# Fertiliser value of biogas slurry for maize and dry bean production and its effect on soil quality and carbon dioxide emissions

By

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#### Abstract

Biogas slurry, a secondary organic material generated from anaerobic digestion of animal waste and crop residues and sometimes considered a waste product (Abubaker, 2012; Ferdous et al., 2018). Understanding how BGS and CM affect dry matter, grain yield, primary macronutrient uptake, residual soil concentration and C exchange in soils is important for improving crop production and also clarifying the contribution of the organic resources to the C budget. BGS and CM could essentially improve organic C accumulation, provides plant essential nutrients in the soil, which supports plant growth, and build up soil reserves. However, BGS and CM could subsequently result in greater CO<sub>2</sub> emissions in soils. The need to understand the effect of organic amendments on agricultural soils and on GHG emissions is relevant to improve agricultural production, minimize and mitigate the effect of agriculture on net CO<sub>2</sub> emissions. The aim of the study was to investigate the effect of BGS, CM relative to CF on dry matter yield, grain yield and soil quality after harvest of maize and dry bean and also the effect of BGS and CF on CO<sub>2</sub> emissions from soils.

Field experiments were conducted with BGS, CM and CF treatments in 2016/2017 and 2017/2018 seasons. The first set of experiments was arranged in a randomized complete block design with four treatments; (i) BGS, (ii) CM, (iii) CF and (iv) unamended control, for both maize and dry bean crops. Each treatment was applied at 40, 80 and 120 kg Nha<sup>-1</sup> for maize and 30, 60 and 90 kg Nha<sup>-1</sup> for the dry bean and 0 kg Nha<sup>-1</sup> (unamended control) for both crops. Another experiment of co-application of BGS with CF was conducted, with six BGS and CF (BGS/CF) treatment combinations based on percentages of recommended N rates of 120 kg Nha<sup>-1</sup> (maize) and 90 kg Nha<sup>-1</sup> (dry bean). The BGS/CF treatments were (i) 0/100, (ii) 20/80, (iii) 40/60, (iv) 60/40, (v) 80/20, (vi) 100/0.

In the first set of experiments CM had higher dry matter yield than both BGS and CF in both seasons for maize. BGS had higher dry bean dry matter than CM and CF in 2016/2017 while in the 2017/2018 the CM treatment had higher dry matter than BGS and CF except at 90 kg Nha<sup>-1</sup>. Maize grain yield of 2.3, 2.4, and 3.0 t ha<sup>-1</sup> at 40, 80 and 120 kg Nha<sup>-1</sup>, respectively, were observed from CF, which was higher than BGS (1.3, 2.0, 2.6 t ha<sup>-1</sup>) and CM (1.6, 1.9, 2.4 t ha<sup>-1</sup>) in 2016/2017. The BGS treatment had higher grain yield than CM except at 40 kg Nha<sup>-1</sup> for maize. Dry bean grain yield of 0.6, 0.9 and 1.2 t ha<sup>-1</sup> from CF was higher than BGS (0.4, 0.5 and 0.9 t ha<sup>-1</sup>) and CM

(0.4, 0.5 and 0.7 t ha<sup>-1</sup>) at 30, 60 and 90 kg Nha<sup>-1</sup>, respectively in 2016/2017 but BGS had similar yield to CM except at 90 kg Nha<sup>-1</sup>. Increasing nitrogen application rate for both maize and dry bean increased soil N, P, K, Ca, Mg and organic C for all treatments although soil pH was not affected.

CO<sub>2</sub> emissions were measured only from the maize trial because of shortage on the number of chambers. Higher CO<sub>2</sub> emissions were from both BGS and CM than CF treatment. The highest CO<sub>2</sub> emissions were observed in the month of February in both seasons. The CO<sub>2</sub> emissions decreased with time (month) over the growing seasons. At 40 kg Nha<sup>-1</sup> BGS had significantly higher CO<sub>2</sub> emissions than CM in both seasons. However, at 80 and 120 kg Nha<sup>-1</sup> the results did not show any significant difference (at p < 0.05) between BGS and CM although both resources had higher CO<sub>2</sub> emissions than the CF treatment. The  $\beta$ -glucosidase and urease activities were higher in the CM treatment than both BGS and CF at all N application rates.

In the co-application experiment, 40/60 (BGS/CF) resulted into higher maize dry matter yield (6.9 t ha<sup>-1</sup>) in 2016/2017 than fertiliser only (0/100) which had 5.0 t ha<sup>-1</sup>, while in 2017/2018, fertiliser alone (0/100) resulted into higher dry matter (10.1 t ha<sup>-1</sup>) than all other treatments. The 40/60 and 0/100 treatments resulted into similar grain yield (2.9 t ha<sup>-1</sup>) in the 2016/2017 season, which was higher than all other treatments. The 40/60, 60/40 and 100/0 (BGS/CF) treatments had higher N, P, K, Ca and Mg uptake than the 0/100, 20/80 and 80/20. For dry bean, the 40/60 and 100/0 resulted into similar dry matter yields of 5.5 and 5.3 t ha<sup>-1</sup>, respectively, in 2016/2017 while in 2017/2018 the 40/60, the 100/0 and 0/100 treatments resulted in 5.2, 5.5 and 5.4 t ha <sup>1</sup>, respectively, that were higher than all other treatments. The 0/100 treatment had higher grain yield 1.1 t ha<sup>-1</sup> than all other treatments while 40/60, 60/40 and 100/0 treatments had similar grain yields in 2016/2017. The 100/0 had higher P, K and Ca uptake than all other treatments in 2016/2017 while in 2017/2018, 40/60 had higher P, Ca and Mg uptake. Residual soil pH, total N, K and Mg were higher in the 100/0 than all other treatments in 2016/2017 while in 2017/2018 the 40/60 treatment had higher OC, P and K after harvest. Co-application of the two resources does not benefits dry bean in terms of dry matter and grain yields. The findings of his study show that coapplication of BGS and CF at 40/60 and 60/40 have maize yield benefits compared to the two resources, separately, while co-application did not improve dry bean yields relative to BGS alone.

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#### List of abbreviations and acronyms

BGS – Biogas slurry

CM – Cattle manure

CF - Chemical fertiliser

C - Carbon

CO<sub>2</sub> - Carbon dioxide

GHG – Greenhouse gas

N – Nitrogen

CC - Climate change

P – Phosphorus

K - Potassium

Ca - Calcium

Mg – Magnesium

OC – Organic carbon

OM – Organic matter

AM - Animal manure

CEC – Cation exchange capacity

SOC - Soil organic Carbon

SOM – Soil organic matter

NSS - Non stead state

MBC - Microbial biomass carbon

ARC-VOP – Agricultural Research Council – Vegetable and Ornamental Plants

GC – Gas chromatography

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#### **CHAPTER 1: INTRODUCTION AND BACKGROUND**

#### 1.1 Introduction and Background

In the twenty-first century, food security and CC mitigation are two main challenges that have been of concern (Migliorati, 2015; Chihambakwe et al., 2019). In contemporary science and politics, global CC has become an important issue (Li, 2007). Debates have highlighted anthropogenic activities as the main causes of CC (Damon and Kunen, 1979; Crowley, 2000; Cuffey, 2004). The IPCC (2001) suggested that the contemporary CC is mainly caused by anthropogenic emissions of GHGs such as N<sub>2</sub>O, CO<sub>2</sub> and CH<sub>4</sub>. As defined by Snyder et al. (2009) GHGs can be defined as those gases that absorb infrared radiation in the atmosphere, warming the surface of the earth by trapping the heat. Fossil fuels, land-use change and human activities, over the past 25 years are thought to have doubled the rate of GHGs emissions (Denman et al., 2007). Snyder et al. (2009) suggested that the major GHG issue for the total economy has been CO<sub>2</sub>, however, in the agricultural sector, N<sub>2</sub>O has been the most important gas especially emissions from the soil together with those from N inputs and crop system.

The agricultural sector has been faced with the challenge of doubling agriculture production at least by 2050 to satisfy the world's growing population (Godfray et al., 2010). Such a challenge can only be overcome through intensive agricultural practices including fertilisation (Fageria et al., 2008). As a result, the use of inorganic fertilisers to meet the higher demand for food production, has been on the rise (Nasir et al., 2015). However, the use of inorganic fertilisers affects soil properties not always in a favourable way (Czekala et al., 2019). Nasir et al. (2015) noted that long-term use of inorganic fertilisers has adverse effects on the environment and soil condition. Some of the major challenges in the agricultural production system are lack of adequate nutrients, soil degradation (Malav et al., 2015) and the potential increase of GHG emissions (Abubaker, 2012). Reducing GHG emissions from the agriculture production system is important for developing a more sustainable food production practice (Gagnon et al., 2016). The use of N fertilisers (organic and or inorganic) and N fertiliser management systems could help reduce GHG emissions and mitigate CC, especially N2O emissions (Snyder et al., 2009; Millar et al., 2010; Gagnon et al., 2016). Inorganic and organic fertilisers have a strong link to N<sub>2</sub>O production in soils, however,

N fertiliser management that avoids the build-up of inorganic N reduces some of the  $N_2O$  emissions (Follet et al., 2005; EPA, 2014). On the other hand, the effect of N fertilisers on  $CO_2$  emissions is very unclear as studies show contrasting results and that  $CO_2$  emissions can potentially contribute between 10-30 % of current total anthropogenic emissions (Gagnon et al., 2015).

Inorganic fertilisers have become increasingly expensive, making them unaffordable especially for small scale farmers (Weltzein 1990; Malav et al., 2015). Nevertheless, overuse of inorganic fertilisers by commercial farmers has now been considered a threat to human health and the environment (Sharma and Singhvi, 2017). The continued decline of soil fertility caused by the loss of nutrients leaves the addition of organic fertilisers as the only alternatives to improve soil fertility and sustain crop productivity (Nasir et al., 2015). Large amounts of organic waste are being produced daily from urban and rural areas from different sources (Abubaker, 2012; WBA, 2018). This creates disposal problems, nevertheless recycling and utilization of organic wastes for renewable energy and source of fertiliser provides a solution. While the need to address soil fertility challenges are immense, smallholder farmers also face challenges with energy sources.

In order to avoid waste or loss to the environment, all organic resources that have a positive influence on soil fertility and crop productivity may need to be utilized (Smith et al., 2014). The organic fertilisers for improving quality of agricultural soils include BGS from the biogas technology, livestock manures, industrial sewage, agricultural material and domestic waste, compost, (Nasir et al., 2012 & 2015; Smith et al., 2014; Islam et al., 2014; Nkoa, 2014). All these organic wastes can also be used in the biogas technology and provide numerous advantages such as renewable energy production, CC mitigation, improving urban air quality and contributing towards food security (WBA, 2018). Although the biogas technology creates the opportunity for production of BGS (fertiliser) a secondary organic material rich in essential plant nutrients and energy (methane), for cooking and lighting (Abubaker, 2012; Debebe and Itana, 2016), there could be competition between use as organic fertiliser and as a feedstock for biogas production. Alternatively, the organic materials can first be used as a feedstock for biogas production and the waste from that process used as an organic fertiliser. The biogas technology, therefore, offers an option for improving soil

fertility, maximizing crop productivity while on the other side offer the option of reducing GHG emissions.

Nasir et al. (2012) suggested that the use of organic wastes materials can serve a double purpose by producing energy as well as providing a valuable nutrient source to the soil that helps in improving soil fertility. Because of the impact of fossil fuels on global warming, biogas technology has gained much interest since the 1970s (Nkoa, 2014). Rutz, (2010) suggested that the number of biogas plants are increasing worldwide and will continue increasing over the coming years to try and meet the demand for energy and offer CC mitigation option. The use of BGS as a nutrient source could reduce the dependency on CF especially in the smallholder sector in the vicinity of biogas plants (Kumar et al., 2015), and add the necessary OC, with the benefits on soil quality and crop productivity (Galvez et al., 2012). While CF, animal manures and crop residues have been extensively studied, studies on the use of BGS are either limited or poorly documented (Abubaker (2012).

The BGS has its own concerns such as it may contain toxic organic compounds like alkenes and halogenated hydrocarbon amongst others, may also have pathogens and can cause phytotoxicity (Nkoa, 2014; Zhang et al., 2019). In short-term use, BGS or any other digestate has been found not to have any effect on soil chemical properties (Odlare et al., 2008), however, the application of BGS influences soil pH, plant-available P and K in a long term. Soil treated with BGS from household waste has been found to have an effect on microbial biomass, where about 100% of the microbial biomass can be active and N mineralization rate increases and potential ammonia oxidation (Nkoa, 2014). This was further supported by results from an incubation study by Grootboom (2019), where the addition of BGS to soil resulted in higher ammonium-nitrogen and extractable P and lower nitrate-N in soil. Furthermore, Garg et al. (2008) highlighted the potential of BGS in reducing soil bulk density and hydraulic conductivity. The effect of BGS on the soil is influenced by the nutrient content of slurry that depends on the original feedstock used during anaerobic digestion (Alburguerque et al., 2012).

In a pot experiment Grootboom (2019), showed that BGS resulted in higher spinach dry matter yield, and uptake of N, P, K, Ca and Fe when compared to CM, particularly when applied at a rate equivalent to 150 kg Nha<sup>-1</sup>. The effects of BGS on crops should

be analyzed a posterriori with respect to three types of controls i.e unfertilized, undigested feedstock and mineral fertiliser (Nkoa, 2014). This means that BGS research results could be categories into three groups, (1) performance similar to the control (Unfertilised), (2) performance similar or higher than undigested feedstock and (3) performance equal or better than mineral fertiliser (Tiwari et al., 2000; Chantigny et al., 2007; Moller et al., 2008; Nkoa, 2014). According to Moller and Muller (2012), the effect of BGS on crop yields shows conflicting reports on literature, hence the need to further study the effect of BGS on crop production.

#### 1.2 Objectives

The main aim of this research was to investigate the effect of biogas slurry relative to chemical fertiliser on carbon dioxide emissions, dry matter, grain yield of maize and dry bean and selected soil quality parameters after harvest.

The specific objectives of this study were to determine the effect of:

- Biogas slurry and cattle manure, relative to chemical fertiliser, on plant dry matter, grain yield and primary macronutrient uptake of maize and dry bean, and soil nutrient composition after crop harvest.
- II. Co-application ratio of biogas slurry with chemical fertiliser on nutrient uptake, dry matter and grain yields of maize and dry bean and soil nutrient concentrations after harvest.
- III. Biogas slurry and cattle manure at different N applications rate on CO<sub>2</sub> emissions and activity of enzymes involved in C and N cycling under maize.

#### 1.3 Structure of dissertation

Each chapter is mostly self-contained, containing a literature review, materials and methods, results, discussion and conclusions.

**Chapter 1** gives an introduction and background.

**Chapter 2** reviews the literature on the importance of biogas technology system, production, and use of biogas slurry from the biogas technology and its effect on crop production relative to chemical fertiliser and cattle manure.

**Chapter 3** focuses on the effects of two organic resources (Biogas slurry and Cattle manure) on dry matter, grain yield, primary nutrient uptake and residual soil nutrients after harvest of maize (*Zea mays L.*) and dry bean (*Phaseolus vulgaris*).

**Chapter 4** is devoted to carbon dioxide emissions measurements over two growing seasons after application of biogas slurry, cattle manure and chemical fertiliser in a Maize (*Zea mays L*).

**Chapter 5** explores the effect of co-application of biogas slurry with chemical fertiliser on dry matter and grain yields of maize (*Zea mays L.*) and dry bean (*Phaseolus vulgaris*) and soil quality after harvest.

**Chapter 6** is the general discussion of the potential use of biogas slurry as a source of nutrients for maize and dry bean production. It also includes the recommendations for future studies

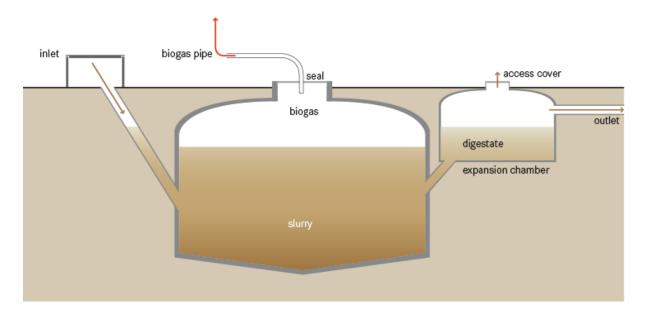
#### **CHAPTER 2: LITERATURE REVIEW**

#### 2.1 Biogas technology

The needs of energy requirements by humankind have led to the search for various resources that could be used (lortyer et al., 2012). The biogas technology has among other technologies been seen as a better alternative for providing clean energy. However, the technology still requires more research into qualitative and quantitative ways for sustainable energy production (lortyer et al., 2012). According to Msibi and Kornelius, (2017), about 625 000 households in South Africa could benefit from the biogas technology (biogas digesters) that are fed with cattle and pig waste on the basis of livestock numbers. lortyer et al. (2012) described the biogas technology as a rural technology that is simpler and cheaper in terms of energy generation, which could help better the livelihood of rural communities. Garfi et al. (2012) reported that biogas technology, as a system, that can improve social development as it reduces the workload of women and children to collect fuelwood or cow dung for household energy. The challenge with the biogas technology system particularly for rural communities is insufficient feedstock, people do not have enough of any type of particular feedstock to feed the system daily for their energy needs and water to use on biogas digesters. In urban communities, this system could be used as a waste management strategy (Nkoa, 2014) and a CC mitigation option that could offer clean air. According to Amigun et al. (2012), the biogas technology system improves the health of low-income households in rural areas while Bond and Templeton (2011) mentioned that the biogas technology has the potential to reduce anthropogenic CH<sub>4</sub> emissions by around 4%.

Increasing industrialization and growing population in South Africa has led to tons of waste being produced with major disposal challenges (Rohrs et al., 1998; Snyman and Van der Waals, 2004). In developed countries, the average solid organic waste produced by a person per day is 0.77kg and increasing (Troschinetz and Mihelcic, 2009). A similar study by Couth and Toris (2011) indicated that in Africa, the waste generated by each person per day is 0.63 kg with an average organic content of 56%. However, with the increasing global concerns about the energy crisis, it has become important to rely on local abundant agriculture bio-resources (Liu et al., 2009). From the past few years, South Africa has greatly benefited from what is known as clean

energy produced from several renewable sources such as wind energy, hydro-energy and biofuels (Sparks et al., 2014).



**Figure 2.1:** Typical representation of a below ground small-scale biogas reactor known as the Anaerobic Contact (complete-mix digesters) systems (Mes et al., 2003).

Research has shown that biogas digesters have the potential to treat organic waste and produce energy out of it while producing a secondary residue rich in nutrients that can be used as a soil amendment (Abubakar, 2012; Smith et al., 2014). Three types of biogas digester, Chinese fixed dome, Indian floating drum and Taiwanese plastic tubular, are popular digesters worldwide (Msibi and Kornelius, 2017). Due to energy shortage and CC issues, the development and sustainability of energy have become an important global strategy across the world (Wu et al., 2014). In developing countries such as South Africa, local authorities have developed municipal waste management strategies for the use of organic waste as an energy source. But the biogas technology in South Africa has not gained much recognition, this has been attributed to limited research (Bond and Templeton, 2011), although a number of projects have been introduced. Organic waste such as plant residues, AMand sewage sludge amongst others can be used in biogas technology and this can be a good source of renewable energy in a country like South Africa that faces major challenges of electricity shortage or outbreaks.

South Africa has enjoyed the benefits of using readily available coal for energy but this has problems that lead to load shedding in the past recent years (DOE, 2011). Studies

conducted in Libya, North Central African showed that municipal solid waste, industrial solid waste and health care waste can be used as a source of bioenergy, producing electricity and playing an important role in municipal solid waste management (Hamad et al., 2014). Research by Wu et al. (2014) showed that in the rural areas of China, the biogas technology system has gained its popularity as it plays an important role in relieving energy shortages and also reducing environmental pollution problems. Worldwide interest has drastically increased on biogas technology as it shows greater potential in the renewable energy industry. Use of cattle, pig and buffalo dug in producing energy in the biogas technology could be a solution to poor energy services, poverty, CC mitigation and soil fertility problems (Warnars and Oppenoorth, 2014).

Three thousand years ago, the biogas technology system was used for heating water by ancient Assyrian (Bond and Templeton, 2011). This then showed that the biogas system was among the earliest energy resource. In 1776, Volta, an Italian physicist, was the first physicist to prove that there is methane in biogas that could be used for energy and since then, the biogas system has been continually studied (Kumar et al., 2015) The biogas technology system has been considered as a cost-effective way of energy generation without increasing atmospheric CO<sub>2</sub> (Smith et al., 2014). Furthermore, Surendra et al. (2014) suggested that biogas technology could be used as a clean renewable energy source for cooking, generating heat and electricity and can be upgraded into bio-methane for use of as a transportation fuel.

In some countries around the world, conventional energy undermines economic and political stability hence renewable energy sources provide more diverse and secure energy (Osalj and Musec, 2010). Renewable energy sources are a better solution for energy production than fossil tools (EC, 2000). Large amounts of organic waste produced either from livestock, industries, agriculture and households can be used as a source in renewable energy production. In developing countries, there is a greater potential for biogas technology for renewable energy production as it can produce clean energy, improve waste management, reducing workload for women and children (Surendra et al., 2014). On the other hand, this also creates employment opportunities for communities at the local level as well as the potential to reduce GHG emissions. In addition to that, a considerable amount of renewable energy in the form of animal manure, crop residues, food and food processing waste can be utilized economically for biogas production. In the European Union, renewable energy sources are an

integral part of trying to mitigate CC while contributing to economic growth, job creation and increasing energy security (Osalj and Mursec, 2010). According to Osalj and Mursec (2010) wet organic waste, larger amounts of AMand BGS represent a constant pollution risk that can negatively impact the environment. In the rural communities of developing countries, the biogas technology has not permeated much, one of the reasons for the widespread diffusion of this are the high costs associated with the installations and maintenance of this biogas technology (Surendra et al., 2014). There are quite a number of challenges associated with the use of biogas technology as a source of renewable energy.

#### 2.2 Biogas slurry production from different feedstock's

Researchers and policymakers feel there is a need for classification of feedstocks and quantification so as to help in the planning and implementation of waste management (Thomas, 2004). The quality of the feedstock determines the amount of CH<sub>4</sub> produced and the nutrient content of the BGS. Nyang'au et al. (2016) described the quality of BGS as being dependent on several factors such as the kind of dung from either animal, human and or any other feedstock, breeds and the age of animals. According to Jordaan (2018), not all possibilities of organic matter production from organic material used in biogas technology are relevant to the South African industry. The number of people involved in farming also determines the availability of feedstock in South Africa.

There are a number of feedstock's that can be used in biogas technology systems such as agriculture residues, fresh CM, pig manure, chicken manure, organic solid waste, sewage sludge, dairy manure and food wastes amongst others. (Gomez et al., 2007; Amon et al., 2007; Drennan and Distefano, 2010; Bond and Templeton, 2011; Surendra et al., 2014; Nkoa, 2015; Insam et al., 2015; Nyang'au et al., 2016). Moller et al. (2008) and Nkao (2012) explained that BGS quality depends upon the configuration of the digester and the feedstock input used. Koszel and Lorencowicz (2015) highlighted the safety of BGS before being used as a fertiliser, as it may contain toxic organic compounds, pathogens and phytotoxicity and has the problem of odour emission (Nkoa, 2012). In the past the main concern of BGS was heavy metals that led to objections against the use of slurry (Insam et al., 2015), especially if the slurries were to be used for agriculture, horticulture and or silvicultural purposes but organic household waste was of little concern (Insam et al., 2015). Production residues or crop

remains such as bruised, undersized, misshaped fruit, vegetables and fruit toppings can also be used in the biogas technology for the anaerobic digestion (Van der Merve, 2014). Van der Merve (2014) further suggested that not all production residues are effective for anaerobic digestion.

**Table 2. 1**: Types of feedstock's that can be used in biogas technology for production of biogas slurry in South Africa

Organic resources				
Agriculture	Municipal	Commercial and industrial		
Crops	Household	Fruit and vegetables		
Grass silage	Municipal solid waste	Industrial		
Sugar beet	Sewage sludge	Supermarkets		
Residues		Tanneries		
<ul> <li>Wheat straws</li> </ul>		Food processors		
Manures		Catering		
Cattle		Dairy		
• Pig		Fish processing		
• poultry		Slaughterhouses		
, ,		Food scraps		

BGS from anaerobic digestion can be produced from different substrates such as agricultural crop waste, animal manure, abattoir waste, municipal biowaste, industrial waste and wastewater. These organic resources can be grouped into agriculture, municipal and commercial and/or industrial waste (Table 2.1). During the anaerobic digestion process a secondary product known as BGS is produced (Abubaker, 2012; Smith et al., 2014). BGS is a by-product from anaerobic digestion of various organic wastes that has received much attention worldwide (Paul and Beauchamp, 1993; Islam, 2006; Abubaker et al., 2012; Smith et al., 2014; Nyang'au et al., 2016).

Scientists have described BGS as an environmentally friendly organic fertiliser that could be used in agriculture (Holm-Nielsea et al., 2009) and avoid any negative impacts to the environment (Insam et al., 2015). BGS contains a significant amount of nutrients (Insam et al., 2015) and as such, it has gained much attention not only

because of the high cost of CF (Surendra et al., 2014). Serendra et al. (2014) further explained that when BGS is used as a fertiliser and or soil amendment, it enhances the physical, chemical and biological attributes of the soil. The use of BGS in agricultural soils has numerous advantages and disadvantages. One of the advantages of BGS is that it contains plant readily available N, ammonium ion content, and pH which are increased because of fermentation while the C content is reduced leading to a low C/N ratio (Teihoeven-Urslmans et al., 2009).

## 2.3 Comparison of biogas slurry and animal manure as a source of fertiliser in soils

A number of studies show variation in the amount of organic matter content, cellulose/lignin ratio, total N and C, C and N ratio, pH contained in the BGS and AM (Guster et al., 2005; Moller et al., 2008; Tambone et al., 2009; Nkoa, 2012; Moller and Muller, 2012). BGS has ammonium that is directly available for plant uptake but susceptible to leaching and volatilization (Bonten et al., 2014). Moller and Muller (2012) described the difference between BGS and raw AM depended on the feedstock and its degradability. The fact that the BGS have elevated pH values than AM is caused by the fact that the digestate is affected by concentration of basic cations that increase the pH because of electric charge balance of the solution having to be neutral decreasing the concentration of H<sup>+</sup> and precipitation of carbonates decreasing pH of AM (Hjorth et al., 2010).

The decomposition of both BGS and AM in soils could be affected by the time of application because OC in both AM and BGS is different. According to Bonten et al. (2014), most of the OC have been removed in the intestine tract of the animal and the remaining organic matter is composed of organic material not readily available for biological degradation. Bonten et al. (2014) further explained that the organically bound nutrients are mineralized during the anaerobic digestion process because of the breaking down of organic matter and increases the content of immediately available N. Moller and Muller, (2012) concluded that AM and BGS are different, mainly because of the type and composition of manure (Table 2.3). Degradable organic compounds in the manure during the anaerobic digestion process are converted to biogas making them more stable and less susceptible to mineralization than AM (Bonten et al., 2014). It should be remembered that BGS produced from fresh AM and solid AM will not differ much in terms of the total nutrient content because of

the same feedstock in some cases. However, Moller and Muller (2012) indicated that the nutrient content in BGS is higher than AM because part of the organic matter is decomposed during anaerobic digestion (Table 2.2).

Table 2. 2: Characterization of digestate and undigested animal manure

Parameter	Absolute value	Change <sup>a)</sup>
Dry matter (%)	1.5-13.2	-1.5 to – 5.5
Organic matter (as % of DM)	63.8-75.0	-5 to -15
Total N (% DM)	3.1-14.0%	b)
Total N (g/kg FM)	1.20-9.10	≈ 0
Total C (% DM)	36.0-45.0	-2 to -3
C/N ratio	3.0-8.5	-3 to -5
NH <sub>4</sub> (% of total N)	44-81%	+10 to +33
Total P (g/kg FM)	0.4-2.6	≈ 0
Water soluble P (% of total P)	25-45	-20 to 47
Total K (g/kg FM	1.9-4.3	≈ 0
Total Ca (g/kg FM	1.0-2.3	≈ 0
Total Mg (g/kg FM)	0.3-0.7	≈ 0
рН	7.3-9.0	+0.5 to +2 units

Source: Moller and Muller, (2012)

DM = dry matter

FM = fresh matter

Improved utilization of AM has a central role in the efforts to decrease the environmental effects of farming as noted by Malav et al. (2015). AM has been reported to contain between 0.4-0.8% N, 0.6-0.82%  $P_2O_5$  and 0.5-065%  $K_2O$  which makes it less rich in micro-nutrients as compared to BGS (Table 2.3) (Nasir et al., 2010). Nasir et al. (2010) further suggested that AM on the open pool, losses nutrients especially N and thus possesses relatively lower manorial value compared to BGS. During the process of anaerobic digestion, the concentration of ammonium-N is increased while the dry matter is reduced, causing lower C concentration and C/N ratio

a) in comparison to undigested liquid animal manure, the absolute value

b) increase with the degree of degradation

while increasing the slurry pH (Wentzel et al., 2015). Wentzel et al. (2015) further reported that comparing BGS with other organic fertilisers such as farmyard manure and sewage sludge, BGS provides more plant-available nutrients (Table 2.3).

Table 2. 3: Nutrient content of organic manures

Organic manure	OM	C: N ratio	N <sub>2</sub> (%)	P <sub>2</sub> O <sub>5</sub> (%)	K <sub>2</sub> O (9%)
Farmyard manure	25-55	15-20	0.40-0.80	0.60-0.82	0.50-0.65
Biogas slurry	60-73	17-23	1.50-2.25	0.90-1.20	0.80-1.20
Vermicompost	9.80-13.40	-	0.19-1.02	0.19-1.02	0.15-0.73

Source: Surendra et al. (2014)

Application of AM to the soil provides N in an organic form but for BGS this element is more easily soluble and used by the plant mostly in the ammonia N form (Nasir et al., 2012). When comparing AM and green manure to BGS, BGS may contain nutrients in an inorganic form (Johansen et al., 2013). These nutrients are easily available for plant uptake. However, according to Kumar et al. (2015), the application of BGS has comparable effects to those of synthetic CF and AM. For example, the BGS can be a good source of nutrients as it contains more of macro and micronutrients that also necessary for plant growth (Table 2.4) (Alam, 2006).

**Table 2. 4:** Nutrient composition of biogas slurry

N (%)	P (%)	K (%)	Reference
1 – 1.8	0.8 – 1.2	0.8 – 1	Gupta, 1991
1.5 - 2	1	1	Tripathi, 1993
1.5	0.4	2.2	Board, 2007
1.3 – 2.5	0.9 - 1.9	1	Myles et al., 1993
0.5 - 1.0	0.5 - 0.8	0.6 - 1.5	Demont et al., 1990
1.5 – 2.0	1	1	Khandelwal et al., 1986

Source: Kumar et al., 2015

#### 2.4 Impact of biogas slurry on physicochemical and biological soil properties

There have been many research studies done on BGS as a soil amendment with conflicting results shown in reviews by Moller and Muller (2012) and Nkoa (2012). Although there have been quite a number of reviews, knowledge from BGS as a soil amendment and/or organic fertiliser is far from complete (Nkoa, 2012). Soil fertility

may be directly or indirectly associated with the physical, biological and chemical soil properties, therefore altering with these properties through the application of fertiliser may have an impact on sustainable crop production (Bulluck et al., 2002; Abubaker, 2012). Application of BGS in soils can be a precious alternative to synthetic CF (Kumar et al., 2015) as it contains valuable essential nutrients. BGS contains nutrients such as N, P and K, it is known to improve soil structure and organic matter accumulation (Nkoa, 2014; Insam et al., 2015). Koszel and Lorencowicz (2015) explained that because BGS contains N, P, K, elements that are easily available for plants, it resembles mineral fertiliser. Chiew et al. (2015) reported that the application of BGS in soils could increase macro and micronutrients in both soil and plant and improve microbial biomass. Syumborg et al. (2007), emphasized that the type of the soil, climate conditions, the frequency of application and the properties of BGS influences the magnitude of organic matter accumulation and other essential elements in the soil.

