NITROGEN AND PHOSPHORUS RELEASE AND POTENTIAL FERTILISER EFFECTS OF BIOGAS SLURRY ON SPINACH YIELD

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ABSTRACT

Use of organic waste to produce biogas aids in waste management and produces organic residue, biogas slurry (BGS), with appreciable quantities plant nutrient and potential to improve soil productivity. Feedstock and retention time during anaerobic digestion influence the quality and fertilizer value of the biogas Slurry. The objective of the study was to determine the release nutrients of biogas slurry, its potential as nitrogen (N) source and effects of co-application with chemical fertilizer (CF) on spinach yields and soil chemical parameters. Samples of biogas slurry, produced from cattle dung, and cattle manure (CM) were collected from Qwa-qwa, in the Free State Province of South Africa. The samples were analysed for pH, electrical conductivity (EC), total carbon (C), nitrogen, phosphorus (P), exchangeable bases and trace elements. Dried biogas slurry and cattle manure were applied to Avalon and Hutton soils at 0, 1, 2 and 3% (w/w).Then moistened to field capacity moisture, and incubated for 8 week, with periodic moisture correction. Destructive sampling was used to collect soil samples at 0, 1, 2, 4 and 8 weeks and the samples were analysed for pH, electrical conductivity, ammonium and nitrate-nitrogen, available phosphorus and exchangeable bases. Three glasshouse experiments were conducted to determine the fertiliser value of BGS for spinach (*Spinacia oleracea*) grown for 8 weeks. In the first experiment, biogas slurry was compared with chemical fertilizer and cattle manure as nitrogen sources at 100 kg N/ha in the Avalon soil. The second experiment involved application of biogas slurry and cattle manure at increasing nitrogen application rates of 0, 100, 150, 200 and 250 kg N/ha in Avalon soil. While the third experiment involved co-application of biogas slurry with chemical fertilizer at 0/100, 40/60, 60/40 and 100/0 kg N/ha (BGS/CF) in Avalon and Hutton soils. Soil pH increased with increasing application rate on both soils, during incubation. The 1% application rate showed the least pH increase for Hutton soil, which was significantly higher by 1.89% for cattle manure and 3.70% for biogas slurry than the control at week 8. Ammonium-N declined by 73.6% in Avalon compared to the 36.7% in Hutton soil at 3% BGS NH_4-N within 2 weeks, then increased steadily up to week 8 at all application rates for both soils. On the other hand, nitrate-nitrogen declined for biogas slurry and increased for cattle manure, with increasing application rate after 2, 4 and 8 weeks of incubation in both soils. Available phosphorus increased with increasing rate of both biogas slurry and cattle manure especially up to two weeks of incubation in soils. Spinach dry matter yield were comparable between biogas slurry (4.04 g/pot) and cattle manure (3.40

g/pot) as a nitrogen sources, even though greater nutrient uptake and soil residual fertility occurred in BGS treatment.. Increasing application of biogas slurry and cattle manure increased spinach dry-matter (DM) accumulation significantly from 100 up to 150 kg N/ha by 32.9% for biogas slurry and 23.1% for cattle manure, beyond that rate there was no notable variation. Higher nutrient uptake was observed at 150 kg N/ha, which supports the higher yields. However, biogas slurry co-application with chemical fertilizer had no synergistic effect and increasing application rate showed no significant improvement in dry matter yield and nutrient uptake. The findings of the study implied that the fertiliser value of biogas slurry was similar to cattle manure and subsequent crop could benefit from residual fertility after biogas slurry and cattle manure.

Keywords: Biogas slurry, cattle manure, nitrogen, nutrient uptake, soil fertility, spinach.

