Total K (mg kg^{-1})
CM	2.60^e	34.94^e	5327.98^{bc}	18629.10^d
BSFL Residue	2.06^d	16.57^d	4582.04^a	10318.53^c
BSFL HR	1.58^c	14.65^c	5785.15^d	8759.04^b
BSFL ResidueCompost	1.17^a	12.53^a	5175.96^b	8125.90^a
BSFL Residue COMBI	1.23^b	13.84^b	5429.39^c	8427.57^{ab}
s.e.d	0.026	0.161	100.29	236.60
p-value	<0.001	<0.001	<0.001	<0.001
c.v (%)	1.8	1.1	2.3	2.7

According to Tukey's test, averages followed by the same letter in the same column do not differ significantly (p<0.05).
CM = Chicken Manure; BSFL Residue = Black Soldier Fly Larvae Residue; BSFL HR = Black Soldier Fly Larvae Heated Residue; BSFL Residue Compost = Black Soldier Fly Larvae Residue Compost; BSFLR Residue COMBI = Black Soldier Fly Larvae Residue Biochar Co-compost.

4.3.1.4 *Exchangeable base cations and CEC of amendments*

Table 4.4 shows exchangeable cations, Calcium (Ca), Potassium (K), and Magnesium (Mg), and cation exchange capacity. All these characteristics differed significantly among amendments (p<0.001) (Table 4). Composting the BSFL Residue decreased all exchangeable bases. Calcium ranged from 12.72 cmol$_c$ kg^{-1} (BSFL Residue Compost) to 50.74 cmol$_c$ kg^{-1} (CM). However, BSFL Residue COMBI had higher Ca (20.74 cmol$_c$ kg^{-1}) compared to BSFLResidue Compost. Potassium ranged from 20.84 to 47.77 cmol$_c$ kg^{-1} (BSFL Residue Compost and CM). BSFL HR had higher K 22.46 (cmol$_c$ kg^{-1}) compared to both composts (BSFL Residue COMBI and BSFL Residue Compost). Magnesium ranged from 47.09 cmol$_c$ kg^{-1} to 21.67 cmol$_c$ kg^{-1} in BSFL Residue

Compost. Both CM and BSFL HR had higher Mg compared to composts (38.08 and 24.97 $cmol_c$ kg^{-1}). Sodium ranged from 10.38 to 28.55 $cmol_c kg^{-1}$ (CM and BSFL Residue, respectively). Cation exchange capacity ranged from 9.41 to 18.51 $cmol_c$ kg^{-1} (CM and BSFL Residue COMBI), respectively. Cation exchange capacity for BSFL HR (16.65 $cmol_c$ kg^{-1}) was comparable to BSFL Residue Compost, BSFL Residue, and BSFL Residue COMBI.

Table 4.4 Exchangeable base cations and CEC of BSFL Residue, BSFL Residue COMBI, BSFL Residue Compost, BSFL HR, and CM.

Sample ID	Ca ($cmol_c$ kg^{-1})	K ($cmol_c$ kg^{-1})	Mg ($cmol_c$ kg^{-1})	Na ($cmol_c$ kg^{-1})	CEC ($cmol_c$ kg^{-1})
CM	50.74[d]	47.77[d]	38.08[d]	10.38[a]	9.41[a]
BSFL Residue	42.90[c]	26.46[c]	47.09	28.55[e]	14.64[b]
BSFL HR	17.69[ab]	22.46[b]	24.97[c]	15.00[d]	16.65[bc]
BSFL Residue Compost	12.72[a]	20.84[a]	21.67[a]	13.95[b]	16.21[bc]
BSFL Residue COMBI	20.74[b]	21.11[ab]	22.06[b]	14.33[c]	18.15[c]
s.e.d	2.004	0.607	1.154	0.176	1.498
p-value	<0.001	<0.001	<0.001	<0.001	<0.001
c.v (%)	8.0	2.7	4.5	1.3	12.3

According to Tukey's test, averages followed by the same letter in the same column do not differ significantly (p<0.05).

4.3.1.5   *Trace element and Fe and Mn concentration of amendments*

Table 4.5 shows the concentration of trace elements in the residue, composts, heated residue, and CM. All trace elements metals differed significantly among amendments (p<0.001) (Table5). Trace element concentrations increased in the BSFL Residue COMBI, BSFL Residue Compost as well as BSFL HR. Chromium ranged from 17.70 mg kg^{-1} (BSFL Residue) to 384.30 mg kg^{-1} (BSFL Residue COMBI). Cadmium ranged from 0.17 to 2.64 mg kg^{-1} in BSFL Residue and CM, respectively. Copper ranged from 89.70 (BSFL Residue) to 281.60 mg kg^{-1} (CM). Composting increased heavy metals to 125.70 mg kg^{-1} in BSFL Residue Compost and 131.0 mg kg^{-1} in BSFL Residue COMBI. Iron ranged from 7529.03 to 12455.93 mg kg^{-1} in BSFL Residue COMBI and CM, respectively. Manganese (Mn) ranged from 408.20 mg kg^{-1} (BSFL HR) to 2447.70 mg kg^{-1} in (CM). Composting increased Mn from 336.20 mg kg^{-1} in the BSFL

Residue to 393.50 and 359.20 mg kg^{-1} in the BSFL Residue Compost and BSFL Residue COMBI, respectively. Nickel ranged from 22.20 in BSFL Residue to 207.90 mg kg^{-1} in BSFL Residue COMBI. Zinc (Zn) ranged from 347.40 mg kg^{-1} (BSFL Residue) to 786.30 mg kg^{-1} in CM. Composting increased Zn to 570.80 and 581.60 mg kg^{-1} in the BSFL Residue Compost and BSFL Residue COMBI, respectively. BSFL HR did not differ significantly from composts, 602.50 mg kg^{-1}.

Table 4.5 Trace element and Fe and Mn concentration of BFL Residue, BSFL Residue Composts, CM, and BSFL HR.

Sample ID	mg kg^{-1}						
	Cr	Cd	Cu	Fe	Mn	Ni	Zn
BSFL Residue	17.70[a]	0.17[a]	89.70[a]	7667.56[a]	336.20[a]	22.20[a]	347.40[a]
BSFL Residue COMBI	384.30[d]	0.39[b]	131.00[b]	7529.03[a]	393.50[bc]	207.90[c]	581.60[b]
BSFL Residue Compost	273.10[c]	0.49[b]	125.70[b]	11288.18[c]	359.20[ab]	135.50[b]	570.80[b]
BSFL HR	284.10[c]	1.35[c]	150.40[c]	9976.46[b]	408.20[c]	142.20[b]	602.50[b]
CM	125.70[b]	2.64[d]	281.60[d]	12455.93[d]	2447.70[d]	129.30[b]	786.30[c]
Minimum recommendation	**350**	**<3**	**120**	**-**	**-**	**150**	**200**
s.e.d	8.84	0.09	2.64	211.80	18.94	11.57	15.36
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
c.v (%)	5.0	10.5	2.1	2.7	2.9	11.1	3.3

According to Tukey's test, averages followed by the same letter in the same column do not differ significantly (p<0.05).

4.3.1.6 Compost stability and maturity

4.3.1.6.1 C: N ratios of amendments

Figure 4.4 shows the C: N ratios of the residue, heated residue, compost, biochar compost (COMBI), and CM. The C: N ratio ranged from 8:1 to 13:1 in residue and CM, respectively. Composting increased the C: N ratio. Composting increased C: N ratio in both BSFL Residue COMBI and BSFL Residue Compost to 11:1.
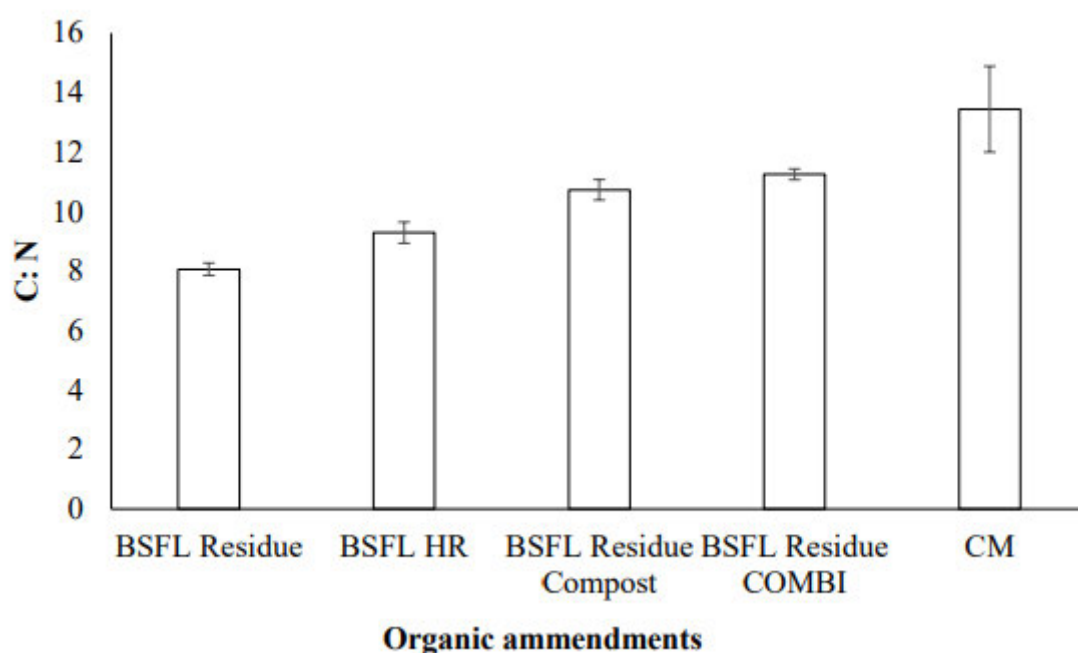
Figure 4.4 Carbon to nitrogen (C: N) ratios of the amendments
CM = Chicken Manure; BSFL Residue = Black Soldier Fly Larvae Residue; BSFL HR = Black Soldier Fly Larvae Heated Residue; BSFL Residue Compost = Black Soldier Fly Larvae Residue Compost; BSFLR Residue COMBI = Black Soldier Fly Larvae Residue Biochar Co-compost.

4.3.1.6.2 Ammonium concentration of amendments

Figure 4.5 shows the ammonium concentration of the compost and COMBI compared to the residue, HR, and CM. Ammonium ranged from 3.33 to 12.66 mg kg^{-1} in the BSFL HR and BSFL Residue, respectively. Composting the BSFL Residue decreased the ammonium concentration of both composts to 5.92 and 6.80 mg kg^{-1}in the BSFL Residue Compost and BSFL Residue COMBI, respectively. The BSFL Residue had the highest ammonium (12.66 mg kg^{-1}). Composting the residue resulted in a decrease in ammonium.
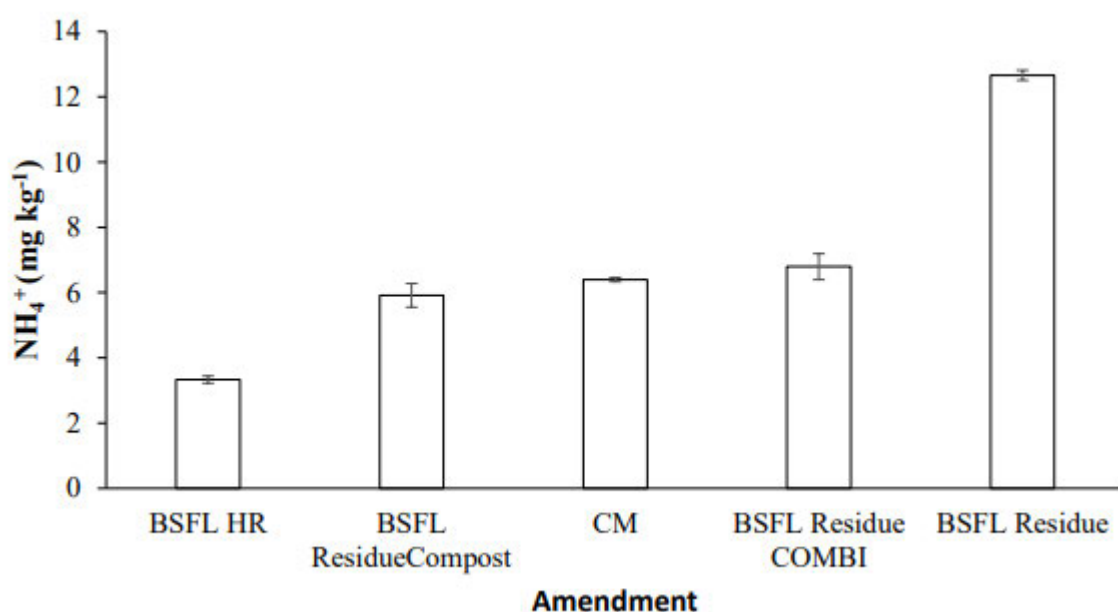
Figure 4.5 Ammonium composition of the organic amendments.

CM = Chicken Manure; BSFL Residue = Black Soldier Fly Larvae Residue; BSFL HR = Black Soldier Fly Larvae Heated Residue; BSFL Residue Compost = Black Soldier Fly Larvae Residue Compost; BSFLR Residue COMBI = Black Soldier Fly Larvae Residue Biochar Co-compost.

4.3.1.7 *Phytotoxic test using germination of radish seeds treated with amendment extracts compared to deionised water.*

The phytotoxic test was done on radish seeds over six days. Figure 4.6 shows the germination percentage graph for BSFL Residue COMBI and BSFL Residue Compost compared to the control, CM, BSFL Residue, and BSFL HR. The control had 88% germination on day one. Germination on day one for BSFL Residue, BSFL HR, BSFL Residue Compost, and BSFL Residue COMBI was 27, 50, 73, and 53%, respectively. At the end of the germination test, the highest germination percentage was recorded in control (100%), while the lowest germination was in the BSFL Residue (40%). Germination increased until a final of 100, 87, 80, 77, 40% for control, BSFL Residue Compost, BSFL Residue COMBI, BSFL HR, and BSFL Residue, respectively.
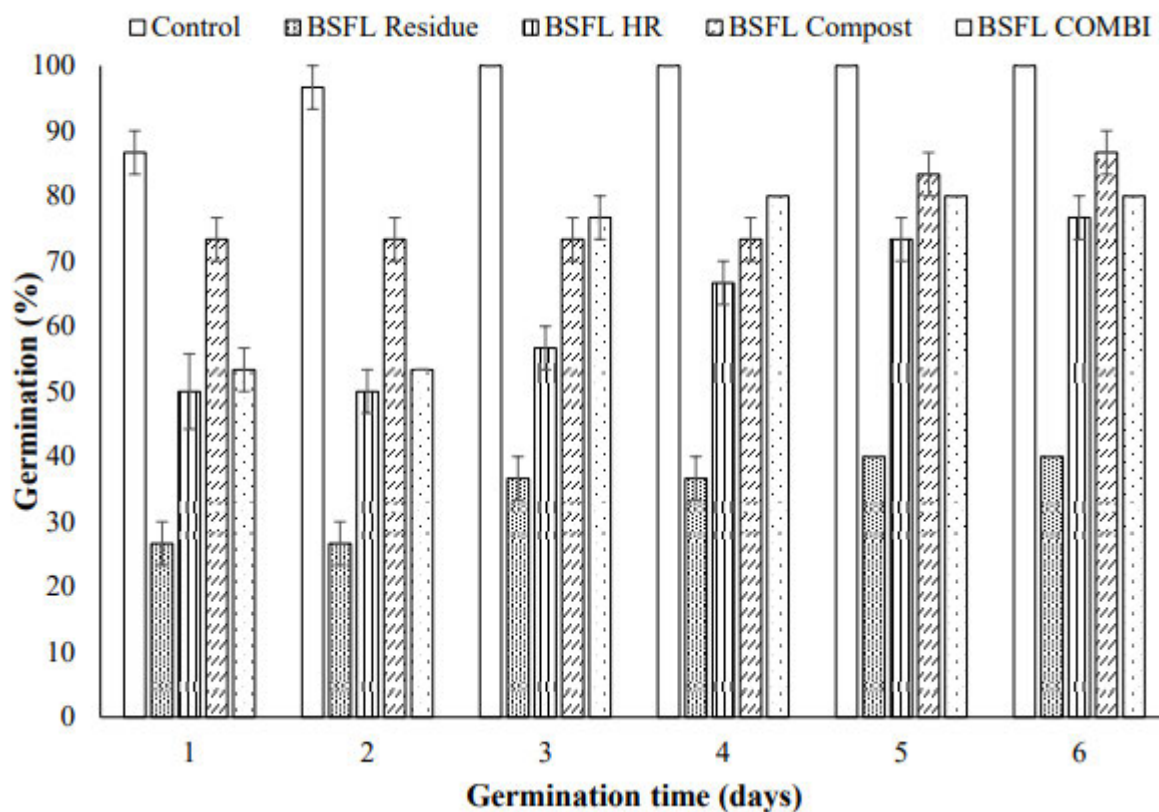
Figure 4.6 Germination of radish seeds treated with deionised water (A) control and organic extracts over a 6 -day period.