#### 2.4.1 The potential for biogas slurry to improve physical properties

Gibson et al. (2005) described South Africa as comprising of drylands, therefore is susceptible to land or soil degradation and desertification. The majority of the land in South Africa is used for agricultural production, 100 million hectares (80 %) and about 11 % of the land is used for crop production (DEAT, 2006). Factors such as drought and CC contribute to soil degradation, but the key causes of soil degradation are poor land management and planning (DEAT, 2009). Lal (2004) suggested that continuous cultivation promotes soil degradation, losses of organic matter that would lead to increased production costs and higher CO<sub>2</sub> emissions.

Meadows and Hoffman (2002), raised a view that in South Africa, CC has not been the major factor of soil degradation, but people were responsible. DEAT, (2009) suggested that soil degradation can result from inappropriate agricultural practices. Grandy et al. (2002) reported that as a result of decreased soil organic matter and excessive cultivation cause degradation of soils, and this can be improved by the addition of organic materials such as BGS (Zheng et al., 2017). Biological degradation, chemical degradation and physical soil degradation are regarded as the major sources of soil degradation. The potential for land to contribute to development is threatened by soil degradation. Soil degradation can be improved by the addition of organic material such as BGS that has a greater potential to improve soil physical properties (Alagoz and Yilmaz, 2009). According to Alagoz and Yilmaz, (2009) for sustainable

crop production in agriculture through the application of BGS, soil aggregate formation plays a major role, therefore, a good soil structure is paramount in agricultural production. Furthermore, the addition of BGS to the soil can have a greater potential in improving physical soil properties. According to Spohn and Gaini (2011) reduced soil erosion, mediating air permeability, water infiltration and nutrient cycling can be achieved through the application of BGS in soil. Zheng et al. (2017) indicated that the application of BGS with CF could lead to increased soil macroaggregates.

#### 2.4.2 The potential for biogas slurry to improve chemical properties

Application of different organic materials including BGS to soils have a major influence in changing physical and chemical properties (Zheng et al., 2017), they have been used for several years to evaluate the effect of such in long term experiments (Tejada and Gonzalez, 2004). Alagoz and Yilmaz (2009) indicated that changes due to the application of organic materials could be seen after years of data collection, which then provide significant results. According to Zheng et al. (2017) decomposition of organic matter from organic material serves as a substrate for microbial activity. Changes that occur in soils are quickly seen on microbiological and biochemical properties which are very responsive and provide immediate and precise information (Dick and Tabatabai, 1993). It is important to note that requirements for nutrients between soil microorganisms and plants have to be maintained through appropriate fertiliser practices (Liu et al., 2009). This cannot be achieved through the use of CF only as the application of CF only has become a treat not only to the soil but also to human health and to the environment (Malav et al., 2015). Studies have shown that BGS has the potential to ameliorate soil quality (Zheng et al., 2017).

BGS from the decomposition of various organic matter has been known to be of good quality organic fertiliser (Islam, 2006). A high percentage of readily available nutrients are contained in BGS, which could be applied directly to the soil to improve the physical components of the soil and the chemical properties as well. BGS contains nutrients such as P, K, Zn, Fe and Mg (Kumar et al., 2015) hence it could then help as a source of nutrients in improving chemical properties of the soil that is deficient from these nutrients. Moreover, BGS is known to contain high organic matter (Zheng et al., 2017), which may then enhance the CEC of the soil (Kumar et al., 2015). Soil that is low in CEC may be susceptible to leaching of soil nutrients by rain but BGS has the potential to increase CEC.

Long-term application of BGS in the soil may have a positive effect on some soil fertility indices such as organic matter, available N, P and K (Zheng et al., 2017). Terhoeven-Urselmans et al., (2009) explained that after the application of BGS, 90% of ammonium nitrogen applied could be used which is a clear indication that the amount of soil ammonium nitrogen has increased. BGS has a high pH which on application to the soil increases soil pH, this then could promote the availability of P in soils. BGS is a slow releaser of nutrients, therefore application to the soil could contribute to organic matter accumulation, available N, P and K, especially in long-term application studies.

#### 2.4.3 The potential of biogas slurry to improve biological properties

In agriculture, the concept of soil biota is very important especially when organic amendments such as BGS and AM are used. According to Arthurson (2005) when there are changes in the environment, soil microbial biomass as a living metabolizing unit responds quickly to such compared to organic matter as an entity. Odlare (2005) highlighted that change in soil microbial parameters after BGS application may be seen long before changes in chemical properties such as C, N and P content are noticed. Limited information is available on BGS impact on soil microbial community (Johansen et al., 2013) but BGS has been found to influence nutrients and microbes in soils. Application of BGS in soils needs to be done with great cure and managed well so that it does not influence soil microbial community negatively. Soil microbial community, growth and activity may be affected by BGS application because of the fact that OC has been decreased significantly during the anaerobic digestion process (Arthurson 2009). Johansen et al. (2013) explained that in a case whereby OC is less available for microbial growth, N immobilization in microbial biomass is allegedly negligible. The microbial community is known to be drivers of processes such as biochemical that help in plant nutrient availability for the growth of mycorrhizas responsible for improving plant uptake of mineral N in soils (Johansen et al., 2013). Ernst et al. (2007) suggested that BGS may have an impact on microbial activity in the soil, biomass, earthworm biomass and in the long term may also have a greater impact on soil OC sequestration, with the low C input and higher recalcitrance of their organic matter. Contradictory to Ernst et al. (2007) findings Guster et al. (2005) reported that the higher recalcitrance of the organic matter remaining in the BGS can be considered as beneficial for soil OC sequestration.

Soil organic matter plays a significant role in improving soil fertility and is an energy source for soil microflora and microfauna (Harris and Bezdicek 1994). The decline in soil organic matter is mainly linked to biological degradation which is brought about by soil tillage (DEAT, 2009). This could have a negative effect on soil quality as tillage enhances aeration and this promotes rapid bacterial oxidation of soil organic matter. Application of organic amendments such as BGS increase SOM and microbial activity (Stumpe et al., 2012; Johansen et al., 2013). Fontaine et al. (2003) suggested that the addition of organic material to the soil has the potential to prompt a priming effect that could result in increased soil OC mineralization. Organic material (plant residues) incorporated to the soil by soil fauna has the potential of increasing C into the soil (Von Lutzow et al., 2006), this transformation process results in a biogeochemical mixture of plants litter compounds and microbial decomposition products in varies stages of decomposition in soils (Paul, 2014). Soil temperature and water content greatly influence soil C storage through their effect on microbial activity. Application of BGS to soils have an influence on various soil enzymatic activities and microbial biomass (Liang et al., 2003) and also affects ammonia-oxidizing activity and composition (Nyberg et al., 2006). Arthurson (2009) concluded that the application of organic material in soils causes a broader range of soil function benefits but anaerobic digestion suppressed the potential rate of ammonia oxidation in soil. This is because the compounds that inhibit ammonia-oxidizing activity are present in the biogas residue.

Studies by Petersen et al. (2003); Nyberg et al. (2006); Odlare et al. (2008) showed that application of BGS enhanced microbial biomass metabolically active microorganisms, N mineralization capacity and specific growth rate constant of denitrifies. Fungal cell membrane component, ergosterol and microbial biomass are sensitive indicators for the effects of organic N fertiliser application in soil and thus for soil fertility (Heunze et al., 2010). According to Raich and Potter, (1995) N application in the soil helps microbes to grow. However, Lu et al. (2011) explained that microbial activity may be reduced by N addition to agricultural soils which have a low C: N ratio <15. There have been research studies done on BGS as a soil amendment with conflicting results shown in reviews by Moller and Muller (2012) and Nkoa (2012) on the microbial community as affected by BGS. Although there has been quite a number of reviews on the use of organic amendments in soils, knowledge about BGS used as

soil amendments or organic fertiliser is far from complete and the impact of BGS on the microbial community has not been extensively studied (Arthurson, 2009). Stumpe et al. (2012) reported that soils amended with organic waste showed enhanced basal respiration, the different activity of the soil microbial biomass, stimulated enzyme activity as well as different responses to substrate-induced respiration. Furthermore, Stumpe et al. (2012) showed no differences between respiration and mineralization when liquid manure and sewage sludge are applied to soils but BGS showed high rates of respiration of mineralization. Although there are restrictions on the use of sewage sludge as a soil amendment in some countries it is important to discuss its impact on the soil as a soil amendment. An incubation study by De Neve et al. (2003) showed that after 30 days, BGS as a treatment showed high rates of mineralization meaning that BGS was most degradable than AM. BGS is known to have high pH and NH<sub>4</sub>+ content, therefore the microbial activity becomes enhanced in soils (Stumpe et al., 2012).

#### 2.5 Biogas slurry as a source of nutrients in soils

Crop growth is limited by a shortage of nutrients such as N and P (Williams and Joseph, 1976). The application of fertilisers containing N and P can significantly increase crop yields (Smith et al., 2014). AM, BGS, composted materials and biochar are among other organic materials that are an alternative to CF (Smith et al., 2014) and can provide essential nutrients to the soil (Soane, 1990; Bernal et al., 1993; Paul and Beauchamp, 1993; Bonten et al., 2014). Addition of BGS to the soil provides OC and other nutrients needed by the plant for crop growth (Zheng et al., 2016), this is one way of returning nutrients back to the soil ecosystem and can also help tackle the widespread loss of SOC as BGS contains high SOC. With humus having a stable C as the main constituent, BGS might be a potential source of SOC. The soil is known to be the major C reservoir (FAO, 2017) as the atmosphere and terrestrial vegetation combined are said to contain less C when compared to the soil. The introduction of developed management practices is a necessity to help in enhancing increased SOC in the soils (Purakayastha et al., 2008). According to Bachmann et al. (2011), large concentrations of soluble inorganic P are contained in the biogas slurry, this may represent a valuable P fertiliser.

The use of BGS with a high content of valuable macro and micronutrients has been proposed as a source of organic fertiliser and can solve fertility problems (Abubaker,

2012). As described by Surendra et al. (2014) and Malav et al. (2015) BGS is a by-product after digestion of dung and other biomass for generation of CH<sub>4</sub>-rich gas from anaerobic digestion which can provide both micro and macronutrients to the soil and can improve physio-chemical and biological attributes of the soil as well as increase crop productivity. Islam (2006) reported that BGS is considered as an effective source of organic fertiliser as it contains considerable amounts of nutrients and organic matter. A study by Gupta (2007), showed that BGS from the digesters was rich in nutritional elements including N, P, K, zinc, nickel, iron, cobalt, cadmium, chromium, boron, calcium and sodium. Furthermore, Surendra et al. (2014) reported that nutrients from BGS are higher compared to other organic manure as seen in Table 2.3.

#### 2.6 Effect of biogas slurry application on crop production

BGS application in soils could have a greater impact on the nutritional status of a crop and improved crop yields (Smith et al., 2014). Moller and Muller (2012) reported conflicting results on the effect of BGS application on crop yields. BGS effect on crop yield could be influenced by the time of application and the nature of the application in soils. Nkoa (2012) noted that inappropriate application and or storage of BGS could lead to loss of fertiliser value of the slurry and that would affect agricultural production if used as a source of the nutrient. According to Yu et al. (2010), decomposition of BGS in soils is slower compared to CF, which makes it a better nutrient uptake assimilator for plants in the long-term. Liu et al. (2010) emphasized that N from BGS directly influences plant yield in a growing season while the effect of P and K can be seen in the next season or several years. This could be attributed to the fact that during the anaerobic digestion process, precipitation of insoluble inorganic P occurs and reduces the concentration of immediately available P and micronutrients (Moller and Muller, 2012). Furthermore, the N is converted to NH<sub>4</sub><sup>+</sup> making it more available for uptake (Smith et al., 2014). Presence of N, P and K in BGS benefits plant as these nutrients are necessary for plant growth. BGS could be beneficial in increasing yields of different crops when compared to the application of BGS at a 100% recommended rate and where no BGS were applied (control) (Table 2.5).

**Table 2. 5:** Effect of biogas slurry on maize, bean, rice, wheat, cabbage and tomato yield

Crops	Yield t/ha (without BGS)	Yield t/ha (with BGS)	Increment
Maize	1.47	4.52	3.05
Bean	1.20	1.80	0.60
Rice	2.49	5.09	2.60
Wheat	1.29	1.84	0.55
Cabbage	1.60	2.17	0.57
Tomato	1.70	2.70	1.00

Source: Gurung, 1997; Nasir et al., 2010; Kumar et al., 2015; Debebe and Itana, 2016; Xu et al., 2019

Smith et al. (2014) described nutrients from the BGS after anaerobic digestion could be partially available for plant uptake but may also be susceptible to lose by physical processes or use by microorganisms, which will then affect the availability of nutrients to the plant. Furthermore, Smith et al (2014) suggested that the application of BGS as a soil amendment may offer a promising win-win opportunity to improve crop productivity. However, according to Malav et al. (2015) BGS may contain appreciable amounts of organic matter (20-30%). Adding to those benefits, Weiland (2010) suggested that due to improved flow properties in the BGS, it can penetrate the soil faster which then reduces the risk for N losses in the form of NH<sub>4</sub><sup>+</sup> improving crop production. However, Arthurson et al. (2009) suggested that the use of BGS on soil cannot only provide good benefits but also provides dis-advantages. Lui et al. (2008) noted that there are key problems associated with the use of BGS as there is the variability of components that can suppress vegetable growth and yields, therefore it is necessary to supplement some other nutrients. Ramirez et al. (2010) suggested that to help understand the mechanisms responsible for the impact of BGS and N fertilization in maize production, it is important to separate the growing season into different periods related to maize physiology and root respiration. One other problem with BGS application is that the C nutrient transformation during decomposition of organic matter in soil intensely interacts with plant nutrient uptake, which could lead to nutrient competition between soil microorganisms and plants (Liu et al., 2009).

#### 2.7 Effect of biogas slurry application on phosphorus availability in soils

Phosphorus is a critical ingredient in food production in agriculture. The main source of P in soils has been mined rock phosphate often combined with sulphuric acid, N and K in mineral fertilisers (Cordell et al., 2009). According to (FAO 2008) for agricultural soils, it has become more useful to use a material that contains P for crop production mainly because of the fact that rock phosphate used as a source of P in soils are finite and non-renewable which could lead to a decline in agricultural production in the near future. Limitations of P in agricultural soils can be alleviated by the application of mineral and organic fertilisers (Bationo and Mokwunye, 1991; Smith et al., 2014). In agriculture, P is one of the valuable elements in soils and is often a major limiting nutrient (Sattari et al., 2012) in crop production. Dery and Anderson (2007); Cordell et al. (2009) have raised concerns about the rapid depletion of P reserves around the world. When accounting for P in soils including residual soil P in P management is critical especially when estimating future P inputs required from mineral fertiliser and manure application (Sattari et al., 2014).

Cordell et al. (2008) explained that approximately 50-100 years P reserves could be exhausted, Sattari et al. (2012) further indicated that by the year 2100, P reserves would be depleted. The use of organic material such as BGS containing P could be useful in improving the sustainability of P cycles (Van Vuuren et al., 2010). According to Sharpley (1996), organic fertilisers have a better effect than P from mineral fertilisers. Eichler-Lobermann et al. (2007) reported that organic fertilisers also improve the availability of P in soils and not only supply nutrients to the soil. It is critical to maintaining P levels at a range that is good for crop yields and maximum crop production. In soils application of BGS increases humus content and improves microbiological activity (Oberson and Joner, 2005). It is a common practice in agriculture to apply AM and compost in soils but little is known about the impact of organic fertilisers on P pools.

AM and BGS amongst other organic materials are known to contain high amounts of P and have become an important source of P because of the fact that P reserves are limited and are on the decline. In a study by Sharpley et al. (2004), P from soils amended with BGS was found to be more than P in soils without manure, which indicated the effect of organic manures on soil P. P availability in soils is influenced by a number of characteristics of soil and also P fertiliser sources (Havlin et al., 2005).

Sharpley and Moyer (2000) suggested that organic and inorganic P fractions in soils and their distribution depend on manure type used. Smallholder farmers in developing countries struggle with sourcing P or cannot afford P fertilisers (Scholz et al., 2013) as P fertilisers from mined rock phosphate have become more expensive. If the levels of readily available P in the soil becomes below the critical level than the rate of P release from residual P becomes insufficient to sustain optimal crop yields. Campos et al. (2019) stated that P in BGS must be recorded for P pollution relief and nutrient recycling.

# 2.8 Effect of co-application of biogas slurry and chemical fertiliser on crop yield and nutrient availability

The increasing population in Sub-Sarahan Africa has led to large demands of food and in the last decades, this has been met by the immense use of CF. Long-term use of CF in agricultural soils leads to increased loss of SOC and total N (Zheng et al., 2017). Nasir et al. (2012) reported that the sole use of CF is not a good practice as they have adverse effects on the environment and on soil conditions also causing soil degradation. Czekala et al. (2019); Nasir et al. (2015) reported that the immense use of CF affects soil properties not always in a favourable way. Among other challenges facing the agriculture sector, soil degradation is by far the most common problem and is mainly caused by the continuous use of CF and intensive cultivation (Zheng et al., 2017). Therefore, to correct such there is no alternative than to add organic material such as BGS plus CF (Nasir et al., 2015). To improve soil fertility and sustain crop productivity, the use of organic amendments such as BGS and raw AM is an alternative option as soil organic matter can be enhanced (Malav et al., 2015), which is the main indicator of soil degradation. Perez-Piqueres et al. (2006), also agreed with other researchers that organic residues application to soils has become an important approach in increasing soil fertility but on the other hand may present challenges and benefits after application into agricultural soils.

Cordell et al. (2009), indicated that using BGS on soil can have many benefits such as mitigating global CO<sub>2</sub> emissions, substitute for expensive CF and the nutritional value of the slurry can be improved by combination with other sources of nutrients such as CF, BGS, and raw AM. Organic and inorganic integration can improve soil physicochemical behaviour, the physiological system of the crop and increased crop yields (Malav et al., 2015). High crop yields, improved soil quality and increased fertility

levels can be achieved through the application of CF together with organic fertilisers (Bharde et al., 2003; Kumar et al., 2015). The combined effect of BGS with CF improves C: N, ratio, transformation on the crop and increases potential yields (Kumar et al., 2015). It is necessary to analyze the BGS before application to the soil and if BGS is found to contain large amounts of heavy metals, treating the slurry before application to the soil may be required. The effect of the co-application of BGS with CF on crops depends on the absorption rate of nutrients by the crop at the time of application.

# 2.9 Effect of organic fertilisers and synthetic chemical fertilisers on greenhouse gas emissions in soils

#### 2.9.1 Carbon dioxide emissions

Mineralization of SOM by microorganisms causes CO2 emissions back to the atmosphere, but there is a potential to mitigate the increased atmosphere GHGs with increased C sequestration in agricultural soils (Purakayastha et al., 2008). Biogas fermentation technology is considered a cost-effective way of renewable energy generation without increasing atmospheric CO<sub>2</sub> concentration as GHG emissions are a major problem for CC. The increasing levels of CO<sub>2</sub> in the atmosphere have instigated more research on evaluating the contributions of agriculture, industries and environmental practice on CO<sub>2</sub> emissions (Al-Kaisi et al., 2008). Al-Kaisi et al. (2008), further suggested that management practices such as tillage, N fertilization, cropping systems and many other practices affect soil C loss mechanisms which can be determined and quantified through measurements of CO2 emissions and other indicators such as a change in C fraction and change in soil microbial biomass C. Yang et al. (2015) described management practices such as nutrient management and conservation agriculture, in soil and vegetation to conserve and sequester C as a practical option to mitigate the atmospheric accumulation of CO<sub>2</sub>. However, according to Post and Kwon, (2000) for that to be achieved an improved C budget is required.

In the soil system, the mechanisms of soil CO<sub>2</sub> emissions to the atmosphere involves the movement of CO<sub>2</sub> through soil pores and can be measured at the soil surface (Rolston, 1986). Gagnon et al. (2015) suggested that a number of mechanisms have been raised to explain the magnitude and direction of the impacts of mineral N fertilization on soil CO<sub>2</sub> emissions. However, Sainju et al. (2010) reported that when

all other factors are the same, fertilized soils are expected to emit more CO<sub>2</sub> emissions due to greater crop residue inputs. But Gagnon et al. (2015) stated that alternative N fertilization was unclear on how it affects CO<sub>2</sub> emissions from soils as studies showed contrasting results. Laboratory studies by Kowalenko et al (1978) and Green et al. (1995) indicated that CO<sub>2</sub> emissions in soil decrease after application of N fertilisers such as ammonium nitrate and potassium nitrate in the absence of crop residues. But conclusions from Al-Kaisi et al. (2008) indicated that soil CO<sub>2</sub> response to N fertilization was site-specific and that CO<sub>2</sub> emissions decrease with ammonium nitrate application.

This was in contradiction with other studies (Halvorson et al., 2010; Halvorson and del Grosso, 2012; Gagnon et al., 2015) where the application of different N sources was found to have little impact on CO<sub>2</sub> emissions either organic or inorganic. However, according to Ramirez et al. (2010); Sainju et al. (2010), studies on urea as a source of N have indicated an increase in CO<sub>2</sub> emissions while other studies have not. Gagnon et al. (2015) suggested that a number of hypotheses have been reported on why N fertilisers may reduce CO<sub>2</sub> emissions, such hypotheses that were indicated were the ones associated with reductions in pH, enzyme activity and microbial biomass. Gagnon et al. (2015) went on to further suggest that CO<sub>2</sub> emissions from soils come both from autotrophic and heterotrophic respiration, this occurs in the presence of vegetation. However, Olsson et al. (2005) reported that upon fertilization, autotrophic and heterotrophic respiration are both reduced.

A study by Gagnon et al. (2015) showed that differences in soil type yielded to different amounts of CO<sub>2</sub> emissions as a result sandy loam and silty clay soils showed high CO<sub>2</sub> fluxes whereas loam and silty clay soils showed low CO<sub>2</sub> fluxes. Furthermore, on their study, different amounts of CO<sub>2</sub> emissions were notable from different N sources such as KNO<sub>3</sub> and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> where CO<sub>2</sub> emissions from KNO<sub>3</sub> were lower than (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> by 22% on average.

#### 2.9.2 Nitrous oxide emissions

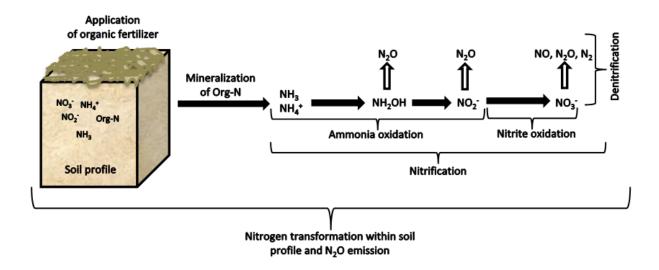
Global CC is brought about by GHG exchange between soils and atmosphere, where N<sub>2</sub>O, CH<sub>4</sub> and CO<sub>2</sub> are the main GHG's from the agricultural system (Rafique et al., 2014). Nitrous oxide is among the most important GHGs because of its global warming potential that is 298 - 310 times than that of CO<sub>2</sub>, it can stand in the atmosphere for more than 114 - 120 years (Signor and Cerri, 2013; IPCC, 2001; IPCC, 2007).

Abubaker, (2012) reported that the greater attention that  $N_2O$  has got is due to the potential of the gas to destroy the ozone layer that protects the earth from ultraviolet radiation from the sun. The FAO (2003) has projected that in 2030 the global  $N_2O$  emissions are set to increase from 35 to 60% due to the increase in global N fertiliser use and livestock farming. Stehfest and Bouwman (2006), reported that fertiliser application and fossil fuel combustion are amongst the human activities that have caused an increase in emissions of  $N_2O$  and NO. Sangeetha et al. (2009) suggested that doubling the concentration of  $N_2O$  in the atmosphere would result in 10% decrease in the ozone layer and this would increase the ultraviolet radiation reaching the earth by 20% causing massive damage to the ozone layer, leading to major global CC in the environment.

In soils, the processes of nitrification, denitrification and chemo-denitrification lead to N<sub>2</sub>O production (Rochette and Eriksen-Hamel, 2008). Bremmer, (1997) suggested that N<sub>2</sub>O can be produced from the soil through nitrification and denitrification as shown in figure 2.1 below. While Galloway et al. (2003) and Hauser (2013) suggested that during the transformation of soil N through mineralization, nitrification and denitrification, NO and N<sub>2</sub>O gases are formed as byproducts which are emitted to the atmosphere. These processes are mainly influenced by soil moisture, temperature, oxygen concentration, nitrate concentration, pH, texture, amount of available organic carbon and nitrogen and soil C/N ratio (Sangeetha et al., 2009; Signor and Cerri, 2013).

Global N<sub>2</sub>O emissions of about 90% are predicted to have resulted from the microbial processes, nitrification and denitrification (Hensen et al., 2013). Nitrous oxide emissions from soils are accelerated by N application and AM in agriculture (Stehfest and Bouwman 2006). The emission patterns are complicated by fertiliser spreading, the method of spreading and also the type of fertiliser that is used. According to Skiba et al. (2013), significant peaks in emissions for N<sub>2</sub>O can be noticed between 0 to 21 days after application of mineral fertiliser, mostly triggered by the rain but for organic compounds, they occur later and are longer lasting (Jones et al., 2007). This is because microbial decomposition must precede nitrification and denitrification. Whalen (2000) reported that emissions of N<sub>2</sub>O from soils do result in N losses and thereby retreating availability of this nutrient from arable land. As stated by Abubaker (2012), there is a number of important factors such as soil moisture, oxygen

concentration, mineral N, available C, soil texture, pH and temperature that play a role in  $N_2O$  emissions. Abubaker (2012) went on to suggest that soil moisture is the most important factor for  $N_2O$  emissions.



**Figure 2.1:** Soil microbial processes leading to emissions of  $N_2O$  in soils (Abubaker,2012).

Snyder et al. (2009), indicated that the form of N present, how N is absorbed and utilized or dispersed in the environment is determined by the process of nitrification. This has huge implications for plant productivity and environmental quality. Tesfai et al. (2015); Nash et al. (2012) reported that one major contributor to soil N<sub>2</sub>O emissions is N lost through the nitrification process were poorly and imperfectly drained soils can potentially have huge amounts of applied N fertiliser. Nash et al. (2012) further concluded that soil N<sub>2</sub>O emissions due to agriculture practices are significant because they contribute to global warming and ozone depletion.

At higher N rates the percentage of N emitted from the soils as N<sub>2</sub>O becomes more variable (Snyder et al., 2009). Eichner, (1990) and Stehfest and Bouwman (2006) and Snyder et al. (2009) emphasized that soil N content, climate, SOC content, soil texture, soil drainage abundance of NO<sub>3</sub>-N, soil pH, N application rates per fertiliser type and type of crop are important factors affecting N<sub>2</sub>O emissions. Abubaker (2012), reported that the use of recycled organic residues on soils can carry the risk of increased GHG emissions especially N<sub>2</sub>O, which can also contribute to CC.

#### 2.9.3 Methane emissions

In soils, CH<sub>4</sub> is produced through the methanogenesis process under anaerobic conditions. The transfer of CH<sub>4</sub> from the soil to the atmosphere occurs mostly through the aerenchyma of aquatic plants and through diffusion from wetland soils via bubbles (Le Mer and Roger, 2001). Anaerobic soils are the most important source of CH<sub>4</sub> (Inubushi et al., 2011). As reported by Oremland and Culbertson (1992) CH4 in the atmosphere is responsible for changes in the chemical formula as it is very reactive. Le Mer and Roger (2001) reported that CH<sub>4</sub> reacts with hydroxyl radicals in the atmosphere which then reduces its oxidative power and ability to eliminate pollutants like chloro-fluoro carbons. Most importantly microbial activity occurring in soils has a major influence on CH<sub>4</sub> emissions. CH<sub>4</sub> is known to have a Global warming potential that is 3.7 times that of CO<sub>2</sub> (Odlare et al., 2012). Studies by Blake and Rowland (1988); Rodhe (1990); Le Mer and Roger (2001) suggested that CH<sub>4</sub> has an ability to absorb infrared radiation (20-30 times) more effectively than CO<sub>2</sub> despite a short residence time in the atmosphere. The IPCC (2000, 2006, 2010) estimated that annual emissions for CH<sub>4</sub> are between 400 to 600 Tg year<sup>-1</sup> for the year 2010, which has grown three times the value estimated in the 15th and 18th centuries. Wetland soils are known to be the main source of natural CH<sub>4</sub> followed by domesticated ruminants and rice fields

The main source of CH<sub>4</sub> emissions are paddy soils, where they contribute about 20% (Purkait et al., 2007), the rest of CH<sub>4</sub> emissions can be attributed to natural wetlands, marshy lands, ruminants, termites, biomass, burning, coal and enteric fermentation. Le Mer and Roger (2001) argued that most of CH<sub>4</sub> comes from agriculture. A few studies have been conducted on CH<sub>4</sub> emissions and uptake after N fertiliser application in cultivated agricultural soils (Amos et al., 2005). Powlson et al. (1997) suggested that N application for over 150 years on wheat plots, where pH was maintained at a neutral level, CH<sub>4</sub> emissions and uptake was reduced by 50%. CH<sub>4</sub> can be produced in the anoxic environment through oxidation by methanotrophic bacteria and methanogenic bacteria during the anaerobic digestion of organic matter, this also includes submerged soils contributing about 5.8% to global atmospheric CH<sub>4</sub> sink (Yang et al., 2015; Le Mer and Roger, 2001; Smith et al., 2003). Furthermore, CH<sub>4</sub> in soils can be eliminated by microbial oxidation, where this can occur in the aerobic zone of methanogenic soils and in upland soils. According to Nesbit and

Breitenbeck (1992), soils that are more efficient in methanotrophy are the watersaturated or sites that are often submerged.

#### 2.9.4 Measuring of greenhouse gas emissions from agricultural soils

In developing countries, about 14% of annual GHG emissions are from the agriculture sector and about 17% is from deforestation (Vermeulen et al., 2012). The agriculture sector has been known to be a great emitter of GHG's and therefore, quantification of GHG emissions is necessary so as to identify management practices that provide opportunities to reduce GHG emissions while providing greater resilience in production systems, food security and rural welfare (Sapkota et al., 2014). Olander et al. (2013) reported that quantification of GHG emissions from soils may also help guild national planning for low emissions development generating and trading C credits, certifying sustainable agricultural practice and supporting farmers in adopting low C-intensive farming practices.

To understand the drivers of CC, accurate measurements of GHG emissions need to be collected, this also helps in supporting well-informed modeling as well as help identifying mitigation opportunities (Collier et al., 2014). The increasing demand for biofuel crops and global fertiliser use has increased the importance of accurate measurements of GHG emission from the soil (Verge et al., 2007; Venterea et al., 2009). This has driven the need for the development of several methods for quantifying exchanges of GHGs between landscape and atmosphere. In most of the measurements that have been used on GHG emissions, non-steady-state chambers have been the most (Rochete, 2011). Under different conditions, different tools and techniques are employed for suitability purposes due to the heterogeneity of agricultural production niches (Sapkota et al., 2014). Quite a number of strategies for measuring GHG emissions exist (Denmead, 2008). Collier et al. (2014) suggested that a number of approaches that exist for measuring GHG emissions from soil, where each measurement strategy has its own strengths and weaknesses.