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LIST OF ACRONYMS

AD	: Anaerobic Digestion
B	: Boron
BGS	: Biogas Slurry
C	: Carbon
Ca	: Calcium
CF	: Chemical fertilizer
CH_4	: Methane
CM	: Cattle Manure
CO_2	: Carbon dioxide
Cu	: Copper
DM	: Dry matter
EC	: Electrical Conductivity
Fe	: Iron
GHG	: greenhouse gas
HCL	: Hydrochloric acid
ICP-OES	: Inductively Coupled Plasma Optical Emission Spectrometric
K	: Potassium
KCL	: Potassium Chloride
Mg	: Magnesium
Mn	: Manganese
MSW	: municipal solid waste
N	: Nitrogen
NH_4F	: Ammonium fluoride
NH_4-N	: Ammonium nitrogen
NO_3-N	: Nitrate nitrogen
P	: Phosphorus
SSA	: sub-Sahara Africa
Zn	: Zinc

CHAPTER 1

GENERAL INTRODUCTION

The expected increase in population from 1.3 billion to 1.9 billion in Africa by 2050 (Lal, 2013) will directly result in significant increases in energy and food demands, which are associated with intensified agricultural and industrial production systems. Currently, the sub-Saharan Africa (SSA) constitutes about 960 million people (Brown *et al.,* 2014; Lal, 2013; Eberhard *et al.,* 2008). Intensified agricultural production will consequently result in generated of large quantities of organic wastes annually, and some of these wastes could result in water, soil and environmental contamination, including greenhouse gas (GHGs) emissions (Odlare *et al.,* 2011). Sustainable and environmentally-friendly approaches, which aid in recycling of waste materials to valuable products, have been proposed as an alternative management strategies (Odlare *et al.*, 2011 and Svensson *et al.*, 2004). Amongst the proposed sustainable strategies is the utilization of organic wastes as resource for renewable energy, such as in biogas production (Abubaker, 2012; Helm-Nielsen *et al.,* 2009). Biogas technology can help to overcome energy poverty, which poses a persistent barrier to economic development in Africa (Bond and Templeton, 2011).

Biogas technology utilizes specialized equipment (biodigesters) for anaerobic digestion (AD) of organic material, including manure, municipal and industrial materials, energy crops and crop residues, to produce a combustible gas with a high proportion of methane (50-70%) (Luosterinen *et al*., 2011). The process is primarily driven by various micro-organisms and comprises of hydrolysis, fermentation, acetogenesis and methanogenesis stages (Moller and Muller, 2012). Temperature, loading rate, pH and biodigester designs amongst others, are factors that significantly influence the AD process. For instance, temperatures higher than the optimum range (30-38^{o}C) may damage proteins and cellular components of microbes thereby affecting their performance, lowering methane production (Gustin and Marinsek-Logar, 2011). In the process of producing green energy an organic waste, biogas slurry, is produced.

Biogas slurry is an organic by-product of AD with the potential to improve soil fertility status and provides a substrate for the microbial communities (Anger *et al.,* 2012). The potential of BGS to be a soil amendment is attributed to appreciable quantities of plant essential nutrients (N, P, K, Mg Ca amongst others) that it contains (Table 2.2). The quantities of these nutrients may vary significantly depending on the feedstock used and retention time among others (Abubaker *et al*., 2012; Alburquerque *et al*., 2012 and Yu *et al*., 2010). A large number of biogas digesters were installed in SSA countries including Botswana, Lesotho, Namibia, Zimbabwe, and South Africa since 2001 (Amigun *et al.,* 2012), to promote use green energy while minimising environmental degradation. Biogas slurry from these digesters could be beneficial to the soil through improving nutrient cycling, availability and storage capacity, biological activity, soil aggregation, drainage and resistance to erosion. Several studies show that applying BGS improves soil quality and increases crop production (Khan *et al.,* 2015; Barbosa *et al.,* 2014; Odlare *et al.,* 2011 and Nasir *et al.,* 2010).