CM = Chicken Manure; BSFL Residue = Black Soldier Fly Larvae Residue; BSFL HR = Black Soldier Fly Larvae Heated Residue; BSFL Residue Compost = Black Soldier Fly Larvae Residue Compost; BSFLR Residue COMBI = Black Soldier Fly Larvae Residue Biochar Co-compost.

4.4 Discussion

This study sought to determine the effect of biochar addition before composting on physical and chemical properties of co-compost during and after composting of composts with and without biochar. The finished composts' characteristics were determined and compared to CM, BSFL Residue, and BSFL HR. Composting the residue using biochar (BSFL Residue COMBI) resulted in a higher thermophilic temperature than the compost during the composting process (Figure 4.2). The results are similar to those of Li et al. (2015) findings, who observed a higher temperature in pig manure composted with biochar and attributed it to less heat loss and higher microbial activity. Antonangelo et al. (2021) reported that temperature increases faster during

the composting process in the presence of biochar. They further stated that the thermophilic phase's duration is also prolonged when biochar is added before composting. However, this study's thermophilic temperature was reached simultaneously for both the BSFL Residue COMBI and BSFL Residue Compost. A higher thermophilic temperature guarantees the killing of pathogens. The heat generated during the thermophilic phase results from intense microbial metabolic activity (Rawat and Johri, 2013). In a study on food waste in-vessel composting using biochar, biochar composted food waste reached higher thermophilic temperatures faster than compost without biochar (Chaher et al., 2020).

During the composting process, pH initially dropped for both the BSFL Residue COMBI and BSFL Residue Compost, then increased to a peak at day 17 and started decreasing again. A similar trend was observed when food waste was co-composted with biochar (Rawat et al., 2019). The initial drop in pH was associated with the production of organic acids and their volatilisation during the thermophilic phase (Chaher et al., 2020). A higher increase in the BSFL Residue COMBI pH could be attributed to the biochar's alkalinity compared to the BSFL Residue Compost, where no biochar was added. After composting, BSFL Residue COMBI had higher pH compared to the BSFL residue, which could be attributed to the high alkaline bases in the biochar. Similar findings were obtained when cow manure was composted using pine bark biochar (Botha, 2016). Most crops grow in pH ranging from 6 to 7.5. Both the BSFL Residue COMBI and BSFL Residue Compost met the requirement for use in soils, especially acidic soils (Oshunsanya, 2018).

The residue's EC increased when the BSFL Residue was composted, mostly when it was composted with BSFL Residue biochar. Pyrolysing the BSFL Residue to produce biochar increased the composts' basic cations that constituted a higher EC. During the composting process, EC generally decreases during the thermophilic stage and increases again during the curing stage when basic cations become concentrated. The minimum optimum EC for crop growth ranges from 2 to 3.5 mS cm^{-1} (Abou-Hadid et al., 1996). Both BSFL Residue COMBI and BSFL Residue Compost and the CM and HR had higher EC values ranging from 4.72 to 6.02 mS cm^{-1}; hence using these amendments might result in toxic salinity effects on plants.

Water holding capacity increased in the BSFL Residue COMBI compared to BSFL Residue Compost, mostly because of biochar availability, which is porous. The pores in biochar can absorb water and reduces its ability to drain. Pyrolysing the BSFL Residue resulted in a

mesoporous material. Mesopores are essential for water storage, and they supply most of the the plant's available water (de Jesus Duarte et al., 2019). Liu et al. (2012) reported that biochar helps maintain moisture content during composting, especially during the thermophilic phase. During aerobic composting, microorganisms need oxygen to degrade organic matter optimally. Biochar helps aerate the compost mixture due to the presence of pores. (Antonangelo et al., 2021). Studies also found increased moisture storage capacity in biochar amended soils (Lehmann and Joseph, 2015).

The increase in organic matter in BSFL Residue COMBI is attributed to the C in the biochar. According to a study carried out by Hagemann et al. (2018), the increase in organic matter in co-compost made from manure and biochar was attributed to an increase in total organic carbon contributed by the biochar. A contrasting study concluded that biochar's addition could enhance SOM degradation, resulting in a more stable, humified final product with less organic matter. In these studies, the composts were less frequently aerated compared to this study, for example, weekly (Sanchez-Garcia et al., 2015). Ash was high in COMBI because the biochar has an ash component. The ash from the residue was only a result of the residue, whereas, in COMBI, it was the additive effect of ash from both the biochar and residue.

The increase in total P in BSFL Residue COMBI could be attributed to the higher P content in the BSFL Residue COMBI. A similar study by Hagemann et al. (2018) found a significantly higher total P in co-compost made using biochar produced from sewage sludge attributed to the high content of phosphorus in sewage sludge. Also, composting decreases the volume of the compost hence concentrating P (Sommer, 2001). However, a different response was observed in a study by Kammann et al. (2015), where P was lower in the biochar co-compost attributed to biochar effectively sorbing nutrients during composting that is only slowly released during extraction.

Total N decreased with composting. Ammonium was high in the BSFL Residue and reduced in the composts. The decrease could be attributed to N losses during the composting process (Yang et al., 2019). Ammonia loss through volatilisation is the main way for N reduction in the final compost. However, BSFL Residue COMBI had a higher total N compared to BSFL Residue Compost, indicating that biochar affected N losses during composting. During the thermophilic stages of composting, ammonia's volatilisation occurs typically due to the decomposition of

nitrogen-containing material (Awasthi et al., 2017). Biochar addition can reduce ammonia (NH_3) emissions. Biochar blended compost showed a reduction in NH_3 emission ranging from 11 to 65% (Awasthi et al., 2017; Chowdhury et al., 2014; Malinska et al., 2014). BSFL Residue COMBI had higher ammonium compared to BSFL Residue Compost, which could be attributed to biochar's ability to adsorb ammonium ions. In a study by Malińska et al. (2014), biochar amendment resulted in a decrease in ammonia loss through volatilisation, ultimately resulting in a higher ammonium content of the biochar co-compost.

Composting increased the C: N ratio of the residue. The starting C: N ratio was 8:1, which was boosted either in BSFL Residue COMBI or BSFL Residue Compost by adding either a combination of biochar and sawdust or sawdust only, respectively, to achieve an initial composting material with a C: N ratio of 26:1. Changes in the C: N is attributed to the losses during composting at thermophilic temperatures when bio-oxidative processes resulting in OM decomposition are at their maximum (Botha, 2016).

The labile carbon fractions in organic residues decompose much more quickly and efficiently than the non-labile fractions, which explained the decrease in C in the composts (Bolan et al., 2012). Compost containing biochar had a higher C: N ratio mainly because a fraction of the total C in BSFL Residue biochar is non-labile and is not prone to microbial decomposition. Hence, the high C: N ratio is attributed to the recalcitrant C. Compost is considered mature when the C/N ratio is less than 15: 1 (Kuo et al., 2004). In this case, both composts (BSFL Residue COMBI and BSFL Residue Compost) had an acceptable C: N ratio of 11.3:1 and 10.7: 1, respectively, which falls under the required C: N ratio for crop production.

Exchangeable bases were higher in BSFL Residue COMBI compared to BSFL Residue Compost. The biochar in BSFL Residue COMBI had additional concentrated alkali bases such as Ca, Mg, and K from pyrolysis, as observed in Chapter 3 of this Thesis. The addition of these exchangeable bases to the BSFL Residue increased the BSFL residue COMBI exchangeable bases concentration. The decrease in size in the compost heap could have contributed to increased exchangeable bases' concentration. Trace metal concentration values were slightly higher than limits for Cu and Cr, relatively high for Ni, and more than double for Zn compared to the safe contaminant concentration limits in biosolids for land application, according to the South African

guidelines (Cd, Cr, Cu, Ni, and Zn;< 3, 350, 120, 150, and 200 mg kg^{-1}, respectively aqua-regia extractable (Herselman and Snyman, 2009).

4.5 Conclusion

The two composts prepared differed in terms of their physical and chemical properties with BSFL Residue COMBI having higher pH, CEC, water holding capacity, and organic matter compared to BSFL Residue Compost. Total P and N are increased by composting using BSFL Residue biochar. Hence BSFL Residue COMBI can be used as a nutrient source for crop production. Trace elements increased in the BSFL Residue COMBI and BSFL Residue Compost compared to BSFL Residue. Composting reduced the volume of the compost resulting in a concentration of trace elements. The presence of trace elements in the composts is attributed to the faecal matter's inherent trace metal characteristics from the UDDT's. The toilets are not used solely for human excretion, but other foreign objects such as batteries, weaves, and iron nails are thrown into the toilets. There is a need for education on the proper use of UDDT's if the faecal matter is to be used for crop production.

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CHAPTER FIVE: NITROGEN AND PHOSPHORUS RELEASE PATTERNS IN A SANDY SOIL AMENDED WITH BLACK SOLDIERFLY LARVAE (BSFL) RESIDUE AND BIOCHAR CO-COMPOST

Black soldier fly larvae (BSFL) biochar compost (COMBI) is made from BSFL faecal residue. Its biochar is an organic amendment with nutrients nitrogen, phosphorus, potassium, and basic cations calcium and magnesium, which can be used for crop production. Information is lacking on nitrogen and phosphorus mineralisation patterns of BSFL Residue COMBI in sandy, acidic soils. A sandy soil was incubated with BSFL Residue COMBI compared to BSFL Residue Compost, BSFL Residue, BSFL Heated Residue, chicken manure, commercial chemical fertilisers, and control. One-kilogram soil was incubated over 112 days based on maize nutrient phosphorus requirements and sampled on days 0, 1, 2, 3, 5, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 105, and 112. Soil samples were analysed for phosphorus and nitrogen (ammonium and nitrates) release patterns. At day 0, P ranged from 0 (Control) to 382.3 mg kg^{-1} (BSFL Residue COMBI). Phosphorus release initially decreased at day 21, ranging from 0 to 167.2 mg kg^{-1} in control and BSFL Residue Compost, respectively. Phosphorus release occurred from day 21 until the end of the incubation study, P ranged from 0 in control to 276.3 mg kg^{-1} in BSFL Residue COMBI. Phosphorus release patterns for both BSFL Residue COMBI and BSFL Residue Compost were statistically similar. Ammonium reached a peak on day 14, with BSFL Residue COMBI and BSFL Residue Compost releasing statistically similar amounts of ammonium. Nitrate release was lower in BSFL Residue COMBI (37.9 mg kg^{-1}) than all the treatments except for the control (31.3 mg kg^{-1}). pH increased for all treatments ranging from 4.5 to 5.7 in control and CM, respectively, and decreased at day 21. The BSFL Residue COMBI and BSFL Residue Compost had statistically similar pH at the start of the incubation and did not differ throughout the incubation study. Therefore, BSFL Residue COMBI can be used as a slow P and N nutrient release fertiliser making it an important tool for a steady supply of nutrients by preventing nutrient leaching and environmental contamination.

Keywords: black soldier fly larvae; co-compost; nitrogen; nutrient release; phosphorus; residue

5.1. Introduction

Co-composting could provide solutions to managing the accumulation of Black Soldier Fly Larvae (BSFL) waste after feeding. The BSFL waste is a residue left behind after BSFL decomposes faecal matter from Urine Diversion Dry Toilets (UDDTs). Black soldier fly larvae faecal residue is a waste of organic origin. The residue has residual nutrients, nitrogen (N), Phosphorus (P), (Potassium) K, (Calcium) Ca, and (Magnesium) Mg, as well as micronutrients that are essential for crop production.

However, there are challenges associated with the direct use of the residue. The residue is characterised by high moisture, about 55% (Mutsakatira et al., 2018), making it bulky and expensive to transport. Also, residual pathogens, as well as high ammonium, can be toxic to plants. Generally, organic waste typically has high moisture and high organic matter, and when used as a fertiliser without composting, seed germination will be inhibited, and crop growth will be affected (Kawasaki et al., 2020). In some cases, the residue is acidic and cannot be used as a soil amendment, especially in poor acidic sandy soils. The residue is also characterised by a low C: N ratio. This challenge can be addressed by adding a bulking agent such as biochar. The same residue can be pyrolysed to biochar and co-composted using the residue to utilise the residue fully.

Biochar is a solid carbonaceous material made from pyrolysis of organic matter under limitedor low oxygen conditions using temperatures ranging from 300 to 700 °C (Blackwell et al., 2009; Lehmann and Joseph, 2015). The addition of biochar during the composting process alters compost physicochemical properties resulting in improved compost quality regarding organic carbon (OC), moisture content, and pH (Frimpong et al., 2020). Biochar is a good bulking agent as it possesses characteristics of porosity, allowing airflow and circulation. By combining the two, it is possible to use the benefits of each to optimise the process and the endproduct (co-compost). The composting process is shortened, and higher thermophilic temperatures are reached when biochar is added to the compost pile before composting (Campsand Tomlinson, 2015). The BSFL Residue CPMBI made, as reported the Chapter 4 of this thesis, had nutritional value regarding total phosphorus (P) and nitrogen (N) characteristics, which could be released when added to the soil. It also increases the availability of nutrients such as nitrogen (N),

phosphorus (K), and other micronutrients(Agegnehu et al., 2016; Prost etal., 2013). However, despite the potential benefits of making co-compost using BSFL faecal matter residue and biochar made from the BSFL residue, there is little or no information on the releasepatterns of the major macro-nutrients (nitrogen and phosphorus) in soils. Hence the objectiveof the study was to investigate the nitrogen and phosphorus release patterns of co-compost made from BSFL residue derived biochar mixed with the BSFL residue in a Cartref soil.

5.2. Materials and methods

5.2.1. Soil collection and preparation

A sandy Cartref soil collected from Kwadinabakubo Hillcrest, Durban (29°43'53.08694"S;30°51'41.51041"E) was used for the pot trial. Before planting, the soil was air-dried and passed through a 2 mm sieve. Topsoil was sampled using an auger at the 0-30 cm depth over the areas where the soil collection for both incubation and pot trial were collected using the zig-zag method. The soil samples were composited, and a composite sample was sent to Cedara for soil nutrient analysis (Appendix 1). A mass of 1 kg of soil was weighed into the plastic incubating containers. The weight of both the soil and container was noted for soil moisture correction.

5.2.2. Experimental material

Soil amendments BSFL residue + BSFL residue biochar + sawdust (COMBI), BSFL residue + sawdust compost (compost), chicken manure (CM), BSFL residue, Chemical Commercial Fertiliser (CCF) in the form of Single Super Phosphate (SSP), a phosphorus source and Urea, a nitrogen source, Heated Residue (HR) and Control. Both COMBI and compost were composted using the in-vessel composting method in bins for 108 days (Chapter 4). The CM was sourced from Ukulinga Farm. The BSFL HR was made by directly heating BSFL residue by blowing a counter-current flow within the rotary drier at a temperature of 200 C to reduce the moisture down to 5%.

5.2.3. Study site

The incubation study was conducted in an incubation room (darkroom at 25 °C) at the Soil Science Laboratories Department, University of KwaZulu-Natal, Pietermaritzburg, SouthAfrica.

5.2.4. Experimental design

The experiment was laid out using a completely randomised design (CRD) as a single factor

analysis with seven treatment factors replicated three times, giving a total of 21 experimental units, each experimental unit represented by a 2 kg perforated container. Sampling was carried out 20 times on days 0, 1, 2, 3, 5, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 105, and 112. The factors were as follows: Control; Chemical Commercial Fertilisers (CCF) (Single Super Phosphate (SSP) and urea); BSFL residue; BSFL heated residue (HR); BSFL residue + BSFL biochar + sawdust compost (COMBI); BSFL residue + sawdust compost (compost) and chicken manure (CM). Urea supplied N, and SSP supplied P. The treatments were applied based on the recommended P application rate (60 kg ha^{-1}) for maize, based on soil analysis results from Cedara (Appendix 2) and maize crop nutrient requirements.

5.2.5. Experimental set-up

The pressure plate method was used to determine field capacity (FC) for initiating the incubation process (Leong et al., 2004). A soil core was filled with soil and saturated with water. The soil core was left to drain off freely for 24 hours. After 24 hours, the soil core was subjected to 33 kPa. The soil and core mass were weighed before and after oven drying to determine the soil water content at field capacity (FC). Soil water content was expressed per kg soil sample used in the incubation study to initiate the incubation study. Soil water content was maintained at 80% FC by weighing pots and replenishing them with water. A 1 kg soil sample was used for the incubation study. The calculation was made based on the P content of the amendments to determine the amount to put in 1 kg soil to meet the fertiliser P requirement of 60 kg P ha^{-1}. The amendments were added at twice the recommended application rate for better determination of the release pattern. Table 5.1 shows the P concentration in each amendment and the amount used at double the recommended application rate to meet the P requirement in 1 kg soil based on maize requirements in the incubation study.

Table 5.1 Phosphorus in amendments and the quantity added to reach P requirements for the incubation study at twice the recommended application rate.

Treatment	P in (g kg^{-1})	Quantity of amendment added g kg^{-1} soil)	Nitrogen supplied by amendment (g kg^{-1})
Control	-	-	-
CM	5.33	6.06	0.16
BSFL Residue	4.58	7.04	0.15
BSFL HR	5.79	5.58	0.09
BSFL Residue Compost	5.18	6.22	0.07
BSFL Residue COMBI	5.43	5.94	0.07

CM = Chicken Manure; BSFL Residue = Black Soldier Fly Larvae Residue; BSFL HR = Black Soldier Fly Larvae Heated Residue; BSFL Residue Compost = Black Soldier Fly Larvae Residue Compost; BSFLR Residue COMBI = Black Soldier Fly Larvae Residue Biochar Co-compost

5.2.6. Analyses

For all analyses (pH, phosphorus, and nitrogen), soils were sampled over 20 sampling days from day 0, 1, 2, 3, 5, 7, and weekly after that until day 112.