About 100 years ago, chamber techniques were developed for measuring soil respiration rates, with no methodological changes made until the 80s where Mathias et al. (1980) and Hutchinson and Moiser (1981) propose several improvements to chamber designs and deployment methods (Lundegurdh, 1926; Rochette and Eriksen-Hamel, 2008). Closed chamber techniques are mostly used for

measurements of trace gas fluxes, in small areas of several treatments in soils but are subject to high coefficients of variation due to spatial variation in soil gas flux and remain the most commonly used approach (Mosier et al., 1998; Rochette and Eriksen-Hamel, 2008; Parkin et al., 2012; Collier et al., 2014). Measuring gas fluxes with soil chamber methods have been mostly used recently (Parkin et al., 2012). However, complexities associated with the chamber approach in terms of accurate flux estimates have been reported by Parkin at al. (2012). Sixteen characteristics criteria were developed by Rochette and Eriksen-Hamel (2008) to assess the reliability of chamberbased estimates. However, several factors, which include the absence of an absolute reference for the gas source, had delayed efforts for accepting a standard methodology for GHG measurements (Rocette and Eriksen-Hamel, 2008). Therefore, there is no agreement among scientists concerning the best method for calculating gas fluxes using chamber concentration versus time. Many researchers argue that decreasing the deployment time and increasing the height of the chamber reduces the non-linearity in the data and reduces the underestimation of the pre-deployment flux (Livingston et al., 2006; Venterea et al., 2009). Parkin et al. (2012) reported that placing a chamber on the soil surface alters the conditions, which then affects the flux of the gas. Adding to that Hutchinson and Mosier (1981), indicated that placing a chamber on the soil surface results in the build-up of gas in the chamber headspace and soil pores, which then reduces the diffusive flux of gas from the soil surface resulting in a non-linear equation.

In the 20<sup>th</sup> century, chamber methods for GHG emission measurements were introduced (Lundegard, 1927; Oertel et al., 2016). A study by Oertel et al. (2016) clearly explains the design of chambers that have been widely used, where a box or cylinder is placed onto the soil and gas accumulates in the headspace. According to Parkin et al. (2012), non-flow through chamber-based methods have been commonly used and are of low cost also suitable for scientific studies. Non flow through non steady-state chambers have a base that is usually inserted in the soil surface to a depth of few centimeters. Most of the research that has been done on GHG emissions has suggested that the base or collar as referred to by some is not necessary, chambers can work better without the base/collar (Pumpanen et al., 2004). Livingston and Hutchinson (1995) and Parkin et al. (2012) and, reported that static chambers are the most used method for measurements of GHG fluxes from the soil. Pihlatie et al.

(2013), furthermore explained that the technique basically covers a known area of the soil which allows for gas exchange between the soil and the headspace.

According to Rochette and Eriksen-Hamel (2008), for comparison of treatments in a given study, chambers were used for GHG emissions. Researchers have debated on the design of an optical chamber and how to calculate the fluxes from the soil (Pihlatie et al., 2013). Several factors including the absence of an absolute reference for the gas have delayed efforts to accepting a standard methodology for measurements of GHG emissions using chambers (Rochette and Eriksen-Hamel, 2008). Furthermore, Rochette and Eriksen-Hamel (2008) emphasized that the deployment of chambers onto the soil surface modifies the fluxes that are intended for the measure and that chambers are an intrusive gas flux measuring method. Studies have highlighted the complexities involving chamber design, deployment time, gas sampling and measurement procedures and data analysis, which can affect the accuracy of flux measurements (Parkin et al., 2012; Rochette and Eriksen-Hamel, 2008). In a study by Rochette and Eriksen-Hamel, (2008) the main issues described was bias associated with estimate induced by the flux calculations in chamber-based measurements while Parkin et al. (2012) noted that placing a chamber on the surface of the soil alters the conditions where the gas flux is affected.

Chamber methods are used for measuring GHGs such as CO<sub>2</sub>, N<sub>2</sub>O and CH<sub>4</sub>, on the other hand, they are used to estimate differences between treatments or explore system dynamics over seasons (Collier et al., 2014). Sapkota et al. (2014) suggested that for smallholder farmers, chamber-based measurement is still the dominant method because of adaptability, portability, cost-effectiveness and flexibility to the diverse production environment. Nonflow through, NSS chamber-based measurements are commonly used to develop and validate empirical and processbased models to quantify the emissions of gas at farm scale and beyond and are also used for comparisons purposes between treatments (Rochette and Eriksen-Hamel, 2008; Sapkota et al., 2014).

A wide variation exists in method protocols used to determine GHG emissions from soil, where details of which can directly affect chamber induced bias (Rochette and Eriken\_Hamel, 2008). One of the major concerns on the use of NSS chambers is the underestimation of the actual pre-deployment flux. Venterea and Parkin (2012)

reported that because of the current emission assessments at the regional, national and global scale they are negatively biased. But several precautions need to be taken to avoid biased flux estimates when chambers are used (Rochette and Eriksen-Hamel, 2008).

#### 2.10 Conclusion

BGS as a source of nutrients in soil appears primarily positive. Studies show that BGS contains large amounts of macro and micronutrients that are essential for plant growth and improved soil quality. The nutrient value of BGS depends on the feedstock used during the anaerobic digestion process. Comparing BGS to other organic fertilisers such as AM amongst others shows that BGS provides nutrients in a more available form than AM. An example, N in BGS is in the ammonium form, which is directly available for plant uptake while the N in AM is organic N that is only available for plant uptake after mineralization. Farmers could take advantage of BGS where CF is not available. However, it could be necessary to supplement CFs to meet crop requirements when using BGS because BGS might be suitable for some crops than other crops. Conflicting results on the effect of BGS on soil quality, plant biomass and crop yields have been reported, hence the need for the establishment of more field studies to demonstrate the effect of BGS as a soil conditioner and the potential of BGS on crop growth so that doubtful farmers may be convinced. The application of BGS in soil could have a priming effect caused by soil organic matter mineralization and other sources of labile C that could have an effect on GHG emissions. Farmers will not use BGS unless it is an accepted fertiliser product, therefore government and other stakeholders (scientists) should work together in improving the regulation and recommendations of making BGS as an accepted fertiliser product.

# CHAPTER 3: NITROGEN FERTILISER VALUE OF BIOGAS SLURRY AND CATTLE MANURE FOR MAIZE (ZEA MAYS L.) AND DRY BEAN (PHASEOLUS VULGARIS)

This paper has been submitted for publication to Heliyon (current status- under review)

#### 3.1 INTRODUCTION

The decline in soil fertility associated with agricultural intensification and continuous cultivation without replenishing nutrients is a major problem for the agricultural sector (Gurung, 1997). Khan et al. (2012) reported that lack of adequate nutrient supply and poor soil quality are the main constraints in low input agriculture. The application of CF on smallholder farms is limited by their high costs. CFs are expensive for small and marginal farmers (Weltzein, 1990; Nasir et al., 2015). To resolve soil fertility and nutrient management problems, organic soil amendments that positively influence soil fertility and crop productivity need to be utilized to avoid waste or loss to the environment (Smith et al., 2014).

A vast range of organic fertilisers such as CM and compost are considered as options to improve soil fertility. However, the use of manure to produce biogas could be more beneficial through the provision of energy (biogas) and a potential organic fertiliser (BGS), from the same waste. BGS is a by-product from anaerobic digestion of various organic wastes through the biogas technology and it has received great attention worldwide (Paul and Beauchamp, 1993; Islam, 2006; Weiland, 2010; Abubaker et al., 2012; Smith et al., 2014; Nyang'au et al., 2016). The BGS from CM could offer a mitigation strategy for GHG emissions and make a contribution to soil fertility (Weiland, 2010).

BGS is reported to be rich in macro- and micronutrients that are essential for plant growth and development and are in the readily available form (Ward et al., 2008; Smith et al., 2014; Kumar et al., 2015; Cao et al., 2016). The high nutrient composition of BGS suggests that it has the potential to be used as a fertiliser (Odlare et al., 2011). The use of BGS from fresh AM provides the potential to recycle nutrients, and influence their uptake by crops, and consequently improve crop yields (Smith et al., 2014; Kumar et al., 2015). BGS could, therefore, be the cheapest and safer alternative source of nutrients compared to CF (Khan et al., 2012).

Several studies have reported that anaerobically digested cattle and pig slurry improved soil fertility, dry matter and grain yield of maize (Paul and Beauchamp, 1993; Nasir et al., 2010; Malav, et al., 2015), cabbage yield (Debebe and Itana, 2016), tomato quality (Yu et al., 2010) compared to CF and compost. Kumar et al. (2015) reported that the level of heavy metals in BGS derived from AM is insignificant compared to CF suggesting low environmental risks. The nutrient cycling and liming effects of BGS could, therefore, improve soil quality and crop yields, with insignificant negative effects (Nyang'au et al., 2016). Zheng et al. (2016) noted that BGS could potentially improve soil structure, and water-holding capacity and crop yields.

When comparing BGS to AM, BGS has been found to contains organic compounds that are more stable and less susceptible to mineralization unlike AM that contains stable organic material that is not readily available for biological degradation (Soane, 1990; Bernal et al., 1993; Paul and Beauchamp, 1993; Bonten et al., 2014). Bonten et al. (2014) explained that BGS contains high organic matter, essential for improving soil health and quality, high N, P, and K that are in forms that are readily available for plant uptake. The chemical composition of BGS could depend on the quality of the feedstock and the biogas production process used. AM composition depends on the feed and storage conditions and ranges between 0.4-0.80% N, 0.6-0.8% P and 0.5-0.65% K (Surendra et al., 2014). In many studies, BGS produced from different production systems were found to contain 0.5-2.5% N, 0.5-1.9% P and 0.6-2.2% (Khandelwal et al., 1986; Demont et al., 1990; Surendra et al., 2014; Kumar et al., 2015). Khan et al. (2012) reported that BGS contains higher N compared to AM. However, Bonten et al. (2014) argued that the N, P, K, Ca and Mg contents of BGS and AM could be similar, on a fresh matter basis, if ammonia volatilization is prevented during anaerobic digestion. Considering the fact that biogas provides energy benefits together with fertiliser (BGS) that is similar or higher nutrient composition compared to AM, BGS would be highly beneficial through nutrient uptake by crops.

Bachmann et al. (2011) and Ernst et al. (2008) indicated that BGS has the potential to improve N uptake, crop growth and yields, and improve soil quality while reducing the cost of fertilisers for farmers. The use of BGS as a nutrient source could reduce the need to use CF especially in the smallholder sector in the vicinity of biogas plants (Kumar et al., 2015), and add the necessary organic C, with the benefits on soil quality and crop productivity (Galvez et al., 2012). Dauden and Quilez (2004), Nasir et al.

(2012) and Kumar et al. (2015) indicated that the effects of CF and BGS are comparable in terms of crop yields. The application of BGS has been shown to have a positive impact on the soil ecosystem, including soil microbial biomass, when compared to non-amended soils (Tiwari et al., 2000). However, cumulative CO<sub>2</sub> emissions have also been reported. A similar or better fertiliser value of BGS compared to that of the feedstock will reduce waste management challenges associated with manure and the slurry and increase benefits from biogas and organic fertiliser. There is a need to compare the fertiliser value of BGS with that of the feedstock. The process of biogas production from organic wastes of the same source as CM could change the chemical composition of the feedstock possibly making the nutrients more labile in the slurry.

Many studies comparing BGS from different sources and AM have been conducted in some cases, with other waste streams added during anaerobic digestion, which then hampers the comparison between BGS and AM. Nevertheless, there is a paucity of studies on the comparison of CM, BGS and the feedstock. Results from preliminary work, with the same BGS and CM used in this study, have shown that the addition of BGS to soil resulted in higher ammonium-N and extractable P and lower nitrate-N in soil than CM, during incubation (Grootboom, 2019). The BGS also showed higher spinach dry matter yield, and uptake of N, P, K, Ca and Fe, than CM, particularly when applied at a rate equivalent to 150 kg Nha<sup>-1</sup> in a pot experiment (Grootboom, 2019). These preliminary studies were conducted under controlled conditions in the laboratory and glasshouse, and the responses could be different under field conditions where temperature and soil moisture are not controlled. Moreover, the use of a different crop could result in variations in the responses to BGS and CM.

Maize and dry bean are among the most commonly grown crops on the smallholder farms of South Africa (Chandhla, 2001; DAFF, 2016). The threshold soil nutrient levels for maize are 8 mg NO<sub>3</sub>-N kg<sup>-1</sup>, 8.5 mg P kg<sup>-1</sup>, 80-125 mg K kg<sup>-1</sup>, 1.5-2.0 cmol(+) Ca kg<sup>-1</sup> and 0.28 cmol(+) Mg kg<sup>-1</sup> (FSSA, 2007; Ayodele and Omotoso, 2008; Biljon et al., 2008). Those for dry bean are 36 mg N kg<sup>-1</sup>, 21 mg P kg<sup>-1</sup>, 120 mg K kg<sup>-1</sup>, 1.25 cmol(+)Ca kg<sup>-1</sup> and 0.5 cmol(+) Mg kg<sup>-1</sup> (FSSA, 2007; Long et al., 2010). Application of BGS and CM could add nutrients to soils that are deficient and improve the productivity of these commonly grown crops.

The objective of the study was to determine the effects of biogas slurry and cattle manure, relative to chemical fertiliser, on plant dry matter, grain yield and primary macronutrient uptake of maize and dry bean, and soil nutrient composition after crop harvest under field conditions.

#### 3.2 MATERIALS AND METHODS

#### 3.2.1 Site description

The study was conducted at Agricultural Research Council's Vegetable and Ornamental Plant (ARC-VOP) experimental site (25°59"S and 28°35"E), in Roodeplaat, Pretoria, South Africa. The area receives a mean annual rainfall of 587 mm, most of it during the summer season (Dyson, 2009). Summer seasons are warm with air temperatures exceeding 29°C while the winter seasons have air temperatures below 24°C and may decrease to 5°C (Kruger and Shongwe, 2004; ARC, 2019). The area is on alluvial deposits. The soil has an effective rooting depth of 1.5 m and was classified as Avalon soil form (SCWG, 1991) and translated to Xanthic Ferralsols (IUSS WRB, 2006).

#### 3.2.2 Soil sampling and initial soil characterisation

Soil samples were collected randomly from the 0-20 cm depth with a bucket auger at the study site before the establishment of experimental plots for initial soil characterization (Table 3.1). After harvest five soil subsamples were collected from each plot, thoroughly mixed to get a composite sample, air-dried at room temperature (approximately 23°C), and sieved (< 2 mm). The samples were stored in plastic containers at room temperature before analysis. The characteristics of the soils are in Table 3.1.

Table 3. 1: Selected chemical characteristics (means ± standard deviation) of the soil.

Parameter	Concentration	
pH (H <sub>2</sub> O)	6.88 ± 0.07	
Total N (g kg <sup>-1</sup> )	$0.30 \pm 0.02$	
Total C (g kg <sup>-1</sup> )	12.2 ± 0.31	
Extractable P (mg kg <sup>-1</sup> )	$6.35 \pm 0.17$	
Exchangeable K (cmol₀/kg)	$0.20 \pm 0.03$	
Exchangeable Ca (cmol <sub>o</sub> /kg)	$3.32 \pm 0.04$	
Exchangeable Mg (cmolc/kg)	$2.21 \pm 0.03$	
Extractable Zn (mg kg <sup>-1</sup> )	$0.37 \pm 0.03$	
Extractable Cu (mg kg <sup>-1</sup> )	$0.30 \pm 0.04$	
Sand (g kg <sup>-1</sup> )	660 ± 1.00	
Silt (g kg <sup>-1</sup> )	$100 \pm 0.58$	
Clay (g kg <sup>-1</sup> )	240 ± 1.15	

#### 3.2.3 Biogas slurry and cattle manure

The BGS and CM used in the experiments were obtained from a concentrated animal Feeding Operation in the Free State Province of South Africa. Fresh AM collected in the morning from animal kraal is mixed with water then fed on to the biogas digesters to produce biogas with BGS as a by-product. The CM was collected from the kraal mixed thoroughly and stored into closed boxes. The liquid BGS was stored in closed drums while the CM was stored in closed boxes until application in the field. The characteristics of BGS and CM are shown in Table 3.2. The BGS and CM had pH above 8, with BGS having higher total C (350 g kg<sup>-1</sup>) and N (25.5 g kg<sup>-1</sup>) compared to CM, which had 282 g kg<sup>-1</sup> total C and 19.0 g kg<sup>-1</sup> total N, leading to lower C/N ratio in the BGS. Total N concentration in CM was 25% lower than that in BGS. The BGS had higher levels of other mineral nutrients than CM.

**Table 3. 2**: Physico-chemical characteristics (mean  $\pm$  standard deviation) of biogas slurry and cattle manure.

Parameter	Biogas slurry	Cattle manure
рН	9.10±0.01	8.57±0.02
EC (dS m <sup>-1</sup> )	3.75±0.01	3.57±0.01
Total N (g kg <sup>-1</sup> )	25.5±0.02	19.0±0.10
Total C (g kg <sup>-1</sup> )	350±0.06	282±0.90
C/N ratio	13.7	14.8
Total P (g kg <sup>-1</sup> )	5.73±0.17	3.37±0.02
Total K (g kg <sup>-1</sup> )	17.7±0.81	16.7±0.04
Total Ca (g kg <sup>-1</sup> )	19.1±0.49	7.12±0.01
Total Mg (g kg <sup>-1</sup> )	9.12±0.24	5.11±0.01
Total Fe (g kg <sup>-1</sup> )	5.18±0.03	5.16±0.02
Total Cu (mg kg <sup>-1</sup> )	40.3±0.03	24.8±0.10
Total Zn (mg kg <sup>-1</sup> )	799±0.21	125±0.70
Total Mn (mg kg <sup>-1</sup> )	788±0.20	464±0.20

## **3.2.4** Effects of biogas slurry and cattle manure as nitrogen sources for maize *Trial set up*

The field experiments were conducted with maize in the 2016/2017 season and were arranged in a randomized complete block design (RCBD) with four replicates for each treatment, and with each maize plot being 4 × 3 m. The maize cultivar planted was BG 5285 hybrid with high prolificacy was planted manually and is recommended for all regions of production in South Africa. The inter- and intra-row spacing was 50 cm. The targeted plant population was 40 000 plants ha<sup>-1</sup>. The inter- and intra-row spacing was considered for easy movement in between the plots.

The treatments used in the study were unamended control, BGS, CM and compound CF - 3:2:1 (28). The BGS, CM and CF were applied at 40, 80, 120 kg Nha<sup>-1</sup>. These amounts corresponded to the recommended rate for maize for potential grain yield of 3, 4 and 5-t ha<sup>-1</sup>, respectively (FSSA, 2007). The spreading of both BGS and CM was performed by hand and incorporated into the top 0-10 cm of the soil with handheld hoe. Due to the difference in fertilisers, P and K were added through straight fertilisers (single superphosphate and potash) so that N became the only limiting primary nutrient.

#### Trial monitoring

The plots were irrigated with sprinkler irrigation for two hours to field capacity every other day with water from the Roodeplaat Dam. The characteristics of the water used are shown in Table 3.3. The water had a high pH (8.40) and low electrical conductivity (0.36 dS m<sup>-1</sup>). The analysis showed that all elements found in the water were lower than the threshold for irrigation water and was suitable for irrigation use. Mechanical weeding was done every third week to control weeds. Methamidofos 585 SL insecticide was applied to control full armyworm only in the 2016/2017 season. Plant sampling was done at the tasselling stage, while soil samples were collected after harvest. The experiment was repeated in the 2017/2018 season in the same experimental plots that were established in the 2016/2017 season, with the same treatment, layout, management and sampling.

Table 3. 3: Water analysis characterization.

Irrigation water	Threshold	
8.40	8.5*#	
0.004	3.0*#	
3.23	30*#	
39.2	175*	
33.8	960 <sup>&amp;</sup>	
46.6	460*	
5.55	78 <sup>&amp;</sup>	
24.1	400 <sup>&amp;</sup>	
12.0	60.8 <sup>&amp;</sup>	
	8.40 0.004 3.23 39.2 33.8 46.6 5.55 24.1	

<sup>\*</sup>DWAF, 1996; #Vomocil and Hart, 1998; & Ayers and Westcott, 1994

#### Plant sampling and preparation for analysis

Leaves of maize crop were sampled separately from five different plants randomly from each plot at the tasselling stage. From each plant sampled, all leaves and plant stocks were collected for analysis. All samples were kept in well-labeled paper bags and oven-dried at 50°C, weighed to determine the dry matter and ground to < 0.5 mm using Fritsch Pulverisette mortar grinder. The ground plant samples were digested following Nitric-Perchloric Acid Digestion Method (Zasoski and Burau, 1977). Briefly, 0.5 g of dried plant material was digested with 7 ml nitric acid and 3 ml perchloric acid at 180°C, brought up to volume in a 100 ml volumetric flask and analysed for P, K, Ca and Mg by ICP-OES. Total N was determined by dry combustion method (Jimenez and Ladha, 1993). Maize grain yield was only determined in the 2016/2017 season because monkeys damaged the cobs before the harvesting period in the 2017/2018 season.

#### Selected physicochemical soil parameters after maize

The soil samples were analysed at the Agricultural Research Council – Institute for Soil, Climate and Water (ARC-ISCW). Soil pH was measured both in water and KCl at a ratio of 1:2.5 (soil: solution) while EC was measured in water (Okalebo et al., 2002). Briefly, a 10 g mixture of soil was thoroughly mixed with 25 mL deionised water in a plastic bottle. The mixture was allowed to stand for 1 hour. Then pH and EC readings were measured using a glass electrode pH 700 meter, Eutech instruments, Singapore and EC meter multi 9310 IDS, Germany respectively.

Total N was determined using the Kjeldahl digestion method (Saez-Plaza et al., 2013). Briefly, a 1g mixture of soil weighed into a glass tube, 2 g of catalyst mixture and 10 ml of sulphuric acid were added and then left overnight. The glass tubes containing samples were heated for 2 hours at 360°C until the sample was clear then removed from the heating block and was left to cool down. Samples were transferred into a 100ml volumetric flask and brought to volume with deionised water, before filtration into sampling bottles and sent for further analysis at ARC analytical service. Plant available P was extracted with Bray 1 extraction solution (Bray and Kurtz, 1945). Briefly, 6.67 g of soil was weighed and placed in 100 mL inert plastic bottle and 50 mL of the extracting solution of 0.5M HCL and NH<sub>4</sub>F was added to the sample. The mixture was shaken in a reciprocating shaker for 60 seconds at 120 rpm. The mixture was

then filtered using a Whatman No 40-filter paper and the filtrate was analysed for P using a Continuous Flow Auto Analyser 3, *SEAL* Analytical, Australia.

The exchangeable bases were extracted with 1M Ammonium acetate solution (NH<sub>4</sub>OAc) adjusted to a pH of 7 (Chapman, 1965 and Hesse, 1971). A 5.0 g sample was weighed into a 100 mL inert plastic bottle and 50 mL of NH<sub>4</sub>OAc solution was added. The mixture was then shaken using a reciprocating shaker at 180 rpm for 30 minutes. The mixture was filtered using Whatman No 40-filter paper and the filtrate was analysed for K, Ca and Mg using an inductively coupled plasma spectrometer (ICP), ICPES-9820, Plasma Atomic Emission Spectrometer, Shimadzu Corporation, Japan.

### 3.2.5 Effects of biogas slurry and cattle manure as nitrogen sources for dry bean

The trial described for maize was also conducted with dry bean on the same site, on the same soil type, using the same trial set up and treatments. The dry bean cultivar planted was Kranskop, which is one of the red speckled sugar beans mostly grown in South Africa. The plot sizes used for dry bean were  $3 \times 2$  m in place of  $4 \times 3$  m used for maize. The inter- and intra-row spacing was 50 cm and the targeted plant population was 40 000 plants ha<sup>-1</sup>. The same treatments as in the maize trial were used and the BGS, CM and CF were applied at different rates of 30, 60, 90 kg Nha<sup>-1</sup>. These amounts corresponded to the recommended rate for dry bean for expected grain yield of 1, 2 and 3 t ha<sup>-1</sup> respectively (FSSA, 2007). The monitoring, sampling and analyses were similar to those described in the maize trial with a few deviations. No pesticides or herbicides were added on dry bean crop for pest control. The experiment was repeated in the 2017/2018 season.

For each sampling, five plant samples from dry bean plots were randomly collected from each plot, dried, weighed, ground and analysed for the same parameters as for maize as detailed in the description of the maize trial. Also similar to maize, grain yield for dry bean was only determined in the 2016/2017 season. In the 2017/2018 season, Guinea fowl birds damaged the experimental site just before the harvesting period. Soil samples from the individual plots were collected after dry bean, dried, sieved and analysed for the same parameters as for the maize trial.

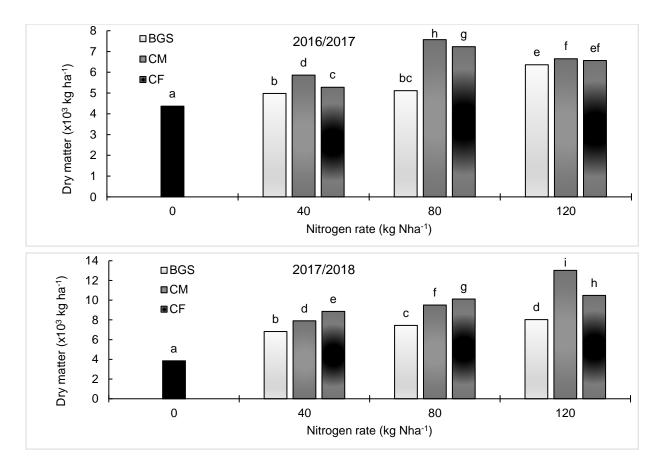
#### 3.3 Statistical analysis

Statistical analysis was performed with Genstat  $18^{th}$  edition (VSN International, 2016). Data on dry matter, grain yield, N, P, Ca and Mg were subjected to a one-way analysis of variance with Tukey test was used to establish any significant responses of treatments (p < 0.05). Correlation analysis for dry matter and uptake and soil concentrations were performed using JMP  $13^{th}$  edition. All analysis of variance for the results was done separately for each crop.

#### 3.4 RESULTS

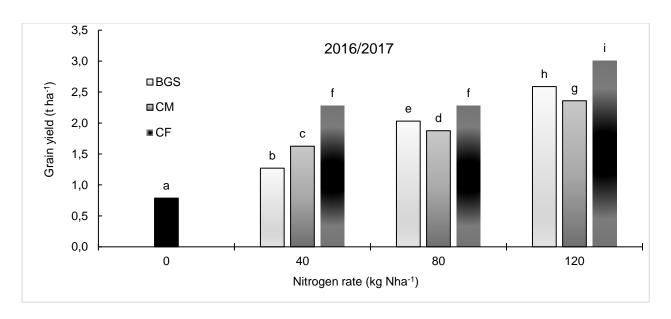
### 3.4.1 Effects of biogas slurry and cattle manure as nitrogen sources for maize Dry matter and grain yield

Maize dry matter yield increased with an increase in N rate for all the treatments (*p* < 0.05), which were higher than the control for both seasons (Figure 3.1). In both seasons, the BGS treatment resulted in the lower dry matter than CM and CF at all N application rates, except at 120 kg Nha<sup>-1</sup> rate in the 2016/2017 season when BGS and CF treatments had similar effects. Cattle manure had higher dry matter than CF except at 120 kg Nha<sup>-1</sup> rate. The highest dry matter in the 2016/2017 season was at 80 kg Nha<sup>-1</sup> and at 120 kg Nha<sup>-1</sup> in the 2017/2018 season for CM followed by CF. Except for the control, dry matter yield results for all treatments were generally higher in the 2017/2018 season than the 2016/2017 season.



**Figure 3. 1**: Effect of biogas slurry, cattle manure and chemical fertiliser on maize plant biomass. The different letters in the figure indicate statistically significant differences according to the Tukey test at the (p < 0.05). One tonne per hectare equals 1000 kilogram per hectare.

Maize grain yield increased with increasing N rate for all treatments (p < 0.05), which were higher than the control (Figure 3.2). The CF treatment had higher grain yield than both BGS and CM, at all rates in the 2016/2017 season. The BGS treatment had higher grain yield that CM, except at 40 kg Nha<sup>-1</sup>.

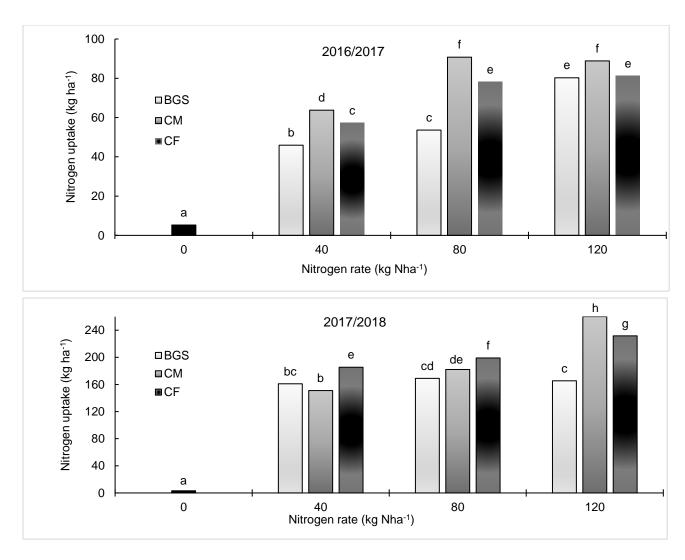


**Figure 3. 2**: Grain yield of maize crop as influenced by different treatments at different application rates for the 2016/2017 planting season. The different letters in the figure indicate statistically significant differences according to the Tukey test at the (p < 0.05).

#### Uptake of nitrogen, phosphorus and potassium

Increasing N application rate caused an increase in N uptake by maize for all treatments (p < 0.05), which were higher than the control in both seasons (Figure 3.3). In the 2016/2017 season, the results of N uptake followed a similar trend to those of dry matter. In the 2017/2018 season, the results of N uptake also followed a similar trend to that of dry matter except that at 40 and 80 kg Nha<sup>-1</sup>, BGS resulted in similar N uptake with CM, with both being lower than CF (Figure 3.3). When applied at 120 kg Nha<sup>-1</sup>, the N uptake was in the order BGS < CF < CM.

All treatments, at all rates, had higher P uptake than the control in both seasons (Table 3.4). At each rate, the BGS treatment had lower P uptake than the CF and CM, which were similar for both seasons. In the 2016/2017 season, increasing N application rates increased P uptake for all treatments with a 120 kg Nha<sup>-1</sup> rate resulting in higher P uptake than the other application rates. The 120 kg Nha<sup>-1</sup> rate had higher P uptake than the other rates for all treatments in the 2017/2018 season. Higher rates of amendments increased K uptake except in the 2017/2018 season where the uptake in the BGS treatment did not change (Table 3.4). The K uptake was in the order BGS < CM < CF in the 2016/2017 season and BGS < CF < CM in the 2017/2018 season.



**Figure 3. 3**: Effect of biogas slurry, cattle manure and chemical fertiliser on maize total N uptake. The different letters in the figure indicate statistically significant differences according to the Tukey test at the (p < 0.05).

Increasing N application rate increased Ca and Mg uptake by maize for all treatments (p < 0.05), when compared with the control in both the 2016/2017 and 2017/2018 season (Table 3.4). The Ca results in the 2016/2017 season showed that BGS resulted in lower Ca uptake than CM at all N application rates. The CF treatment had higher Ca uptake than BGS but lower than CM treatment at all N application rates. In the 2017/2018 season, BGS resulted in similar Ca uptake with CM at 40 kg Nha<sup>-1</sup>. When applied at 80 and 120 kg Nha<sup>-1</sup> BGS resulted in lower Ca than CM. The CF results showed that CF resulted in higher Ca uptake than both BGS and CM at 40 and 80 kg Nha<sup>-1</sup> but at 120 kg Nha<sup>-1</sup> Ca uptake in the CF treatment was similar to the CM.

**Table 3. 4:** Effect of increasing nitrogen rate from biogas slurry, cattle manure and chemical fertiliser on uptake of phosphorus and bases by maize.