Odlare *et al.* (2008) noted 20% more N mineralisation in soils treated with BGS than cattle manure (CM). Abubaker *et al.* (2012), who compared N mineralisation between BGS and pig manure, reported similar results. Arthurson (2009) indicated that NH_4-N in BGS is approximately 25% greater than untreated manure. The higher N mineralisation of BGS compared to CM may be associated with alterations of polymers such lipids and carbohydrates into monomers during AD, which aids in faster release of plant nutrients (Moller and Muller, 2012). This suggests that, there could greater N mineralisation in BGS produced at different plants in southern Africa, which could enhance crop growth and yields. Batiano and Makwunye (1991) noted 250% increase in millet yield following co-application of BGS and chemical fertiliser. In addition to the supply of nutrients, Seka (2012) noted a 10% increase in soil organic matter in sandy-loam soil treated with BGS from the co-digestion of maize silage and cow manure.

South Africa is currently encountering energy shortages, land degradation, climate change, increased fuel prices and increased GHGs emissions (Pollet *et al.,* 2015 and Xulu, 2014). The biogas technology has a potential to minimise the strain on energy generation (Abubaker, 2012; Yu *et al.,* 2010). In addition, BGS could possibly be a solution to nutrient cycling and improvement of degraded land to enhance stability through improved carbon sequestration (Tilman *et al.,* 2002). The use of BGS could enhance food security of small-scale farmers, combat land degradation and reduces GHG emissions in South Africa (Rutz, 2010). The fertiliser value of BGS relative to commonly used nutrient sources such as CM and CF needs to be established. The appropriate application rates for optimal benefit from BGS also need to be determined. Little research work has been done on the use of BGS as a soil amendment so far both at commercial and communal scales in South Africa. Although good results are expected from BGS as a source of nutrients, the outcomes are not likely to be better than CF. Effects of co-application of CF with BGS also need to be established considering that smallholder farmers do not generally apply CF at recommended rates. The utilization of BGS is still limited in South Africa possibly because of inadequate assurance on the safety, value and foreignness of the product. Hence, it is essential to evaluate the BGS for both short and long-term use in arable lands. Three main research questions were formulated for this study:

i) How does the release of plant available N and P from BGS compare with the feedstock (original manure) in different soils?

ii) Will the use of BGS as fertilizer improve crop yields and soil quality compared to CM?

iii) Could co-application of BGS and CF improve crop yields and soil quality compared to the individual amendments?

The overall objective of the study is to assess the short-term effects of BGS (produced from cattle manure) on soil fertility, crop growth and yield in South Africa. The specific objectives will be:

i) To determine the nutrient release patterns of BGS and CM from incubated Avalon and Hutton soils.

ii) To determine the effect of BGS as a nitrogen source against cattle manure and chemical fertilizer on spinach yield, nutrient uptake and soil residual fertility.

ii) To determine the effects of increasing application rates of BGS on spinach yields, nutrient uptake and soil residual fertility compared to cattle manure.

iii) To determine effect of co-application of BGS with chemical fertilizer at different mixing ratios on spinach yield, nutrient uptake and soil residual fertility

The hypotheses of the study will be:

i) The nutrient release patterns of BGS and CM do not differ in Avalon and Hutton soils

ii) BGS as an N-source does not perform better than CF and CM with respect to spinach yield, nutrient uptake and soil residual fertility

iii) Increasing application rates of BGS does not affect spinach yield, nutrient uptake and soil residual fertility.

iv) Spinach yield, nutrient uptake and soil residual fertility are not affected by co-application of BGS with chemical fertilizer.

The findings of this study will add values on baseline information and data for future study on the application of biogas slurry in agricultural soils. This study will also contribute to increased knowledge of BGS and raise farmers' awareness of biogas technology in South Africa.