5.2.6.1. *Phosphorus determination*

Phosphorus was extracted following the AMBIC method (Van der Merwe et al., 1984). The extracted solution was mixed with a colour reagent and changed to a blue colour. The extractable P was thus determined after reading the blue liquid's absorbance at 670 nm using an ultraviolet spectrophotometer (Varian Alpha UV-VIS spectrophotometer, Spectronic Unicam, Berlin, Germany). Phosphorus standards (0, 10, 20, 40, and 60 mg L^{-1}) were made, read on the ultraviolet spectrophotometer, and used to draw a calibration curve. The equation of the calibration curve was used to determine the concentration of the extracts.

5.2.6.2. *Nitrogen (ammonium and nitrates) determination*

Mineral nitrogen (NH_4^+-N and NO_3-N) was extracted with a 2M KCl solution using a 1:10 soil extraction solution volume ratio. From each sample, 2 g of sample was added to 50 mL plastic

tubes with 20 mL of the extracting solution (Maynard et al., 1993). An overhead shaker was used to shake the mixture for 30 minutes. The mixture was filtered using Whatman 1 Filter paper and transferred to storage bottles. The NH_4^+-N and NO_3-N were analysed using the Thermo-Scientific Discreet Gallery (Scientific Therm Fisher, Waltham, Massachusetts, USA,2014).

5.2.6.3. *pH determination*

The soil samples were extracted using 1M KCl for pH. A 1:5 weight: volume mixing ratio (2g soil in 5 ml KCl) was used for pH determination. Samples were stirred for 30 minutes using a magnetic stirrer, and pH was read using an H198129 pH meter (HANNA Instruments,Romania).

5.2.7. Data analysis

Analysis of variance (ANOVA) was used for assessing changes in pH and nutrient N and P release in a sandy using GenStat 18th (VSN International 2015). Means were separated using Tukey's least significant difference (LSD) at a 5% significance level.

5.3.   Results

5.3.1. Phosphorus release pattern

The pattern of phosphorus release over time differed significantly among amendments (p<0.001) (Figure 5.1). Phosphorus content (mg kg^{-1}) at the beginning of the incubation study ranged between 0 (Control) and 382.3 mg kg^{-1} (BSFL Residue COMBI). Initially, there was a decrease in P during the first 21 days and ranged between 172.6 mg kg-1 for BSFL residue compost and 0 mg kg^{-1} (control). On day 21, BSFL COMBI decreased to 172.6 mg kg^{-1} and was not different statistically from BSFL residue compost (167.2 mg kg^{-1}). After day 21, there was a sudden rapid increase in extractable P in all treatments, reaching a maximum at day 28.However, the control remained at 0 mg kg^{-1}. Phosphorus release started on day 63 for all treatments except for control and CM and was gradual until the end of the incubation study. At the end of the 112-incubation period, extractable phosphorus ranged from 0 mg kg^{-1} (Control)to 276.3 mg kg^{-1} (BSFL Residue COMBI).

Figure 5.1 Changes in phosphorus among different soil amendments added to a Cartref soil over a 112-day incubation period.

CM = Chicken Manure; CCF = Chemical Commercial Fertiliser; BSFL Residue = Black Soldier Fly Larvae Residue; BSFL HR = Black Soldier Fly Larvae Heated Residue; BSFL Residue Compost = Black Soldier Fly Larvae Residue Compost; BSFLR Residue COMBI = Black Soldier Fly Larvae Residue Biochar Co-compost

5.3.2. Nitrogen (ammonium and nitrate release pattern)

There was a significant difference ($p<0.001$) in the ammonium release pattern among the different soil amendments over the 112-day incubation period (Figure 5.2). At the beginning of the incubation study, ammonium ranged from 0 mg kg-1 (control, BSFL Residue Compost. BSFL Residue COMBI, BSFL HR, and BSFL Residue) to 2.7 mg kg^{-1} (CM). Ammonium initially increased during the incubation study's early days, reaching a peak on day 14, ranging from 3.02 to 5.5 mg kg^{-1} in control and BSFL Residue, respectively. However, maximum ammonium release was attained on day 2 for CM (7.8 mg kg^{-1}). On day 14, compost and COMBI ammonium concentrations were comparable (4.72 and 4.73 mg kg^{-1}). After day 14, there was a general decrease in ammonium in all treatments to reach 0 mg kg^{-1} at day 112.

Figure 5.2 Changes in ammonium among different soil amendments added to a Cartref soil over a 112-day incubation period.

CM = Chicken Manure; CCF = Chemical Commercial Fertiliser; BSFL Residue = Black Soldier Fly Larvae Residue; BSFL HR = Black Soldier Fly Larvae Heated Residue; BSFL Residue Compost = Black Soldier Fly Larvae Residue Compost; BSFLR Residue COMBI = Black Soldier Fly Larvae Residue Biochar Co-compost.

Figure 5.3 shows the nitrate release pattern of amendments over the 112-day incubation period. The nitrate release pattern was significantly different ($p<0.001$) among the different soil amendments on each sampling day. At the beginning of the incubation study, the nitrate concentration for all organic amendments was 0 mg kg^{-1}. An increase in nitrate concentration was observed on day 14 from BSFL residue and day 21 for all the other treatments. At the endof the incubation, nitrate ranged from 31.3 to 96.8 mg kg^{-1} in the control and CM, respectively.Both BSFL Residue COMBI (37.9 mg kg^{-1} and BSFL Residue Compost (41.3 mg kg^{-1}) were comparable at the end of the 112-day incubation period.
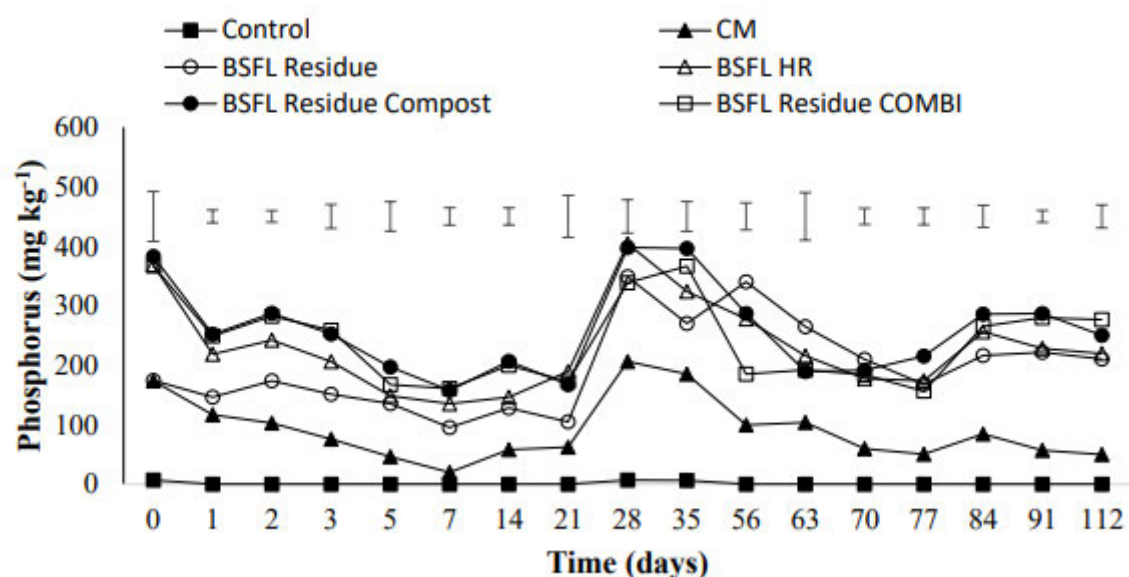
Figure 5.3 Changes in nitrate among different soil amendments added to a Cartref soil over a 112-day incubation period.

CM = Chicken Manure; CCF = Chemical Commercial Fertiliser; BSFL Residue = Black Soldier Fly Larvae Residue; BSFL HR = Black Soldier Fly Larvae Heated Residue; BSFL Residue Compost = Black Soldier Fly Larvae Residue Compost; BSFLR Residue COMBI = Black Soldier Fly Larvae Residue Biochar Co-compost

5.3.3. pH measurements

There was a significant difference in $pH_{(KCl)}$ (p<0.001) among the different treatments over the 112-day incubation period (Figure 5.4). At the beginning of the incubation, day 0, pH ranged from 4.5 to 5.7 in the control and CM. There were slight increases in the pH in all treatments except for the control, which maintained the same pH. The highest pH of 5.3 wasreached on day 14 for BSFL Residue COMBI. However, for BSFL Residue Compost and CM highest pH was reached on day 21 (5.3 and 6.2, respectively). After the peak, pH started to decrease for all treatments to day 56. At the end of the incubation, pH ranged from 4.2 in control and 5.4 in BSFL Residue COMBI.

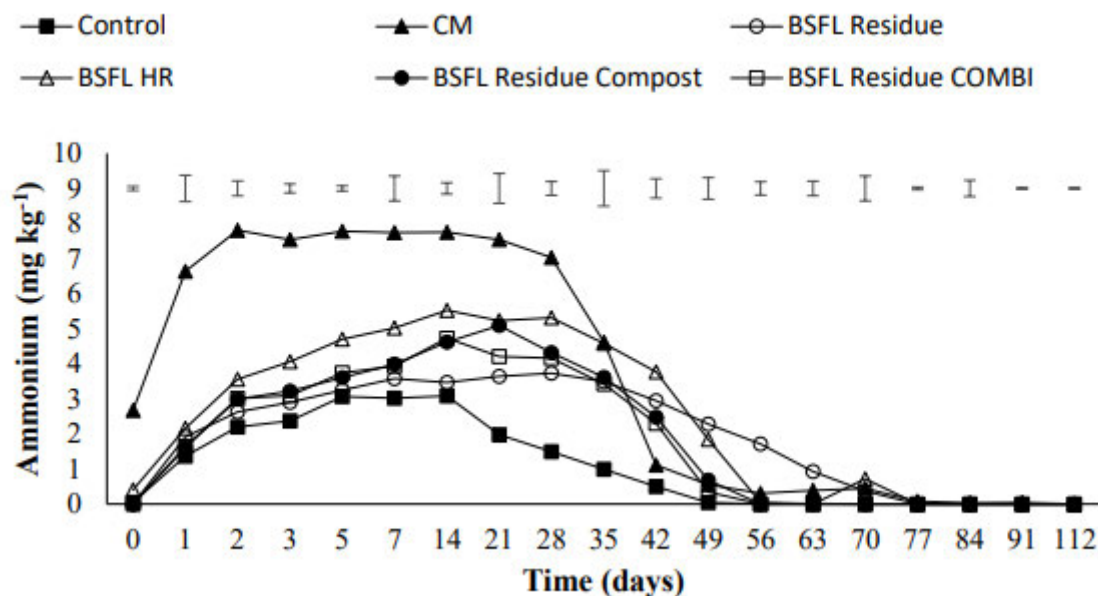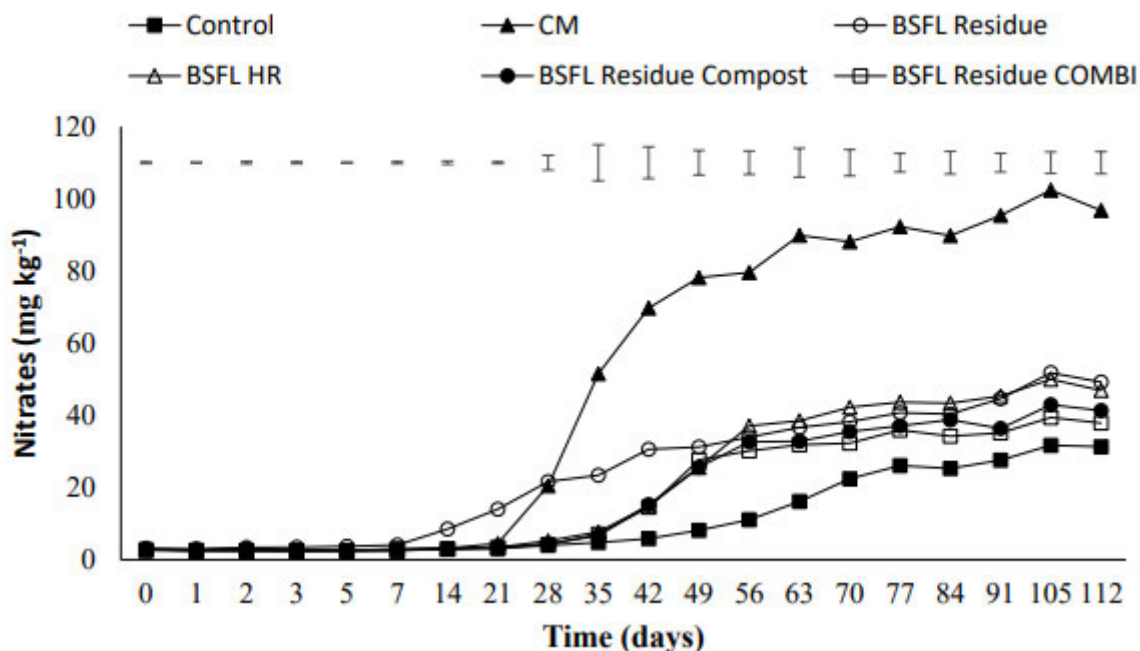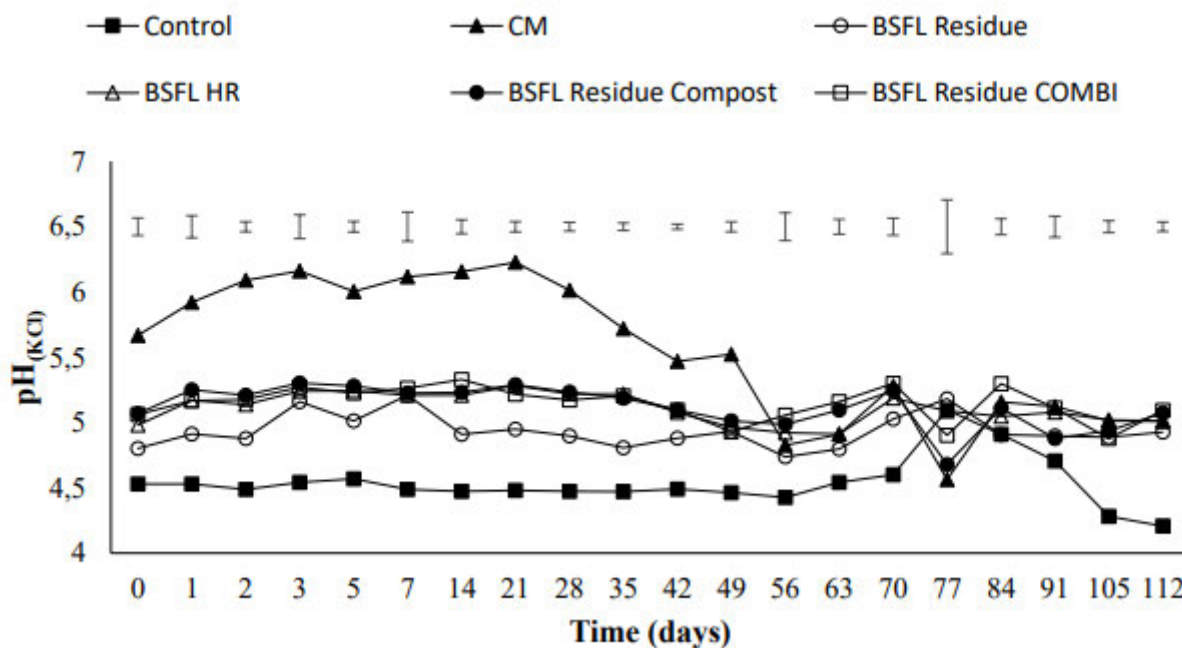**Figure 5.4** Changes in pH among different soil amendments added to a Cartref soil over a 112-day incubation period.

CM = Chicken Manure; CCF = Chemical Commercial Fertiliser; BSFL Residue = Black Soldier Fly Larvae Residue; BSFL HR = Black Soldier Fly Larvae Heated Residue; BSFL Residue Compost = Black Soldier Fly Larvae Residue Compost; BSFLR Residue COMBI = Black Soldier Fly Larvae Residue Biochar Co-compost

5.4. Discussion

This study investigated the nutrient release patterns (nitrogen and phosphorus) and pH in Cartref soil amended using co-compost made from BSFL residue faecal derived biochar mixed with the BSFL faecal residue. A decrease in extractable P (Figure 5.1) at the beginning of the incubation in all treatments could be attributed to the immobilisation of available P by microbes (Enwezor and Soil, 1966). Brtnicky et al. (2019) reported that organic amended soils had higher soil microbial biomass P (SMB-P), although SMB-P was not measured in this study. These microbes utilise available P as a source of food resulting in a decrease in extractable P (Garg and Bahl, 2008). It is unclear why there was an abrupt increase in extractable P from day 21 to w h e n ammonium was converted to nitrate. Initially, ammonium gets converted to nitrite (NO_2^-) by *Nitrosomonas*. The nutrients then get rapidly oxidised to form nitrates by Nitrobacter. Both

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Nitrosomonas and *Nitrobacter* are sensitive to acidity (Freedman, 2018). However, there was a lag between ammonium decrease on day 14 and nitrate release a week later on day 21 for CM, BSFL Residue compost, BSFL Residue COMBI, and BSFL HR. An increase in nitrates is due to the oxidisation of ammonium to NO_2^- by *Nitrobacter* and then to NO_3^- by *Nitrosomonas*, thereby increasing the concentration of NO_3^- towards the end of the incubation (Freedman, 2018).