Treatment	0	40	80	120	
		season 2016/2017			
Phosphorus uptake	(kg/ha)				
Control	3.60a				
BGS		4.58 <sup>b</sup>	5.87°	8.62e	
CM		7.22 <sup>d</sup>	8.77 <sup>e</sup>	10.38 <sup>f</sup>	
CF		7.71 <sup>d</sup>	8.02 <sup>de</sup>	10.75 <sup>f</sup>	
Potassium uptake (k	kg/ha)				
Control	14.60 <sup>a</sup>				
BGS		43.84 <sup>b</sup>	55.99 <sup>d</sup>	57.83 <sup>de</sup>	
CM		50.88 <sup>c</sup>	62.06 <sup>e</sup>	70.33 <sup>f</sup>	
CF		62.21 <sup>e</sup>	72.20 <sup>f</sup>	<b>77.74</b> <sup>9</sup>	
Calcium uptake (kg/	ha)				
Control	8.54 <sup>a</sup>				
BGS		16.35 <sup>b</sup>	16.74 <sup>b</sup>	19.85 <sup>d</sup>	
CM		20.55 <sup>e</sup>	23.80 <sup>g</sup>	25.70 <sup>h</sup>	
CF		17.97°	22.20 <sup>f</sup>	22.13 <sup>f</sup>	
Magnesium uptake	(kg/ha)				
Control	8.40 <sup>a</sup>				
BGS		24.48 <sup>d</sup>	29.65 <sup>f</sup>	31.77 <sup>g</sup>	
CM		24.16 <sup>d</sup>	34.24 <sup>h</sup>	29.34 <sup>f</sup>	
CF		15.85 <sup>b</sup>	26.79e	20.32°	
		season 2017/2018			
Phosphorus uptake	(kg/ha)				
Control	3.14 <sup>a</sup>				
BGS		19.71 <sup>b</sup>	21.81 <sup>b</sup>	25.34°	
CM		27.63 <sup>cd</sup>	27.63 <sup>cd</sup>	44.53 <sup>f</sup>	
CF		28.83 <sup>de</sup>	28.74 <sup>de</sup>	30.77 <sup>f</sup>	
Potassium uptake (k	(g/ha)				
Control	9.38ª				
BGS		50.68 <sup>b</sup>	50.30 <sup>b</sup>	46.63 <sup>b</sup>	
CM		62.64°	72.50 <sup>d</sup>	153.41 <sup>h</sup>	
CF		107.21 <sup>e</sup>	115.97 <sup>f</sup>	125.21 <sup>g</sup>	
Calcium uptake (kg/	ha)				
Control	3.37ª				
BGS		22.62b	26.93 <sup>d</sup>	35.19 <sup>f</sup>	
CM		22.44 <sup>b</sup>	28.91 <sup>e</sup>	44.00 <sup>g</sup>	
CF		24.81°	36.35 <sup>f</sup>	43.61 <sup>g</sup>	
Magnesium uptake	(kg/ha)	·			
Control	19.75ª				
BGS		36.54 <sup>c</sup>	41.88 <sup>ef</sup>	49.73 <sup>g</sup>	
CM		35.55 <sup>c</sup>	40.98 <sup>de</sup>	52.61 <sup>h</sup>	
CF		27.50 <sup>b</sup>	39.21 <sup>d</sup>	43.15 <sup>f</sup>	

The different letters in the table indicate statistically significant differences according to the Tukey test at the (p < 0.05).

In the 2016/2017 season, BGS resulted in similar Mg uptake with CM at 40 kg Nha<sup>-1</sup> (Table 3.4), while at 80 kg Nha<sup>-1</sup> BGS resulted in lower uptake. When applied at 120 kg Nha<sup>-1</sup> BGS resulted in higher Mg uptake than CM. The CF treatment resulted in lower Mg uptake than both BGS and CM at all rates in both seasons. In the 2017/2018 season BGS resulted in similar Mg uptake with CM at 40 and 80 kg Nha<sup>-1</sup>. When applied at 120 kg Nha<sup>-1</sup> BGS resulted in lower Mg uptake than CM.

#### Residual nutrients after maize

Soil pH after maize harvest increased with increasing N application rate for BGS and CM (p < 0.05) and were higher than the control for both seasons (Table 3.5). The soil pH was in the order BGS > CM > CF for both reasons at each rate. The CF treatment at 120 kg Nha<sup>-1</sup> had lower soil pH than the control.

The BGS resulted in higher SOC than CF, except at 80 kg Nha<sup>-1</sup>, while there were no differences between BGS and CM in the 2016/2017 season (Table 3.5). In 2017/2018 season, at all N application rates, BGS resulted in higher SOC than CF. The BGS treatment had higher SOC than CM, except at 80 kg Nha<sup>-1</sup>. When applied at 120 kg Nha<sup>-1</sup> there were no differences between CM and CF treatments. Increasing N application rate increased total N, P and K in the soil for all treatments when compared with the control in both seasons (Table 3.5). The BGS treatment resulted in a lower total N than CM but was similar to CF treatment when applied at 40 and 80 kg Nha<sup>-1</sup>, in the 2016/2017 season. At 120 kg Nha<sup>-1</sup> BGS resulted in a higher total N than CM and there was no difference between CM and CF treatments. In 2017/2018 season, BGS resulted in a higher total N in the soil after harvest at all N application rates, except at 120 kg Nha<sup>-1</sup> where BGS resulted in a similar total N with CF treatment.

**Table 3. 5:** Effect of increasing nitrogen rate from biogas slurry, cattle manure and chemical fertiliser on soil pH and total carbon nitrogen after maize.

Treatment	0	40	80	120	
	season 2016/2017				
рH					
Control	6.12 <sup>b</sup>				
BGS		7.01 <sup>ef</sup>	7.04 <sup>ef</sup>	7.09 <sup>f</sup>	
CM		6.85 <sup>cd</sup>	6.82 <sup>c</sup>	6.94 <sup>de</sup>	
CF		6.12 <sup>b</sup>	6.10 <sup>b</sup>	5.84 <sup>a</sup>	
Organic carbon (%)					
Control	0.12 <sup>a</sup>				
BGS		0.93 <sup>cd</sup>	$0.98^{e}$	1.06 <sup>f</sup>	
CM		0.88 <sup>bc</sup>	0.96 <sup>de</sup>	1.07 <sup>f</sup>	
CF		0.87 <sup>b</sup>	0.96 <sup>de</sup>	0.97 <sup>de</sup>	
Total nitrogen (%)					
Control	0.03 <sup>a</sup>				
BGS		0.06 <sup>b</sup>	0.06 <sup>b</sup>	$0.08^{e}$	
CM		0.07 <sup>d</sup>	$0.07^{d}$	0.07 <sup>d</sup>	
CF		0.06 <sup>c</sup>	$0.08^{e}$	$0.07^{d}$	
		season 2017/2018			
рН					
Control	6.26 <sup>b</sup>				
BGS		7.35 <sup>g</sup>	7.09 <sup>f</sup>	7.55 <sup>h</sup>	
CM		7.15 <sup>f</sup>	6.83 <sup>e</sup>	6.76 <sup>e</sup>	
CF		6.15°	6.07 <sup>b</sup>	5.93 <sup>a</sup>	
Organic carbon (%)					
Control	0.10 <sup>a</sup>				
BGS		1.17 <sup>e</sup>	1.20 <sup>e</sup>	1.24 <sup>f</sup>	
CM		1.08 <sup>d</sup>	1.19 <sup>e</sup>	1.10 <sup>d</sup>	
CF		0.86 <sup>b</sup>	0.95 <sup>c</sup>	1.10 <sup>d</sup>	
Total nitrogen (%)					
Control	0.03ª				
BGS		0.07e	0.08 <sup>f</sup>	0.10 <sup>h</sup>	
CM		0.06 <sup>b</sup>	0.06°	0.07 <sup>de</sup>	
CF		0.06 <sup>cd</sup>	0.07 <sup>f</sup>	0.09 <sup>g</sup>	

The different letters in the table indicate statistically significant differences according to the Tukey test at the (p < 0.05).

Soil extractable P was higher in the BGS treatment than CM in the 2016/2017 season (Table 3.6). Both BGS and CM treatments had lower extractable P than the CF treatment across all application rates. In 2017/2018 season, the BGS treatment resulted in higher soil extractable P than CM except at 40 kg Nha<sup>-1</sup>. Both BGS and CM treatments resulted in lower P than CF treatment when applied at 40 and 120 kg Nha-<sup>1</sup> but at 80 kg Nha<sup>-1</sup> BGS treatment had higher P than CF treatment. The K results showed lower K in the BGS treatment than CM treatment across all N application rates, with similar exchangeable K between CM and CF treatments, in the 2016/2017 season (Table 3.6). In the 2017/2018 season, BGS and CM treatments had similar exchangeable K at all N application rates, while the CF treatment had higher K than BGS and CM treatment. Increasing the N application rate of all the treatments caused an increase in exchangeable Ca and Mg in the soil after harvest (p < 0.05) when compared with the control (Table 3.6). The BGS treatment had higher Ca and Mg than CM at all rates for both seasons, except for Ca at 40 kg Nha<sup>-1</sup> in the 2017/2018 season. The CF treatment had lower levels of Ca and Mg than both BGS and CM treatments, except when applied at 120 kg Nha<sup>-1</sup>. However, soil Ca in the CM treatment in the 2017/2018 season was lower than CF treatment.

**Table 3. 6**: Effect of increasing nitrogen rate from biogas slurry, cattle manure and chemical fertiliser on soil extractable phosphorus and exchangeable potassium after maize.

Treatment	0	40	80	120
	season 2016/2017			
Extractable phosp	:			
Control	3.60 <sup>a</sup>			
BGS		4.59 <sup>c</sup>	9.66 <sup>e</sup>	14.89 <sup>h</sup>
CM		3.87 <sup>b</sup>	6.56 <sup>d</sup>	9.50e
CF		10.54 <sup>f</sup>	12.20 <sup>g</sup>	15.37 <sup>i</sup>
Exchangeable po	tassium (cmol <sub>c</sub> /kg)		-	
Control	0.14 <sup>a</sup>			
BGS		0.19 <sup>b</sup>	0.21 <sup>c</sup>	$0.22^{d}$
CM		0.23 <sup>e</sup>	0.29 <sup>f</sup>	0.35 <sup>h</sup>
CF		$0.32^{g}$	$0.35^{h}$	0.36 <sup>h</sup>
Exchangeable ca	lcium (cmol <sub>c</sub> /kg)			
Control	3.08 <sup>a</sup>			
BGS		5.90 <sup>e</sup>	6.25 <sup>f</sup>	8.67 <sup>h</sup>
CM		5.65 <sup>d</sup>	5.65 <sup>d</sup>	6.45 <sup>g</sup>
CF		4.25 <sup>b</sup>	4.89 <sup>c</sup>	6.32 <sup>fg</sup>
Exchangeable ma	agnesium (cmol <sub>c</sub> /kg)			
Control	2.81a			
BGS		4.53 <sup>f</sup>	5.12 <sup>h</sup>	5.57 <sup>i</sup>
CM		3.85 <sup>d</sup>	4.10 <sup>e</sup>	4.53 <sup>f</sup>
CF		3.08 <sup>b</sup>	3.55 <sup>c</sup>	4.83 <sup>g</sup>
		season 2	2017/2018	
Extractable phosp	ohorus (mg/kg)			
Control	2.54 <sup>a</sup>			
BGS		9.03 <sup>b</sup>	17.18 <sup>9</sup>	17.38 <sup>h</sup>
CM		9.74°	11.84 <sup>d</sup>	12.85 <sup>e</sup>
CF		12.72 <sup>e</sup>	13.97 <sup>f</sup>	18.98 <sup>i</sup>
Exchangeable po	tassium (cmol <sub>c</sub> /kg)		-	
Control	0.12 <sup>a</sup>			
BGS		0.28 <sup>b</sup>	0.34 <sup>f</sup>	0.33 <sup>e</sup>
CM		0.30°	0.31e	0.45 <sup>g</sup>
CF		0.46 <sup>h</sup>	1.02 <sup>i</sup>	1.21 <sup>j</sup>
Exchangeable ca	lcium (cmol <sub>c</sub> /kg)			
Control	4.22a			
BGS		6.28 <sup>b</sup>	9.08 <sup>h</sup>	9.96 <sup>i</sup>
CM		6.43 <sup>c</sup>	6.65 <sup>d</sup>	6.78 <sup>e</sup>
CF		6.63 <sup>d</sup>	8.03 <sup>f</sup>	8.28 <sup>g</sup>
	agnesium (cmolc/kg)		<u> </u>	
Control	3.08 <sup>a</sup>			
BGS	-	5.04 <sup>f</sup>	5.48 <sup>i</sup>	5.50 <sup>i</sup>
CM		4.73 <sup>e</sup>	5.11 <sup>g</sup>	5.25 <sup>h</sup>
CF		4.28 <sup>b</sup>	4.45°	4.64 <sup>d</sup>

The different letters in the table indicate statistically significant differences according to the Tukey test at the (p < 0.05).

There was a strong positive correlation between maize dry matter and uptake of all elements in both the 2016/2017 and 2017/2018 seasons. Dry matter and soil concentrations of the different elements showed a weak positive correlation. Soil N and exchangeable K were the only parameters that showed a slightly stronger positive correlation in the 2016/2017 season while in the 2017/2018 season only P showed a slightly strong positive correlation analysis.

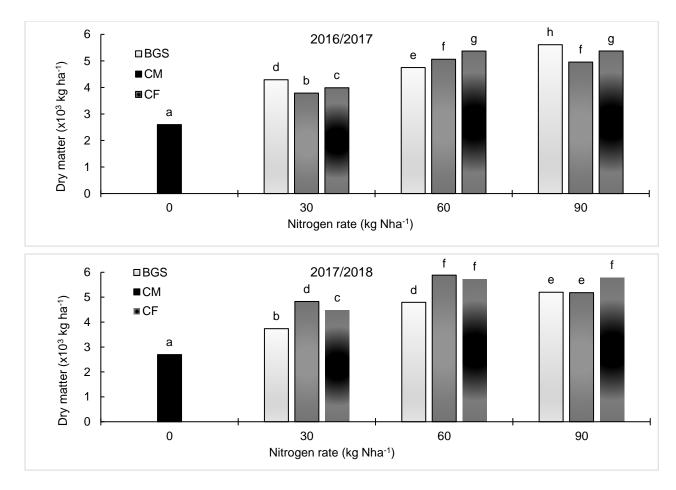
**Table 3. 7:** Correlation analysis of dry matter with plant uptake and selected soil parameters after maize.

	season 2016/2017	season 2017/2018
	Dry matter	Dry matter
Plant uptake		
N	0.786	0.956
Р	0.763	0.968
K	0.965	0898
Ca	0.802	0.931
Mg	0.672	0.779
Soil parameters		
N	0.606	0.517
Р	0.388	0.639
K	0.582	0.521
Ca	0.364	0.483
Mg	0.385	0.387

#### 3.4.2 Effects of biogas slurry and cattle manure as nitrogen sources for dry bean

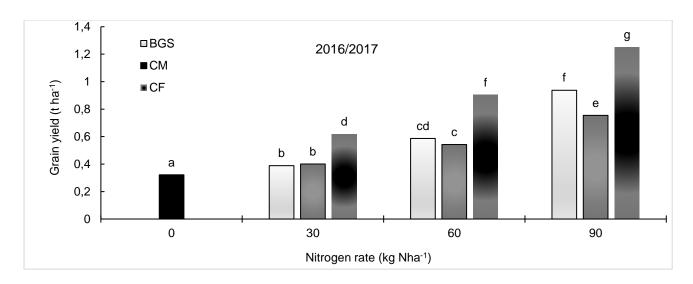
#### Dry matter and grain yield

Dry matter yield of dry bean increased with an increase in N rate for all the treatments (p < 0.05) when compared with the control in both seasons (Figure 3.4). Dry matter was in the order BGS > CF > CM, except in at 60 kg Nha<sup>-1</sup> in the 2016/2017 season. In the 2017/2018 season, the BGS treatment had lower dry matter than both the CF and CM except at 90 kg Nha<sup>-1</sup> where it had similar levels with CM treatment. The dry matter in the CM treatment was higher at 30 kg Nha<sup>-1</sup> and lower at 90 kg Nha<sup>-1</sup> than the CF treatments, with no differences at 60 kg Nha<sup>-1</sup>.



**Figure 3. 4:** Effect of biogas slurry, cattle manure and chemical fertiliser on dry bean plant biomass. The different letters in the figure indicate statistically significant differences according to the Tukey test at the (p < 0.05).

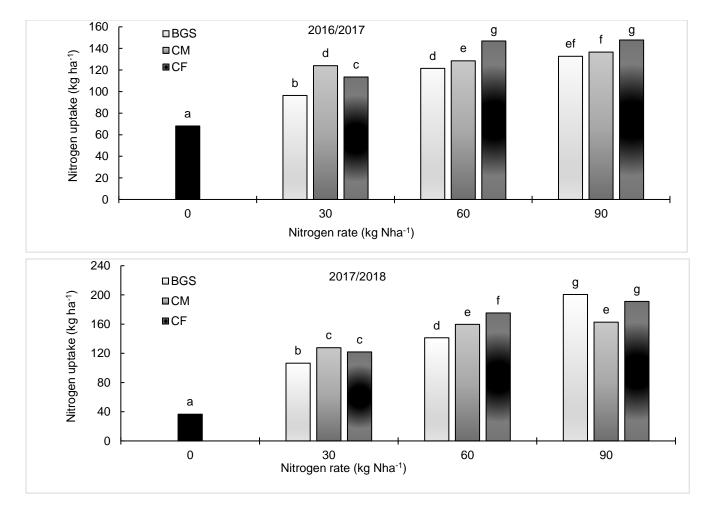
Grain yield of dry bean increased with increasing N rate for all treatments (p < 0.05), which were higher than the control in the 2016/2017 season (Figure 3.5). The CF treatment had higher grain yield than both BGS and CM treatments, at all rates. The BGS treatment had similar grain yield with CM, except at 90 kg Nha<sup>-1</sup>, where BGS had higher grain yield. The BGS treatment at 60 kg Nha<sup>-1</sup> had similar grain yield as CF at 30 kg Nha<sup>-1</sup> while at 90 kg Nha<sup>-1</sup> the BGS treatment resulted in a similar yield as CF treatment at 60 kg Nha<sup>-1</sup>.



**Figure 3. 5**: Grain yield of dry bean as influenced by different treatments at different application rates for the 2016/2017 planting season. The different letters in the figure indicate statistically significant differences according to the Tukey test at the (p < 0.05).

#### Uptake of nitrogen, phosphorus and potassium

Increasing N application rate increased N uptake in dry bean for all treatments (p < 0.05), when compared with the control in both seasons (Figure 3.6). The BGS resulted in lower N uptake than CM in both seasons except at 90 kg Nha<sup>-1</sup>, where there were no differences in 2016/2017 season while BGS had higher N uptake in the 2017/2018 season. The CF treatment had higher N uptake than the CM except at 30 kg Nha<sup>-1</sup>, in both seasons.



**Figure 3. 6:** Effect of biogas slurry, cattle manure and chemical fertiliser on dry bean total N uptake. The different letters indicate statistically significant differences according to the Tukey test at the (p < 0.05).

All treatments, at all rates, had higher P uptake than the control in both seasons (Table 3.7). In the 2016/2017 season, BGS resulted in higher P uptake than both CM and CF treatments, except at 60 kg Nha<sup>-1</sup>. There were no differences in P uptake between CM and CF at all rates. In the 2017/2018 season, the P uptake results were in the order BGS < CM < CF, except at 60 kg Nha<sup>-1</sup> where CM had equal uptake with CF.

An increase in the N application rate increased K uptake for all treatments for both seasons except that K uptake did not respond to the rate of BGS in the 2017/2018 season. All the treatments at all rates had higher K uptake than the control in both seasons. In the 2016/2017 season, at 30 kg Nha<sup>-1</sup>, BGS application resulted in higher K uptake than both CM and CF treatments that were similar in K uptake. When applied at 60 kg Nha<sup>-1</sup> BGS resulted in similar K uptake with CM but CF resulted in much

higher K uptake than the other two treatments. At 90 kg Nha<sup>-1</sup> BGS resulted in similar K uptake with CF, which was higher than CM. In the 2017/2018 season, BGS resulted in K uptake which was higher at 30 kg Nha<sup>-1</sup>, similar at 60 kg Nha<sup>-1</sup> and lower at 90 kg Nha<sup>-1</sup> than CM treatment. The BGS treatment resulted in lower K uptake that CF treatment at all rates.

The increasing N application rate increased Ca and Mg uptake by dry bean for all treatments (*p* < 0.05) when compared with the control in both seasons (Table 3.7). The Ca results in the 2016/2017 season showed that BGS resulted in higher Ca uptake than CM except at 60 kg Nha<sup>-1</sup>. Higher Ca uptake was observed in the BGS treatment than CF at all N application rates. In the 2017/2018 season, Ca uptake in BGS treatment was lower at 30 kg Nha<sup>-1</sup>, similar at 60 kg Nha<sup>-1</sup> and higher at 90 kg Nha<sup>-1</sup> than CM treatment. The CF treatment had higher Ca uptake than both BGS and CM at all application rates. In the 2016/2017 season, BGS resulted in higher Mg uptake than CM except at 60 kg Nha<sup>-1</sup>. The CF treatments had lower Mg uptake than both BGS and CM, except at 90 kg Nha<sup>-1</sup> where CF had similar Mg uptake with CM but still lower than BGS treatment. In the 2017/2018 season, Mg uptake in BGS treatment was lower at 30 kg Nha<sup>-1</sup>, similar at 60 kg Nha<sup>-1</sup> and higher at 90 kg Nha<sup>-1</sup> than CM treatment. The BGS treatment had higher Mg uptake than CF at all rates.

**Table 3. 8:** Effect of increasing nitrogen rate from the biogas slurry, cattle manure and chemical fertiliser on uptake of phosphorus and bases by dry bean.

Treatment	0	30	60	90		
		season 2016/2017				
Phosphorus uptake						
Control	5.36 <sup>a</sup>					
BGS		12.95 <sup>c</sup>	13.34°	19.03 <sup>f</sup>		
CM		11.51 <sup>b</sup>	16.26 <sup>d</sup>	16.53 <sup>de</sup>		
CF		11.46 <sup>b</sup>	17.07 <sup>de</sup>	17.73 <sup>e</sup>		
Potassium uptake (k	g/ha)					
Control	24.91 <sup>a</sup>					
BGS		53.14 <sup>d</sup>	53.17 <sup>d</sup>	76.81 <sup>f</sup>		
CM		47.60 <sup>c</sup>	50.97 <sup>cd</sup>	65.00 <sup>e</sup>		
CF		43.47 <sup>b</sup>	68.23 <sup>e</sup>	74.13 <sup>f</sup>		
Calcium uptake (kg/l	ha)					
Control	18.40 <sup>a</sup>					
BGS		60.31 <sup>d</sup>	64.79 <sup>f</sup>	95.48 <sup>i</sup>		
CM		46.16 <sup>c</sup>	67.06 <sup>g</sup>	71.56 <sup>h</sup>		
CF		36.23 <sup>b</sup>	62.45 <sup>e</sup>	67.93 <sup>g</sup>		
Magnesium uptake (	(kg/ha)					
Control	8.25 <sup>a</sup>					
BGS		27.62 <sup>e</sup>	26.84 <sup>d</sup>	35.68 <sup>h</sup>		
CM		21.79°	28.47 <sup>f</sup>	31.04 <sup>g</sup>		
CF		18.28 <sup>b</sup>	26.86 <sup>d</sup>	31.56 <sup>g</sup>		
		season 2	2017/2018			
Phosphorus uptake	(kg/ha)					
Control	7.19 <sup>a</sup>					
BGS		13.71°	11.96 <sup>b</sup>	11.32 <sup>b</sup>		
CM		11.57 <sup>b</sup>	16.20 <sup>e</sup>	14.67 <sup>d</sup>		
CF		19.83 <sup>g</sup>	16.10 <sup>e</sup>	17.43 <sup>f</sup>		
Potassium uptake (k	g/ha)		-			
Control	23.96 <sup>a</sup>					
BGS		60.74 <sup>d</sup>	51.50≌	50.50 <sup>c</sup>		
CM		42.47 <sup>b</sup>	53.28°	84.31 <sup>e</sup>		
CF		111.6 <sup>f</sup>	115.4 <sup>f</sup>	143.9 <sup>g</sup>		
Calcium uptake (kg/l	ha)					
Control	14.18 <sup>a</sup>					
BGS		72.48 <sup>b</sup>	88.54 <sup>e</sup>	99.95 <sup>h</sup>		
CM		84.41 <sup>d</sup>	88.11 <sup>e</sup>	94.45 <sup>9</sup>		
CF		91.96 <sup>f</sup>	114.8 <sup>i</sup>	115.3 <sup>j</sup>		
Magnesium uptake (	kg/ha)					
Control	9.11 <sup>a</sup>					
BGS		37.27 <sup>c</sup>	43.87 <sup>ef</sup>	51.65 <sup>9</sup>		
CM		42.99 <sup>de</sup>	43.17 <sup>def</sup>	43.77 <sup>ef</sup>		
CF		28.77 <sup>b</sup>	42.58 <sup>d</sup>	44.08 <sup>f</sup>		

The different letters in the table indicate statistically significant differences according to the Tukey test at the (p < 0.05).

#### Soil nutrients after dry bean

Soil pH after dry bean was increased for BGS and CM treatments and declined in CF treatment as a result of increasing N application rate, when compared to the control (p < 0.05), for both seasons (Table 3.9). In the 2016/2017 season, BGS resulted in higher pH while in 2017/2018 season, it had a similar pH to CM at all N application rates. In both seasons, BGS and CM treatments resulted in higher soil pH than CF treatment. SOC results after dry bean harvest were higher in the BGS treatment than the CM and CF treatments at all rates for both seasons, except that there were no differences between BGS and CM treatments at 90 kg Nha<sup>-1</sup> in the 2017/2018 season (Table 3.9). While there was not a clear trend in total soil N in the 2016/2017 season, the concentration increased with the rate in the 2017/2018 season (Table 3.9). No clear trend was observed among the treatments in the 2016/2017 season, while the trend was BGS > CM > CF in the 2017/2018 season.

Soil extractable P in the BGS treatment was lower in the 2016/2017 season and higher in the 2017/2018 season than the CM treatment (Table 3.10). Both CM and BGS treatments were higher than CF treatment across all rates. Soil exchangeable K in the BGS treatment was higher at 30 kg Nha<sup>-1</sup>, similar at 60 kg Nha<sup>-1</sup> and higher at 90 kg Nha<sup>-1</sup> that CM treatment (Table 3.10) in the 2016/2017 season, while in the 2017/18 season the BGS treatment had higher levels at all application rates. The K level was lower in CF treatment in 2016/2017 season and higher in the 2017/2018 season than both BGS and CM treatments. While the effect of application rate was not clear in the 2016/2017 season, higher application rates increased exchangeable K in the 2017/2018 season.

Increasing N application rates caused an increase in exchangeable Ca and Mg in the soil after dry bean (p < 0.05), which were higher than the control in both seasons (Table 3.10). The BGS treatment had lower exchangeable Ca than CM treatment except at 90 kg Nha<sup>-1</sup> in the 2016/2017 season while in the 2017/2018 season BGS had higher levels at all application rates. In the 2016/2017 season, the exchangeable Ca in the BGS treatment was similar at 30 kg Nha<sup>-1</sup>, lower at 60 kg Nha<sup>-1</sup> and higher at 90 kg Nha<sup>-1</sup>. The CF treatment had lower exchangeable Ca than the BGS treatment at all application rates in the 2017/2018 season. Soil exchangeable Mg in the BGS treatment was higher at 30 kg Nha<sup>-1</sup>, similar at 60 kg Nha<sup>-1</sup> and lower at 90 kg Nha<sup>-1</sup>

that CM treatment in the 2016/2017 season while in the 2017/2018 season the BGS treatment had higher levels at all rates (Table 3.10). The CF treatment had lower exchangeable Mg than BGS and CM treatments at all N application rates in both seasons.

**Table 3. 9:** Effect of increasing nitrogen rate from biogas slurry, cattle manure and chemical fertiliser on soil pH and total carbon and nitrogen after dry bean.

Treatment	0	30	60	90
	season 2016/2017			
рН				
Control	6.26 <sup>d</sup>			
BGS		7.06 <sup>i</sup>	6.97 <sup>h</sup>	6.91 <sup>g</sup>
CM		6.84 <sup>f</sup>	6.86 <sup>f</sup>	6.71 <sup>e</sup>
CF		6.19°	6.14 <sup>b</sup>	6.10 <sup>a</sup>
Organic carbon (%)				
Control	0.82a			
BGS		0.97℃	1.03 <sup>de</sup>	1.09 <sup>f</sup>
CM		0.90 <sup>b</sup>	0.98 <sup>cd</sup>	1.01 <sup>cd</sup>
CF		0.88 <sup>b</sup>	0.97°	1.06 <sup>ef</sup>
Total nitrogen (%)				
Control	0.027 <sup>a</sup>			
BGS		0.087 <sup>g</sup>	0.087 <sup>g</sup>	0.068 <sup>b</sup>
CM		0.087 <sup>g</sup>	0.083 <sup>f</sup>	$0.079^{e}$
CF		0.073°	0.078 <sup>d</sup>	$0.080^{e}$
	season 2017/2018			
рΗ				
Control	6.30°			
BGS		7.05 <sup>e</sup>	7.06 <sup>e</sup>	7.13 <sup>f</sup>
CM		6.88 <sup>d</sup>	7.06 <sup>e</sup>	7.12 <sup>f</sup>
CF		6.16 <sup>b</sup>	6.12 <sup>b</sup>	5.84 <sup>a</sup>
Organic carbon (%)				
Control	0.85 <sup>a</sup>			
BGS		1.27 <sup>e</sup>	1.33 <sup>f</sup>	1.95 <sup>h</sup>
CM		1.15 <sup>d</sup>	1.16 <sup>d</sup>	1.93 <sup>h</sup>
CF		1.07℃	0.97 <sup>b</sup>	1.43 <sup>g</sup>
Total nitrogen (%)				
Control	0.032a			
BGS		0.087 <sup>d</sup>	0.097 <sup>g</sup>	0.119 <sup>h</sup>
CM		0.081°	0.089 <sup>e</sup>	0.093 <sup>f</sup>
CF		0.074 <sup>b</sup>	0.087 <sup>d</sup>	0.097 <sup>g</sup>

The different letters in the table indicate statistically significant differences according to the Tukey test at the (p < 0.05).

**Table 3. 10:** Effect of increasing N rate from biogas slurry, cattle manure and chemical fertiliser on soil extractable phosphorus and exchangeable potassium after dry bean.

Treatment	0	30	60	90
	season 2016/2017			
Extractable phosphore	:			
Control	4.85 <sup>a</sup>			
BGS		5.96°	6.65 <sup>e</sup>	7.55 <sup>f</sup>
CM		6.73 <sup>e</sup>	7.59 <sup>f</sup>	7.93 <sup>g</sup>
CF		5.46 <sup>b</sup>	5.86°	6.49 <sup>d</sup>
Exchangeable potass	sium (cmol <sub>c</sub> /kg)			
Control	0.22 <sup>a</sup>			
BGS		0.31 <sup>e</sup>	0.28 <sup>d</sup>	0.32 <sup>e</sup>
CM		$0.33^{g}$	0.28 <sup>d</sup>	$0.28^{d}$
CF		0.23 <sup>b</sup>	0.23 <sup>b</sup>	0.27c
Exchangeable calciu	m (cmol <sub>c</sub> /kg)		•	
Control	4.15 <sup>a</sup>			
BGS		5.09 <sup>c</sup>	5.40 <sup>d</sup>	6.40 <sup>h</sup>
CM		5.40 <sup>d</sup>	5.85 <sup>f</sup>	6.28 <sup>g</sup>
CF		4.76 <sup>c</sup>	5.68e	6.25 <sup>g</sup>
Exchangeable magn	esium (cmolc/kg)			
Control	2.95 <sup>a</sup>			
BGS		3.67 <sup>d</sup>	4.18 <sup>f</sup>	4.61 <sup>g</sup>
CM		3.61°	4.22 <sup>f</sup>	4.69 <sup>h</sup>
CF		3.22 <sup>b</sup>	3.57°	4.06e
		season 2	2017/2018	
Extractable phosphor	rus (mg/kg)			
Control	3.84a			
BGS		8.87 <sup>d</sup>	9.67 <sup>e</sup>	11.95 <sup>h</sup>
CM		9.15 <sup>d</sup>	10.08 <sup>f</sup>	11.92 <sup>h</sup>
CF		7.14 <sup>b</sup>	8.44°	10.57 <sup>g</sup>
Exchangeable potass	sium (cmolc/kg)			
Control	0.20a			
BGS		0.27 <sup>c</sup>	0.32e	0.34 <sup>9</sup>
CM		0.25 <sup>b</sup>	0.27°	0.31 <sup>d</sup>
CF		0.30 <sup>d</sup>	0.33 <sup>f</sup>	0.35 <sup>h</sup>
Exchangeable calciu	m (cmol <sub>c</sub> /kg)			
Control	5.40 <sup>a</sup>			
BGS		6.69 <sup>d</sup>	7.29 <sup>i</sup>	7.65 <sup>j</sup>
CM		5.68 <sup>b</sup>	6.84 <sup>e</sup>	7.09 <sup>9</sup>
CF		6.55°	7.05 <sup>f</sup>	7.21 <sup>h</sup>
Exchangeable magn	esium (cmol <sub>c</sub> /ka)	-	-	
Control	3.44 <sup>a</sup>			
BGS		5.03 <sup>h</sup>	5.36 <sup>i</sup>	5.43 <sup>j</sup>
CM		4.55 <sup>d</sup>	4.69 <sup>f</sup>	4.90 <sup>9</sup>
CF		4.15 <sup>b</sup>	4.33°	4.63 <sup>e</sup>

The different letters in the table indicate statistically significant differences according to the Tukey test at the (p < 0.05).