CHAPTER 2: LITERATURE REVIEW

BIOGAS SLURRY CHARACTERISTICS, NUTRIENT RELEASE AND POTENTIAL FERTILIZER VALUE: A REVIEW

2.1. Introduction

The estimated world energy utilization is at 15 terawatts of which only 0.94 is emanating from renewable energy sources (Cho, 2011). Of the 15 TW, sub-Saharan Africa is responsible for merely 4% (Roopnarain and Adeleke, 2017). However, Demirbas and Demirbas (2007) indicated that about 50% Africa's energy is produce from fuel wood, which is associated with health risks and deforestation, amongst other disadvantages. Africa has a potential to enhance energy production through utilizing the eco-friendly biogas technology, using readily available, reliable and affordable organic wastes (Roopnarain and Adeleke, 2017). The AD of organic materials to generate bioenergy has become a well-known practice in various parts of the world. Abubaker *et al.* (2012) projected that biogas could account for approximately 25% of world's energy generation by 2020 since 2005. The growth in biogas industry has led to increased levels of by-products such as BGS. The management of BGS, an organic residue produced during CH_4 generation through the AD of organic material has been on the eye of research and environmental protection (Garg *et al.,* 2005). Globally, BGS is being used mainly as a soil conditioner (applied in various forms) (Gil *et al.,* 2007; Tambone *et al.,* 2010; Abubaker *et al.,* 2012 and Chen *et al.,* 2012). The utilization of BGS as a soil amendment is attributed to its nutrient composition that entails appreciable quantities of essential plant nutrients (Garg *et al.,* 2005). Supporting findings reported by Insam *et al*. (2015) displayed that the application BGS increases the mineralisation of N and plant available P in treated soils thereby improving crop uptake. Contrary, Bachmann *et al*. (2014) indicated that AD does not modify the P fertilization value for crops in a sustainable manner. It is, however, important to note that the BGS may contain heavy metals but at lower concentrations, when compared to untreated cattle manure (Jin and Chang, 2011). This is due to the solubility and availability of heavy metals is significantly reduced as a result of precipitation processes with sulphide (S^{2-}), carbonates (CO_3^{2-}) and phosphate (PO_4^{3-}) (Moller and Muller, 2012). With the ability of BGS to supply essential plant nutrients without posing soil contamination threats, BGS can potentially be co-applied with chemical fertilizers or alone as nutrient source. This chapter aims to review literature on the production and characterization of BGS, its utilization as a soil amendment and crop yield improvement.

2.2. Biogas slurry production, characterization and use as soil amendment

2.2.1. Major feedstock used and the process of anaerobic digestion

Feedstock in AD refers to biomass or organic material that can be used as a starting material in the biogas production. To date, a wide range of feedstock used in biogas production have been documented and include municipal solid waste (MSW), animal manure, industrial by-products and selected energy crops amongst others (Table 1) (Okhadoh *et al.,* 2014; Weiland, 2010; Balat *et al.,* 2009). In the sub-Saharan Africa, the mostly used feedstock is cattle manure, energy crop residues and household, industrial and slaughterhouse wastes (Parawira, 2009). Unlike other parts of the world where human excreta is also used, only a few sub-Saharan Countries (Burundi, Bolivia, Ivory Coast and Tanzania) use human excreta as a feedstock. This may be due to socio-economic, cultural and health related issues. These countries use the Chinese fixed-dome and India floating cover digesters to produce biogas (Tumwesige *et al.,* 2011). The major requirement for the feedstock to produce large quantities of biogas is that it must contain a high quality of degradable organic matter, which includes crude protein, crude fat, crude fibre, cellulose, hemi-cellulose, starch and sugar (Drapcho *et al.,* 2008). Over the past two decades, extensive research has been done on AD of organic material (Pino *et al.,* 2014; Abubaker *et al.,* 2012; Moller and Muller, 2012). The process involves breaking down of organic matter in the absence of oxygen which yields a by-product BGS with improved inorganic nutrient content than the digested material (Loria and Sawyer, 2005). All the stages of AD namely hydrolysis or degradation, fermentation or acidogenesis, acetogenesis and methanogenesis are driven by complex bacterial and archaea communities (Ziganshin *et al.,* 2012). Success or failure of any stage is influenced significantly by operational conditions such as temperature, type and composition of the biomass used, pH and moisture (Khalid *et al.,* 2011).