Nitrate release in BSFL Residue COMBI was low compared to all the other treatments except for the control. Biochar can capture water-soluble nitrates and store them in its mesopores, releasing them slowly over time, making biochar a slow nitrate releasing fertiliser to prevent oversupplying nitrates than the plants can take. In a study carried out by (Hagemann et al., 2017), biochar addition either alone or added to compost released ammonium and nitrates slower than non-biochar amended treatments. Kammann et al. (2015) confirmed similar findings where co-composted biochar compost at 2% w/w biochar improved agronomic value by reducing nutrient losses through nitrate leaching. In their experiment, after sequential biochar washing and electro-ultrafiltration, it was observed that co-composted biochar was nutrient-enriched, predominantly with the nitrate anion. Thus, BSFL Residue COMBI reduces the risk of nitrate leaching and ground-water contamination by slowly releasing sufficient nitrates that are quickly taken up by the plants as they are released.

5.5. Conclusion

Black soldier fly Residue COMBI can release nitrates slowly into the soil. Slow-release of nutrients into the soils prevents leaching, especially when sandy soil is used for crop production. As a result, groundwater contamination is prevented, and the plants effectively use nutrients as they are taken up as soon as they are released. There is also a possibility of residual fertility due to slow-release, which can grow a second cycle of crops without adding a nutrient source.

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CHAPTER SIX: MAIZE RESPONSE TO BLACK SOLDIER FLY LARVAE BIOCHAR CO-COMPOST UNDER GREENHOUSE CONDITIONS

The use of co-composted biochar compost (COMBI) made by adding biochar at the beginning of the composting process can supply nutrients, especially phosphorus, potassium, and nitrogen, while simultaneously improving soil health and resilience. The effect of COMBI and compost without biochar (BSFL Residue Compost) produced from the residue after the decomposition of faecal waste using BSFL on the growth, and nutritional value of maize grown in sandy soil under controlled conditions was evaluated. The study's objectives were to assess the growth and yield performance and determine the nutrient uptake of maize grown using BSFL Residue composts compared to other nutrient sources. A 7 x 3 x 3 factorial design with the following treatments was used; Factor 1: fertiliser sources – 7 levels (Control, Chemical Commercial Fertiliser (CCF, (Urea and Single Super Phosphate)), BSFL Residue, BSFL Heated Residue (BSFL HR), BSFL Residue Compost, BSL Residue biochar compost (BSFL Residue COMBI); Factor 2: application rate – 2 levels (recommended, double recommended based on phosphorus); Factor 3: cutting interval – 3 levels (seedling, ear leaf, maturity) replicated three times. Maize was planted in 20 kg pots after administering the phosphorus sources. In treatments where nitrogen was a limiting factor, urea was added. Data was analysed using Genstat Statistical analysis software 18th edition at a 5% level of significance. Stover and grain macro and trace elements were analysed using the Principal Component Analysis (PCA) (FactoMineR, factoextra, lattice, gdata, and metan) using the R Version 3.6 statistical package. BSFL Residue COMBI at the recommended rate had comparable yields (9.6 t ha^{-1}) to chemical fertiliser (10 t ha^{-1}) at double the recommended rate and compost (9.1 t ha^{-1}). However, using BSFL Residue COMBI at double the recommended application rate significantly reduced yield (1.4 t ha^{-1}). Control plants did not yield harvestable grain. Nutrient uptake was lowest in control plants compared to all the treatments. All BSFL residue-derived amendments had higher trace elements in grain compared to CM and CFF at both application rates. Therefore, BSFL Residue COMBI applied at the recommended application rate based on phosphorus yielded similar yield output as chemical fertilizers

and local organic amendments such as CM.

6.1. Introduction

There is increasing interest in finding sustainable management practices to find sustainable methods to replenish nutrients to the soil (Gowing and Palmer, 2008; Sanchez- Monedero et al., 2019). Incorporating organic amendments such as compost and biochar into the farming practice is one approach that can be used (D'Hose et al., 2020; Oldfield et al., 2018; Sanchez-Monedero et al., 2019). When individually added to the soil, biochar and compost can improve soil quality (Mensah and Frimpong, 2018). Both biochar and compost can add soil organic carbon (SOC) to the soil due to the high amount of stable C. Soil organic C is an important indicator of soil health (Biancalani et al., 2012). In addition to C addition, compost is also known to improve soil chemical, physical and biological characteristics (D'Hose et al., 2016). In addition to having labile C, biochar is also characterised by recalcitrant C, with effects on soil quality being dependent on the status of the soil, with a higher positive influence on fertile soils than unfertile soils because it is not a nutrient source unless when it is pyrolysed from a manure feedstock (Ding et al., 2016).

It has been suggested that mixing biochar and compost can be a strategy to gain benefits from both compost and biochar (Liu et al., 2012). Synergistic effects were observed when biochar and compost were combined before application, where sandy soil had improved soil organic- matter content, nutrients levels, and water-storage capacity (Liu et al., 2012).

On the other hand, biochar can be added at the onset of the composting process to produce biochar compost (COMBI). Co-composting is achieved by combining two or more organic materials such as kitchen waste, manure, sewage sludge at the beginning of the composting process. Co-compost is made from various feedstock, including human faecal matter (Barthod et al., 2018). Biochar can also be used as a bulking agent in the composting process. The use of biochar combined with compost in the initial composting process is better compared to either biochar or compost used separately. The addition of biochar and composts synergise during the composting process (Antonangelo et al., 2021; Camps and Tomlinson, 2015). Several studies had shown remarkable performance in plant growth when biochar compost (COMBI) was used as a soil amendment (Awasthi et al., 2017; Oldfield et al., 2018; Wang et al., 2019)

Recently, biochar has been studied as an alternative or complementary to compost in

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amendments for crop production. In an experiment on a large-scale field trial in Lower Saxony, Germany, Glaser et al. (2015) observed that 10 Mg ha^{-1} biochar before composting increased maize yield by 26% compared to pure compost. The biochar used was made from woody material and compost from kitchen waste, biosolids, and fruit waste.

Black soldier fly larvae faecal residue can also be pyrolysed to make biochar. Little information is available on studies on biochar co-compost from BSFL faecal matter residue on crop productivity. Hence, this study investigated maize growth and nutrient response using BSFL Residue Biochar Compost (BSFL Residue COMBI) at different application rates based on maize Phosphorus (P) requirements under controlled conditions to other organic amendments and chemical fertiliser. The study's objectives were to (i) assess the growth and yield performance of maize grown with BSFL Residue COMBI and (ii) determine the nutrient, N, and P uptake by maize plants fertilised using BSFL Residue COMBI as a plant nutrient source.

6.2.   Materials and method
6.2.1.   Experimental material
The experimental material comprised of maize seed SC 403 sourced from Seed Co. Zimbabwe. Soil amendments were BSFL residue + BSFL residue biochar + sawdust (BSFL Residue COMBI), BSFL residue + sawdust compost (BSFL Residue compost), chicken manure (CM), BSFL residue, Chemical Commercial Fertiliser (CCF (Urea + Single Super Phosphate)) in the form of Single Super Phosphate (SSP), a phosphorus source and Urea, a nitrogen source, BSFL Heated Residue (BSFL HR) and Control. The BSFL HR was made using hot air blown at 200 °C through an inlet valve into a rotary drier with BSFL Residue. The CM was sourced from Ukulinga Farm. This thesis gives a detailed description of co-compost using the BSFL Residue and biochar production in Chapters 3, 4, and 5.

6.2.2.   Experimental site
A sandy Cartref soil, similar to the one used in the incubation study (Chapter 5, in this thesis), was used in the pot experiment. The study was carried out in tunnel L at the Controlled Environment Research Unit (CERU) at the University of KwaZulu-Natal (UKZN) Pietermaritzburg campus.

6.2.3.   Experimental design
The experiment was set up as a 7 x 3 x 2 randomised factorial treatment structure replicated three

times, giving a total of 126 experimental units (a 20 kg pot represented each experimentalunit), with the following treatments: Fertiliser source – 7 levels (control, CM; CCF; BSFL Residue; BSFL HR; BSFL Residue Compost; BSFL Residue COMBI); cutting interval – 3 levels (four weeks after planting, tasselling, maturity) and application rates – 2 levels (recommended and twice recommended rate based on phosphorus). The treatments (Appendix 3) were administered based on maize's phosphorus (P) requirement after soil fertility analysis (Appendix 2). Appendix 3 shows the amount of P in each amendment, the quantity used to meet the P requirement at the recommended rate and double the recommended rate and the quantityof N supplied by that amendment. In all treatments where the amendment did not suffice the N nutrient requirement, N was externally added using Urea (46%). For the chemical fertiliser treatment, SSP (10.5%), Urea (46%), and KCl (50%) were used at planting. Based on soil analysis, potassium was applied as KCl in all treatments per nutrient requirement (Appendix 2). After mixing the soil with respective treatments, two maize seeds (SC 403) were planted 2 mm below the soil surface in each pot. A week after emergence, the smaller, less robust seedling was uprooted, leaving one experimental plant. All pots were irrigated to field capacity using tap water. Weeding was carried out every two weeks during the experiment.

6.2.4. Data collection

After emergence, measurements on plant height were taken weekly. Two weeks after emergence, whole seedlings were harvested and dried. Whole plant harvesting was done at the ear leaf stage (eight weeks after planting) and dried. The remaining maize was left to reach maturity. At maturity, whole plants were harvested, and cobs were separated. Stover and grain mass was determined using a laboratory scale. The grain was air-dried before measuring dry mass. Harvest index was calculated using the formula below:

$$\text{Harvest Index} = \frac{Grain\ mass\ (g)}{Total\ mass\ (g)}$$

Where total mass = grain mass + cob mass + stover mass

6.2.5. Chemical analyses for nutrient determination

Total N was determined on the grain, stover, ear leaf roots, and soil using the Leco-TruMac CNS Autoanalyser (Leco-cooperation, LECO CNS-2000, St Joseph MI, USA, 2012). A sample was ground to 2 mm, and a 0.2 g sample was analysed using the Leco CNS Analyser. A 0.5 g plant sample was weighed and digested for total nutrients and trace metals using 10 mL aqua regia

preparedusing HCl and nitric acid (3: 1). The mixture was filtered using Whatman No. 1 filter paper filled to 25 mL volume using distilled water and analysed for heavy metals using the AAS. Total P on the grain, stover, ear leaf, roots, and soil were done on samples digested on a hot sand bath using sulfuric acid, hydrogen peroxide, and hydrofluoric acid. Briefly, a 0.25 g well-mixed sample was weighed into a 100 mL Teflon beaker. A volume of 5mL of concentrated sulphuric acid was added and swirled gently. A volume of 3 mL of 30% hydrogen peroxide was added in 0.5 mL portions and swirled vigorously to avoid foam overflow. When the reactionwith hydrogen peroxide had subsided, 1 mL of concentrated hydrofluoric acid was added in 0.5mL portions and swirled gently. The beaker was placed on a hot plate at 150 °C for 20 minutesto eliminate excess hydrogen peroxide. After slight cooling, the beaker was washed down the sides with approximately 15 mL of distilled water. The mixture was mixed and allowed to cool to room temperature. The beaker contents were quantitatively transferred to a 50 mL volumetricflask, passing it through a Whatmann 1 filter paper. Two additional washings of the beaker were made with 10 mL of distilled water, filtered, and made up to volume. The P concentrationof the extract was measured using the molybdenum blue method. The blue extract's absorbancewas read at 670 nm on an ultraviolet spectrophotometer (Varian Alpha UV-VIS, Spectronic Unicam, Berlin, Germany. The total concentration of P (wPT; mg/kg oven-dried soil) was calculated using the following equation:

$$W\text{pt} = \frac{P\text{p x } 50}{\text{m}} \text{ X } \frac{(100 + \text{Ww}}{100}$$

Pp - concentration of P (mg L^{-1}) measured according to the molybdenum blue method

m - the mass of air-dried soil (g)

Ww - percentage of water content (mass fraction) based on oven-dried samples.

6.2.6. Data analysis

Data for growth and yield was analysed using GenStat 18th Edition Statistical Package, and means were separated at a 5% level of significance using Fisher's least significant difference. The Principal Component Analysis (PCA) (FactoMineR, factoextra, lattice, gdata, and metan) was used to analyse macronutrient and trace element uptake data in the grain and stover using the R Version 3.6 statistical package. A dendrogram and a cluster plot were used to group similar treatments into clusters.

6.3 Results

6.3.1 Plant growth and yield

During the early stage of the experiment, visible nitrogen and phosphorus deficiency symptoms were already showing in control. Figure 6.1 shows the control plants two weeks after emergence. The controls showed nitrogen and phosphorus deficiency symptoms indicated by chlorosis, stunted growth, and purpling of leaves.
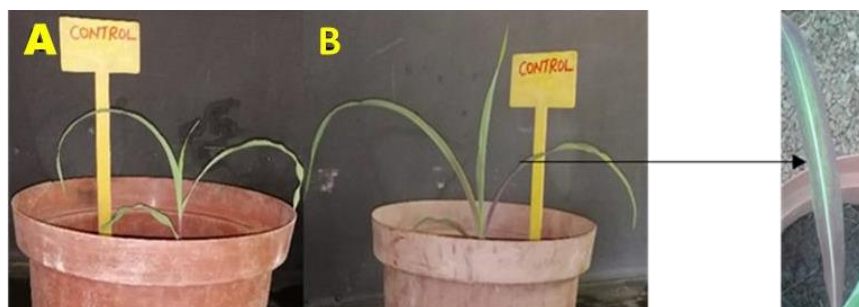
Figure 6.1 Control plants showing stunted growth and chlorosis symptoms of nitrogen deficiency (A) and purpling of leaves, a symptom of phosphorus (B) two weeks after emergence.

Figure 6.2 shows a comparison of the control plants and the CM, CCF, BSFL residue, BSFL HR, BSFL Residue COMBI, and BSFLR Residue Compost treated plants at the recommended (A) and double recommended application rate (B). Both BSFL Residue COMBI and BSFL Residue compost were comparable to CM and CCF at the recommended rate. However, BSFLHR grew taller than all the treatments, reaching a maximum of 2.33 and 2.69 m at the recommended and double recommended application rate, respectively, at the end of theexperiment. Application at twice the recommended rate resulted in stunted growth in CM and CCF compared to the control (Figure 6.2B). BSFL Residue COMBI, BSFL Residue Compost,and BSFL HR were not affected by doubling the rate and performed comparably to similar treatments at the recommended rate (Figure 6.2B).

Figure 6.2 Comparison of maize plants grown with different nutrient sources two weeks after emergence at the recommended rate (A) and twice recommended rate (B) two weeks after emergence.

BSFL Residue= Black Soldier Fly Larvae Residue; BSFL HR = Black Soldier Fly Larvae Heated Residue; BSFL Residue compost = Black Soldier Fly Residue Compost; BSFL ResidueCOMBI = Black Soldier Fly Larvae Biochar compost.

Figure 6.3 shows a comparison of the treatments four weeks after emergence. The purpling of leaves in control continued up to week four, with the negative control having stunted growth compared to the planted with soils treated with organic amendments (CM, BSFL residue, BSFL HR, BSFL Compost, and BSFL Residue COMBI) at the recommended rate. Plant height in plants treated with chemical fertilisers was comparable to organic amendments at the recommended rate. When the rate was doubled, the height of maize plants grown with BSFL Residue and heated residue were higher than CM, CM, BSFL Residue COMBI, and BSFL Residue Compost. The control plants remained stunted at four weeks after emergence.

Figure 6.3 Comparison of maize plants grown with different nutrient sources four weeks after emergence at the recommended rate (A) and twice recommended rate (B) two weeks after emergence.

BSFL Residue= Black Soldier Fly Larvae Residue; BSFL HR = Black Soldier Fly Larvae Heated Residue; BSFL Residue compost = Black Soldier Fly Residue compost; BSFL Residue COMBI = Black Soldier Fly Larvae biochar compost.