#### Microbial biomass carbon

Increasing the N application rate increased MBC in both planting seasons (Table 3.11). The control treatment had the lowest MBC than any other treatments in both 2016/2017 and 2017/2018 seasons. In 2016/2017 season, CM had higher MBC than both BGS and CF treatments across all application rates. BGS was higher than the CF treatment across all application rates. In the 2017/2018 season, at 30 and 60 kg Nha<sup>-1</sup> CM treatment had higher MBC than BGS treatment while at 90 kg Nha<sup>-1</sup> BGS had higher MBC than CM treatment. Both BGS and CM treatments had higher MBC than CF treatment across all application rates. The general the order CM > BGS > CF in both seasons.

**Table 3.11**: Effect of biogas slurry, cattle manure and chemical fertiliser on soil microbial biomass carbon in soil under maize in 2016/2017 and 2017/2018 seasons.

Treatment	0	30	60	90	
	season 2016/2017				
MBC (mgC/kg)					
Control	88.6ª				
BGS		296,4 <sup>e</sup>	307.5 <sup>f</sup>	321.7 <sup>g</sup>	
CM		334.7 <sup>h</sup>	350.4 <sup>i</sup>	494.6 <sup>j</sup>	
CF		138.9 <sup>b</sup>	192.8 <sup>c</sup>	201.5 <sup>d</sup>	
		season 2017	7/2018		
MBC (mgC/kg)					
Control	93.9 <sup>a</sup>				
BGS		304.7 <sup>e</sup>	373.5 <sup>g</sup>	459.2 <sup>j</sup>	
CM		320.8 <sup>f</sup>	386.3 <sup>h</sup>	391.0 <sup>i</sup>	
CF		178.8 <sup>b</sup>	194.0°	226.1 <sup>d</sup>	

The different letters in the table indicate statistically significant differences according to the Tukey test at the (p < 0.05).

Strong positive correlations were observed between dry bean dry matter and uptake of all elements in both 2016/2017 and 2017/18 seasons (Table 3.12). Dry matter and soil concentrations of the different elements were positively correlated in the 2016/2017 season with K showing a weak correlation. In the 2017/2018 season, all elements showed weak positive correlation between dry matter and soil concentrations (Table 3.12).

**Table 3.12:** Correlation analysis of dry matter with plant uptake and selected soil parameters after dry bean.

	season 2016/2017	season 2017/2018
	Dry matter	Dry matter
Plant uptake		
N	0.973	1.00
Р	0.983	0.851
K	0.938	0.576
Ca	0.889	0.841
Mg	0.939	0.798
Soil parameters		
N	0.649	0.431
Р	0.578	0.473
K	0.376	0.443
Ca	0.897	0.359
Mg	0.718	0.242

#### 3.5 DISCUSSION

The increase in dry matter with the increasing rate of the amendment was explained by higher available N, P and K, among other nutrients, which resulted in increased uptake. Uptake of N, Ca and Mg, in both seasons, and to a lesser extent P and K (particularly in the 2016/2017 season), followed the same trend as that of dry matter yield. The greater availability of the nutrients resulted in greater uptake and growth of both maize and dry bean at higher application rates. The positive correlation of dry matter and uptake of the different nutrients for both dry bean and maize in both 2016/2017 and 2017/2018 seasons, also confirms the importance of uptake on dry matter accumulation. The results of this study were in agreement with Rahman et al. (2008) who showed that increasing N application rates increased dry matter of maize fodder after application of cattle slurry at different N levels. Islam et al. (2010) reported

that increasing N application rates could increase soil N, P and K, and some of the other macronutrients that could increase meristematic growth leading to higher dry matter yields.

The lower dry matter of maize in both seasons and dry bean (2017/2018 season) in the BGS than the CF treatment was in response to lower uptake of N, P and K. On the other hand, the lower dry matter of maize (both seasons) and dry bean (2017/2018 season) in the BGS than CM treatment was a result of lower uptake of P, K, Ca and Mg for the maize and lower uptake of P and K for dry bean. Although the results for this study indicate that the increase in dry matter in BGS treatment was not more than CM and CF treatments, its addition increased the dry matter for both dry bean and maize. The higher dry matter of dry bean in the BGS treatment in the 2016/2017 season than CM and CF treatments was due to higher uptake of P, K, Ca and Mg, and not N uptake. Amin (2011) suggested that an increase in plant growth and plant height can be attributed to N contained in treatment. The results of this study indicate that all nutrients contained in the treatment contribute to the growth and dry matter yield of the plant.

The different trends in dry bean dry matter in the 2017/2018 season than the 2016/2017 season could be because the soil levels of P, Ca, Mg were higher after the two applications (two seasons) than after first application, to a soil that was particularly deficient of P. The soil concentrations of these nutrients were relatively higher after that second season than after the first, even after accounting for the nutrients taken up by the plants. Only a fraction of nutrients become available in the soil after the first season of application of organic fertilisers (Hartl et al., 2003), and in the second season, nutrients become more relatively available for plant uptake, as a result of longer time for mineralisation. Tittarelli et al. (2007) reported that about 30-35 % of total N content becomes available from manure in the first season of application. Zhang et al. (2006) reported that the release of nutrients from organic waste and or manure mostly occurs in the second season after application.

Where there were no nutrient additions (control), soil nutrients were lower in the second season as a result of nutrient mining, even when the nutrient uptake was lower than all other treatments. These results further supported the view that the addition of these amendments enriched the soil. The 4 t ha<sup>-1</sup> (maize) and 2.6 t ha<sup>-1</sup> (dry bean)

dry matter yield in the control, where fertiliser material was not added, was relatively higher than expected under low input systems. This relatively high dry matter yield could be because the experiment was conducted under irrigation, with water that had some nutrients, compared to dryland conditions that are used in the smallholder settings. However, the high dry matter did not translate to high grain yield that was 0.8 t ha-1 for maize and 0.32 t ha-1 for dry bean. Such low grain yields were as expected under resource-poor smallholder settings. Fanadzo et al. (2009) reported that for maize only about 20 to 30% of relative yield potential of 9 to 12 t ha-1 can be expected from resource-poor smallholder farmers while DAFF (2018) reported that an average of 4.0 t ha-1 can be expected from commercial farmers under dryland farming. The lower dry matter and grain yields in the controls resulted from lower uptake of N, P, K, Ca and Mg than all other treatments that had higher levels.

The increase in grain yield with an increase in amendment rate could be explained by increased availability N, P and K, which increased their uptake, growth and grain filling. These results were in line with results reported by Malav et al. (2015b), Yu et al. (2010) and Henson and Bliss (1991). The similarity in the trends between dry matter and grain yield, in relation to an increase in N rate, suggested that higher dry matter resulted in higher grain yield. The higher grain yield for both maize and dry bean in the CF than the BGS and CM treatments in the 2016/2017 season was a result of higher uptake of P and K, for maize and higher K uptake in dry bean. The BGS and CM treatments release nutrients slowly than the CF treatment (Xu et al., 2019). The release of nutrients slowly by BGS could be beneficial in meeting the nutritional requirements of the crop in the long term (Bharde et al., 2003).

The higher K uptake was possible because CF supplied the nutrient in more readily available form, although it supplied lower total quantities at each application rate than the other two resources. For example, at 80 kg Nha<sup>-1</sup>, CF supplied 20 kg Kha<sup>-1</sup> while BGS and CM treatments supplied 54.2 and 70.2 kg Kha<sup>-1</sup>, respectively. The higher P uptake in maize and, to some extent, dry bean, was because of the higher P added through CF treatment than the other two materials. For example, at 80 kg Nha<sup>-1</sup>, CF treatment supplied 40 kg Pha<sup>-1</sup> while BGS and CM treatments supplied 18.0 and 14.2 kg Kha<sup>-1</sup>, respectively. This suggests that the benefits of treatments on nutrient supply and uptake was dose-dependent. The target yields on which the N application was based were 5 t ha<sup>-1</sup> (120 kg Nha<sup>-1</sup>) for maize and 3 t ha<sup>-1</sup> for dry bean (90 kg Nha<sup>-1</sup>),

while the highest grain yield realised, was lower with 3.4 t ha<sup>-1</sup> for maize and 1.25 t ha<sup>-1</sup> for dry bean. The lower yields than target yields were a result of lower P and K uptake especially from the BGS and CM resources than CF treatment. Although P and K were corrected for both BGS and CM treatments however based on the soil K and the target yields, the required P was 88 kg Pha<sup>-1</sup> for maize and >38 kg Pha<sup>-1</sup> for dry bean, while the required K for dry bean was 20 kg Kha<sup>-1</sup> for maize and 40 kg Kha<sup>-1</sup> for dry bean. The similarity in maize yield between BGS at 80 kg Nha<sup>-1</sup> (60 kg Nha<sup>-1</sup> for dry bean) and CF at 40 kg Nha<sup>-1</sup> (30 kg Nha<sup>-1</sup> for dry bean) suggests that it could be necessary to double the rate of BGS (based on N) if yields comparable with those of CF are to be achieved.

The higher maize grain yield in the BGS than CM treatments at higher rates could not be explained by uptake of any of the nutrients measured. The higher dry bean grain yield in the BGS than CM at higher application rates was a result of higher uptake of P, Ca and Mg, with a limited relationship with N uptake. This effect was because these elements, and possibly others not studied, were added in larger quantities and could have been in more readily available forms due to the digestion process, while those in CM required mineralisation in the soil. The nutrient content of BGS is higher than that of manure (Moller and Muller, 2012). For example, at 80 kg Nha<sup>-1</sup>, BGS supplied 18.0 kg Pha<sup>-1</sup>, 60 kg Ca ha<sup>-1</sup> and 28 kg Mg ha<sup>-1</sup> compared to 14.2 kg P ha<sup>-1</sup>, 30 kg Ca ha<sup>-1</sup> and 22 kg Mg ha-1 for CM. Organically bound nutrients are also mineralized into available form during the digestion process (Bonten et al., 2014). Phosphorus was corrected for both BGS and CM, the lower P uptake in BGS than CM treatment explain the higher extractable P in the soil treated with BGS particularly after maize for both seasons and after dry bean in the second season (2017/2018). The higher pH in BGS and CM treatments could have reduced extractable P due to the formation of calcium phosphates when compared to CF treatment. The high exchangeable Ca and Mg in soil under maize and treated with BGS than CM could be because of higher rates added (higher Ca and Mg in BGS), possibly in more available forms, together with lower uptake.

At 30 kg Nha<sup>-1</sup>, BGS added 22.5 kg Ca ha<sup>-1</sup> and 10.7 kg Mg ha<sup>-1</sup> compared to 11.3 kg Ca ha<sup>-1</sup> and 8.2 kg Mg ha<sup>-1</sup> for CM. The high extractable Ca in a soil with high pH, particularly the one treated with BGS, could have resulted in precipitation with P. This view was supported by the lower Ca and P uptake for maize in both seasons.

Phosphorus results showed that the application of N sources influenced the amount of P in soils after harvest. BGS resulted in lower P than CM but both BGS and CM were higher than CF treatment (2016/2017), BGS resulted in similar P with CM treatment although both treatments had higher than CF (2017/2018), in the dry bean field. In the maize field, BGS resulted in higher P than CM but both treatments had lower than the CF treatment in both seasons except at 40 kg Nha<sup>-1</sup> in 2017/2018. Tarkalson and Leyten (2009) reported that soils treated with organic fertilisers result in greater build-up of P than inorganic fertiliser, and that soils treated with either liquid or solid CM showed higher availability and mobility of P. The retention of P in soils is affected by many factors such as Ca, Fe and Mn concentration in soil, which might have influenced the amount of P found in this study although Fe and Mn were not studied.

The higher soil pH in the BGS and CM treatments than the CF treatment and the control after both maize and dry bean was a result of the liming effect of the original organic materials due to their high pH. The original BGS had higher pH 9.1 while CM treatment had pH 8.57, which explains the higher soil pH in BGS treatments after crop harvest (both crops) than CM. The results of the study were in agreement with Malav et al. (2015) findings. However, Ndayegamiye and Cote, (1989) reported no significant increase in pH after application of pig slurry and farmyard manure at different rates in soils, which contradicted the results of this study. The digestion process during biogas production could have increased production of ammonia, causing an increase in pH of the BGS (Moller and Muller, 2012; Bonten et al., 2014). The lower pH in the CF treatments, which decreased with an increase in application rate, was a result of nitrification, which resulted in greater acidity. The results of this study were in agreement with Xu et al. (2019), who in a study conducted in China reported lower pH from CF treatment than BGS that originated from anaerobically digestion of pig waste. Xu et al. (2019) indicated that the addition of BGS to the soil increases soil pH, this is beneficial for reducing soil acidification. While nitrification would also be expected for BGS and CM treatments, it may have occurred at a lower rate than ammonia production. An incubation study conducted by Grootboom (2019) with the same slurry and soil showed that nitrification was extremely lower (high ammonium-N and low nitrate-N) in the BGS treatment than CM treatment, suggesting that more nitrate-N was available possibly explaining the higher N uptake in the CM treatments than BGS.

Loussaert et al. (2018) suggested that uptake of nitrate-N would not significantly affect maize especially at high application, but ammonium-N would. Jackson and Volk (1992) concluded that uptake of nitrate-N and ammonium-N in maize was subject to independent internal controls and that relative proportion at which nitrate, and ammonium are absorbed could be influenced by internal as well as external conditions. In dry bean if ammonium-N is the dominant form of N, plant species show growth depression (Claussen and Lenz, 1999). Guo et al. (2002) reported that ammonium-N influences production and partitioning of dry bean and also water uptake. The higher total N, extractable P and exchangeable K in soil with the increasing rate was due to higher additions through the amendments.

The lower exchangeable K for CF than both BGS and CM treatments could be a result of lower mineralization of CF treatment, in the first season (2016/2017) as K was corrected for both BGS and CM to equal CF. However, the lack of differences (maize) or higher exchangeable K in BGS than CM at the end of the second season (2017/2018), was because of two seasons of enrichment with BGS having added nutrients in more readily available forms. These results were consistent with Xu et al. (2019) findings. Potassium results showed that increasing N application rates for both seasons increased K value in the soil after harvest. In the dry bean field, the results showed that in both seasons there were no significant differences among the treatments although increasing N application rate increased K in soil. In the maize field, BGS resulted in lower exchangeable K than the CM treatment in the first season but similar exchangeable K results were observed in the second season between BGS and CM, while CF treatment resulted in higher exchangeable K than both BGS and CM in both seasons.

The higher SOC in both the BGS and CM treatments than the CF treatment was because of additions from the organic materials. Organic material added as BGS and CM, which influences OC in soils (Ladha et al., 2014). The explanation for the higher OC in the soil amended with BGS than CM (Tables 3.5, and 3.9) was not clear. A possible explanation could be that the C in the BGS was more recalcitrant after the labile fraction was converted to biogas during the digestion process, while in the CM the C in labile fraction mineralised and got lost as CO<sub>2</sub>, resulting in higher mass loss from the soil. Bonten et al. (2014) explained that during the digestion process the degradable organic compounds are converted into biogas making the organic

compounds in the BGS more stable and less susceptible to mineralization than original manure. However, Abubaker et al. (2013) reported that the application of BGS to the soil did not strongly affect SOM content because SOM varied with the original soil C. The increase in soil C due to BGS could improve the soil physical condition and possibly overall soil productivity. BGS resulted in high OC than CM treatment but both BGS and CM were higher than CF in both seasons for both dry bean and maize. The results of the second season (2017/2018) showed the build-up of OC in the soil after BGS and CM application. The results for this study were in agreement with Ndayegamiye and Cote, (1989), who reported that the application of pig slurry and farmyard manure increased SOM in the soil. Although there was a slight increase in SOC in the short-term, the results show a potential build-up of OC in a long-term application. Nikoli and Matsi (2011) reported that application of slurry over a period of nine years at rates equivalent to recommended inorganic fertilisers for crops, increased OC, which could be true under the conditions of this study, carried out over two seasons (2016/2017 and 2017/2018). However, Ndayegamiye and Cote, (1989) reported that long-term application of pig slurry, with low carbohydrate content, could result in reduction of native OM in the soil leading to reduced OC levels.

#### 3.6 CONCLUSION

Application of the organic wastes to soil provided plant essential nutrients, which supported plant growth and built up soil reserves, compared to the control. Biogas slurry resulted in lower dry matter and grain yield (first season), and uptake of N, P, K and Ca by maize when compared with the 3:2:1 (28) CF. The BGS application resulted in similar (first season) or lower dry matter (second season) and lower grain yield (first season), lower uptake of N (both seasons), P, K and Ca (second season) for dry bean when compared with CF. The BGS resulted in higher grain yield than CM at N application rates of 80 and 120 kg Nha<sup>-1</sup> for maize and at 60 and 90 kg Nha<sup>-1</sup> for dry bean in the first season (only season studied).

The value of BGS, in terms of dry matter and uptake of nutrients, compared with CM depended on the crop and the number of seasons of application. Biogas slurry application resulted in lower maize dry matter and P, K, Ca and Mg uptake than CM. When applied to dry bean, BGS increased dry matter and uptake of P, K, Ca and Mg more than CM in the first season, while in the second season BGS had lower dry matter and uptake of P and K. Biogas slurry needs to be applied at double the rate of

CF, based on N, to achieve similar dry matter and grain yield to CF. Based on the comparison of the results of the two seasons (2016/2017 and 2017/2018), continuous application of BGS and CM limes the soils and provides nutrient and OC enrichment., Farmers may need to use CM to produce biogas and use the BGS with the same or even better nutrient value to improve soil fertility and crop yield especially where CF is not readily accessible.

# CHAPTER 4: SHORT-TERM EFFECT OF APPLICATION OF BIOGAS SLURRY, CATTLE MANURE AND CHEMICAL FERTILISER ON CO<sub>2</sub> EMISSIONS IN A MAIZE (ZEA MAYS L.) FIELD.

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#### 4.1 INTRODUCTION

Around the world, global warming is a major concern (Fares et al., 2017), caused by the increased atmospheric concentration of GHGs, particularly CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O. Studies have predicted a drastic increase in anthropogenic GHGs (IPCC, 2007; Munoz et al., 2010), which will cause an increase in temperature of the atmosphere and oceans. Understanding the drivers behind CC is critical in both managed and unmanaged ecosystems in order to identify opportunities for the reduction of GHG emissions and mitigating CC (Collier et al., 2014). For the past two decades, mitigation of CC and global warming has motivated research on GHGs (Parkin et al., 2012; Hatfield and Parkin, 2012; Nguyen et al., 2014). Understanding C and N cycles have become critical for mitigating global CC and its effects on the future of the environment (Jassal et al., 2005). There has been a greater interest in quantifying the impact of different agricultural practices on C dynamics and the amount of C sequestrated in soil (Hatfield and Parkin, 2012).

Contributions from both human activities and natural systems to GHGs must be studied thoroughly, in order to better develop mitigation strategies for anthropogenic contributions to GHGs especially CO<sub>2</sub> (Myhre et al., 2013; Collier et al., 2014). According to IPCC (2006), annual atmospheric concentrations of N<sub>2</sub>O, CO<sub>2</sub> and CH<sub>4</sub> are increasing at a rate of 0.25%, 0.4% and 0.6%, respectively. About 10-12% of the total estimated GHG emissions are reported to be from agriculture, each year (Niggli et al., 2009). Abubaker (2012) and Pelster et al. (2017) reported that about 14-25% of the total anthropogenic CO<sub>2</sub> is contributed by agricultural soils and/or agricultural practices. According to Roberston et al. (2000), SOM from organic farming significantly influences the anthropogenic CO<sub>2</sub> emissions.

Research has shown that N fertilization, irrigation, soil moisture, soil temperature, land use type, oxygen concentration, available C, soil texture and pH are major factors affecting GHG emissions from the soils (Ishizuka et al., 2002; Lang et al., 2003; Morell et al., 2010; Abubakar, 2012; Hatfield and Parkin, 2012; Yang et al., 2015). Sainju et al. (2010) suggested that fertilization of soils is expected to result in more CO<sub>2</sub> emissions keeping all other factors the same. In agricultural ecosystems, N transformations are reported to contribute to the emission of GHG, particularly CO<sub>2</sub> and N<sub>2</sub>O (Alluvione et al., 2010; Venterea et al., 2010; Rochette, 2011). Ramirez et al. (2010) indicated that urea fertilization increased CO<sub>2</sub> emissions in soils. Abbas and Fares (2009) reported that organic amendments increase soil CO<sub>2</sub> emissions, and the increase varies with weather conditions than amendment type and application level.

Amendments including organic and inorganic fertilisers are used to improve the nutrient status of the soil for better crop production (Migliorati et al., 2015). Organic amendments such as CM, BGS and compost, amongst others can help improve soil quality but could also potentially contribute to soil CO<sub>2</sub> emissions (Abbas and Fares, 2009). Fares et al. (2017) suggested that the use of organic fertilisers through organic farming can help mitigate CO<sub>2</sub> emissions through C sequestration into the soil. However, little is known about the impact of organic N fertilisers on CO<sub>2</sub> emissions in soils. A question remains on whether the use of organic N fertilisers presents an alternative to CF in terms of minimising emissions of GHGs and accumulation of soil C in agricultural ecosystems (Heintze et al., 2017).

Biogas slurry is a by-product from anaerobic digestion of organic wastes that has received much attention worldwide (Paul and Beauchamp, 1993; Islam, 2006; Abubaker et al., 2012; Smith et al., 2014; Nyang'au et al., 2016). Recently BGS has been used as an amendment to maintain soil fertility and productivity (Eickenscheudt et al., 2014). BGS can be considered a nutrient-enriched organic fertiliser for crop production as it is a good source of plant nutrients (Xu et al., 2019). After the addition of BGS to the soil more nutrients become available (Zirkler et al., 2014), more mineralization of C and N, N use efficiency and crop yields improved (Abubaker et al., 2012; Sieling et al., 2013). Terhoeven-Ureslmans et al. (2009) reported that GHG emissions could be reduced with the application of BGS, however, the effect of BGS on CO<sub>2</sub> emissions has not yet received much attention. According to Holly et al. (2017),

the use of anaerobic digestion and solid-liquid separation wastes on GHG emissions remain to be inconsistent. AM is known to release CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O to the atmosphere upon application to the soil (Collins et al., 2011). This release of CO<sub>2</sub> in soils from AM can be attributed to the decomposition of OC content in the manure by soil microorganisms (Fangueiro et al., 2008). Recently the focus on GHG emissions has been on the reduction of CO<sub>2</sub> emissions (Font-Palma, 2019). Anaerobic digested slurry like BGS with a lower C:N ratio than raw AM could result in higher CO<sub>2</sub> emissions (Holly et al., 2017; Salehi et al., 2017).

Due to its huge direct effect on GHG fluxes from the soils (Snyder et al., 2010), N fertilization should be done according to N recommended rates (Gagnon et al., 2016). Studies on CO<sub>2</sub> emissions have shown contradicting results, leading to unclear impacts of organic N fertilization on CO<sub>2</sub> emissions (Mosier et al., 2006; Venterea et al., 2010). Most of the research done on GHG emissions has focused on the effect of vegetative cover, soil tillage methods on CO<sub>2</sub> fluxes but not on N addition to soils from different organic sources such as BGS and CM at different application rates. Studies have been well documented on the increase observed in the atmospheric concentration of GHG's but little has been documented on emissions during N fertiliser application from different organic fertilisers sources in an agricultural eco-system in the sab-Saharan African region (Huang et al., 2014; Pelster et al., 2017).

The hypothesis of this study was that the application of BGS and CM at different N rates would lead to high CO<sub>2</sub> emissions than CF because of higher available C contents in the two resources although CF has higher mineral N than the two resources (BGS and CM). Furthermore, enzyme activity and OC from these resources would cause significant variations in CO<sub>2</sub> emissions in soils. The objective of the study was to evaluate the short-term effect of BGS and CM at different N applications rates on CO<sub>2</sub> emissions and the activity of enzymes involved in C and N cycling in maize.

#### **4.2 MATERIALS AND METHODS**

#### 4.2.1 Site description

The study was conducted at ARC-VOPI experimental site in Roodeplaat, Pretoria, Gauteng province of South Africa. The site characteristics and soil are as described in the Materials and Methods of Chapter 3.

#### 4.2.2 Soil sampling and initial soil characterization

Soil samples were collected randomly from each plot and taken to the laboratory within 2 hours. The sampling, preparation of the soil samples is as detailed in Chapter 3

#### 4.2.3 Biogas slurry and cattle manure sampling

The BGS and CM used in the experiments were obtained from a concentrated animal Feeding Operation in the Free State province of South Africa. Their characteristics are as detailed in Chapter 3.

#### 4.2.4 Effects of biogas slurry and cattle manure on CO<sub>2</sub> emissions under maize

#### Trial set up

The field experiment was conducted in the 2016/2017 and 2017/2018 seasons. The experimental details are in Chapter 3.

#### Trial monitoring

The trials were irrigated with water from the Roodeplaat dam, and irrigation was done every after two days for two hours. The characteristics of the water used are as detailed in Chapter 3.

#### 4.2.5 Measurement of CO<sub>2</sub> emission rate from soil

Rate of CO<sub>2</sub> fluxes was measured weekly every month starting from January to May in both 2016/2017 and in the 2017/2018 seasons using closed chamber technique (Hutchison and Mosier, 1981; Hutchinson and Livingston, 1993). Each month four measurements were collected and combined to get a mean CO<sub>2</sub> flux measurement for a month. The chamber dimensions were 50 cm x 50 cm with a height of 40 cm. The chambers were positioned over the plant row that included the treatments. The placement strategy was based on the methodology established by Parkin and Kaspar (2006) in order to measure CO<sub>2</sub> fluxes from the field. Air samples were collected from the chamber headspace using SGE gastight syringe with Luer lock valve, (Chromspec cc, South Africa) and transported to the laboratory. Air samples were collected between 10 am and 12 pm on the day of sampling, and this was done so as to minimize bias associated with diurnal variations of flux patterns. The air samples were analyzed on the same day of sampling using GC, (SRI instrument, 8610C, Manufacturer in Torrance, California, USA), equipped with an electron capture detector (ECD) and

flame ionization detector (FID). During the analysis, a certified gas standard of 10 ppm CO<sub>2</sub> (Air Liquide, South Africa) was pumped into the GC every 15 minutes. Finally, the CO<sub>2</sub> fluxes were calculated from the linear change in gas concentration over time.

#### 4.2.6 Calculation of soil CO<sub>2</sub> Fluxes

The CO<sub>2</sub> fluxes were calculated following equations Eq 2 and Eq 3.

$$CO_2(gC m^{-2}) = b * (M/V_m) * (V_{CH}/A_{CH})$$
 Equation 1

where  $CO_2$  is the emission (gC m<sup>-2</sup>), b is the change in concentration over time inside the chamber, M is the gas molar mass of  $CO_2$ ,  $V_m$  is the molar volume of the gas corrected for temperature,  $V_{CH}$  is the volume of the chamber headspace, and  $A_{CH}$  is the surface area covered by the chamber.

$$V_m = 22.4 * (273 + averag \, T^{\circ}C) \div 273$$
 Equation 2

 $V_m$  is defined as above: 22.4L mol<sup>-1</sup>; T is the average temperature during the measurement (°C) on that particular day.

Average CO<sub>2</sub> fluxes over the growing season were determined by summing up the individual flux measurements divided by the number of months during the measurement period. The fluxes expressed in gCm<sup>-2</sup> were then converted into gCkg<sup>-1</sup>, this was done to normalise the effect of C present on the CO<sub>2</sub> emission.

CO<sub>2</sub> fluxes were only measured on the maize field only. This was as a result of limited resources together with the amount of data being collected at the same time, which required attention to maintain good quality data. Based on the results of uptake and concentrations of N, P, K, Ca and Mg, for both crops, the CO<sub>2</sub> flux measurements may be reasonably representative of expected results under dry bean.

#### 4.2.7 Measurements of enzyme activity

The activity of β-glucosidase was assayed using 1 g of soil with the appropriate substrates and incubated for 1 hour (37°C) at an optimal pH as described by Dick et al. (1996). Urease enzyme activity was estimated according to Kandeler and Gerber (1988). This method was based on the estimation of urea hydrolysis in soils. Briefly,

this method involves mixing 5 g of soil with a urea solution and incubating it for 2 hours at 37°C. Enzyme activities were assayed in duplicate with one control, to which substrate was added after incubation.

#### 4.2.8 Microbial biomass

The MBC was determined following the procedure as described by Anderson and Ingram (1993). Briefly, 10g of soil weighed into 50ml beaker then extracted with extracting solution and fumigated. The extract is filtered and retained for analysis. The extract is analysed for dissolved OC.

Microbial biomass  $C = (Extracted C_{t1} - Extracted C_{t0}) \times 2.64 (Vance et al., 1988)$ 

#### 4.2.9 Statistical analysis

Data for all measured parameters, for each crop, were subjected to a one-way analysis of variance using Genstat  $18^{th}$  edition (VSN International, 2016) and the Turkey test was used to separate treatment means (p < 0.05). Correlation analysis was carried out to determine the relative importance of various enzyme activities, pH and organic matter in regulating  $CO_2$  fluxes from soil using JMP  $13^{th}$  edition.

#### 4.3 RESULTS

#### 4.3.1 CO<sub>2</sub> fluxes

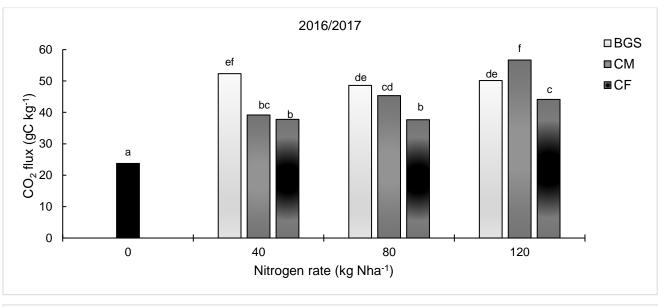
The CO<sub>2</sub> fluxes in all treatments generally declined as the season progressed. An increase in N application rate significantly increased CO<sub>2</sub> fluxes in the organic treatments but not in CF treatment. The treatments showed significant (*p* < 0.05) effects on CO<sub>2</sub> fluxes in both 2016/2017 season and 2017/2018 season (Table 4.1). In both seasons, the control had lower CO<sub>2</sub> flux than all other treatments throughout. The BGS treatment had higher CO<sub>2</sub> flux than the CF treatment throughout both seasons at each application rate, except at 40 kg Nha<sup>-1</sup> in February of both seasons (Table 4.1). The CM treatments also had higher CO<sub>2</sub> fluxes than the CF treatment throughout both seasons when applied at 80 and 120 kg Nha<sup>-1</sup>. However, at 40 kg Nha<sup>-1</sup>, the CM treatment had lower flux than CF treatment for January and February of both seasons and for April of the second season, (2017/2018), while in May they

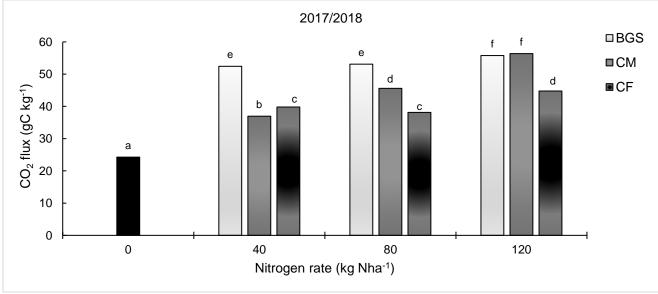
were similar (Table 4.1). When applied at 40 and 80 kg Nha<sup>-1</sup>, the BGS treatment also had higher CO<sub>2</sub> fluxes than CM for all the months of both seasons except April and May of the 2016/2017 season where CM treatment had higher CO<sub>2</sub> fluxes than BGS treatment. However, the BGS treatment had lower monthly fluxes that CM treatment when applied at 120 kg Nha<sup>-1</sup>, except in January of both seasons when BGS had higher fluxes (Table 4.1).