Table 2.1: The generally used feedstock in biogas production with selected quality parameters that are important for AD

Categories	Quality parameter						Reference
	Crude protein (%)	Fat (%)	Crude Fibre (%)	Volatile solids (%)	Ash (%)	Dry Matter (%)	
				Agricultural			
Maize Stover	10.9	-	80.8	95.0	5.00	93.0	Nzila *et al.,* 2015 and Adebayo *et al.,* 2014
Maize	7.8	2.6	47.1	-	-	30.2	Herrmann *et al.,* 2016
Cotton Residues	15.6		63.4	88.0	4.00	91.0	Nzila *et al.,* 2015
Winter Wheat	7.5	2.2	41.2	-	-	48.7	Herrmann *et al.,* 2016
				Industrial			
Sugarcane Bagasse	1.17	-	94.5	90.0	4.00	94.0	Nzila *et al.,* 2015
Apple Processing waste	2.80	<7.5	7.3	90.0	-	-	Kafle and Kim, 2013
				Municipal			
Sludge	60.21	17.99	12.43	72.46	-	157.4*	Liu *et al*., 2012
Food waste	17.26	15.27	0.3	-	3.21	61.6	Nizami *et al.,* 2016

*Where value expressed by * are in g/kg.*

2.2.2. Characterization of different biogas slurry

Biogas slurry a by-product of AD rich in plant nutrients and presents alternative to chemical fertilizers (Abubaker *et al.,* 2012; Arthurson, 2009 and Garg *et al.,* 2005). Preceding research (Pino *et al.,* 2014; Alburquerque *et al.,* 2012; Warnar and Oppenoorth, 2014; Abubaker, 2012; Garg *et al*., 2005) point out some of plant essential nutrient found in BGS (Table 2.2). The proportion of the nutrients entailed in BGS may vary significantly from one slurry to the next as a number of factors, which include feedstock, temperature and retention time among other factors (Nkoa *et al.,* 2014; Provenzano *et al.,* 2011 and Deublein and Steinhauser, 2008), influences them.

Findings by Apples *et al.* (2011) indicated that within the same feedstock, nutrient composition of BGS may still vary. Similarly, results obtained by Abubaker *et al.* (2012) where four different types of BGSs have varying nutrient concentrations (Table 2.2). For example, BGS emanating from fatty feedstock has higher nutrient content followed by carbohydrates and then proteins (Smith *et al.,* 2014). In addition, rural and urban MSW composition could vary significantly due to differences in lifestyle, seasonal changes as well as the type of food waste generated. In general, the low nutrient content in BGS of SSA countries is attributed to the inadequate quality of animal feeds (Moller and Stinner, 2010). Generally, pH of BGS is higher than that of feedstock used, the raise pH is attributed to the formation of $(NH_4)_2CO_3$ and NH_3 (Georgacakis *et al.,* 1992) and is governed by a series of reactions occurring during AD (eqns. 1-3) (Hjorth *et al.,* 2010 and Moller and Muller, 2012).

$$NH_4^+ \leftrightarrow NH_{3(aq)} \qquad \text{Eqn. 1}$$

$$CO_2^{2-} \leftrightarrow HCO_3^{2-} \leftrightarrow CO_3^{2-} \qquad \text{Eqn. 2}$$

$$CH_3COOH \leftrightarrow CH_3COO- \qquad \text{Eqn. 3}$$

According to Pino *et al.* (2014), the degree of pH increase varies from feedstock to feedstock characteristics and digestion process. Attesting results, Makadi *et al.* (2012) noted a significant increase in pH from 4.0 to 8.3 during co-digestion of cattle manure with energy crop and agro-industrial waste. However, Loria and Sawyer (2005) reported marginal pH increase following AD of pig manure thus 7.8 to 8.1, which is close to the pH of 7.95 noted by Alburquerque *et al*. (2012) on BGS emanating from pig slurry. Plaza *et al.* (2004) indicated that BGS from pig manure is generally alkaline due to the presence of salts and carbonates present in the feed. The alkaline nature of the material would then be beneficial in amelioration of soil acidity in poorly resourced areas of SSA that receive high rainfall, which has a potential to induce soil acidity, aluminium toxicity and macro nutrient immobilization amongst others (Jasen *et al.,* 2010). In most instances, such soils are low in organic matter content.