Figure 6.4 shows the plants eight weeks after emergence. This was the stage when the ear leaf and stover were harvested for the second harvesting stage.
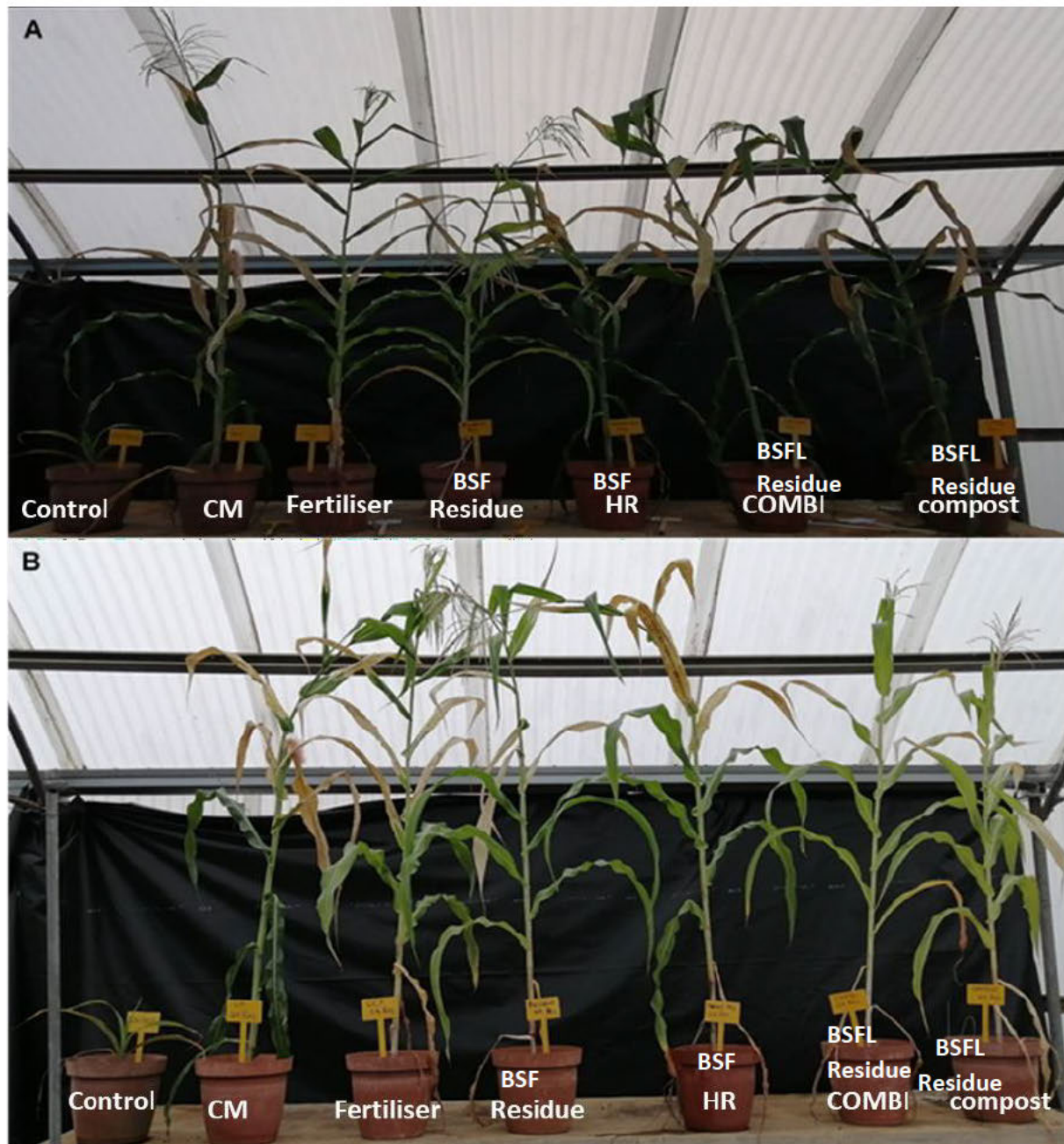

Figure 6.4 Comparison of maize plants grown with different nutrient sources, eight weeks after emergence at the recommended rate (A) and twice recommended rate (B) two weeks after emergence.

BSFL Residue= Black Soldier Fly Larvae Residue; BSFL HR = Black Soldier Fly Larvae Heated Residue; BSFL Residue compost = Black Soldier Fly Residue compost; BSFL ResidueCOMBI = Black Soldier Fly Larvae biochar compost.

Figure 6.5 shows an increase in plant height over time at the recommended rate and double the recommended rate when maize was grown using BSFL Residue, BSFL Residue Compost, BSFL Residue COMBI, CCF, and control under greenhouse conditions. There was an increasein plant height in plants planted with organic amendments at both application rates. The control had the least height at the end of the growing period (0.77 m). At the recommended application rate, plant height was comparable for CM, BSFL Residue Compost, BSFL HR, and BSFL Residue COMBI. However, doubling the fertiliser rate resulted in a decrease in plant height inBSFL Residue COMBI. Plant height was comparable to control at both application rates. At double the recommended rate, BSFL HR had the highest maize plant height (2.69 m). Plants at the recommended application rate were shorter than plants at double the recommended application rate. Maize grown using BSFL Residue COMBI at both application rates was lower than CM, BSFL HR, and BSFLR Residue Compost. At both the recommended and double recommended rate, BSFL Residue COMBI had a statistically similar height of 2.17 and

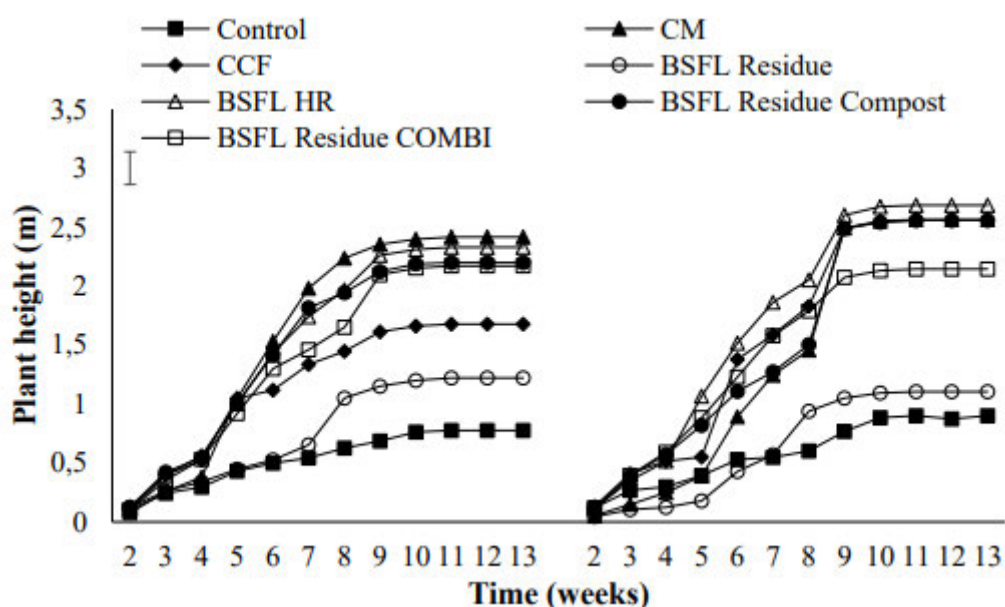2.14 m at the recommended and double recommended rate, respectively.

Figure 6.5 Plant height at harvesting stage of maize plants grown with different nutrient sources at the recommended rate (left) and twice recommended rate (right).

BSFL Residue= Black Soldier Fly Larvae Residue; BSFL HR = Black Soldier Fly Larvae Heated Residue; BSFL Residue compost = Black Soldier Fly Residue Compost; BSFL ResidueCOMBI = Black Soldier Fly Larvae Biochar compost.

Figure 6.6 shows dry mass maize planted with BSFL Residue COMBI, BSFL Residue compost, BSFL Residue HR, BSFL residue, CM, and CCF compared to the control plants at different application rates and different growth stages. There was no significant difference in dry mass in plants planted with all the amendments at both application rates at the seedling stage. At the ear leaf stage, the highest stover dry mass was obtained in maize planted with CCF (191.2 g pot^{-1}) at the recommended application rate. Stover masses for CM and BSFL HR at both application rates, CCF at double the recommended application, and BSFL Residue compost the recommended application rate were not significantly different. Doubling the application rate for BSFL Residue COMBI at the ear leaf resulted in a lower stover mass (67.9 g pot^{-1}) compared to the recommended application rate (95.3 g pot^{-1}).

At maturity, CCF had the highest stover mass at double application rate (156.6 g pot^{-1}). At the recommended application rate, stover mass for CM, CCF, BSFL HR, BSFL Residue Compost, and BSFL Residue COMBI did not differ significantly (112.4, 120.8, 124.6, 112.6, and 123.6 g pot^{-1}), respectively. Doubling the application rate resulted in increased stover mass at maturity in BSFL Residue and CCF (92.7 and 156.6 g pot^{-1}), respectively. However, doubling the application rate for CM, BSFL HR, BSFL Residue Compost, and BSFL Residue COMBI resulted in a decrease in stover mass to 79.1, 93.9, 68.7, and 108.6 g pot^{-1}, respectively.
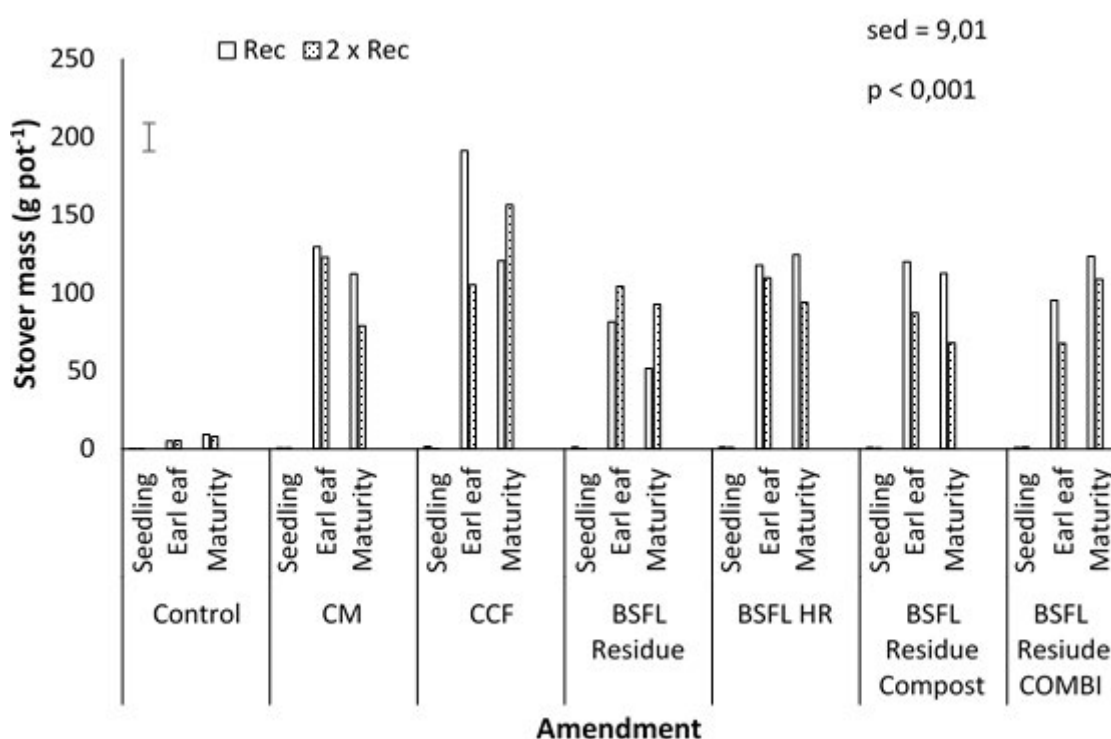
Figure 6.6 Stover mass of maize plants at different maize growth stages (seedling, ear leaf, and maturity stage) of maize grown with different phosphorus nutrient sources at the recommended rate and twice recommended rate.

BSFL Residue= Black Soldier Fly Larvae Residue; BSFL HR = Black Soldier Fly Larvae Heated Residue; BSFL Residue compost = Black Soldier Fly Residue Compost; BSFL Residue COMBI = Black Soldier Fly Larvae Biochar compost.

Figure 6.7 shows the grain yield, stover mass, and harvest index of maize grown with CM, CCF, BSFL Residue, BSFL HR, BSFL Residue Compost, BSFL Residue COMBI, and control.There was a significant interaction ($p<0.001$) between amendment and application rate on maize yield. The highest yield was obtained from seed planted with CCF at double the recommended application rate (10.2 t ha^{-1}). It was comparable to BSFL Residue COMBI at the recommended application rate (9.6 t ha^{-1}) and BSFL Residue Compost (9.1 t ha^{-1}) at the recommended application rate. Chicken manure and CCF were comparable at the recommended application rate. BSFL Residue at double the recommended rate and BSFL HR at both application rates were comparable. The recommended application rate using BSFL Residue yields was lower (3.2 t ha^{-1}) than at the double recommended application rate but lower than BSFL Residue COMBI at the same application rate. The lowest yield (1.4 t ha^{-1}) was obtained from plants grown with BSFL Residue COMBI at double the recommended application rate.

There was a significant interaction (p<0.001) between amendment and application rate on maize stover mass (Figure 6.7). At the recommended fertilizer application rate, the highest stover yield was obtained in maize grown with CM (0.031 t ha-1). Stover mass was comparable for BSFL HR and BSFL Residue COMBI (0.023 and 0.024 t ha^{-1}), respectively, at the recommended application rate. BSFL Residue COMBI at both application rates (0.025 and 0.026 t ha^{-1}) was comparable to BSFL Residue Compost at the recommended P applicationrate (0.026 t ha^{-1}). The control had the least stover mass (0.002 t ha^{-1}), followed by residue at double the recommended application rate (0.01 t ha^{-1}).

There was a significant interaction (p<0.001) between amendment and application rate on maize HI. Harvest index ranged from 0.48 to 0.13 in BSFL Residue and BSFL Residue COMBI, respectively, at double the recommended application rate. At both application rates, harvest indices for CM, CCF, BSFL Residue, BSFL HR, BSFL Residue Compost were comparable. A significant decrease in HI was observed when the fertiliser application rate was doubled in BSFL Residue COMBI from 0.46 to 0.13.
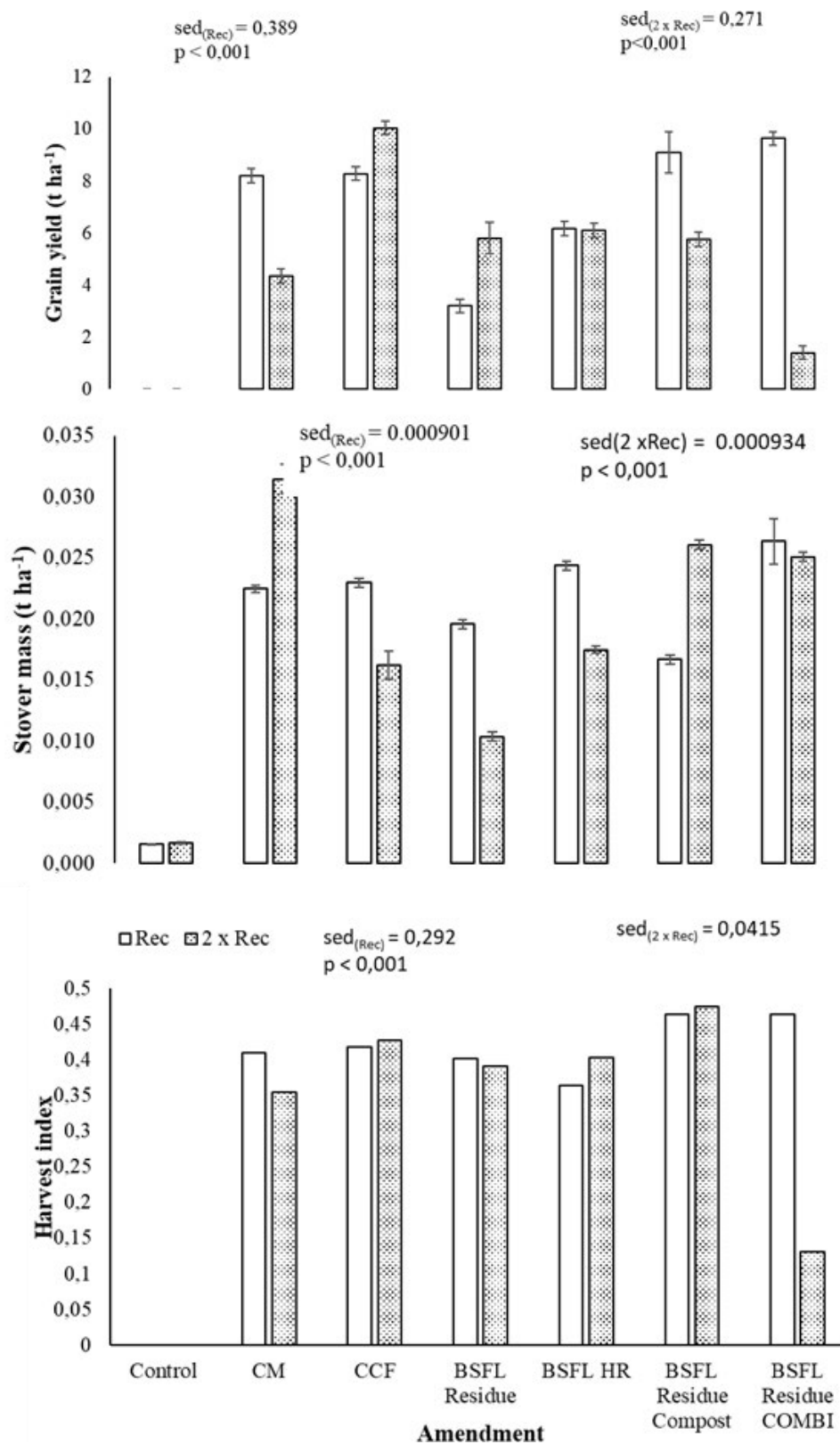
Figure 6.7 Stover mass, grain yield, and harvest index of maize grown with different nutrient sources at the recommended rate and twice recommended rate based on phosphorus.