**Table 4.1:** Monthly variations of  $CO_2$  fluxes (gC m<sup>-2</sup>) measured from the maize field treated with BGS, CM, and CF. The different letters in the table indicate statistically significant differences according to the Tukey test at the (p < 0.05).

Treatment		Season	2016/2017			Seaso	n 2017/2018	
kg Nha <sup>-1</sup>	0	40	80	120	0	40	80	120
			<u>J</u> a	<u>an</u>				
Control	39.8a				34.86a			
BGS		103 <sup>de</sup>	104 <sup>e</sup>	113 <sup>f</sup>		104 <sup>d</sup>	104 <sup>d</sup>	117 <sup>e</sup>
CM		73.3 <sup>b</sup>	85.0 <sup>cd</sup>	105 <sup>e</sup>		73.8 <sup>b</sup>	88.2 <sup>c</sup>	104 <sup>d</sup>
CF		85.2 <sup>c</sup>	97.6 <sup>d</sup>	86.6°		85.7°	74.9 <sup>b</sup>	85.5°
			Fe	<u>eb</u>				
Control	36.8a				36.13ª			
BGS		80.8 <sup>c</sup>	106 <sup>f</sup>	127 <sup>g</sup>		81.1 <sup>b</sup>	111 <sup>f</sup>	128 <sup>g</sup>
CM		71.9 <sup>b</sup>	91.6 <sup>d</sup>	124 <sup>9</sup>		78.6 <sup>b</sup>	92.5 <sup>c</sup>	125 <sup>g</sup>
CF		97.8e	75.0 <sup>b</sup>	103 <sup>ef</sup>		98.0 <sup>d</sup>	81.7 <sup>b</sup>	102 <sup>e</sup>
			<u>M</u>	<u>ar</u>				
Control	34.6a				36.58a			
BGS		<b>72.7</b> <sup>g</sup>	75.3 <sup>h</sup>	63.9 <sup>e</sup>		91.5 <sup>fg</sup>	97.6 <sup>h</sup>	90.2 <sup>f</sup>
CM		51.7 <sup>d</sup>	67.9 <sup>f</sup>	83.0 <sup>i</sup>		68.3 <sup>d</sup>	78.7 <sup>e</sup>	93.6 <sup>g</sup>
CF		43.3°	39.3 <sup>b</sup>	37.8 <sup>b</sup>		62.1°	50.6 <sup>b</sup>	47.6 <sup>b</sup>
			<u>A</u>	<u>pr</u>				
Control	36.3 <sup>a</sup>				29.59a			
BGS		<b>69.4</b> <sup>g</sup>	<b>71.7</b> <sup>9</sup>	52.4 <sup>e</sup>		79.9 <sup>i</sup>	78.1 <sup>i</sup>	63.1 <sup>f</sup>
CM		57.3 <sup>f</sup>	77.9 <sup>h</sup>	79.9 <sup>h</sup>		50.1 <sup>d</sup>	67.1 <sup>g</sup>	74.3 <sup>h</sup>
CF		45.4 <sup>c</sup>	40.3 <sup>b</sup>	49.1 <sup>d</sup>		54.1 <sup>e</sup>	35.1 <sup>b</sup>	38.3°
			<u>M</u>	<u>ay</u>				
Control	34.8 <sup>a</sup>				28.64a			
BGS		63.6 <sup>f</sup>	58.1 <sup>e</sup>	45.4°		69.2 <sup>f</sup>	65.5 <sup>e</sup>	51.6 <sup>d</sup>
CM		49.7 <sup>d</sup>	68.8 <sup>g</sup>	74.0 <sup>h</sup>		45.7°	53.8 <sup>d</sup>	68.5 <sup>f</sup>
CF		46.0°	38.2 <sup>b</sup>	41.9 <sup>b</sup>		45.9 <sup>c</sup>	35.2 <sup>b</sup>	32.5 <sup>b</sup>

All the treatments increased average CO<sub>2</sub> fluxes in both seasons when compared with the control. Average CO<sub>2</sub> fluxes followed a similar trend in both seasons all treatments. At 40 kg Nha<sup>-1</sup>, the average CO<sub>2</sub> flux was higher in the BGS than both CM and CF treatments for both seasons, CM and CF did not show any significant difference in the first season (Figure 4.1). At 80 kg Nha<sup>-1</sup> BGS and CM had similar CO<sub>2</sub> fluxes in the first season (2016/2017) while in the second season BGS had higher CO<sub>2</sub> than CM treatment. At 120 kg Nha<sup>-1</sup> CM had higher CO<sub>2</sub> than BGS treatment in the first season while in the second season both BGS and CM had similar CO<sub>2</sub> fluxes (Figure 4.1).





**Figure 4. 1:** Average  $CO_2$  fluxes measured from maize field treated with biogas slurry, cattle manure, and chemical fertiliser. Bars with different letters significantly different at (p < 0.05).

#### 4.3.2 Enzyme activity

The control had the lowest activity of both enzymes studied. At 40 kg Nha<sup>-1</sup>, the CM treatment resulted in higher  $\beta$ -glucosidase than BGS and CF treatment in both seasons while at 80 kg Nha<sup>-1</sup> the BGS had higher activity than both CM and CF treatments. When applied at 120 kg Nha<sup>-1</sup>, the CF treatment had higher  $\beta$ -glucosidase than both BGS and CM, with BGS higher than CM treatment in both the 2016/2017 and 2017/2018 seasons. Increasing the N application rate decreased urease activity in the soil. Urease activity, at each application rate, was in the order CF > CM > BGS in the first season and CM > BGS > CF in season two. Urease activity was lower for BGS and CM and higher for CF in the first season than the second (Table 4.2).

**Table 4.2:** Effect of biogas slurry, cattle manure and chemical fertiliser on activity of  $\beta$ -glucosidase and urease in soil under maize in 2016/2017 and 2017/2018 seasons.

Treatment	0	40	80	120
	Season 2016/2017			
β-Glucosidase activi	ty (mg/kg/h)			
Control	374.1a			
BGS		760.6°	1135 <sup>i</sup>	841.9 <sup>d</sup>
CM		917.9 <sup>g</sup>	970 <sup>h</sup>	684.9 <sup>i</sup>
CF		792.6 <sup>d</sup>	884.9 <sup>f</sup>	1139 <sup>j</sup>
Urease activity (mg/l	kg/h)			
Control	5.07 <sup>a</sup>			
BGS		49.72 <sup>e</sup>	39.53°	37.54 <sup>b</sup>
CM		101.64 <sup>i</sup>	64.0 <sup>f</sup>	44.93 <sup>d</sup>
CF		110.53 <sup>j</sup>	77.51 <sup>h</sup>	67.75 <sup>g</sup>
		Season 2	2017/2018	
β-Glucosidase activi	ty (mg/kg/h)			
Control	412 <sup>a</sup>			
BGS		1017 <sup>c</sup>	1349 <sup>9</sup>	1542 <sup>i</sup>
CM		1025 <sup>d</sup>	1321 <sup>f</sup>	1363 <sup>h</sup>
CF		962 <sup>b</sup>	1192 <sup>e</sup>	1706 <sup>j</sup>
Urease activity (mg/l	kg/h)			
Control	30.03 <sup>a</sup>			
BGS		92.29 <sup>g</sup>	72.16 <sup>e</sup>	74.04 <sup>e</sup>
CM		97.01 <sup>h</sup>	96.52 <sup>h</sup>	85.36 <sup>f</sup>
CF		66.67 <sup>d</sup>	45.72°	42.69 <sup>b</sup>

The different letters in the table indicate statistically significant differences according to the Tukey test at the (p < 0.05).

#### 4.3.3 Microbial biomass carbon

In both 2016/2017 and 2017/2018 seasons, the control had the lowest MBC. Increasing N application rate increased MBC in soil. At all N application rates, CM resulted in higher MBC than BGS treatment in both planting seasons. In both seasons the highest MBC was in the 120 kg Nha<sup>-1</sup> from the CM treatment. The CF treatment resulted in lower MBC than both BGS and CM treatments in both 2016/2017 and 2017/208 seasons at all N application rates. At each N application rate, MBC was in the order CM > BGS > CF in both seasons (Table 4.3).

**Table 4.3:** Effect of biogas slurry, cattle manure and chemical fertiliser on soil microbial biomass carbon in soil under maize in 2016/2017 and 2017/2018 seasons.

Treatment	0	40	80	120	
	Season 2016/2017				
MBC (mgC/kg)					
Control	116.7ª				
BGS		307.8e	323.2 <sup>f</sup>	434.8 <sup>g</sup>	
CM		446.4 <sup>h</sup>	454.2 <sup>i</sup>	492.3 <sup>j</sup>	
CF		195.2 <sup>b</sup>	202.8°	234.0 <sup>d</sup>	
		Season 2	2017/2018		
MBC (mgC/kg)					
Control	173.9ª				
BGS		430.7 <sup>d</sup>	476.5 <sup>e</sup>	568.5 <sup>f</sup>	
CM		642.4 <sup>g</sup>	654,7 <sup>h</sup>	708.0 <sup>i</sup>	
CF		270.8 <sup>b</sup>	424.6°	424.8°	

The  $CO_2$  fluxes showed a positive correlation with pH, OM,  $\beta$ -glucosidase and urease activity in both 2016/2017 and 2017/2018 seasons (Table 4.4). In the 2016/2017 season, only OM showed a positive correlation with  $CO_2$  fluxes.

**Table 4.4:** Correlation coefficients (r) for parameters affecting CO<sub>2</sub> fluxes in the maize field.

	CO <sub>2</sub> fluxes			
Soil parameters	2016/2017 season	2017/2018 season		
рН	0.4733*	0.3801*		
Organic Carbon	0.5588*	0.5401*		
β-glucosidase P	0.3048*	0.5131*		
Urease	0.3098*	0.4692*		

<sup>\*</sup>significant at p < 0.05

#### 4.4 DISCUSSION

A distinct seasonal variation in soil CO<sub>2</sub> fluxes in 2016/2017 and 2017/2018 seasons was observed with the highest fluxes in January/February and the lowest in April/May (Table 4.1). The study was conducted in summer, dry time, which was ideal for irrigation. There was more irrigation during the start of the growing season, which then meant that there was enough soil moisture that led to higher CO<sub>2</sub> fluxes at the beginning of the growing season. The decline in CO<sub>2</sub> fluxes during the growing season especially close towards crop maturity or harvesting time could be attributed to less irrigation applied during this period. These lower CO<sub>2</sub> fluxes could also be explained by the decline in the concentration of the labile OM due to decomposition during the growing season. Chantigny et al. (2001); Collins et al. (2011) reported that the high CO<sub>2</sub> fluxes from organic amendments such as the ones studied (BGS and CM) could be expected early in the season and that could be attributed to the decomposition of the labile C sources from the organic sources that would release CO<sub>2</sub> to the atmosphere.

The CF treatment had higher CO<sub>2</sub> fluxes than control (no fertilisation) in both seasons. According to Sainju et al. (2008), the application of N fertilisers increases CO<sub>2</sub> fluxes by 14% compared to no N fertilisation, which was observed in the current study. The CF treatment had lower CO<sub>2</sub> fluxes than both BGS and CM treatments. This could be attributed to the fact that both organic amendments contained OC which then accelerates CO<sub>2</sub> fluxes in soil. The results of the current study were in line with Collins et al. (2011), who reported high CO<sub>2</sub> fluxes from solid manure and liquid slurry compared to mineral fertilisers and unfertilized soils. The fact that BGS and CM treatments at all N application rates had high CO<sub>2</sub> fluxes than CF treatment across the growing season could be attributed addition of labile OM, due to the two organic resources, which increased microbial activity. The results of the current study were in line with the findings of Fares et al. (2017) who reported that higher CO<sub>2</sub> fluxes were observed from two organic amendments treatments i.e chicken manure and bone meal more than inorganic amendments. Similarly, Cayuela et al. (2010) reported that high CO<sub>2</sub> fluxes were observed from chicken manure compared to mineral fertiliser. Abbas et al. (2012) found that organic amendments such as compost and chicken manure resulted in higher CO<sub>2</sub> fluxes than the control (unfertilized) which was the case in the current study.

Application of CF treatment does not accelerate decomposition rate in soil hence the lower CO<sub>2</sub> fluxes than BGS treatment and CM treatments. There was a positive correlation between soil CO<sub>2</sub> fluxes and β-glucosidase, urease, OC and pH (Table 4.4). Higher CO<sub>2</sub> fluxes were observed from the organic treatments (BGS and CM) than the CF treatment over the 5-month measuring period. Schuβler (2000); Yi et al. (2007) noted that the application of organic treatments to the soil led to the production of more amounts of roots returns to the soil and more C respiration. Therefore, as expected CO<sub>2</sub> fluxes from BGS and CM treatments would be much higher than CF treatment and the control. Because of the higher C:N ratio in the organic amendments, decomposition is much faster because of microorganism in the former and active and release of more CO<sub>2</sub> through respiration than those with lower C:N ratio (Fares et al., 2017). Furthermore, Fares et al. (2017) reported that soil CO<sub>2</sub> fluxes were significantly affected by the application of organic amendments, amendment type, rate of application and their interaction.

The highest CO<sub>2</sub> fluxes were recorded in the month of February from BGS and CM, 127 and 124 gC m<sup>-2</sup> in 2016/2017 season, respectively, and 128 and 125 gC m<sup>-2</sup> in 2017/2018 season, at 120 kg Nha<sup>-1</sup>, compared to CF treatment that was 103 and 104 gC m<sup>-2</sup> in 2016/2017 season and 2017/2018 season, respectively. This could be attributed to SOC that was observed to be high from both BGS and CM than CF treatment in both seasons (Table 3.5). The time of application of the amendments affected CO<sub>2</sub> fluxes that decreased with time (month) over the growing season. The results of the current study showed higher CO<sub>2</sub> fluxes in BGS treatment earlier on and higher CO<sub>2</sub> fluxes in CM treatment supporting findings by Rochette et al. (2004), who indicated that the addition of solid manure increased CO<sub>2</sub> fluxes more than manure slurry that produces short-lived spikes of emissions over several weeks. Pelster et al. (2017) reported that soil CO<sub>2</sub> fluxes may increase over a growing season and slowly decrease. Rochette et al. (1999) highlighted that the increase in CO<sub>2</sub> fluxes over the growing season can be from 0% up to 45% then decrease which was the case in the current study.

Regardless of the treatment, increased SOC accumulation occurred over the growing season (Table 3.5), and the major portion of CO<sub>2</sub> originates from the oxidation of SOC (Abbas et al., 2012). For an example, the increase in CO<sub>2</sub> fluxes for every 1% increase in SOC accumulated from BGS and CM treatments in the 2016/2017 season was 32.6,

28.6 and 29.5 gC m<sup>-2</sup> at 40, 80 and 120 kg Nha<sup>-1</sup> respectively for BGS while for CM, 19.5, 26.0 and 36.2 gC m<sup>-2</sup> were recorded. However, this was much lower from the CF treatment where 16.2, 14.4 and 20.7 gC m<sup>-2</sup> at 40, 80 and 120 kg Nha<sup>-1</sup> respectively were recorded in the 2016/2017 season. In the 2017/2018 season this increase in CO<sub>2</sub> fluxes for every 1% increase in SOC accumulated decreased as the BGS treatment at 40, 80 and 120 kg Nha<sup>-1</sup> recorded 23.9, 25.4 and 28.6 gC m<sup>-2</sup> while for CM treatment 12.3, 19.5 and 33.2 gC m<sup>-2</sup> was recorded and for CF treatment this was much lower with 17.4, 13.9 and 17.4 gC m<sup>-2</sup> at 40, 80 and 120 kg Nha<sup>-1</sup> respectively was recorded. This decrease could be attributed to relatively low soil disturbance in the 2017/2018 season as plots were not tilled in the second season, the only soil disturbance was from manual incorporation of amendments and hand weeding. Sanger et al. (2011) reported that the application of BGS treatment increased CO2 fluxes for a few weeks than after 3 to 7 weeks after application decreased to levels of the control which was not the case in the current study. The difference between the current study and Sanger et al. (2011) findings could be attributed to climatic conditions and the characteristics of the slurries from both studies. The current study was conducted in the summer season with annual minimum and maximum temperatures ranging between 24 and 29°C respectively and pH of slurry was 9.1 with C:N ratio of 13.7 while the temperature conditions in the study by Sanger et al. (2011) was controlled at 13.5°C and 23.5°C with slurry having pH of 8.9 and C:N of 8.

According to Abbas et al. (2012), the time after application of organic amendments affects CO<sub>2</sub> emissions especially during the summer season as in the current study. Although in the current study, to have CO<sub>2</sub> fluxes from BGS and CM treatments that were almost similar to that of the CF treatment took several months but CO<sub>2</sub> fluxes decreased with time (month) over the growing season. At the start of the season, CO<sub>2</sub> fluxes were high and decreased during the growing season with the month of May resulting in lower CO<sub>2</sub> fluxes compared to the first month of measurement (January). This could be explained by the decline in the substrate, soil temperature, soil pH and depletion of the readily oxidisable substrate with the approaching winter. Pretoria in summer is characterized by warm and wet days with mean annual air temperature exceeding 21 °C and winter seasons are characterized by cold and dry days with mean annual air temperature decreasing to below 10 °C in June (ARC, 2019).

The higher C:N ratio from both BGS and CM treatments than CF could also explain the reason for higher CO<sub>2</sub> fluxes from the two organic amendments. Ding et al. (2007) reported a significant correlation between CO<sub>2</sub> fluxes and SOC during maize growing season with the main factor influencing CO<sub>2</sub> fluxes being SOC. Furthermore, Zhang et al. (2014) reported that in general, higher CO<sub>2</sub> fluxes be expected because of a higher OC input and SOC content. De Nobili et al. (2001) explained that the addition of organic amendments in the soil increases the soil microbial numbers, therefore increasing CO<sub>2</sub> fluxes. Pelster et al. (2017) suggested that CO<sub>2</sub> fluxes could be influenced by possibly the combined effect of heterotrophic decomposition of SOM and root respiration. According to Zhang et al. (2014) application of organic amendments such and manure and straw increase the content of SOM and nutrients that stimulate microbial activity which maintains higher microbial biomass, enzymatic activity, and soil fertility. In the current study, β-glucosidase and urease were higher from BGS and CM treatment than CF in both seasons (Table 4.2). This could also be explained by MBC results that were higher in CM and BGS treatments than CF (Table 4.3). Increase in N application rate increased MBC with CM resulting in higher MBC than BGS and CF treatment, although BGS was also higher than CF treatment at all N application rates. Rochette et al. (1991), explained that the microbial population and activity are associated with increasing CO<sub>2</sub> emissions. BGS and CM were from the same source however, they differed in terms of physical, chemical and biological composition which led to differences in CO<sub>2</sub> fluxes between the two resources. The differences between the two (BGS and CM) indicated the importance of autochthonous C sources to the bacterial mineralization which influences CO2 emissions to the atmosphere.

Das et al. (2017) suggested that the main fuel of  $CO_2$  fluxes in soils is SOC during soil respiration which according to  $\beta$ -glucosidase and urease activity from the current study was high in both seasons for treatments of BGS and CM than CF treatment. The  $\beta$ -glucosidase activity was the enzyme responsible for high  $CO_2$  fluxes at 40 and 80 kg Nha<sup>-1</sup> for maize in both seasons, while the urease activity was the enzyme responsible for high  $CO_2$  fluxes from CF in the first season (2016/2017). The urease activity was also responsible for higher  $CO_2$  fluxes in the second season (2017/2018) in the BGS and CM plots. This view was supported by correlation analysis of  $CO_2$  fluxes with OC that showed a positive correlation. Taylor et al. (2002) explained that OC and nutrient

availability are correlated to soil enzyme activity which could play a role in the release of CO<sub>2</sub> fluxes. Fares et al. (2017) suggested that microorganisms are responsible for decomposition of OM which has an influence in CO<sub>2</sub> especially in organic amendments that have a lower C:N ratio. Raich and Tufekciolu (2000) reported that under favourable conditions increasing the supply of OC may increase microbial activity significantly and subsequently soil CO<sub>2</sub> emissions.

The results of CO<sub>2</sub> fluxes from the current study contradicts Mapanda et al. (2011), who hypothesised that organic amendment such as composted manure-N may be an option for mitigation of GHG emissions than mineral fertiliser N as these results of the current study showed low CO2 emissions from CF treatments than the high CO2 fluxes from BGS and CM treatments. The difference in Mapanda et al. (2011) and the current study could be explained by more stabilized OM in the compost compared to BGS and CM treatments. It is important to note that GHG emissions measurements may be affected by the sampling design, which may not correctly identify the hotspots of GHG production associated with the application of fertiliser (Mapanda et al., 2011). Pelster et al. (2017) highlighted that placement of chambers might slightly have an effect of microbial activity and root activity in the soils underneath the chamber and this might also cause some biases in the flux calculations because of the mixing ratios. However, the chambers were placed the same way for all treatments in this study. Some studies have highlighted that aboveground vegetation respiration and root respiration influence on CO2 emissions in a growing season (Rochette, et al., 2000; Pelster, et al., 2017). In the current study, root respiration and microbial respiration were not differentiated hence the results of CO<sub>2</sub> emissions from maize might have been slightly different from other studies that differentiated root respiration and microbial respiration.

#### 4.5 CONCLUSION

The results of the current study showed that the application of BGS, CM, and CF at different application rates to the soil increased CO<sub>2</sub> emissions, MBC, ß-glucosidase and urease activities. Higher soil CO<sub>2</sub> emissions were observed from both organic amendments (BGS and CM) treatments than CF treatment. The highest N application rate from both BGS and CM yielded to high CO<sub>2</sub> emissions, but the CO<sub>2</sub> emissions decreased with time. The application of organic amendments had a positive effect on

soil microbial biomass and enzyme activity. Enzyme activity regulated by the OC showed positive feedback toward CO<sub>2</sub> fluxes. The activity of enzymes could be better used for interpretation of CO<sub>2</sub> emissions from the soil as soil enzymes play an important role in microbial activity and population. The data collected in the current study could be used for better management practices for reducing CO<sub>2</sub> emissions in South Africa, as there is limited information available especially direct measurements of CO<sub>2</sub> emissions in soils. There is a need for more field research on CO<sub>2</sub> emissions under different organic amendments types and different application rates to help understand the effect of N fertilization on CO<sub>2</sub> fluxes especially from BGS, which has gained much interest worldwide.

CHAPTER 5: EFFECT OF CO-APPLICATION OF BIOGAS SLURRY WITH CHEMICAL FERTILISER ON DRY MATTER AND GRAIN YIELDS OF MAIZE (ZEA MAYS L.) AND DRY BEAN (PHASEOLUS VULGARIS) AND SOIL QUALITY.

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#### **5.1 INTRODUCTION**

Global pressure on agriculture for higher food production (Godfray et al., 2010) has increased agricultural intensification, to maximize crop yields per unit area (Muhmood et al., 2014). Intensification of agricultural production without replenishment of soil nutrients reserves causes soil fertility declines and poor soil quality (Gurung, 1997), a major constraint for crop productivity (Macauley, 2015; Gomiero, 2016; Vanlauwe et al., 2017). Synthetic CF have been to used supply essential soil nutrients and increase crop yields for many decades (Shahbaz et al., 2014). However, long-term excessive use of CF decreases organic soil C, microflora and fauna and overall soil quality, increase GHG emissions (FAO, 2005; MacCarthy et al., 2018) and could contaminate water bodies (Rahman et al., 2008; Shahbaz et al., 2014). In addition, each CF provides only particular essential nutrients to the crop (Kumar et al., 2015). Smallholder farmers, including those in South Africa, apply little or no CF due to high costs (Kumar et al., 2015). The reality is that the majority of smallholder farmers are finding it very difficult to access CF due to distant to markets, transport costs and the price that keeps on fluctuating. Research has shown a huge demand for eco-friendly practices to achieve sustainable food production (Malav et al., 2015; Ashenafi and Tewodros, 2018). Organic fertilisers are believed to be beneficial in improving soil quality and reduce fertilisers costs, for marginal farmers (Malav et al., 2015). The use of locally available organic fertilisers could reduce dependence on CF (Eichler-Lobermann et al., 2007; Islam et al., 2008; Rahman et al., 2008; Islam et al., 2010). Biogas slurry (BGS) produced is one of the locally available organic fertilisers.

The biogas technology produces energy through anaerobic digestion of organic wastes, like AM, thereby reducing environmental pollution (Islam et al., 2010). The BGS, a by-product after anaerobic digestion of organic waste, contains large amounts of micro and macronutrients, necessary for plant growth (Islam et al., 2010; Abubaker et al., 2012; Muhmood et al., 2014; Kumar et al., 2015). The slurry can act as a soil conditioner, while its decomposition mineralises essential nutrients, increasing their

availability in soil and crop biomass accumulation and yield (Cameron et al., 2004). While the use of CF alone decreases soil quality, desired crop yields are difficult to achieve by only using organic fertilisers (Liu et al., 2009). High crop yields, improved soil fertility levels and overall quality may be achievable through supplementing organic fertilisers with CF, especially on smallholder farms where resources are limited (Bharde et al., 2003; Kumar et al., 2015). The combined application of BGS with CF (co-application) could improve C:N ratio, nutrient transformations and possibly increase crop yields (Kumar et al., 2015). However, there are contradictory findings in the literature on the effects of co-application of BGS with CF on soil quality, nutrient availability and crop yields.

Zheng et al. (2016) reported that the co-application of BGS mainly from a mixture of pig manure and urine with CF at different ratios of BGS/CF (15/85); (30/70); (45/55) resulted in higher peanut grain yield than CF only (100% CF) in a Ultisol. Shahbaz et al. (2014) also reported increased N content in plant parts and total N uptake of okra (*Hibiscus esculentus L.*) after the co-application of BGS mainly from anaerobically digested cattle dung with CF. From their study, BGS was applied at 600 kg ha<sup>-1</sup> BGS and only CF was applied at different levels i.e (50, 75 and 100%) of recommended CF dose for okra plant. In contrast, Kumar et al. (2015) reported that co-application of BGS from anaerobically digested cattle dung with CF at ratios of 50/50, and 75/25 (BGS/CF) % resulted in lower wheat yield than CF only (100%CF). Bharde et al. (2003) suggested that rice and wheat growth and yield attributes differed significantly because of the different N sources and their combinations and or ratios.

Malav et al. (2015) reported that the addition of BGS from anaerobically digested cattle dung with CF at 50/50 ratio in an Inceptisol gave a 20% greater number of leaves, leaf area, plant biomass, and cob yield of maize, compared to CF alone. The contradictions in the literature could be a result of differences in the quality of the BGS, which depends on feedstock, combinations of BGS-to-CF, and soils and crop types used in the different studies. It is essential to determine the optimal combination for coapplying BGS, from locally available organic materials, and CF to produce higher yields of crops commonly grown on smallholder farms in Sub-Saharan Africa.

There are indications of a decline in crop productivity in the sub-Saharan Africa region (Macauley, 2015), particularly cereals and legumes, which are commonly grown on

smallholder farms. The decline in soil fertility, including low OM and nutrients, CC and high cost of CF, explain the low productivity of cereal and legume crops on marginal farms. Most biogas plants in Southern Africa use CM as a feedstock, and the BGS produced could be a useful organic fertiliser. The results of Chapter 3 indicated that CF resulted in higher maize and dry bean biomass and N uptake except at 120kg Nha-1 for maize, and lower soil pH OC, Ca and Mg after both crops, in both seasons, when compared to BGS. In addition, soil extractable P was higher in the BGS treatments after dry bean in the second season. Co-application of the two resources could result in both increased crop yield and improved soil quality. There is a paucity of literature on work done on co-application of CF with BGS for increasing soil nutrient reserves and overall quality, and cereal and legume crop yields in South Africa. The objective of this study was to determine the effects of the co-application ratio of biogas slurry with chemical fertiliser on nutrient uptake, dry matter and grain yields of maize and dry bean and soil nutrient concentrations after harvest.

#### **5.2 MATERIALS AND METHODS**

#### 5.2.1 Site description

The study was conducted at ARC-VOP) experimental site in Roodeplaat, Pretoria, Gauteng province of South Africa. The site and soil characteristics are as described in the Materials and Methods of Chapter 3 (Sections 3.2.1 and 3.2.2).

#### 5.2.2 Biogas slurry sampling

The BGS used in the experiments was obtained from a concentrated animal Feeding Operation in the Free State province of South Africa. Their characteristics are as detailed in Chapter 3 (Section 3.2.3).

### 5.2.3 Co-application of biogas slurry and chemical fertiliser as nitrogen sources for maize

Trial set up

The field experiments were conducted with maize and dry bean as detailed in Chapter 3 (Section 3.2.4). The treatments were combinations of CF 3:2:1 (28) and BGS. The BGS/CF treatments, based on percentages of recommended N rate (120 kg N/ha), were (i) 0/100, (ii) 20/80, (iii) 40/60, (iv) 60/40, (v) 80/20, (vi) 100/0. The application of treatments was performed by hand in the field. The P and K were corrected using

single superphosphate and potassium chloride so that N becomes the only limiting factor.

#### Trial monitoring

The trials were irrigated with water from the Roodeplaat dam, and irrigation was done every two days for two hours. The characteristics of the water used are as detailed in Chapter 3 (Table 3.3).

#### Plant sampling and analysis

Leaves for maize and plant samples of dry bean were collected randomly from each plot and taken to the laboratory within 2 hours. The sampling, preparation of the plant and soil samples are as detailed in Chapter 3.

## 5.3 Effects of co-application of biogas slurry and chemical fertiliser as nitrogen sources for dry bean

The trial described for maize was also conducted with dry bean at the same site as detailed in Chapter 3 (Section 3.2.5). The treatments were combinations of CF 3:2:1 (28) and BGS. The BGS/CF treatments, based on percentages of recommended N rate (120 kg N/ha), were (i) 0/100, (ii) 20/80, (iii) 40/60, (iv) 60/40, (v) 80/20 (vi) 100/0. The sampling, handling and analysis of plant and soil samples are as detailed for dry bean in Chapter 3.

#### 5.4 Statistical analysis

Data for all measured parameters, for each crop, were subjected to a one-way analysis of variance using Genstat 18<sup>th</sup> edition (VSN International, 2016) and the Turkey test was used to separate treatment means (p < 0.05). Correlation analysis for dry matter and uptake and soil concentrations was performed using JMP 13<sup>th</sup> edition.