Organic matter content in BGS is mainly influenced by digestion period as well as the nature of the feedstock used. According to Apples *et al.* (2011), prolonged retention time yields BGS with low organic matter content. The low organic matter content is associated with degradation of organic compounds to produce CO_2 and CH_4 (Teglia *et al.,* 2010). This process is a result of metabolic interactions between diverse groups of microbes. Hydrolysis of polymers such as proteins, carbohydrates and lipids yield monomers which are subsequently converted to volatile fatty acids, dihydrogen and acetic acid. These are further altered to CO_2 and acetate by the acetogenic bacteria where lastly the methanogens convert them to mainly CH4 and CO_2 (Moller and Muller, 2012). Most of the carbon in BGS is found in the microbial biomass pool which is the major facilitator soil organic matter formation in treated soils (Coban *et al.,* 2016). Thus, BGS land application may mitigate land degradation through improved carbon (C) sequestration within SSA (Smith *et al.,* 2014). Biogas slurry also contains appreciable quantities of macronutrients.

In general, BGS generally contains N in the form of ammonium-nitrogen (NH_4^+-N) and which is approximately 25% more than the untreated slurry of the same feedstock (Arthurson, 2009). In a similar study, Makadi *et al.* (2012) reported that, of the total N, NH_4-N accounts for 60-80%. This is observed mostly in BGS emanating from feedstock with degradability potential and diet high in concentrates such as poultry, pig and feedlot cattle manure (Fouda, 2011). Again, in the co-digestion of cattle slurry with maize-oat silage, the NH_4-N proportion with respect to total N was 61.2% (Alburquerque *et al*., 2012). In addition, Loria and Sawyer (2005) noted a significant proportion of $NH4^+$-N which constituted 56.4% of the total N following digestion of pig manure.

Based on findings by Alburquerque *et al*. (2012), Makadi *et al.* (2012) and Arthurson (2009), it is evident that during AD N conservation occurs which then further suggest that C/N ratio is reduced leaving behind a more organically stable material. The high percentage of mineral N content contained in the BGS may be utilized as indicator for estimating the instant fertilizing effect of BGS and its comparability with CF (Vogel, 2009). Amongst the important characteristics of BGS is its ability to enhance biological activity immediately upon oxygen exposure (Fuchs *et al.,* 2008). Such effect enhances nutrient cycling of the treated soils thus improving nutrient availability for crop uptake which also includes P amongst others.

Phosphorus, a component of nucleic acids and phospholipids is enhanced by AD. Availability of P for plant uptake following application is debatable and may sometimes vary from residue to residue thus depending on their physio-chemical properties (Delin and Nyberg, 2015). The availability of P is favoured by the raised pH which has the ability to shift the equilibrium to favour the formation of phosphates (HPO_4^{2-} and PO_4^{3-}) (Moller and Muller, 2012). However, Gungor *et al*. (2007) indicated that increase in pH of BGS favours the formation of struvite ($MgNH_4PO_4*6H_2O$) and hydroxylapatite ($Ca_5(PO_4)_2OH$) which have a potential to reduce P solubility in BGS and thus may affect plant available P during the growing period of a plant. In the co-digestion of dairy slurry with maize silage and wheat grain, the average available P content observed was 12% of the total P (Bachmann *et al*., 2011). Based on an incubation study conducted by Bachmann *et al*. (2016) results displayed no significant difference in the digested and undigested pig manure after 28 days of incubation. Therefore, such findings open room for further interrogative experiment to assess whether or not does digested manure performs better than undigested.