BSFL Residue= Black Soldier Fly Larvae Residue; BSFL HR = Black Soldier Fly Larvae Heated Residue; BSFL Residue compost = Black Soldier Fly Residue Compost; BSFL Residue

COMBI = Black Soldier Fly Larvae Biochar compost.

Figure 6.8 shows the cobs after harvesting on soils treated with CM, CCF, BSFL residue, BSFL HR, BSFL Residue compost, and BSFL Residue COMBI. At harvesting, all the other treatments had filled cobs except for the control treatments. The control plants tasseled and fertilised but could not produce harvestable grain yield.
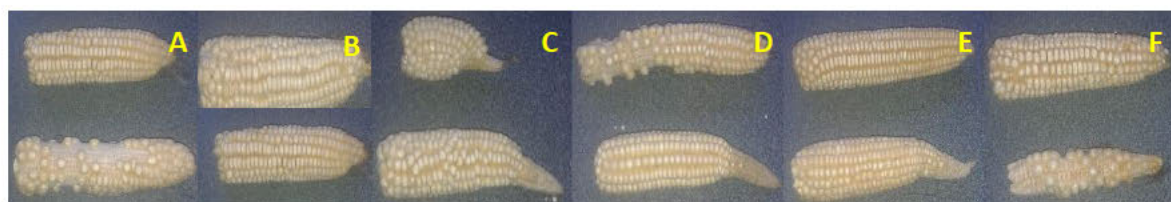
Figure 6.8 Maize cobs harvested for CM (A), CCF (B), BSFL residue (C), BSFL HR (D), BSFL Residue compost (E), and BSFL Residue COMBI (F) for recommended rate (top row) and double recommended rate, bottom row).

BSFL Residue= Black Soldier Fly Larvae Residue; BSFL HR = Black Soldier Fly Larvae Heated Residue; BSFL Residue compost = Black Soldier Fly Residue Compost; BSFL ResidueCOMBI = Black Soldier Fly Larvae Biochar compost.

6.3.2 Chemical characteristics for nutrient determination

Figure 6.9 shows the grain nitrogen concentration in the maize plant at different growth stagesin maize planted with CM, CCF, BSFL Residue COMBI, BSFL Residue Compost, and BSFL HR, compared to the control at the recommended application and double fertiliser application rate based on P. There was a significant interaction (p = 0.002) between amendment and application rate with respect to total grain nitrogen. Total grain nitrogen ranged from 1.68 to 0.76% in BSFL Residue COMBI at the recommended application rate and BSFL Residue Compost at the recommended application rate, respectively. Maize plants treated with CCF (1.38%) at double recommended application rate and BSFL Residue HR (1.40%) and BSFL Residue (1.40%) at recommended application rate had comparable grain nitrogen. The controlplants did not have harvestable grain to analyse.

Figure 6.9 Grain total N of maize grown with different nutrient sources at the recommended rate and twice recommended rate based on phosphorus.

BSFL Residue= Black Soldier Fly Larvae Residue; BSFL HR = Black Soldier Fly Larvae Heated Residue; BSFL Residue compost = Black Soldier Fly Residue Compost; BSFL ResidueCOMBI = Black Soldier Fly Larvae Biochar compost.

Figure 6.10 shows the total N content of the whole plant (grain, stover, and roots) for maize planted using BSFL Residue COMBI, compared to BSFL Residue compost, BSFL HR, CCF, CM, and control at the recommended and double P application rate. There was a significant interaction ($p<0.001$) between amendment and application rate on total nitrogen in whole maize plants at harvesting. Whole plant total N ranged from 1.51 to 3.69 % in BSFL Residue COMBIat double application rate and BSFL Residue Compost at the recommended application rate. The control (1.61%) had comparable whole plant total N to BSFL Residue at both application rates (1.71 and 2.03%) and BSFL Residue COMBI (1.51%) at the recommended application rate.

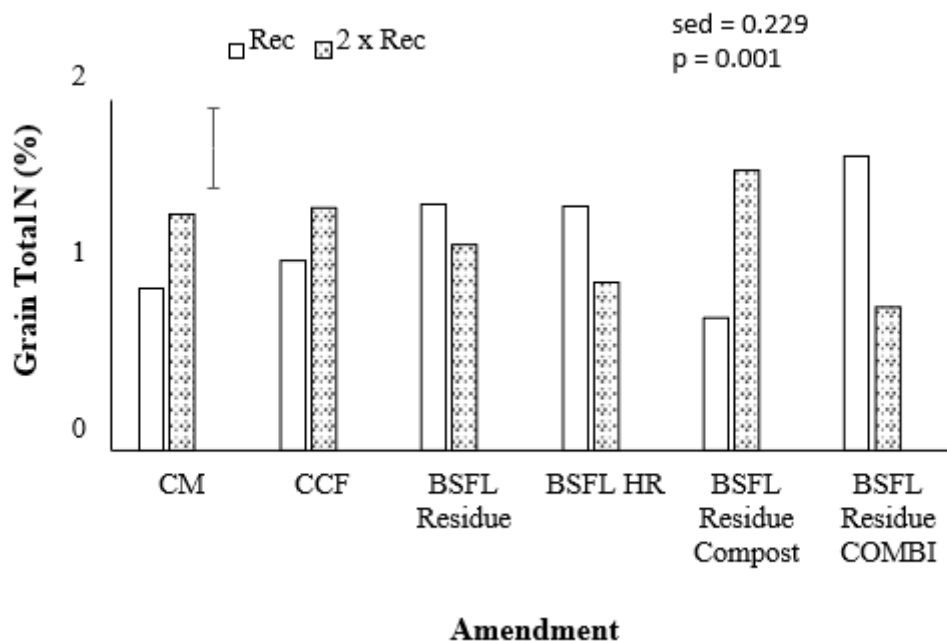**Figure 6.10** Whole plant total N of maize grown with different nutrient sources at the recommended rate and twice recommended rate based on phosphorus.

BSFL Residue= Black Soldier Fly Larvae Residue; BSFL HR = Black Soldier Fly Larvae Heated Residue; BSFL Residue compost = Black Soldier Fly Residue Compost; BSFL ResidueCOMBI = Black Soldier Fly Larvae Biochar compost.

Figure 6.11 shows grain total P and whole plant total P of maize grown using CM, CCF, BSFL Residue, BSFL HR, BSFL Residue Compost, and BSFL Residue COMBI compared to control. There was a significant interaction ($p<0.001$) between amendment and fertiliser application rate on grain total P. Grain total P ranged from 1007.8 to 53.5 mg kg^{-1} in CM and CCF at the recommended application rate, respectively. Doubling the application rate for BSFL Residue Compost yielded more grain total P (735.9 mg kg^{-1}). Chicken manure at the recommended application rate was 526.5 mg kg^{-1}. Grain total P in maize treated with BSFL Residue COMBI was comparable at recommended (375.5 mg kg-1) and double the recommended application rate (440.7 mg kg^{-1}). Grain P for plants treated with residue at the recommended rate (284.2 mgkg^{-1}) was higher than at double the recommended application rate (93.0 mg kg^{-1}). There was no harvestable seed for the control to analyse for P.

There was a significant interaction ($p<0.001$) between amendment and fertiliser application rate on whole plant total P. Total P for the whole plant at harvesting ranged from 3 434.2 to 177.3

mg kg^{-1} for CCF and BSFL Residue at the recommended application rate. BSFL Residue COMBI had comparable total P at the recommended application rate (2 853.7 mg kg^{-1}) and double application rate (2 605.3 mg kg^{-1}), which was also comparable to BSFL Residue Compost at double the recommended application rate (2 664.0 mg kg^{-1}). The lowest total P wasin residue at double the recommended application rate (177.3 mg kg^{-1}). Very low phosphorus was observed in the control at undetectable levels.
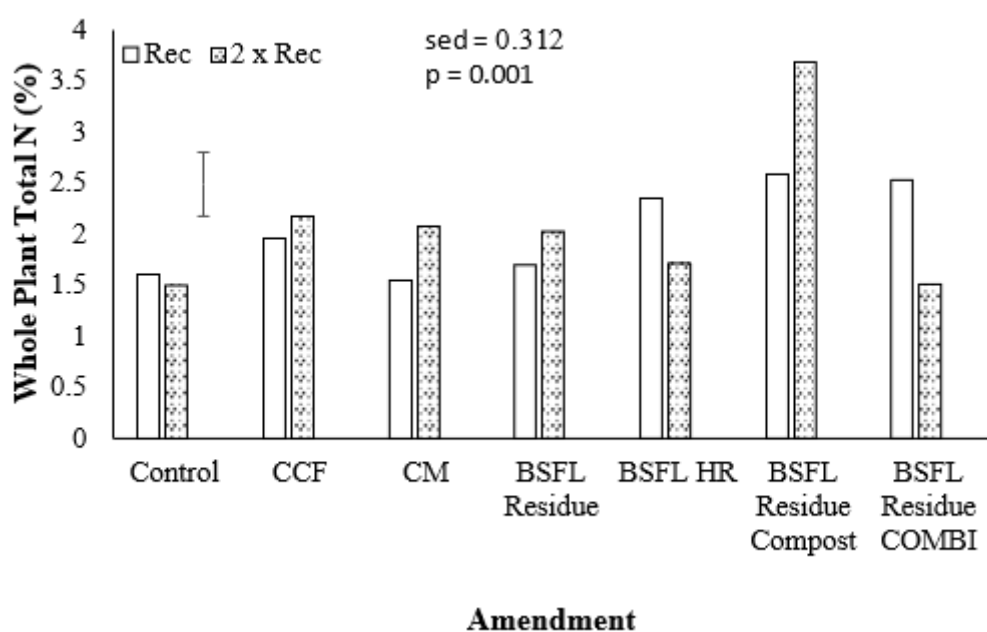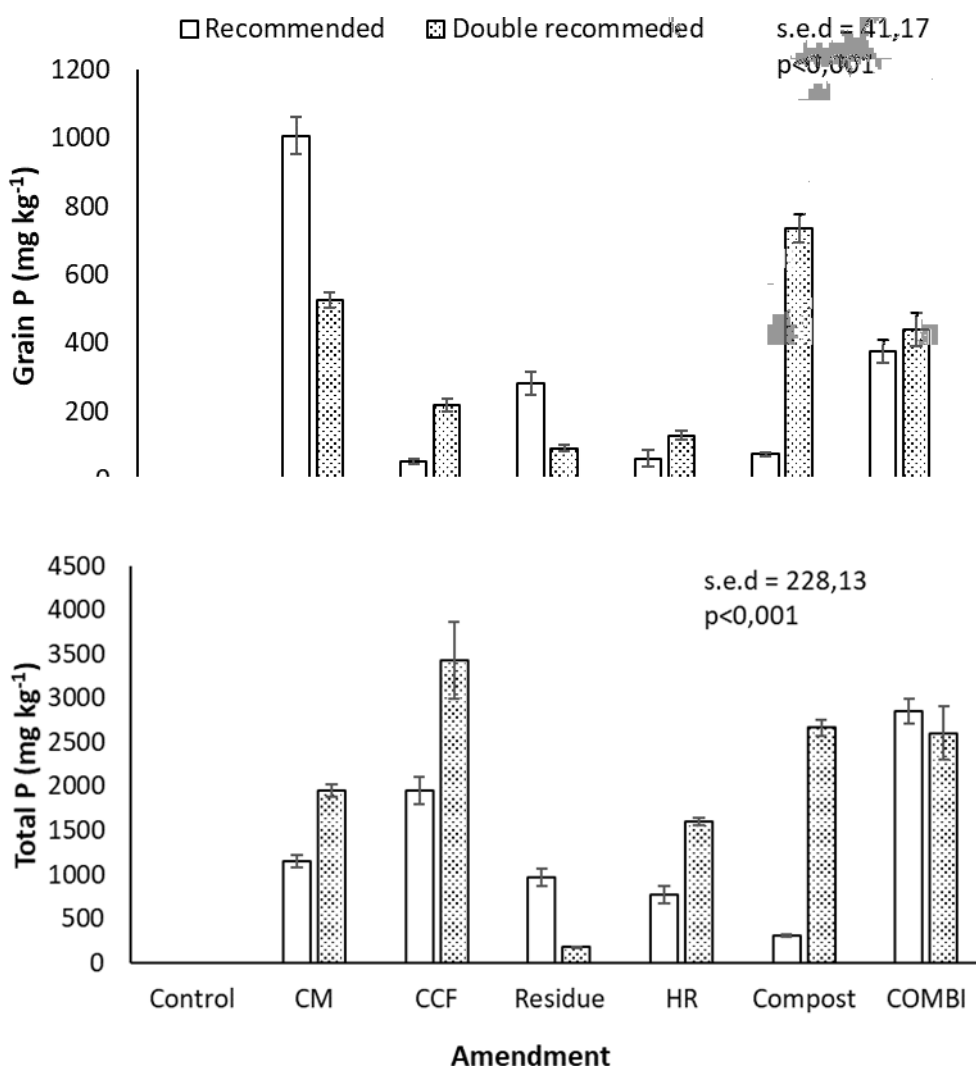

Figure 6.11 Grain and whole plant total P of maize grown with different nutrient sources at the recommended rate and twice recommended rate based on phosphorus.

BSFL Residue= Black Soldier Fly Larvae Residue; BSFL HR = Black Soldier Fly Larvae Heated Residue; BSFL Residue compost = Black Soldier Fly Residue Compost; BSFL ResidueCOMBI = Black Soldier Fly Larvae Biochar compost.

Figure 6.12 shows a cluster dendrogram showing the clustering of treatments for macronutrientsin maize plants. There were four clusters, namely:

1- COMBI 1, HR 2, HR 1, COMP 1, COMP 2, COMBI 2
2- CM 1, CCF 1, Residue 1, CCF 2
3- Residue 2, Control 1
4- CM 2, Control 2

Cluster Dendrogram

Figure 6.12 Dendrogram is based on cluster analysis, assigning different phosphorus sources to different application rates for macro elements.

Figure 6.13 shows a Principal Component Analysis (PCA) biplot showing the scores of P source treatments (Control, CM, CCF, Residue, HR, Compost, COMBI) at recommended and double recommended application rates, relative to macro element uptake in maize grain and stover. The first two components (PC1 and PC2) with an eigenvalue greater than 1.0 accounted for 79.7% of the total PCA. PC1, which accounted for 50.8 % of the total variance, showed a significant positive correlation with G_Ca, G_K, G_Na, G_Mg, and T_K. On the other hand, PC2, which accounted for 28.9 % of the total variance, displayed a significant positivecorrelation with T_Ca, T_Mg, and T_Na. Control 1 Control 2 and CM 2 were negatively correlated to all the other treatments. Higher T_Na and T_Mg characterised maize plants treatedwith COMBI 1, HR 2, HR 1, COMP 1, COMP 2, and COMBI 2 (cluster 1) compared to the overall mean. However, T_K was lower than the overall mean. Cluster 2, represented by CM 1, CCF 1, Residue 1, and CCF 2, was characterised by lower G_N, G_Ca, and G_K than the overall mean. Maize planted with Residue 2 and Control 1 (cluster 3) had higher T_Ca compared to the overall mean. Control 2 and CM 2 maize treated plants represented by cluster 4 (Figure 6.12) performed the poorest compared to all treatments with regards to total macronutrient uptake, T_Na, T_Mg, and T_Ca, except T_K. Only G_Mg was higher than the overall mean in this cluster. In the bi-plot, the influences of the various descriptors are given asarrows. The length of an arrow is an indication of the descriptors' variance. The arrows' angle measures the correlation between the descriptors—a small angle expressing a high correlation and vice versa. The output for the PCA is shown in Appendix 4.