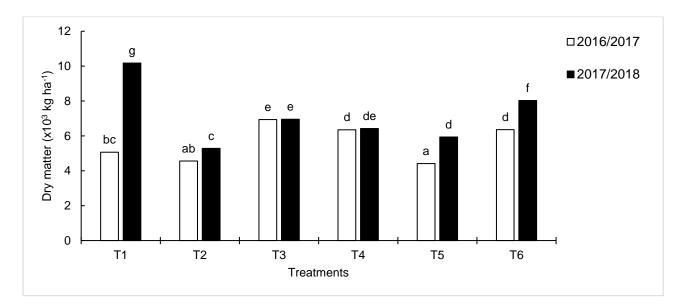
#### 5.5 RESULTS

### 5.5.1 Maize dry matter, grain yield and uptake of primary macronutrients

Dry matter and grain yield

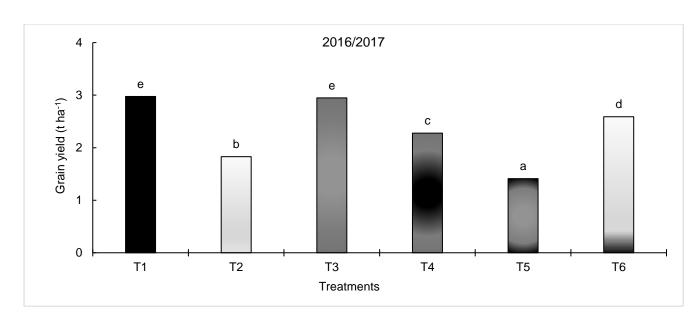
Maize dry matter yields in treatments were significantly different (p < 0.05) in both seasons (Figure 5.1). In the 2016/2017 season, the highest dry matter was in the 40/60

BGS/CF treatment followed by the 60/40 and 100/0, and the 20/80 and the 80/20 being the least. Only the 80/20 treatment had a lower dry matter than the CF only treatment (0/100) (Figure 5.1). In the 2017/2018 season, the highest was the CF only treatment (0/100) followed by the BGS only treatment (100/0), with the least being 20/80. The 40/60 and 60/40 BGS/CF treatments had similar yields. The 60/40 and 80/20 also had similar dry matter yields in the 2017/2018 season. Maize dry matter yield was higher in the 2017/2018 season, than the 2016/2017 season for most treatments except the 40/60 and 60/40 treatments, which had the same yields for both seasons.



**Figure 5. 1:** Maize dry matter as affected by biogas slurry/chemical fertiliser mixtures. Bars with different letters significantly different at (p < 0.05). The treatment are BGS/CF ratios in terms of nitrogen supplied, with T1 = 0/100, T2 = 20/80, T3 = 40/60, T4 = 60/40, T5 = 80/20 and T6 = 100/0.

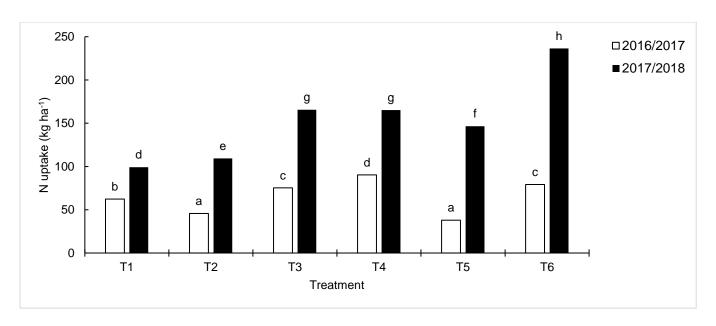
The treatments showed significantly different (p < 0.05) maize grain yields in the 2016/2017 season (Figure 5.2). The highest grain yield was in BGS/CF treatments of 0/100 (CF only) and 40/60 and the least being the 80/20. The other treatments were in the order 100/0 > 60/40 > 20/80 (Figure 5.2).



**Figure 5. 2:** Maize grain yield as affected by biogas slurry/chemical fertiliser mixtures. Bars with different letters significantly different at (p < 0.05). The treatments are BGS/CF ratios in terms of nitrogen supplied, with T1 =0/100, T2 = 20/80, T3 = 40/60, T4= 60/40, T5 =80/20 and T6 =100/0.

Uptake of nitrogen, phosphorus, potassium, calcium and magnesium

In both seasons, the treatments showed significantly different (p < 0.05) N uptake by maize (Figure 5.3). In 2016/2017 season, the highest N uptake was from treatment of 60/40 followed by the other treatments in the order 40/60 = 100/0 > 0/100 > 20/80 = 80/20. In 2017/2018 season, the highest N uptake was from the BGS only (100/0) treatment and the least being CF only (0/100). The other treatments were in the order of 40/60 = 60/40 > 80/20 > 20/80. The 2017/2018 season had a higher uptake of N by maize than the 2016/2017 season. However, the 40/60 and the 60/40 treatments were among the top three in both seasons.



**Figure 5. 3:** Nitrogen uptake by maize as affected by biogas slurry/chemical fertiliser mixtures. Bars with different letters significantly different at (p < 0.05). The treatments are BGS/CF ratios in terms of nitrogen supplied, with T1 = 0/100, T2 = 20/80, T3 = 40/60, T4 = 60/40, T5 = 80/20 and T6 = 100/0.

The BGS/CF treatments showed significant differences (p < 0.05) in the uptake of P, K, Ca and Mg for both seasons. The 2017/2018 season had higher uptake of P, K, Ca and Mg for each treatment than the 2016/2017 season, with the 40/60 and the 60/40 being among the top three in both seasons. Phosphorus uptake in 2016/2017 season was highest in the 60/40 treatment followed by CF only (0/100) and the least was in the 20/80 treatment. The other treatments were in the order 40/60 = 0/100 > 80/20. In 2017/2018 season, the highest P uptake was from the BGS only (100/0) treatment followed by 40/60 and 60/40 and the least was in the CF only treatment (0/100). The other treatments were in the order 80/20 > 20/80. In 2016/2017 season, the highest K uptake was from 60/40 treatment and the least was from the 20/80 and 80/20 treatments. The other treatments were in the order 100/0 > 40/60 > 100/0. In 2017/2018 season, the highest K uptake was from BGS only (100/0) treatment and the least was from treatment and the least was from BGS only (100/0) treatment and the least was from treatment of 20/80. The other treatments were in the order of 40/60 > 60/40 > 0/100 = 80/20.

The highest Ca uptake was from BGS only (100/0) treatment followed by 40/60, while the least was from CF only (0/100) in both seasons. The 80/20 treatment had similar Ca uptake with the CF only in the 2016/2017 season. The other treatments were in the order 60/40 > 20/80. In the 2017/2018 season, the other treatments were in the

order 40/60 > 60/40 > 20/80 = 80/20. In 2016/2017 season, Mg uptake was high in the BGS only (100/0) treatment followed by the 60/40 and the least was the CF only (0/100). The other treatments were in the order 20/80 = 40/60 > 80/20. In 2017/2018 season, the highest Mg uptake was from the 60/40 treatment followed by 40/60 and the least was from CF only (0/100). The other treatments were in order 20/80 = 100/0 > 80/20. Overall, the CF only treatment had the lowest Ca and Mg uptake for both seasons.

**Table 5.1:** Effect of co-application of biogas slurry and chemical fertiliser on uptake of phosphorus and bases by maize for 2016/2017 and 2017/2018 planting season.

		Р	K	Ca	Mg	
Season	Treatments	kg/ha				
	T1	8.40°	39.07b	14.41 <sup>a</sup>	15.60 <sup>a</sup>	
2016/2017	T2	5.12 <sup>a</sup>	30.85 <sup>a</sup>	16.07 <sup>b</sup>	21.81°	
	Т3	8.75°	40.39 <sup>b</sup>	18.98 <sup>d</sup>	21.03°	
	<b>T4</b>	10.85 <sup>e</sup>	63.32 <sup>f</sup>	17.60°	29.54 <sup>e</sup>	
	Т5	6.08 <sup>b</sup>	30.60 <sup>a</sup>	14.65ª	18.57 <sup>b</sup>	
	Т6	9.88 <sup>d</sup>	57.99 <sup>d</sup>	19.88 <sup>e</sup>	31.82 <sup>f</sup>	
	<b>T</b> 1	13.41 <sup>f</sup>	60.05 <sup>e</sup>	16.06 <sup>b</sup>	25.65 <sup>d</sup>	
2017/2018	T2	14.67 <sup>9</sup>	46.60°	25.29 <sup>f</sup>	39.36 <sup>h</sup>	
	Т3	25.34 <sup>j</sup>	68.65 <sup>g</sup>	34.80 <sup>h</sup>	49.18 <sup>i</sup>	
	<b>T4</b>	21.55 <sup>i</sup>	64.13 <sup>f</sup>	32.32 <sup>9</sup>	54.62 <sup>j</sup>	
	Т5	18.65 <sup>h</sup>	59.81 <sup>de</sup>	25.50 <sup>f</sup>	33.53 <sup>g</sup>	
	Т6	31.05 <sup>k</sup>	127 <sup>h</sup>	44.04 <sup>i</sup>	39.63 <sup>h</sup>	

Means with different letters in the same column are significantly different at p < 0.05. The treatments are BGS/CF ratios in terms of nitrogen supplied, with T1 =0/100, T2 = 20/80, T3 = 40/60, T4= 60/40, T5 =80/20 and T6 =100/0.

#### Residual soil nutrients after maize

The BGS/CF treatments showed significant differences (p < 0.05) in soil pH, total N, organic C, extractable phosphorus and exchangeable K, Ca and Mg (Table 5.2). The soil after the harvest had higher pH (except for CF only), organic C, exchangeable Ca and Mg in the 2017/2018 season than in the 2016/2017 season. Soil pH increased with an increase in the proportion of BGS in both seasons, with the highest pH in the BGS only (100/0) treatment and the lowest in the CF only (0/100) treatment. The highest soil C was in BGS only (100/0) treatment for both seasons while the least was in the 20/80 in 2016/2017 season and CF only (0/100) in the 2017/2018 season. The other treatments were in the order 60/40 > 40/60 = 0/100 > 80/20 in the 2016/2017 season and 60/40 > 40/60 > 20/80 = 80/20 in the 2017/2018 season. Total soil N was highest in the CF only (0/100) treatment with the least in the 20/80 and 80/20 in the 2016/2017 season. The other treatments were in the order BGS only (100/0) > 40/60 = 60/40. In the 2017/2018 season, the highest total N was in the 60/40 treatment and the least in the 80/20 and CF only (0/100). The other treatments were in the order 40/60 > 100/0 > 20/80. Where the proportion of BGS was higher than CF, the total soil N after harvest was higher in the 2017/2018 season than the 2016/2017 season. Microbial biomass C after harvest was higher in the 2017/2018 season than 2016/2017 season. The highest MBC was 40/60 in both seasons and the least in 0/100 treatment. The treatments were in the order 40/60 > 100/0 > 60/40 > 80/20 > 20/80 > 0/100 in 2016/2017 season and in 2017/2018 season the order was in 40/60 > 60/40 > 80/20> 20/80 > 0/100. The highest soil extractable P was 40/60 treatment followed by 60/40 in both seasons with the least being 20/80, the only one lower than the CF only (0/100) in the 2016/2017 season, while in the 2017/2018 season the 20/80 and 80/20 had the least. The other treatments were in the order 100/0 > 80/20 > 0/100 in 2016/2017 and 0/100 > 100/0 > 80/20 in the 2017/2018 season. Overall extractable P was lower in the 2017/2018 season than the 2016/2017 season, except the CF only (0/100) and 20/80 treatments. The exchangeable K was highest in the BGS only (100/0) treatment and least in the CF only (0/100), while the others were in the order 60/40 > 40/60 >20/80 = 80/20 in both seasons. Overall exchangeable K was lower in the 2017/2018season than the 2016/2017 season, except the CF only (0/100) and BGS only (100/0) treatments. In 2016/2017 season, the highest exchangeable Ca and Mg were in the CF only (0/100) treatment and the least being 80/20. The other treatments were in the order 40/60 > 100/0 > 60/40 > 20/80 for Ca and 40/60 = 60/40 = 100/0 > 20/80 for Mg. The highest exchangeable Ca and Mg were in the CF only (0/100) treatment and the least being 20/80, in 2017/2018 season. The other treatments were in the order 100/0 > 40/60 = 60/40 > 80/20 for Ca and 100/0 > 60/40 > 40/60 > 80/20 for Mg. Overall exchangeable Ca and Mg were lower in 2017/2018 season than the 2016/2017 season for each treatment.

**Table 5.2:** Soil chemical properties after maize harvest for 2016/2017 and 2017/2018 planting season.

		pH (H₂O)	TN	ОС	MBC	Extractable P	Exchangeable K	Exchangeable Ca	Exchangeable Mg
Season	Treatments			%	mgC/kg	mg/kg		cmol(+)/kg	
	T1	5.84 <sup>ab</sup>	0.103 <sup>h</sup>	0.98°	247.5ª	14.89 <sup>c</sup>	0.224 <sup>b</sup>	9.96 <sup>k</sup>	5.56 <sup>j</sup>
	T2	5.94°	0.056ª	0.93ª	260.2 <sup>b</sup>	7.50a	0.267 <sup>d</sup>	6.95 <sup>f</sup>	4.60 <sup>g</sup>
	Т3	5.93 <sup>bc</sup>	0.060 <sup>bc</sup>	0.99 <sup>cd</sup>	438.2 <sup>f</sup>	53.01 <sup>i</sup>	0.339 <sup>f</sup>	8.57 <sup>i</sup>	4.84 <sup>h</sup>
2016/2017	Т4	6.41 <sup>d</sup>	0.063 <sup>b</sup>	1.06 <sup>e</sup>	359.1 <sup>d</sup>	49.29 <sup>h</sup>	0.350 <sup>g</sup>	7.39 <sup>g</sup>	4.85 <sup>h</sup>
	Т5	6.66 <sup>f</sup>	0.054ª	0.96 <sup>b</sup>	270.8°	15.89 <sup>d</sup>	0.263 <sup>d</sup>	6.69 <sup>e</sup>	4.31 <sup>e</sup>
	Т6	7.54 <sup>i</sup>	0.069 <sup>d</sup>	1.10 <sup>f</sup>	430.7e	18.98 <sup>f</sup>	0.357 <sup>h</sup>	8.26 <sup>h</sup>	4.81 <sup>h</sup>
	T1	5.83ª	0.074 <sup>cd</sup>	1.01 <sup>d</sup>	195.2ª	17.27 <sup>e</sup>	0.227 <sup>b</sup>	8.67 <sup>j</sup>	5.05 <sup>i</sup>
	T2	6.53 <sup>e</sup>	0.067 <sup>e</sup>	1.14 <sup>g</sup>	339.0°	11.18 <sup>b</sup>	0.190 <sup>a</sup>	4.01 <sup>a</sup>	2.93 <sup>a</sup>
	Т3	6.78 <sup>g</sup>	0.096 <sup>g</sup>	1.23 <sup>i</sup>	830.0 <sup>f</sup>	31.56 <sup>g</sup>	0.238°	5.32°	3.60°
2017/2018	Т4	7.04 <sup>h</sup>	0.118 <sup>i</sup>	1.19 <sup>h</sup>	500.2e	31.42 <sup>9</sup>	0.324 <sup>e</sup>	5.30°	4.03 <sup>d</sup>
	Т5	7.08 <sup>h</sup>	0.064 <sup>bc</sup>	1.13 <sup>g</sup>	368.2 <sup>d</sup>	10.59 <sup>b</sup>	0.195 <sup>a</sup>	4.70 <sup>b</sup>	3.20 <sup>b</sup>
	Т6	7.75 <sup>j</sup>	0.086 <sup>f</sup>	1.24 <sup>i</sup>	328.2 <sup>b</sup>	15.37 <sup>cd</sup>	0.590 <sup>i</sup>	6.32 <sup>d</sup>	4.45 <sup>f</sup>

Means with different letters in the same column are significantly different at p < 0.05. The treatments are BGS/CF ratios in terms of nitrogen supplied, with T1 =0/100, T2 = 20/80, T3 = 40/60, T4= 60/40, T5 =80/20 and T6 =100/0.TN=Total nitrogen, OC=Organic carbon, MBC=microbial biomass carbon.

Maize dry matter showed a strong positive correlation with the uptake of N, P, K, Ca and Mg in both seasons except for the 2017/2018 season when a weak positive correlation with Mg uptake was observed (Table 5.3). The different soil parameters measured showed positive correlations with plant uptake and residual soil parameters after harvest in both seasons. In the 2016/2017 season, only exchangeable K showed a strong positive correlation with dry matter, while in the 2017/2018 season all measured soil parameters showed a strong positive correlation with dry matter, except total N (Table 5.3).

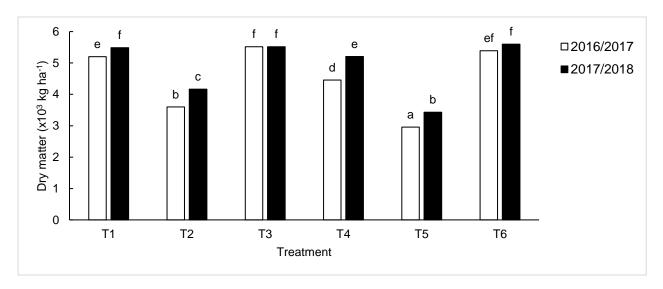
**Table 5.3:** Correlation coefficients (*r*) for parameters affecting dry matter yield in the maize field.

Parameter	Plant uptake				
<del>-</del>	2016/2017 season	2017/2018 season			
N Uptake	0.886	0.958			
P Uptake	0.814	0.974			
K Uptake	0.877	0.888			
Ca Uptake	0.872	0.936			
Mg Uptake	0.902	0.534			
	Soil	parameters			
Total N	0.336	0.296			
Extractable P	0.390	0.859			
Exchangeable K	0.822	0.899			
Exchangeable Ca	0.349	0.649			
Exchangeable Mg	0.227	0.771			
Exchangeable Mg	0.227	0.771			

# **5.5.2 Dry bean dry matter, grain yield and uptake of primary macronutrients** *Dry matter and grain yield*

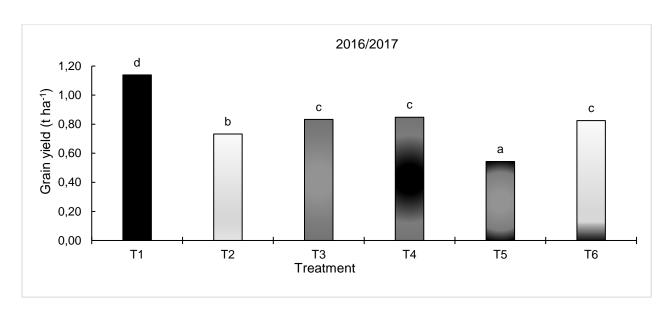
There were significant treatment differences (p < 0.05) in the dry matter of dry bean in both seasons (Figure 5.4). The dry matter yield was higher in the 2017/2018 season than the 2016/2017 season, except the 40/60 treatment, which had similar yields for both seasons. In the 2016/2017 season, the highest dry matter was in the BGS/CF of 40/60 and 100/0 treatments, the only ones higher than the CF only (0/100), and the least was in the 80/20. The other treatments were in the order 0/100 > 60/40 > 20/80 (Figure 5.4). In the 2017/2018 season, the highest dry matter was from the 0/100 = 40/60 = 100/0 treatments, followed by the 60/40 and 20/80, with the least being 80/20.

Essentially, the CF only (0/100), BGS only (100/0) and the 40/60 had the highest yields followed by the 60/40 then the 20/80, with the 80/20 being least for both seasons.



**Figure 5. 4:** Dry matter of dry bean as affected by biogas slurry/chemical fertiliser mixtures. Bars with different letters significantly different at (p < 0.05). The treatments are BGS/CF ratios in terms of nitrogen supplied, with T1 =0/100, T2 = 20/80, T3 = 40/60, T4= 60/40, T5 =80/20 and T6 =100/0.

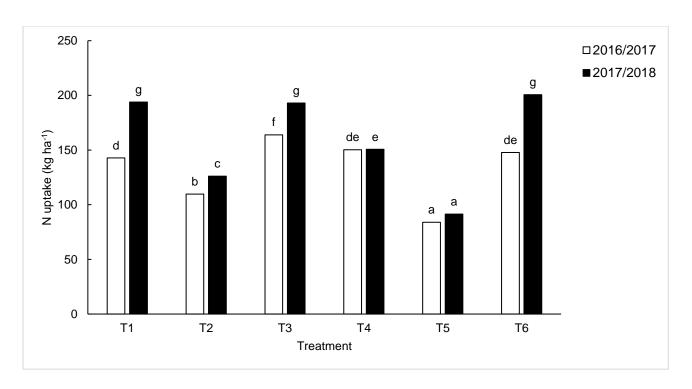
Treatments differences were significant (p < 0.05) on dry bean grain in the 2016/2017 season (Figure 5.5). The highest grain yield was in BGS/CF treatments of 0/100 and the least being the 80/20. The other treatments were in the order 40/60 = 60/40 = 100/0 > 20/80 (Figure 5.5).



**Figure 5. 5:** Dry bean grain yield as affected by biogas slurry/chemical fertiliser mixtures. Bars with different letters significantly different at (p < 0.05). The treatments are BGS/CF ratios in terms of nitrogen supplied, with T1 =0/100, T2 = 20/80, T3 = 40/60, T4= 60/40, T5 =80/20 and T6 =100/0.

Uptake of nitrogen, phosphorus, potassium, calcium and magnesium

The treatments showed significant differences (p < 0.05) on N uptake by dry bean in both seasons (Figure 5.6). The N uptake was higher in the 2017/2018 season than the 2016/2017 season for each treatment, except the 60/40 and the 80/20, which were not affected by season. In the 2016/2017 season, the highest uptake was from the 40/60 treatment and the least being 80/20. The other treatments were in the order 0/100 = 60/40 = 100/0 > 20/80. In the 2017/2018 season, the highest N uptake was from 0/100, 40/60 and 100/0 treatments followed by the 60/40 and then the 20/80, with the least being 80/20.



**Figure 5. 6:** Nitrogen uptake by dry bean as affected by biogas slurry/chemical fertiliser mixtures. Bars with different letters significantly different at (p < 0.05). The treatments are BGS/CF ratios in terms of nitrogen supplied, with T1 =0/100, T2 = 20/80, T3 = 40/60, T4= 60/40, T5 =80/20 and T6 =100/0.

There were significant (p < 0.05) BGS/CF treatment effects on uptake of P, K, Ca and Mg in both seasons. For each treatment, the uptake of P was lower and that of K, Ca and Mg was higher in the 2017/2018 season than the 2016/2017 season. In both seasons the highest P uptake was from the 40/60 followed by the 100/0 treatment and the least being 80/20. The other treatments were in the order 100/0 > 0/100 > 60/40 > 20/80 in the 2016/2017 season and 100/0 = 0/100 > 60/40 > 20/80 in 2017/2018 season.

In the 2016/2017 season, the highest K uptake was from BGS only (100/0) and CF only (0/100) treatments followed by 40/60, and then 60/40, with the least being 20/80 and 80/20. In the 2017/2018 season the highest K uptake was in the BGS only (100/0) treatment, followed by the others in the order 0/100 > 40/60 > 60/40, with the least being 20/80 and 80/20.

The Ca results showed that in the 2016/2017 season, the highest Ca uptake was from the BGS only (100/0) treatment, followed by the other treatments in the order 0/100 >

40/60 > 60/40, with 20/80 and 80/20 being the least. In the 2017/2018 season, the highest Ca uptake was from the 40/60 treatment followed by the other treatments in the order 100/0 > 0/100 > 60/40 > 20/80, with the least being 80/20. In the 2016/2017 season the highest Mg uptake was from 40/60 (BGS/CF), followed in order by 100/0 > 0/100 > 60/40 and the least being 20/80 and 80/20. In the 2017/2018 season, the highest Mg uptake was from treatment of 40/60 and 60/40 followed by the other treatments in the order 100/0 > 0/100 > 20/80, with the least being 80/20.

**Table 5.4:** Effect of biogas slurry, cattle manure and chemical fertiliser on nutrient uptake by dry bean for 2016/2017 and 2017/2018 planting season.

		Р	K	Ca	Mg	
Season	Treatments	kg/ha				
	T1	17.84 <sup>ef</sup>	74.61 <sup>e</sup>	68.62 <sup>e</sup>	31.56°	
	T2	14.75°	36.49 <sup>a</sup>	21.84ª	18.56 <sup>a</sup>	
	Т3	23.23 <sup>h</sup>	58.41 <sup>d</sup>	63.39 <sup>d</sup>	37.90e	
2016/2017	T4	17.00 <sup>de</sup>	48.08°	42.00 <sup>b</sup>	22.31 <sup>b</sup>	
	Т5	9.21ª	34.46 <sup>a</sup>	24.28ª	18.31a	
	Т6	18.82 <sup>fg</sup>	76.28 <sup>e</sup>	93.94 <sup>hi</sup>	35.86 <sup>d</sup>	
	T1	16.60 <sup>d</sup>	139 <sup>9</sup>	90.78 <sup>h</sup>	41.06 <sup>f</sup>	
	T2	12.60 <sup>b</sup>	40.85 <sup>b</sup>	77.91 <sup>f</sup>	37.27 <sup>de</sup>	
	Т3	19.41 <sup>g</sup>	81.73 <sup>f</sup>	100 <sup>j</sup>	46.89 <sup>h</sup>	
2017/2018	T4	15.29°	58.88 <sup>d</sup>	86.08 <sup>g</sup>	45.42 <sup>gh</sup>	
	Т5	8.67ª	40.34 <sup>b</sup>	56.34°	31.19°	
	Т6	17.18 <sup>de</sup>	143 <sup>h</sup>	95.93 <sup>i</sup>	44.66 <sup>g</sup>	

Means with different letters in the same column are significantly different at (p < 0.05). The treatments are BGS/CF ratios in terms of nitrogen supplied, with T1 = 0/100, T2 = 20/80, T3 = 40/60, T4 = 60/40, T5 = 80/20 and T6 = 100/0.

## Soil nutrients after harvest of dry bean

There were significant differences (p < 0.05) among BGS/FS treatments on pH, total N, OC, extractable P, exchangeable K, exchangeable Ca and exchangeable Mg for both seasons (Table 5.5). Total soil C and N and exchangeable K and Mg were higher while extractable P was lower in the 2017/2018 season than in the 2016/2017 season, except for soil C and K in the CF only treatment. In the 2016/2017 season the highest soil pH was from BGS only (100/0) treatment followed by other treatments in the order 60/40 > 40/60 = 80/20 > 20/80 and the least being 0/100. In the 2017/2018 season the highest pH was from treatment of BGS only (100/0) followed by other treatments in order 80/20 > 60/40 > 40/60 > 20/80 and the least being 0/100.

The highest OC was from treatment of 40/60 followed by other treatments in the order 100/0 > 80/20 > 20/80 and 60/40 and the least being 0/100 in the 2016/2017 season. In the 2017/2018 season, the highest OC was from the BGS/CF treatments of 100/0, 40/60, 60/40 followed by 80/20 and the least being 0/100 similar to 20/80. In both seasons the highest total N was from the BGS only (100/0) treatment and the least being 0/100 with other treatments in the order 80/20 > 60/40 = 20/80, in the 2016/2017 season, 40/60 = 60/40 = 80/20 > 20/80 in 2017/2018. Microbial biomass C after harvest was higher in the 2017/2018 season than 2016/2017 season. In the 2016/2017 season, the highest MBC was in 40/60 and the least was in 0/100. In 2017/2018 season the highest MBC was in 40/60 and the least was in 20/80 treatment. The treatments were in the order 100/0 > 40/60 > 60/40 > 100/0 > 80/20 > 20/80 in 2016/2017 season and in the 2017/2018 season the order was in 40/60 > 60/40 = 100/0 > 80/20 > 100/0 > 20/80.

Extractable P in the BGS/CF treatments was in the order 60/40 > 40/60 > 100/0 > 0/100 > 20/80 and the least being 80/20 in both seasons. Exchangeable K in 2016/2017 season was high from of BGS only (100/0) and 0/100 treatments, followed by 80/20 and the least being 20/80, 40/60 and 60/40. In the 2017/2018 season, the highest exchangeable K was from treatments of BGS only (100/0) and the others were in order 40/60 > 60/40 > 0/100 > 20/80 and the least being 80/20.

The highest exchangeable Ca in the 2016/2017 season was from the BGS/CF treatments of 100/0, 40/60, 60/40 followed by 0/100 and 80/20 and the least being 20/80. In the 2017/2018 season, the highest exchangeable Ca was from the BGS only

(100/0) treatment with the other treatments were in order 60/40 > 40/60 = 0/100 > 80/20 and the least being 20/80. The highest exchangeable Mg was from the BGS only (100/0) treatment followed by other treatments in order 0/100 > 40/60 = 60/40 > 20/80 and the least being 80/20 in the 2016/2017 season. In the 2017/2018 season, the highest exchangeable Mg was from the BGS only (100/0) treatment followed by other treatments in order 60/40 > 40/60 > 0/100 > 80/20 and the least being 20/80.

**Table 5.5:** Soil nutrient analysis after harvest in a dry bean field for 2016/2017 and 2017/2018 planting season.

		рН	ΤN	ОС	MBC	Extractable P	Exchangeable K	Exchangeable Ca	Exchangeable Mg
Season	Treatments		%		mgC/kg	mg/kg		cmol(+)/kg	
	T1	6.00 <sup>b</sup>	0.057 <sup>a</sup>	1.06 <sup>a</sup>	194.0°	7.73 <sup>f</sup>	0.265 <sup>ef</sup>	6.33 <sup>de</sup>	4.06 <sup>d</sup>
	T2	6.18 <sup>c</sup>	0.058 <sup>ab</sup>	1.09 <sup>a</sup>	140.6ª	6.65 <sup>e</sup>	0.209 <sup>a</sup>	5.73 <sup>b</sup>	3.57 <sup>b</sup>
	Т3	6.24 <sup>d</sup>	0.061 <sup>ab</sup>	1.46 <sup>d</sup>	339.0e	10.91 <sup>j</sup>	0.217 <sup>b</sup>	6.40e	3.77°
2016/2017	T4	6.34 <sup>e</sup>	0.068 <sup>bc</sup>	1.43 <sup>cd</sup>	214.1 <sup>d</sup>	11.92 <sup>l</sup>	0.203 <sup>a</sup>	6.40e	3.80°
	Т5	6.23 <sup>d</sup>	0.071°	1.17 <sup>b</sup>	166.7 <sup>b</sup>	5.67°	0.206 <sup>a</sup>	6.20 <sup>cd</sup>	3.41ª
	Т6	7.13 <sup>j</sup>	0.097 <sup>e</sup>	1.40°	373.5 <sup>f</sup>	9.85 <sup>h</sup>	0.270 <sup>ef</sup>	6.40 <sup>e</sup>	4.61 <sup>i</sup>
	T1	5.84ª	0.077 <sup>cd</sup>	1.07 <sup>a</sup>	201.5b	5.90 <sup>d</sup>	0.260 <sup>e</sup>	6.18°	4.33 <sup>9</sup>
	T2	6.41 <sup>f</sup>	0.085 <sup>d</sup>	1.08 <sup>a</sup>	174.1ª	5.31 <sup>b</sup>	0.239 <sup>d</sup>	5.48a	4.13 <sup>e</sup>
	Т3	6.41 <sup>f</sup>	0.104 <sup>e</sup>	1.93 <sup>e</sup>	341.7e	10.57 <sup>i</sup>	0.350 <sup>i</sup>	6.20 <sup>cd</sup>	4.53 <sup>h</sup>
2017/2018	T4	6.48 <sup>g</sup>	0.105 <sup>e</sup>	1.93 <sup>e</sup>	289.3 <sup>d</sup>	11.59 <sup>k</sup>	0.321 <sup>g</sup>	7.21 <sup>f</sup>	4.65 <sup>i</sup>
	Т5	6.91 <sup>h</sup>	0.099e	1.19 <sup>b</sup>	237.1°	4.93ª	0.227°	5.84 <sup>b</sup>	4.20 <sup>f</sup>
	Т6	6.99 <sup>i</sup>	0.119 <sup>f</sup>	1.95 <sup>e</sup>	296.4 <sup>d</sup>	8.38 <sup>g</sup>	0.336 <sup>h</sup>	7.65 <sup>g</sup>	5.36 <sup>j</sup>

Means with different letters in the same column are significantly different at (p < 0.05). The treatments are BGS/CF ratios in terms of nitrogen supplied, with T1 =0/100, T2 = 20/80, T3 = 40/60, T4= 60/40, T5 =80/20 and T6 =100/0. TN=Total nitrogen, OC=Organic carbon, MBC=Microbial biomass carbon.

A strong positive correlation was observed between dry bean dry matter and plant nutrient uptake for all elements in both seasons except for Mg uptake in the 2017/2018 season where a weak negative correlation was observed. Dry bean dry matter and different soil parameters showed a positive correlation for both seasons except Mg, in the 2016/2017 season, and total N, in the 2017/2018 season, showed a weak positive correlation (Table 4.6).