Figure 6.13 Principal component analysis biplot of the treatments in relation to growth macronutrient uptake

Key

1 – recommended application rate   2- double recommended application rate

T – Total   G - Grain

Figure 6.14 shows a cluster dendrogram showing the clustering of treatments with respect to micronutrients in maize plants. There were five clusters, namely:

 1- Residue 2
 2- CM 2
 3- Residue 1, CCF 1, CCF 2, CM 1
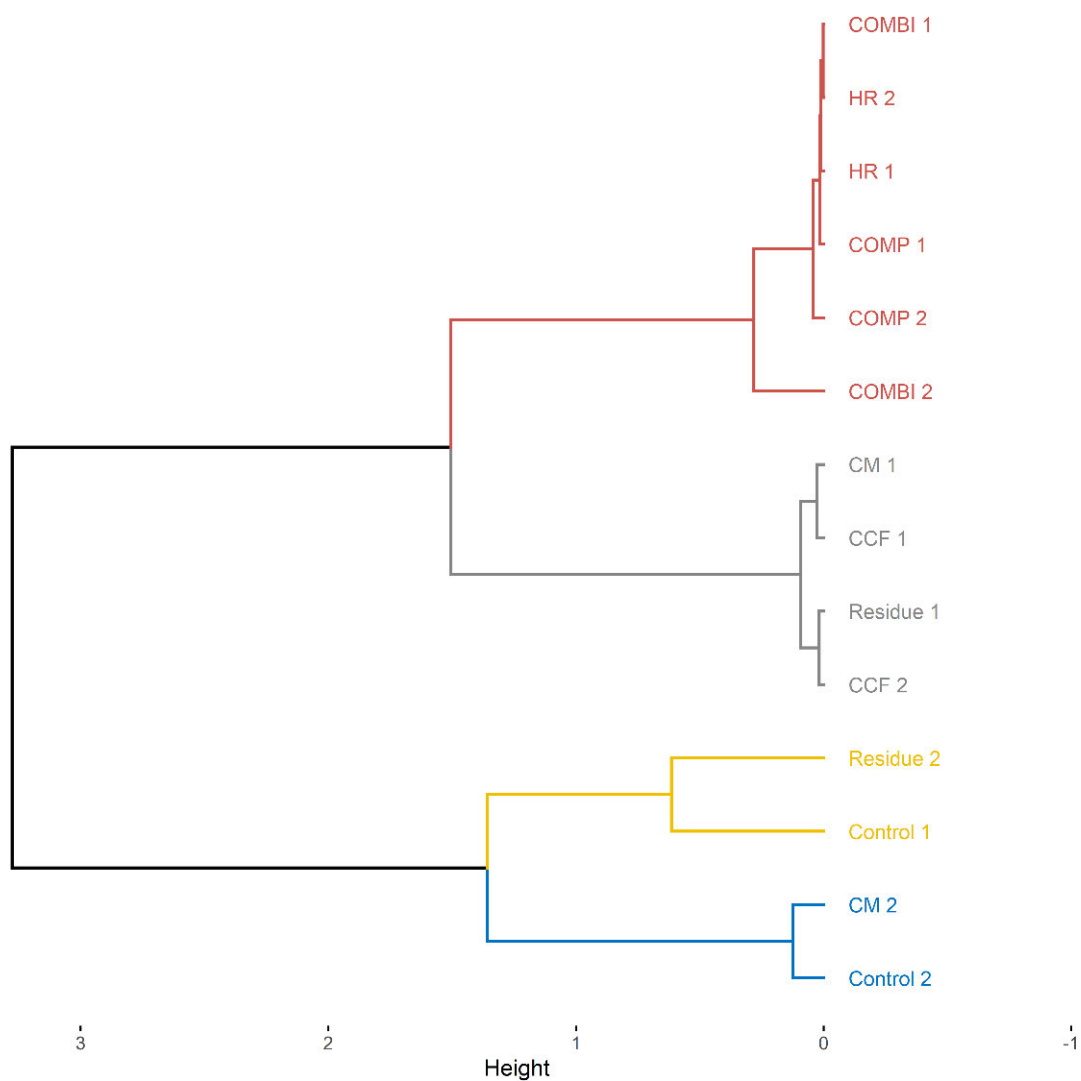 4- COMBI 1, COMP 2, HR 1, HR 2, COMBI 2, COMP 1
 5- Control 1, Control 2
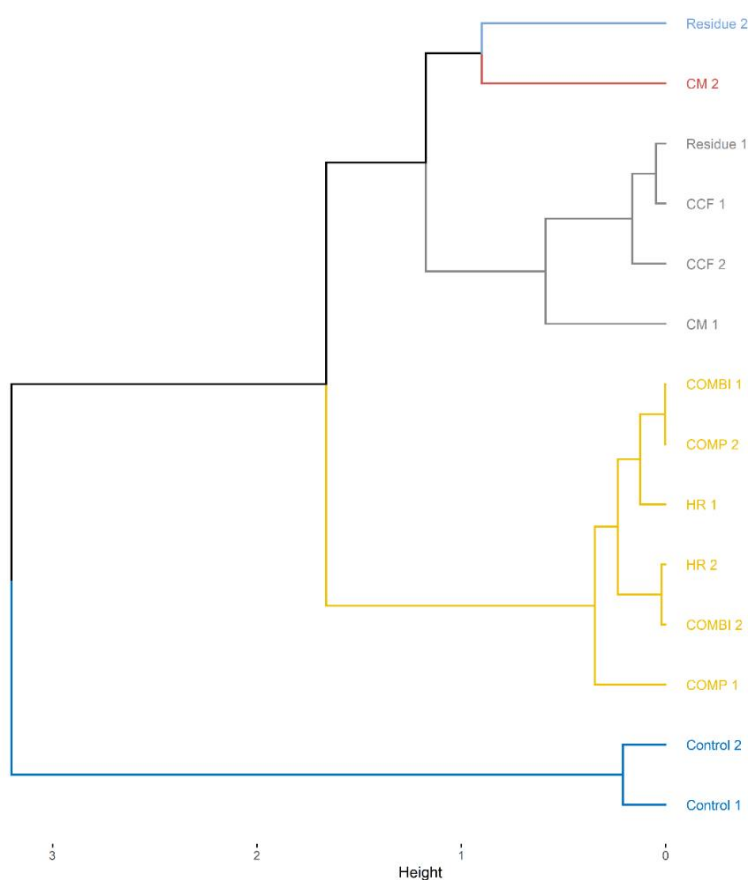
Figure 6.14 Dendrogram based on cluster analysis, assigning different phosphorus sources to different application rates for micronutrients.

Figure 6.15 shows a PCA biplot showing the scores of P source treatments (Control, CM, CCF, Residue, HR, Compost, COMBI) at recommended and double recommended application rates, relative to microelement uptake in maize grain and stover. The first two components (PC1 and PC2) with an eigenvalue greater than 1.0 accounted for 52.7 % of the total PCA. PC1, which accounted for 34.5 % of the total variance, showed a significant positive correlation with G_Cd, G_Mn, G_Cu, G_Zn, and G_Cr. However, PC2 had accounted for a lower total variance of 18.2 %, which displayed a positive correlation with T_Cu, T_Fe, T_Cd, and T_Zn. The controls were negatively correlated to all the other treatments. Cluster 1, represented by maize treated with Residue 2, had lower G_Cu, and G_Zn compared to the overall mean. Cluster 2,represented by CM 2, had higher G_Fe. Higher T_Zn characterised maize planted with Residue1, CCF 1, CCF 2, and CM 1 represented by cluster 3 compared to the overall mean. Cluster 4 with maize planted using COMBI 1, COMP 2, HR 1, HR 2, COMBI 2, and COMP 1 had higher

T_Mg and G_Cd compared to the overall mean. Lastly, cluster 5, consisting of maize planted without any nutrient source (Control), showed T_Cu uptake lower than the overall mean. The output for the PCA is shown in Appendix 5.

Higher T_Na and T_Mg characterised maize plants treated with COMBI 1, HR 2, HR 1, COMP1, COMP 2, and COMBI 2 (cluster 1) compared to the overall mean. However, T_K was lowerthan the overall mean. Cluster 2, represented by CM 1, CCF 1, Residue 1, and CCF 2, was characterised by lower G_N, G_Ca, and G_K than the overall mean. Maize planted with Residue 2 and Control 1 (cluster 3) had higher T_Ca compared to the overall mean. Control 2 and CM 2 maize treated plants represented by cluster 5 (Figure 6.14) performed the poorest compared to all treatments with regards to total macronutrient uptake, T_Na, T_Mg, and T_Ca,except T_K. Only G_Mg was higher than the overall mean in this cluster.
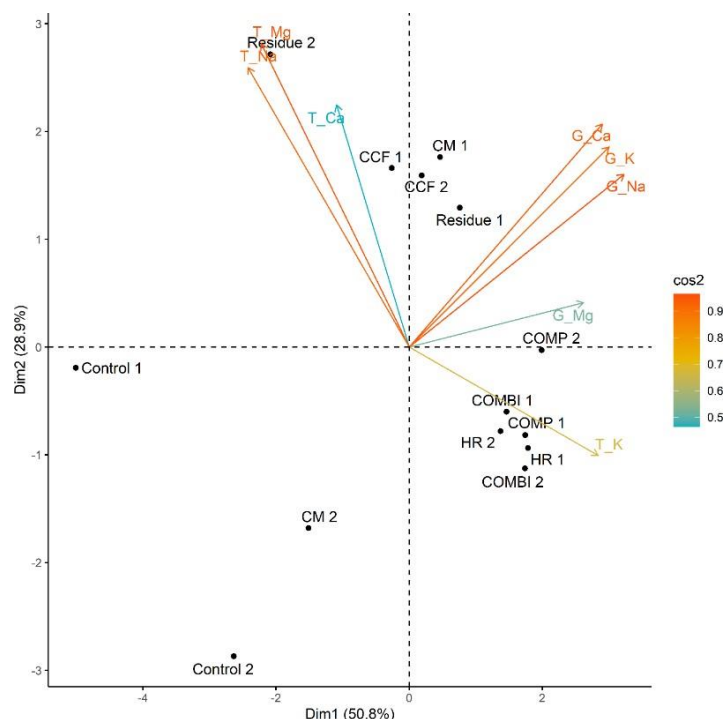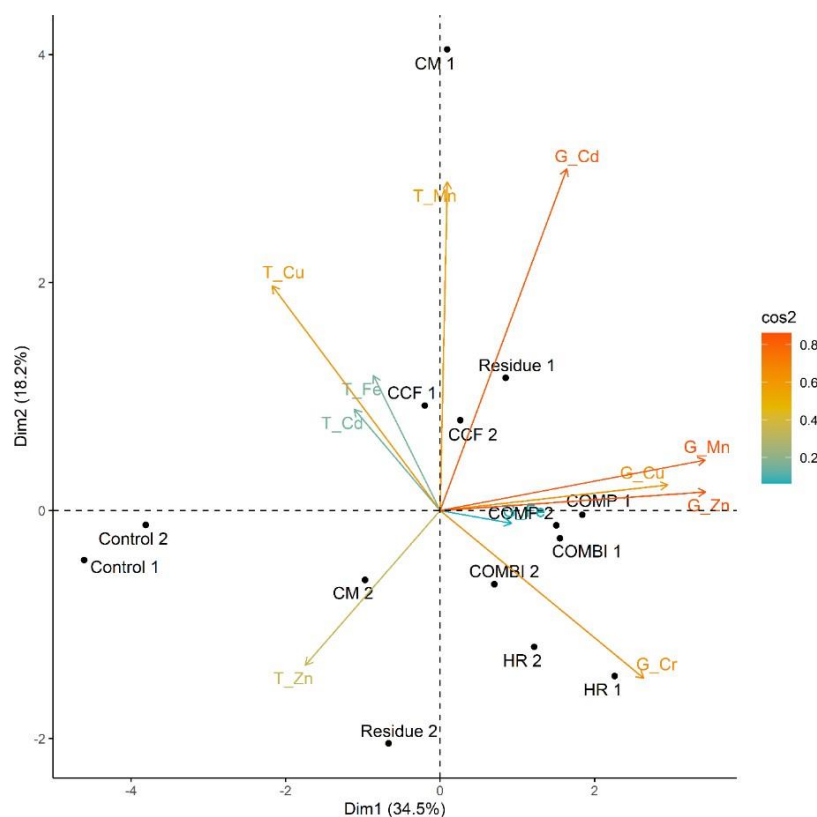
Figure 6.15 Principal component analysis biplot of the treatments in relation to growth macronutrient uptake

Key

1 – recommended application rate	2- double recommended application rate
T – Total	G - Grain

6.4   Discussion

The study sought to assess whether the Human Excreta Derived Materials (HEDM's) composted in Chapter 4 could supply nutrients under a greenhouse study, with maize as a test crop, and to identify which application rate resulted in the highest maize grain yield. The findings from this experiment show that all HEDM's promoted maize's growth and productivity. Both total biomass and grain yield were increased significantly relative to the control. At two weeks, control plants already started to show yellowing of leaves (chlorosis), indicating N deficiency. Control plants also had purple leaves (Figure 1) and leaf margins, a phosphorus deficiency sign (Sawyer, 2004). These symptoms could be attributed to low soil fertility (Appendix 2). Maize plants treated with CCF, CM, BSFL Residue, BSFL HR, BSFL Residue Compost, and BSFL Residue COMBI

were healthy and similar in size at both application rates, except for CM and CCF at the recommended application rate (Figure 2). The health status of the maize grown with BSFL residue-derived composts could be explained because the nutrients in these amendments were available for plant uptake (Agegnehu et al., 2016). Plants need P in the initial stages of development for root formation (Hajabbasi and Schumacher, 1994). BSFL Residue, COMBI BSFL Residue Compost, and BSFL HR could supply this required initial P during early crop establishment.

Untreated BSFL Residue had lower plant height at both application rates, which is likely due to the initially high ammonium levels in the BSFL Residue as observed in Chapter 4. High ammonium levels could have caused ammonium toxicity. Similar results were observed by Alattar et al. (2016) when maize plants planted with BSFL residue from food waste showed stunted growth, being 39% shorter and having 19% fewer leaves on average. However, Zahn and Quilliam (2017) found contradicting results when onions were planted using BSFL residue from fruit waste. Onions planted with BSFL fruit residue had comparable growth to fertilisers but only started to show stunted growth at a higher residue application rate. This positive crop growth response was attributed to the potential presence of nutrients, high microbial biomass, chitin, growth hormones, and soil enzymes (Zahn and Quilliam, 2017). Alattar et al. (2016) suggested post-processing technologies for the residue, and it was observed that in this current study, after heating, composting, and co-composting to produce BSFL HR, BSFL Residue Compost, and BSFL Residue COMBI, plant height significantly increased at both fertiliser application rates (Figure 6.5).

Comparable yields of BSFL Residue COMBI to BSFL Residue Compost and CCF (9.6, 9.1, and 10.2 t ha^{-1}, respectively) could be attributed to the fact that the co-composting process had a positive effect on the BSFL Residue properties. However, the effect of biochar was not visible during one cycle of planting. In an experiment by Agegnehu et al. (2016), a comparable outcome was observed where maize grain yield was 8.08 t ha^{-1} in the COMBI + fertiliser treatment. However, this yield was statistically lower than the Biochar + fertiliser (9.21 t ha^{-1}) fertiliser treatment. In their experiment, biochar + Compost + Fertiliser (8.57 t ha-1) was also higher than COMBI + fertilizer but not statistically different. The higher yield in these treatments with biochar was not attributed to the biochar in this experiment. However, a different outcome

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was observed in a study on cow dung manure where biochar increased yield due to additional P in the biochar, which improved root-fertilizer contact, optimising P availability to plants (Mau et al., 2014). This is important because P uptake affects N availability. Phosphorous is important for root formation, preparing the plant for maximum N uptake.

These results complement a study where holm oak biochar amendment before composting did not affect annual ryegrass yield (Vandecasteele et al., 2016). A study by (Bass et al., 2016) also showed no significant difference in papaya yield with COMBI (made from combining biochar from willow wood, green waste, bagasse, and chicken manure), compared to compost made from the previously mentioned feedstock. However, in the same study, the banana yield was significantly reduced by 24% using the BSFL Residue COMBI amendment. Although soil properties such as soil water content, CEC, K, Ca, NO_3^-, NH_4^+, and soil carbon content were improved by COMBI addition, this did not translate to yield (Bass et al., 2016).

On the contrary, in a study carried out by Schulz et al. (2013), an increase in oats yield was observed when the biochar application was increased in the composting process because of an increase in total N and C. Another study carried out on peanuts using fertiliser + COMBI made from biochar from waste willow wood and a mixture of green waste, bagasse,and chicken manure for co-composting at an application rate of 25 t ha^{-1}, yield increased by 24% (Agegnehu et al., 2015). The P fertiliser's potential of biochar compost is determined by the feedstock used for pyrolysis (Glaser and Lehr, 2019). However, adding biochar to the compost at the recommended application rate in this study added more P to the compost. It is speculated that the increase in P was not high enough to result in significant changes comparedto BSFL Residue Compost.

In an experiment carried out by (Awasthi et al., 2017), an application rate of 8% based on the weight of biochar made from wheat straw was added to biosolids. A co-compost application rate of 150 kg ha^{-1} resulted in the highest yield of Chinese cabbage. However, a 4% biochar application rate resulted in a comparable yield to the BSFL Residue Compost in this experiment. At the recommended application rate, there was enough P for seed filling. One of thecrucial stages where P is important during the crop's growth cycle is grain formation (Liu et al., 2011; Wang et al., 2019). A decrease in grain mass in the BSFL Residue COMBI treatment at double the recommended rate can be attributed to an increase in biochar present in the COMBI at this rate. Increasing the fertiliser application rate could have increased the biochar amount resulting in

decreased bioavailable nutrients content due to nutrient locking (Bass et al., 2016). Another reason for the decline in yield has been attributed to immobilisationof N onto biochar surfaces, making them unavailable to plants and the release of toxic or volatile compounds such as polyaromatic hydrocarbons (PAH)s and polychlorinated biphenyls(PCBs), although these were not measured in this study (Deenik and Cooney, 2016; Laghari etal., 2015).