**Table 5.6**: Correlation coefficients (*r*) for parameters affecting dry matter yield in dry bean.

	Plant uptake			
<del>-</del>	2016/2017 season	2017/2018 season		
N Uptake	0.992	0.530		
P Uptake	0.889	0.484		
K Uptake	0.916	0.537		
Ca Uptake	0.777	0.642		
Mg Uptake	0.859	-0.009		
	Soil	parameters		
Total N	0.927	0.282		
Extractable P	0.942	0.739		
Exchangeable K	0.828	0.813		
Exchangeable Ca	0.937	0.774		
Exchangeable Mg	0.453	0.888		

#### 5.6 DISCUSSION

# Effects of co-application of BGS and CF on maize dry matter, grain yield and nutrient uptake and soil nutrients after harvest

Dry matter for maize was attributed to uptake of N, P, K, Ca, and Mg that supported plant growth in both 2016/2017 season and 2017/2018 seasons but to a lesser extent Mg in the 2017/2018 seasons. This was supported by a strong positive correlation between dry matter and N, P, K, and Ca. The BGS/CF treatments of 40/60 and 60/40 resulted in more readily available plant nutrients, which supported nutrient uptake and plant growth in both seasons. Dry matter yield for maize in BGS/CF treatments of 40/60 and 60/40 resulted in 6.9 t ha¹ and 6.3 t ha¹ compared to 5.0 t ha¹ of CF only (0/100) in 2016/2017 season. These results were in agreement with findings of Malav et al. (2015), which indicated that plant biomass yield of baby corn was higher in the BGS/CF treatment at 50/50 ratio than the other corresponding treatments, especially CF only treatment. Farmers could apply BGS/CF at 40/60 to take advantage of the reduced CF in the first season of planting. However, the advantage in terms of dry matter yield is lost in the second season with BGS/CF treatments of 40/60 and 60/40 having 6.9 and 6.4 t ha¹¹ respectively, compared to 10.1 t ha¹¹ in CF (0/100).

The huge difference from the CF only (0/100) treatment compared to 40/60, 60/40 in the 2017/2018 season can be attributed to the fact that the CF only (0/100) treatment provided nutrients at a more available form than 40/60 and 60/40 BGS/CF treatments. The lack of differences between the two seasons for BGS/CF treatments 40/60 and 60/40, showed that the benefits of the application of BGS with CF and would help in sustaining crop production and subsequently would lead to higher dry matter yields in a long term. The fact that BGS/CF treatments 20/80 and 80/20 were among the lowest for all parameters measured, suggests that these ratios may not be the best for BGS use, with limited variations as a result of seasonal differences.

The higher maize grain yield, particularly in the BGS/CF treatments of 0/100 (2.9 t ha<sup>-1</sup>) and 40/60 (2.9 t ha<sup>-1</sup>) in the 2016/2017 season, could be attributed to uptake of N, P, K, Ca and Mg. This view was supported by the increased dry matter yield and high correlation between nutrient uptake and dry matter in the first season of planting. The higher dry matter yields and plant nutrient uptake obtained from the BGS/CF treatments 40/60 and 0/100 translated to higher grain yield. There is a significant

benefit of co-applying the two resources at 40/60 than BGS alone in terms of maize grain yield. The reduction of CF by 40%, (40/60), resulted in a similar yield as CF alone, which could significantly reduce fertiliser costs while maintaining grain yields. Higher grain yields from BGS/CF treatments of 40/60 and 100/0 than any other treatment could be explained by higher N uptake from these particular treatments. However, the higher grain yields from BGS/CF treatment 0/100 in the first season could not be explained by the lower N uptake observed from in that treatment. These results agreed with Nasir et al. (2012) and Debebe and Itana (2016), who reported that the application of BGS/CF at 50/50 ratio resulted into similar cabbage yields when compared to CF only (100%) treatment. Contrary to these results, Malav et al. (2015) reported that the ratio of 50/50 (BGS/CF) in an Inceptisol yielded higher grain yield for baby corn than CF only (100%) treatment, where BGS was from anaerobic digested cattle dung. The differences between the results in the literature could be a result of the guality of the BGS used.

Higher N uptake from BGS/CF treatments of 40/60, 60/40 and 100/0 than the CF only (0/100) treatment in both seasons demonstrates the benefits of BGS. Higher N uptake observed from these treatments especially in the 2016/2017 season translated into higher dry matter yields. These results were in agreement with the findings by Malav et al. (2015), who reported that the application of BGS/CF at 50/50 ratio resulted in higher N uptake than CF only (0/100) treatment. The higher N uptake in the second season, than the first, could be attributed to possible build-up of soil total N in the second season that resulted in higher N uptake and increased dry matter yields. Higher soil total N after maize harvest in the 2017/2018 season suggests that BGS results in build-up of soil N. The results of this study were contrary with findings of Debebe and Itana (2016), who reported that variation in rate of BGS/CF did not have any effect on the amount of residual total N especially after cabbage. Debebe and Itana (2016), explained that because cabbage in nature is a heavy feeder of N and P, therefore, the change in residual N is difficult to monitor. Shahbaz et al. (2014) suggested that in a single season (one), the application of BGS/CF could not result in major effects, which was the case in the first season for maize.

Higher P uptake by maize from BGS only (100/0), 40/60 and 60/40 treatments than the CF only (0/100) treatment could be explained by the increase in soil pH, which made P available for plant uptake. Soil pH observed in the first season from BGS/CF

treatments of 40/60 and 60/40 was 5.93 and 6.41, respectively, that resulted in increased P availability. Most P becomes available at a pH range of 6.5 and 7.5 (Havlin et al., 2004) and hence higher extractable soil P was observed from these treatments. The pH was in the optimal range in these two treatments than any other treatments with a higher and lower proportion of BGS. The highest extractable P in the 40/60 and 60/40 treatments supports the view that high P uptake by maize in these treatments, was a result of greater availability. However, higher soil pH (pH > 7.0) observed in the second season from BGS/CF treatments of 100/0, 40/60 and 60/40 could have decreased extractable P than the first season for these treatments, due to precipitation of Ca phosphates (Havlin et al., 2004).

Higher K uptake from BGS/CF treatments of 40/60 than 0/100 can be attributed to high K that was contained in BGS, hence higher K uptake from maize. The higher uptake of K, Ca and Mg in the second season than the first, for all treatments suggested rapid accumulation of these elements that subsequently led to higher dry matter. The higher Ca uptake from BGS only (100/0), 40/60 and 60/40 treatments than CF only (0/100) could be attributed to the total Ca supply by the BGS resource that resulted in higher dry matter yield in maize than CF only treatment. The higher exchangeable Ca and Mg in soil from the CF only treatment in the 2016/2017 season could be explained by lower uptake by the maize. High Mg uptake from BGS/CF treatments of 40/60, 60/40 and 100/0 in both seasons could be attributed to Mg supplied by BGS.

The higher OC, exchangeable Ca and Mg in the 2017/2018 season than 2016/2017 shows the inclusion of BGS will result in the increase in these parameters, and improve soil quality, over time. The higher soil organic C in maize from the treatment of BGS (100/0) than CF only (0/100) and other treatment combinations can be attributed to higher organic C supplied BGS. The addition of organic amendments enhances soil organic C concentration that is an important indicator of soil quality and crop productivity (Brar et al., 2015). Merbach and Schulz (2013) and Simon et al. (2015) reported that an increase in soil organic C occurs after a long-term application of organic fertiliser and that decomposition of soil organic matter in soil depends on soil pH (Weintraub and Schimel, 2003). The relatively higher soil pH in treatment that contained BGS was because of the liming effect of BGS that had pH 9.10 when compared to CF only (0/100) treatment that was relatively lower in both seasons. The digestion process during biogas production could have increased the production of

ammonia, causing an increase in pH of the BGS (Moller and Muller, 2012; Bonten et al., 2014). The lower pH from the treatment of CF only (0/100) could be attributed to the nitrification process.

# Effects of co-application of BGS and CF on dry bean dry matter, grain yield and nutrient uptake and soil nutrients after harvest

The trends observed for maize dry matter also applied for dry bean, with higher dry matter observed from the BGS/CF treatment 40/60 (5.5 t ha<sup>-1</sup>), compared to 5.3 t ha<sup>-1</sup> for CF only (0/100) in 2016/2017 season, while in 2017/2018 season similar dry matter yields of 5.5 t ha<sup>-1</sup> (40/60) and 5.5 t ha<sup>-1</sup> (0/100) were observed. Dry bean dry matter yield could be attributed to uptake of N, P, K, Ca, and Mg, as supported by a strong positive correlation between dry matter and uptake of these plant nutrients in both seasons, except for Mg in 2017/2018 where a weak negative correlation was observed. The higher dry matter 40/60 treatment than CF only (0/100) in the first season was evidence that the application of BGS/CF increased the production of assimilates, and no difference between the two treatments in the second season could be attributed to the accumulation of nutrients supplied by BGS resource. Similar dry matter of BGS only (100/0) had with CF only (0/100) in both seasons, shows that there was no yield advantage of co-applying BGS with CF than applying these resources separately. Although the treatment of 40/60 in 2016/2017 resulted in a higher dry matter than CF only (0/100), there was no difference with 100/0 in both seasons. It is essential to point out that the co-application of BGS and CF does not result in any added dry matter and grain yield of dry bean compared to the application of the two resources separately. In essence, the application of BGS alone results in the same dry matter, and 83% of the grain yield, as CF only treatment. Some ratios actually reduce the yields significantly. These results were in line with Haile and Ayalew (2018), who reported higher dry matter yields of kale (Brassica oleracea L.) from BGS/CF treatments of 100/0 and 0/100 than 25/75, 50/50 and 75/25 treatment combinations. The lower dry matter from treatments of BGS/CF (20/80) and 80/20 compared to 0/100 suggests that dry bean utilized whatever nutrients that were more available to support plant growth. The higher maize dry matter yield in the 40/60 and 60/40 treatments, while there was no dry matter yield advantage of co-application, suggests that the effects depend on the crop.

The increase in grain yield of dry bean particularly from BGS/CF treatments of 0/100 (1.1 t ha<sup>-1</sup>), 40/60 (0.8 t ha<sup>-1</sup>), 60/40 (0.8 t ha<sup>-1</sup>) and 100/0 (0.8 t ha<sup>-1</sup>) can be attributed to nutrient uptake of N, P, K, Ca and Mg. The higher N, P, K, Ca and Mg uptake possible increased grain yield. Nutrients from BGS are less readily available for plant uptake than CF, which might have influenced the lower yields from treatment combinations that were high in BGS than CF. These results suggest that farmers may benefit from higher grain yields in the first season of the application of BGS/CF. The lower grain yields from BGS/CF treatments of 20/80 and 80/20 could possibly be explained by the effect of soil pH that might have limited P availability and uptake while it is optimum for 40/60 and 60/40 treatments. Although this behavior is not clear, it is consistent for all parameters studied.

Higher N uptake from the BGS/CF treatment 40/60 in the 2016/2017 season than treatments of CF only (0/100) and BGS only (100/0) showed the benefits of coapplication of BGS with CF. This view was supported by dry matter yield that was high from 40/60 treatment than CF only treatment. The BGS/CF treatment of 40/60 is the only co-application treatment that increases N uptake by dry bean than BGS only and CF only treatments. These results showed that applying BGS only gives the same N uptake and dry matter yield as CF only, such that farmers may not lose out on dry bean when they apply BGS only instead of CF. Shahbaz et al. (2014) noted that the application of BGS with CF increases N uptake, which was the case in the current study, particularly in the first season. Higher N uptake from BGS/CF treatments of 0/100, 40/60 and 100/0 in the second season could be attributed to the build-up of residual soil N that was found to be high from these particular treatments in the second season. The higher N uptake from these particular treatments resulted in similar grain yields observed in the first season although they were not higher than the CF only treatment. There is no advantage of co-application of the two resources compared to BGS alone, in terms of total soil N. Application of BGS alone increases total soil N (and exchangeable K, Ca and Mg) than the co-application and the CF only.

The higher P uptake in the dry bean from BGS/CF treatment 40/60 in both seasons could be attributed to optimum soil pH, which increased P uptake than when the two products BGS only and CF only are added separately. The lower P uptake values from BGS/CF treatment of 80/20 than all others could be explained by soil pH that might have limited P availability especially in the second season where lower P uptake and

lower soil extractable P values were observed. The higher soil extractable P after dry bean in both seasons in the BGS/CF treatments of 60/40 and 40/60 could be attributed accumulation of P from the balanced BGS/CF ratio of the two treatments. This view was supported by high P uptake and extractable P results from the treatment of BGS (100/0) which was higher than CF (0/100) for dry bean in both seasons. Co-application of the two resources at 40/60 and 60/40 increased soil extractable P compared to BGS alone and CF alone. The results of this study contradict findings by Muhamood et al (2014); Shahbaz et al. (2014), who reported that the application of BGS/CF at any combination ratio showed no significant change in extractable P content in the soil. The reason for the difference between the results of the current and those of Muhamood et al (2014); Shahbaz et al. (2014), could be explained by the pH levels observed in the studies after application of BGS with CF.

The higher K uptake observed from the BGS only (100/0) treatment in both seasons suggests the benefits of BGS compared with the CF. There appears to be no advantage in co-applying BGS and CF on K uptake and exchangeable K after harvest of dry bean under the conditions studied. Application of BGS alone is more beneficial in terms of increasing K uptake soil exchangeable K than the co-application and the CF only. This could be attributed to the amount of K that was contained in the original BGS. Co-application at 40/60 and 60/40 increases soil exchangeable K than CF only, indicating the contribution of BGS. The results of this study were in agreement with Malav et al. (2015) who reported a positive contribution to the availability of K in the soil after the application of BGS.

The higher Ca uptake from the BGS only (100/0) treatment in the first seasons for dry bean could be attributed to Ca contained from the original resource (BGS). However, in the second season, the co-application of BGS and CF at the 40/60 increases Ca uptake than the two products when applied separately. This could be because of higher dry matter accumulation, resulting in more Ca being taken up in this treatment. The higher soil exchangeable Ca that was high from the 40/60; 60/40 treatments suggest the benefits of co-application of BGS with CF. However, the increase in exchangeable Ca from the treatment of 100/0 in the second season be attributed to Ca in BGS and potential build-up of Ca. The results from the second season show that there is no added advantage of the co-application of the two resources compared to BGS alone, in terms of exchangeable Ca. The higher Mg uptake from BGS/CF

treatment of 40/60 in the first season and 60/40 in the second season suggest the potential of co-application of the two resources. The higher soil exchangeable Mg in the BGS only (100/0) treatment in both seasons than any other treatment suggested that there is no added advantage of co-application of the two resources on exchangeable Mg after harvest when compared with BGS alone. Co-application at 40/60 and 60/40 increases soil exchangeable Mg than CF only.

The highest soil pH BGS only (100/0) treatment in both seasons suggests that BGS could be beneficial in reducing soil acidity and increase the availability of P. Soil pH increased with an increase in the proportion of BGS. Application of BGS alone increases soil pH, total soil N, exchangeable K, Ca and Mg than the co-application and the CF only. Xu et al. (2019) noted that lower pH levels from CF treatment inhibited soil ecological function compared to soil treated with treatments containing BGS. Malav et al. (2015) reported no improvement in soil pH when soils were treated with BGS/CF at the different combinations in one season of application, which was not the case in the current study. Long-term application of BGS/CF could be beneficial to farmers in increasing their soil pH, in addition to the improvement of soil quality.

The higher soil organic C from BGS/CF treatment of 40/60 in the 2016/2017 season and in 40/60, 60/40 and 100/0 in 2017/2018 could be attributed to higher OC in BGS. The higher concentrations suggest a build-up of soil quality over time. This could also improve soil biological life, including enzyme activity.

### 5.7 CONCLUSION

Co-application of BGS with CF at 40/60 and 60/40 ratios improved maize dry matter than the two resources applied separately, while only the 40/60 had the highest grain yield, similar to CF alone. In the first season, co-application at 40/60, 60/40 and BGS only (100/0) increased maize uptake of N, P, K, Ca and Mg when compared with CF, and all treatments with BGS resulted in greater uptake of these nutrients than CF. There is no advantage of co-application on the uptake of other nutrients by maize when compared to BGS alone. Co-application of BGS with CF at ratios of 40/60 increased dry matter, grain yield and uptake of N, P and Mg of dry bean in the first season, than the two resources separately while no benefit was realised on dry matter, grain yield and uptake of N, P, Ca and Mg in the second season compared to BGS alone.

After maize and dry bean harvest, soil pH and total N increased with the proportion of BGS, and treatments with BGS, particularly the BGS only, 60/40 and 40/60 had higher organic C, extractable P, and exchangeable K. While there were no benefits of coapplication on exchangeable Ca and Mg after maize, the BGS only, 60/40 and 40/60 had higher exchangeable Ca and Mg. Smallholder farmers could take advantage of BGS by applying at 40-60% of the recommended N ha<sup>-1</sup> of and supplement with CF for greater dry matter and grain yields of maize and dry bean and improved soil quality. Long-term research is needed on the effect of co-application of BGS and CF on crop yields, soil quality and greenhouse gas emissions, particularly CO<sub>2</sub>, under dryland field conditions, as practised by smallholder farmers in Sub-Saharan Africa.

## CHAPTER 6: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS 6.1 GENERAL DISCUSSION

Globally there has an increasing interest in BGS produced from anaerobic digestion. A large number of scientific literature has focused on the use of biogas digester for production of energy than the complexity of BGS use on agricultural soils. According to FAO (2013), smallholder farmers, extension services, universities and non-governmental organisations are not fully aware of the numerous benefits of BGS. Several studies on BGS has shown the positive effect on yields of grains and vegetable. However, no studies have analysed the implications of BGS on maize and dry bean and the effect of BGS on CO<sub>2</sub> emissions under field conditions. The comparison of BGS to other organic fertilisers like CM amongst others or CF still remains very unclear.

Application of organic waste to soil provided essential plant nutrients that supported plant growth and increased soil reserves. Large amounts of organic wastes are produced worldwide in urban and rural areas from different sources such as livestock, industries, households and agriculture (Abubaker, 2012). The biogas technology involves anaerobic digestion of any organic waste, a process that produces energy (methane), and also BGS as a by-product that needs to be disposed of. The BGS contains a higher NH<sub>4</sub>+-N/total N ratio, decreased dry matter and total C content, reduced biological oxygen demand, elevated pH values, smaller C:N ratio and reduced viscosity (Moller et al., 2008). As a result of its nutrient composition BGS may have significant value as a source of nutrients, separately or when co-applied with CF for a variety of crops with the improvement of soil quality, on smallholder farms. Soil fertility and nutrient management problems could be resolved with the application of BGS and or organic fertilisers that positively influence soil fertility and crop productivity (Smith et al., 2014). It is important to note that, the chemical composition of BGS could depends on the quality of the feedstock and the biogas production process used. The effect of its decomposition and mineralization of constituent elements may also contribute to CO<sub>2</sub> emissions. The overall objective of the study was to investigate the effect of BGS relative to CM and CF, on dry matter, grain yield, soil quality after harvest of maize and dry bean crops and on CO<sub>2</sub> emissions in soils under field conditions.

The results of the study showed that N uptake in BGS was lower than that of CF for both maize and dry bean in both seasons (2016/2017 and 2017/2018) and this could mainly be attributed to the part of the N in BGS which is present as organic N and N mineralization required. The higher N uptake in the CF treatment translated into higher maize dry matter yields observed in the second season, although in the first season CM had higher dry matter than both BGS and CF treatments. Grain yield from the CF treatment was higher than both BGS and CM which could be attributed to higher N uptake. However, the lower uptake from BGS and CM did not affect dry matter yield of dry bean as higher dry matter yields from BGS were observed for N application rate of 30 and 90 kg Nha-1 in the 2016/2017 season while CM had higher dry matter yield at 30 and 60 kg Nha<sup>-1</sup> in the 2017/2018 season. According to Janssen (1996); Alburquerque et al. (2012), the N mineralization of organic products depends on the decomposability and the C:N ratio of the OM. Moller and Muller (2012) found that in a pot experiment where BGS and AM were applied equivalently, BGS gave higher N uptake by about 10-25% than AM and this was explained by the higher ammonium-N/total N in BGS than in AM.

Contrary to their findings, results of the current study showed that N uptake from CM was higher than BGS for both maize and dry bean at all N application rates, and except only at 90 kg Nha<sup>-1</sup> for dry bean, BGS resulted into similar N uptake with CM in the first season (2016/2017) and higher N uptake than CM in 2017/2018 season. BGS, when applied alone will not supply enough N to support biomass production, particularly in the first season. As a result, co-application with CF may increase the availability of N throughout the growing season. The treatments of BGS/CF (40/60) and BGS/CF (60/40) led to higher N uptake than BGS/CF (0/100) in both planting seasons for maize while for dry bean 40/60 had higher N uptake than 0/100 in the first season and in the second season, 40/60 and 0/100 were similar. This could be attributed to the organic N added by BGS and also the chemical composition of the BGS and CF combination.

The higher N uptake from the treatment of 40/60 translated into higher dry matter and grain yield than 0/100 for maize in the first season although, in the second season, 0/100 had higher dry matter than any other treatment. The same was true for dry bean as higher N uptake from 40/60 resulted in higher dry matter yields than 0/100 in the first season. In the second season 40/60, 100/0 and 0/100 resulted in similar dry matter

yields. Higher N uptake from the treatment of 40/60 did not translate into higher grain yields as 0/100 treatment resulted in higher grain yields than all other treatment combinations. It can be deduced from the results of this study that the best combination of 40/60 as it relates to N uptake yielded to better dry matter and grain yields than any other treatment combination at least in a short-term application. The treatment combination of 40/60 could prove to better soil quality and add value in the future.

The pH of the BGS used in the study was higher than that of CM, which increased the soil pH to a range that was favourable for nutrient uptake for both maize and dry bean. Velthof et al. (1998) stated that P in the organic products may be available in many forms which then makes it differ in the availability and uptake by plants. The amounts of P, K, Mg and Ca contained in BGS are similar to those of AM (Bonten et al., 2014). Islam et al. (2010) explained that increasing the N application rate increases the residual P and K, and some of the other micronutrients that could increase meristematic growth which could lead to higher dry matter yields. Soil extractable P was lower in BGS and CM than CF (at all N application rates) in both seasons when applied separately for maize while for dry bean, both BGS and CM had higher extractable P than CF treatment. For maize, extractable P in BGS was higher than in CM for both seasons. Higher extractable P from BGS did not influence P uptake for maize as CM resulted in higher P uptake than BGS.

The CF treatment had higher P uptake than both BGS and CM. This higher P uptake from CF translated into higher grain yield as the CF treatment at all N application rates had higher grain yield than both BGS and CM for maize. Xu et al. (2019) reported that BGS and CM release nutrients slowly than CF which than results in lower yields. Although BGS had lower extractable P than CF, BGS resulted in higher grain yields than CM and 80 and 120 kg Nha<sup>-1</sup> for maize. The low extractable P from BGS could be attributed to the fact that BGS is a slow releaser of nutrients but could be beneficial in the long-term which could help in meeting the nutritional requirements of the crop (Bharde et al., 2003). Warnars and Oppenoorth (2014) concluded that comparing CF and BGS, the biodegradation of OM in BGS is a slow process that is better for nutrient assimilation by plants, which would mean that in a long-term BGS would be of better value.

In the dry bean field only in the second season (2017/2018), BGS had higher extractable P than CM. The higher extractable P in BGS did not influence P uptake as higher P uptake was observed in CM more than BGS although the overall P uptake was higher in CF treatment. BGS resulted in lower exchangeable K than CM and although CM and CF were similar in the first season. However, in the second season, both BGS and CM were similar in exchangeable K although CF was higher than both BGS and CM in maize. For dry bean extractable K in BGS and CM were similar in 2016/2017 season and in 2017/2018 season BGS had higher exchangeable K than CM. The CF treatment had lower exchangeable K in 2016/2017 season and higher in 2017/2018 season than BGS and CM. Higher exchangeable K influenced K uptake as higher K uptake as observed from BGS than both CM and CF in 2016/2017 season but in 2017/2018 season, CF resulted in higher K uptake than BGS and CM at all N application rates. Although CF had lower K uptake, grain yield was influenced by K as higher grain yield for dry bean was observed from the CF treatment than both BGS and CM.

The availability of nutrients in both BGS and CM resulted in greater uptake of P and K which then influenced the growth of both maize and dry bean. The lower dry matter yield for maize (both seasons) and dry bean (2017/2018) in the BGS and CM than CF as a result of lower nutrient uptake of P, K, Ca and Mg for maize and lower uptake of P and K for dry bean. Availability of nutrient from organic sources is based on the total nutrient content, efficiency of those sources to meet nutrient requirements of crops is not as assured as CF, however application of organic sources with CF is capable of sustaining higher crop productivity, improving soil quality and productivity in a long-term basis (Yadav et al., 2013). The higher availability of P and K, among other nutrients after co-application of BGS with CF for both maize and dry bean could explain the increase of P and K uptake and increased dry matter yields after application with the combination ratios. The fact that nutrients from BGS are not readily available for plant uptake might have influenced the lower yields from treatment combinations that were higher in BGS than CF e.g 100/0 and 80/20, especially for maize.

During co-application of BGS with CF, treatment of BGS/CF (40/60) and 60/40 resulted in higher dry matter than 0/100 in the first season of planting for maize while for dry bean only the treatment of BGS/CF (40/60) was higher than 0/100 in the first season. The higher dry matter yields and plant nutrient uptake obtained from treatment

of 40/60 in maize translated to higher grain yields, however for dry bean higher dry matter yields and nutrient uptake did not translate to higher grain yields. Debebe and Itana (2016) obtained similar findings where the application of BGS/CF at 50/50 ratio resulted into similar cabbage yields from BGS of cattle dung when compared to CF only (100%) treatment. However, Malav et al. (2015) reported that the ratio of 50/50 (BGS/CF) yielded higher grain yield for baby corn than CF only (100%) treatment. This suggests that the farmers could take advantage of reduced CF in the first season of planting. Application of BGS with CF at a ratio of almost 50/50 gives an advantage in the first season of planting as the treatment of 0/100 (BGS/CF) resulted in higher dry matter than any other treatment combination for maize meant that farmers could still benefit from coapplication of BGS with CF in the long-term as this advantage was not lost for dry bean the crop.

The huge difference from the treatment of CF only (0/100) compared to 40/60 and 60/40 treatments in the second season of planting for maize could be attributed to the fact that treatment of 0/100 provides nutrients at a more available form than 40/60 and 60/40 (BGS/CF) treatments. After co-application of BGS with CF, the extractable P, K and exchangeable Ca and Mg in both planting seasons showed that inclusion of BGS can result in increased maize and dry bean parameters and improve soil quality over time. Although other nutrients were not studied, soil quality may also have been improved by increasing soil micronutrient concentrations including Cu, Zn, Mn and Fe. Availability of nutrients in soil particularly the nutrients studied increased their uptake from the soil leading to increased grain yields for both maize and dry bean crops. Henson and Bliss (1991) suggested that the effect on increased uptake of any nutrient in soil responsible for higher dry matter and grain yield varies with the plant, type of fertiliser and timing of application.

In addition to adding plant essential nutrients, the BGS and the CM add C to the soil, unlike the CF. Wentzel et al. (2015) reported that the addition of BGS or undigested cattle manure had no significant effect on soil OC and or N in a long-term application. However, the amount of OC in the soil after harvest from the current study was affected by the addition of BGS or CM with higher OC observed from treatments of BGS than both CM and CF at all N application rates for both maize and dry bean in both seasons. The differences between the two studies might be attributed to the different sources

of feedstock for the production of BGS. In Wentzel et al. (2015), the feedstock was 95% cattle slurry (cattle faeces and urine), 5% whole crop silage and a mixer of rye, wheat and rapeseed while the BGS from the current study was mainly from fresh cattle manure only. Another effect might be the different soils in which both studies were conducted under, with the study by Wentzel et al. (2015) conducted under three types of clayey loam soils i.e Haplic Cambisols, Argic Cambisols and Stagnic Luvisols while the current study was conducted in a Xanthic Ferralsols according to IUSS WRB (2006).

Co-application of BGS with CF in maize increased the OC content although the highest OC was from 100/0 in both seasons, but the application of 40/60 was nearly similar to 100/0 in both seasons. The effect of increased OC as the result of co-application was evident in MBC content as the treatment of 40/60 had higher MBC than all other treatments in both seasons. In the dry bean, the influence of co-application was clear as the treatment of 40/60 had higher OC than all other treatments in both seasons. This higher OC translated into higher MBC as 40/60 had higher MBC in the 2017/2018 season while 100/0 was higher in the 2016/2017 season.

The increased MBC and enzyme activities may contribute to mineralisation of soil C resulting in CO<sub>2</sub> emissions. The CO<sub>2</sub> flux measurements from BGS and CM resulted in higher CO<sub>2</sub> emissions than CF over the growing season. Increase OC accumulation coupled with increased MBC and increased enzyme activity influenced CO<sub>2</sub> emissions from treatments of BGS and CM. Pelster et al. (2017) suggested that microbial decomposition of OC and root respiration have a greater influence on CO<sub>2</sub> emissions. The addition of organic amendments, such as the two resources studied, to soil influences soil microbial number that could lead to increased CO<sub>2</sub> emissions (De Nobili et al., 2001) as seen in the current study with BGS and CM resulting in higher CO<sub>2</sub> emissions than CF at all N application rates. The study showed that higher CO<sub>2</sub> emissions can be expected after the application of organic fertiliser sources such as BGS and CM. Thangarajan et al. (2013) indicated that the reason for higher CO<sub>2</sub> emissions after the addition of easily decomposable substance was because of microbial activity response to altered amounts of C and N in soils. While the CO<sub>2</sub> emissions may be released, the bulk of C is retained in the soil.

#### **6.2 CONCLUSION**

Application of organic waste to soil provided plant nutrient essential nutrients that supported plant growth and build-up of soil reserves. The value of organic fertiliser in terms of improved dry matter and nutrient uptake depends on the crop and the number of seasons of application. Application of BGS in maize resulted in lower dry matter and uptake of P, K, Ca and Mg than CM but in dry bean, BGS increased dry matter and uptake of P, K, Ca and Mg. The two organic amendments (BGS and CM) increased soil CO<sub>2</sub> fluxes than CF. BGS and CM had an positive effect on soil MBC and enzyme activity that resulted in increased CO<sub>2</sub> emissions from both BGS and CM than CF treatment. Enzymes of ß-glucosidase and urease could better used to interpret CO<sub>2</sub> emissions from the soil after application of organic amendments. Soil enzymes play an important role in microbial activity and microbial population in soil.

Co-application of BGS with CF at N application rates approximately 50/50 improves maize dry matter. In the first season of planting co-application of BGS with CF at 40/60 increased dry matter grain yield and uptake of N, P and Mg. After dry bean harvest, soil pH and total N increased with the proportion of BGS, and treatments with BGS, particularly the BGS only (100/0), 60/40 and 40/60 had higher organic C, extractable P, and exchangeable K, Ca and Mg. Smallholder farmers could apply 40-60% of the recommended Nha<sup>-1</sup> of CF and supplement with BGS for maximum dry matter and grain yields. Smallholder farmers could take advantage of using BGS with the reduced CF for higher maize and dry bean yields and improved soil quality. Where CF is not readily accessible farmers can use CM to produce biogas and use BGS with the same or even better nutrient value to improve soil fertility.

### **6.3 RECOMMENDATIONS**

The study shows that BGS can be used as a source of nutrient for crop production especially for smallholder farmers of South Africa. Application of BGS alone does not necessary benefit the crop especially dry bean grain yield. Co-application of BGS with CF has numerous benefits that could help the smallholder farmers by reducing costs of synthetic CF. From the results of the current study co-application of BGS with CF at ratios of 40/60 and 60/40 are recommended for crop production. There is potential of increased CO<sub>2</sub> emissions from use of BGS in soils. Long-term studies on the effect of BGS on CO<sub>2</sub> and N<sub>2</sub>O emissions under controlled environmental conditions are necessary to gather insight and the behaviour of BGS under control environmental conditions where issues of such as volatilization would be reduced. There is a need to study more of enzyme activities that influence the release of CO<sub>2</sub> emissions in long-term studies.

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