Higher stover mass observed for CCF could be attributed to the ready availability of nutrients in both SSP and Urea (Barker and Pilbeam, 2015) (Figure 6.7). Most of the nutrients taken up by maize plants using BSFL Residue COMBI at double application rate were used for stover mass in preference to grain filling. These results contradict the findings of Agegnehu et al. (2016), who observed similar stover mass yield of the COMBI + fertiliser treatments and treatments where either compost was used with fertiliser or fertiliser + biochar except for the control.

Harvest index is a measure of the efficiency of plants to produce seeds. Comparability of harvest indices for BSFL Residue, BSFL HR, BSFL Residue Compost indicates that the decrease in HI for BSFL Residue COMBI at double the recommended application rate could be attributed to the decrease in grain mass relative to the total plant mass at harvesting.

The study showed a preferential allocation of N to the grain from composted organic N sources (Figure 6.9). BSFL Residue COMBI at the recommended application rate had the highest total N (1.68%), comparable to BSFL Residue Compost (1.68%) at double the recommended application rate. These results are comparable to the findings of Agegnehu et al.(2016), who observed that using biochar co-compost + fertiliser yielded 1.69% kernel N, whichwas lower than treatments without biochar (1.75%), although the difference was not significant. At double, the recommended application rate BSFL Residue COMBI had lower grain N. The lower grain N values for BSFL Residue COMBI at double the recommended application rate could be attributed to the adsorption of N onto the biochar surface, making it unavailable for plant uptake. In the stover, total N was highest in maize treated with BSFL Residue Compost at double the recommended application rate, probably due to more nutrient availability. Like grain N allocation, doubling the BSFL Residue COMBI application rate could have resulted inthe biochar adsorbing the N and making them unavailable for maximum plant growth (Figure 6.10)

Grain P was higher in BSFL Residue Compost at double the recommended application rate. Doubling the application rate could have resulted in the release of more nutrients compared to

the recommended application rate (Figure 6.11). BSFL Residue COMBI had higher grain P compared to CCF at both application rates, indicating an ability to translocate P to the grain. Chicken manure had the highest grain P at the recommended application rate. The trend shows that P from organic sources is better translocated to the grain than inorganic fertilisers. It was expected that grain P would be higher in BSFL Residue COMBI due to the higher total P in the BSFL Residue COMBI. The lower P in BSFL Residue COMBI could be because the P in the BSFL Residue COMBI was preferentially translocated to the stover than the grain. The BSFL Residue at double the recommended application rate had the least total P, mainly due to ammonium toxicity, which did not favour early vigorous root development; hence, these plants took up less available P. In the control plants, undetectable levels of total P indicated low or no uptake since the soil was P deficient.

Based on the PCA, the accumulation of both macro and trace elements was in the grain harvested from pots with BSFL Residue COMBI, BSFL Residue Compost, BSFL Residue, and BSFL HR at both application rates except for potassium. However, normal organic CM, CCF, and the control tended to accumulate both macro and trace elements in the stover. Reasons for this preferential nutrient allocation are not known and were beyond the scope of this study.

6.5   Conclusion

The results show that BSFL Residue COMBI yield performance, when applied at the recommended application rate based on P on maize plants in a greenhouse study, was statistically similar to CCF and CM; hence biochar addition is an unnecessary step during the first growth cycle of maize. However, it is unknown if the biochar treatment has residual effects on the soil and subsequent crop growth. Both composting processes were able to produce compost with the ability to provide nutrients. Lower yields were obtained when biochar was used at double the recommended application rate, indicating that the presence of more biochar could have locked up nutrients, making them unavailable for maize uptake in the first season. Accumulation of grain N and P was more pronounced in BSFL Residue COMBI grain at double the recommended application rate indicating higher nutritional value although at the expense of yield. Preferential accumulation of macronutrients and trace metals were observed in BSFL Residue, and BSFL processed amendments (BSFL HR, BSFL Residue COMBI, and BSFL Residue Compost) at both application rates

6.6 Recommendations

Future research is needed to investigate biochar compost (COMBI) mechanisms long-term application to promote awareness of its role over time through alterations in its surface chemistry, ionic nutrient adsorption, supply (ageing effect), and environmental implications. There is also a need to understand how biochar pyrolysed at different temperatures using different feedstock affects the co-composting process when different composting methods such as aerated (turned) windrow and aerated pile static composting, and vermicomposting are used. Further investigation is required to investigate the preferential allocation of macronutrients and trace elements to the grain in BSFL Residue, and BSFL Residue processed amendments.

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CHAPTER SEVEN: GENERAL CONCLUSION AND RECOMMENDATIONS

7.1. General conclusions

The study sought to understand the factors and processes that may influence the use of Black Soldier Fly (BSFL) Residue from the decomposition of faecal matter collected from Urine Diversion Dry Toilets (UDDT's) as an organic fertiliser source and its effect on soils and crop growth. It is imperative to understand how different methods for processing the BSFL Residue into useable products can affect nitrogen (N) and phosphorus (P) release pattern and use as an organic soil amendment. To date, little is known on the response of plants to co-composted biochar compost made from BSFL Residue from faecal matter decomposition as a feedstock. Hence the overall objective of this study was to determine the response of maize planted with BSFL Residue COMBI (made by co-composting BSFL Residue biochar, BSFL Residue, and sawdust) compared to other Human Excreta Derived Materials (HEDMs) from BSFL Residue at different application rates based on P on the growth and nutrient quality of maize under controlled conditions.

The first objective investigated the effect of pyrolysis temperature and residence time on the production of high-quality biochar. The study revealed that BSFL Residue biochar pyrolysed at 500 °C for 30 minutes compared to biochars pyrolysed at 300 °C and 400 °C for 60 and 45 minutes respectively had the desirable characteristics to be used as a bulking agent for co-composting with regards to the surface area, porosity, pH, cation exchange capacity (CEC), exchangeable bases and total phosphorus (P). However, total N was reduced significantly. These results are in accordance with Gold et al. (2018), who observed an increase in P, CEC, pH, trace metals with increasing pyrolysis temperature. Biochar pyrolysed at 500 °C also had higher pH, CEC, porosity, and surface area, C: N ratio. Based on these qualities, it was chosen as a bulking agent for the co-composting process.

Co-composting the BSFL Residue biochar with the BSFL Residue was undertaken in Chapter 4 of this Thesis. The study sought to compost BSFL Residue with and without BSFL Residue biochar and determined the chemical and physical characteristics of the compost produced. Results showed that BSFL Residue COMBI, made by mixing the BSFL Residue and BSFL Residue COMBI before composting, reached a higher maximum thermophilic temperature compared to BSFL Residue composted without biochar, as observed in Chapter 4. These

findings agree with Li et al. (2015), who found that pig manure composted with biochar reached higher thermophilic temperatures than pig manure composted without biochar. A higher thermophilic temperature is important because it guarantees the killing of microorganisms resulting in a safe compost that can be applied to fields.

Water holding capacity increased in the BSFL Residue COMBI compared to BSFL Residue Compost, mostly because of biochar availability, which is porous and able to absorb water. This is important when BSFL Residue COMBI is used in sandy soils that easily drain and leach nutrients. Studies by Lehmann and Joseph (2015) also showed that biochar amended soils hadincreased moisture storage capacity. Organic matter was higher in BSFL Residue COMBI, probably due to the recalcitrant C in the biochar that microorganisms could not degradeduring composting. These results are in harmony with Hagemann et al. (2018), who attributedthe increase in organic matter to the increase in total organic carbon contributed by the biochar.

Composting using biochar resulted in BSFL Residue COMBI with a higher total P compared to BSFL Residue Compost, where no biochar was added. The increase in P in the BSFL Residue COMBI could be attributed to the increased P concentration in BSFL Residue biochar due to pyrolysis. It has been shown that pyrolysis is a way to recover and reuse phosphorus, especially when the feedstock is of manure origin (Bergfeldt et al., 2018; Sun et al., 2018; Szaja, 2013). Although P increased, total N decreased with composting. However, the decrease was less in BSFL Residue COMBI. Biochar addition can reduce ammonia emissions during the composting process resulting in increased total N in the BSFL Residue COMBI. These findings agree with studies done by Awasthi et al. (2017) and Malinska et al. (2014), who observed a reduction in ammonia emission in composts where biochar was added before composting. Higher ammonium in the BSFL Residue COMBI compared to BSFL Residue Compost could be attributed to biochar's ability to adsorb cations such as ammonium.

Compost containing biochar had a higher C: N ratio mainly because of the non-labile C fraction contributed by biochar, which microorganisms cannot degrade. Exchangeable bases (Ca,K, Mg, and Na) increased with composting, with BSFL Residue COMBI having higher values. Biochar added to the BSFL Residue before composting was exchangeable base enriched after pyrolysis, as observed in Chapter 4.

In Chapter 5, the nutrient release pattern showed that BSFL Residue Biochar could release both N and P comparably to BSFL Residue Compost. BSFL Residue COMBI's ability to release nutrients implies that it can be released for uptake by plants. Initially, the P decreased due to immobilisation by microbes. Upon death, the microbes released the P on day 21 onwards. Although no documented studies have been done on nutrient release in biochar compost (COMBI), the addition of organic amendments has been reported to contain high microbial biomass phosphorus (Brtnicky et al., 2019). Ammonium and nitrate release were investigatedin Chapter 5. During the initial stages of incubation, ammonium decreased with a concomitant increase in nitrates. After adding moisture to all amendments except the control, organic N was converted to ammonium during ammonification and further converted to nitrates through nitrification. Although ammonium release was comparable for both BSFL Residue COMBI and BSFL Residue Compost, nitrate release was lower in BSFL Residue COMBI compared to BSFL Residue Compost. The lower values of nitrates in BSFL Residue COMBI could be attributed to pores in the biochar that can store water-soluble nitrates and release them slowly over time. Hagemann et al. (2017) found similar results when biochar was added to compost or alone released both ammonium and nitrates slower than non-biochar amended treatments. The slow-release nature of BSFL Residue COMBI implies that it can be used as a slow-release fertiliser to supply nutrients while preventing oversupply and leaching, which result in contamination of groundwater bodies.

The increase in yield due to the addition of organic amendments conforms with the expected improvements associated, such as water-holding capacity, nutrient addition, and improvementin soil physical and chemical properties (Chapter 6). This study shows that the addition of composted BSFL Residue to the soil can provide the nutrients needed for harvestable yield to be obtained in poor sandy soils. The addition of BSFL Residue COMBI at the recommended application rate based on P yielded comparable yield to BSFL Residue Compost, CCF, and CM, indicating that biochar pyrolysis is not a necessary step in composting of the residue. However, it can have residual effects, which were not tested in this study. Doubling the application rate using BSFLResidue COMBI drastically reduced yield.

The BSFL Residue and its processed products can be used as macronutrient sources to grow

maize. However, the accumulation of trace metals in the grain is of concern. In the CM, CCF, and control, preferential accumulation of macronutrients was in the stover.

7.2. Recommendations and prospects

It is recommended that farmers can use the BSFL Residue Compost at the recommended application rate based on P as it yields comparable to normal practices. However, this experiment's findings were under controlled conditions, and there is a need for more studies to be carried out under field conditions, using different soils and different crops. Furthermore, most of the findings on the use of biochar co-compost (COMBI) are still scarce. Therefore, there is a need for more research dedicated to evaluating the potential of COMBI ageing in agricultural soils, i.e., the fate of biochar in the COMBI over a long period. There is also a need to determine the residual nutrient effect of COMBI in amended soils. The safety of BSFL Residue COMBI needs to be assessed by microbial tests for faecal coliforms, total coliforms, *Ascaris*, *Helminths*, *Salmonella spp,* and *E. coli*. Modeling studies should be done to evaluate the long-term effects of BSFL Residue COMBI on crops using different soils in different climatic regions.

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APPENDICES

Appendix 1 Soil chemical properties of Newlands Mashu sampled before planting, within the top 30 cm soil profile.

Property	Value
Density (g mL^{-1})	1.24
Organic C (%)	0.8
N (%)	0.005
P (mg kg-1)	4.96
K (cmol$_c$ kg^{-1})	0.093
Ca (cmol$_c$ kg^{-1})	1.25
Mg (cmol$_c$ kg^{-1})	1.05
Exchangeable acidity (cmol$_c$ kg^{-1})	0.52
Total cation (cmol$_c$ kg^{-1})	2.39
Acid sat. (%)	15
pH (KCl)	4.2
Zn (mg kg^{-1})	43
Mn (mg kg^{-1})	4.81
Cu (mg kg^{-1})	0.88

Appendix 2 Recommended fertiliser application rate for irrigated maize after soil analysis by Cedara.

Nutrient	Amount recommended (kg ha^{-1})
Nitrogen	200
Phosphorus	60
Potassium	185

Appendix 3 Total P content of amendments and the amounts added to 20 kg pots to meet the recommended and double recommended applicationrate based on P.

Amendment	P (g kg1)	Amendment to meet P recommended rate (g of the amendment (20kg)⁻¹)	Amendment to meet double P recommended rate (g of the amendment (20kg)⁻¹)	Nitrogen supplied by amendment (g (20kg⁻¹)) P at the recommended Rate	Nitrogen supplied by amendment (g (20kg⁻¹)) at the double recommended rate	Potassium supplied by amendment (g (20kg⁻¹)) at the recommended rate	Potassium supplied by amendment (g (20kg⁻¹)) at the double recommended rate
Control	-	-	-	-	-	-	-
CCF (SSP and urea)	105	3.1	6.2	2.3	4.6	2.0	4.0
CM	5.33	60.6	121.2	1.6	3.2	0.11	0.22
BSFL Residue	4.58	70.4	140.8	1.5	3.0	0.07	0.14
BSFL HR	5.79	55.8	111.6	0.9	1.8	0.05	0.10
BSFL Residue Compost	5.18	62.2	124.4	0.7	1.4	0.05	0.10

| BSFL Residue COMBI | 5.43 | 59.4 | 118.8 | 0.7 | 1.4 | 0.05 | 0.10 |

CCF = Chemical Commercial Fertiliser; SSP = Single Super Phosphate; CM = Chicken Manure; BSFL Residue = Black Soldier Fly Larvae Residue; BSFL HR = Black Soldier Fly Larvae Heated Residue; BSFL Residue Compost = Black Soldier Fly Larvae Residue Compost; BSFL Residue COMBI = Black Soldier Fly Larvae Residue Biochar Compost.

Appendix 4 Macronutrient characteristic variables for each cluster.

> res.hcpc1$desc.var$quanti
$`1`

	v.test	Mean in category	Overall mean	sd in category	Overall sd	p.value
T_Na	2.794089	78250.986	40784.340	1002.8838	19737.8860	0.0052046150
T_Mg	2.596270	1968.082	1081.131	100.6462	502.8589	0.0094242003
T_K	-3.540108	3028.580	11129.356	399.9925	3368.2650	0.0003999638

$`2`

	v.test	Mean in category	Overall mean	sd in category	Overall sd	p.value
G_Na	-2.383210	3982.15344	15307.7259	3982.15344	6995.09896	0.01716239
G_Ca	-2.430077	33.38243	148.3792	33.38243	69.65648	0.01509561
G_K	-2.510645	127.94679	1385.6474	127.94679	737.37441	0.01205109

$`3`

	v.test	Mean in category	Overall mean	sd in category	Overall sd	p.value
T_Ca	2.836649	3056.439	2142.564	458.8638	734.6544	0.004558971

$`4`

	v.test	Mean in category	Overall mean	sd in category	Overall sd	p.value
G_Mg	1.985477	652.9750	461.8318	301.6007	300.6051	0.047091404
T_Na	-2.562467	24586.5213	40784.3400	4632.9590	19737.8860	0.010393160
T_Mg	-2.641775	655.6892	1081.1305	115.1518	502.8589	0.008247276
T_Ca	-2.782251	1487.9620	2142.5637	297.4041	734.6544	0.005398327

>

Appendix 5 Trace elements characteristic variables for each cluster.

> res.hcpc$desc.var$quanti
$`1`

	v.test	Mean in category	Overall mean	sd in category	Overall sd	p.value
G_Cu	-2.507756	0	3.382877	0	1.985623	0.012150044
G_Mn	-3.066247	0	4.965042	0	2.383482	0.002167640
G_Zn	-3.188999	0	19.399959	0	8.954523	0.001427663

$`2`

	v.test	Mean in category	Overall mean	sd in category	Overall sd	p.value
G_Fe	3.199212	153.0763	38.15734	0	35.92103	0.001378039

$`3`

	v.test	Mean in category	Overall mean	sd in category	Overall sd	p.value
T_Zn	2.900056	59.79611	23.4508	0	12.53262	0.003730955

$`4`

	v.test	Mean in category	Overall mean	sd in category	Overall sd	p.value
T_Mn	2.605751	80.5319193	58.4377701	16.906367	19.3350857	0.009167318

G_Cd 2.271599   0.7657818   0.4134037   0.403402 0.3537358 0.023110749

$`5`
     v.test Mean in category Overall mean sd in category Overall sd   p.value
G_Cr 2.760095   11.155848   6.398088   4.3237665 5.382460 0.005778462
G_Mn 2.416800   6.809846   4.965042   0.6154582 2.383482 0.015657639
T_Cu -2.777751   4.818186   5.934155   0.7562558 1.254473 0.005473650