2.2.3. Potential of BGS as a soil amendment for improved soil quality

The use of inorganic fertilizer in cultivated land needs elevated levels of fertilizer synthesis that require substantial quantities of energy (Davis and Haglund, 1999 and Patyk, 1996). Along with the fertilizer synthesis is the constant production of waste, which constantly pollutes the environment (Svensson *et al.,* 2004). According to Steinfeld *et al.* (2006), an estimate of 65% nitrous oxides (N_2O) and 64% ammonia (NH_3) of anthropogenic emissions emanate from animal production systems worldwide. Oenema *et al.* (2006) further emphasised that every kg of N loss through volatilization correspond to approximately 30-70kg of CO_2 emission, which then negatively affect the atmosphere with possible effects on climate change. Smith *et al.* (2007) quantified the direct contribution of agricultural practises to climate change to be approximately 14%. Thus, synthesis, application of inorganic fertilizers and untreated animal manure consumes energy and promotes greenhouse gas (GHG) emissions, respectively, and are therefore unsustainable and pose environmental concerns (Arthurson, 2009).

Use of BGS concurrently with CF may aid in minimizing the amount of CF produced annually as well as the concomitant environmental effect. In most communal areas of Africa, untreated animal manure is utilised as nutrient replenishment strategy caused by lack affordability. However, the main challenges that are concomitant to utilization of untreated or poorly managed animal manure are the ammonia volatilization and contamination with pathogens (Helm-Nielsen *et al.*, 2009). Whereas, AD kills viability pathogens and mineralizing a major proportion of N thereby resulting in more environmentally friendly organic amendment unlike undigested animal manure (Abubaker *et al.,* 2013).

Table 2.2: Typical selected nutrient composition of different BGSs emanating from various feedstocks.

Parameter	**SHW**	**EW&C**	**OHW**	**SOHW**	**AW**	**C&PM**	**APW**	**CRG**	**CPM**	**IW**
pH	7.9	7.9	8	8.7	8.5	-	7.8		8.3	8.0
DM (%)	6.1	3.7	1.7	5.9	53.1	-	-	20.3	1.9	44
Tot C (kg/t)	19	9	7	24	-	-	-	42.8	4.8	330
Tot N (kg/t)	7.9	5.9	2.6	5.3	15.3	0.069	5.88	0.488	3.8	20
C/N (-)	7.1	4.2	11.0	12.1	-	-	-	19.3	12	16.5
NH_4^+-N (kg/t)	5.3	3.7	2.0	3.3	-	159	-	0.81	-	-
Tot P (kg/t)	0.9	0.7	0.2	0.4	3.6	0.048	2.72	0.39	0,5	6.4
Tot K (kg/t)	1.6	2.8	1.1	3.7	12.5	0.34	1.33	1.68	2.4	2.4
Zn (mg/kg)	474	465	299	396	-	<1	-	-	30	94
Mn (mg/kg)	201	266	91.8	287	-	<1	-	-	3	158
Cu (mg/kg)	69.7	69.4	39.8	97.4	-	-	-	-	4	26
Fe (mg/kg)	-	-	-	-	-	<1	-	-	20	2508
Ni (mg/kg)	38.8	35.5	1.7	1.7	-	-	-	-	0.2	-
Cr (mg/kg)	13.0	14.9	11.1	19.5	-	-	-	-	0.1	-
Source	Abubaker *et al.* (2012)				Funchs *et al.* (2008)	Yu *et al.* (2010)	Rafiqul *et al.* (2010)	Stinner *et al.* (2008)	Alburquerque *et al.* (2012)	Pino *et al.* (2014)

Where feed-stocks used were: *SHW= Slaughter house waste; EW&C= Ethanol waste and cereals; OHW= Organic household waste; SOHW= Silage and separated organic household waste; AW= Agricultural waste; C&PM Cattle and pig manure; APW= Alcohol production waste; C&RG= clover and rye grass; and IW =industrial waste; Tot= total; DM= dry matter.*

Several researchers (Abubaker *et al.,* 2012; Chen *et al.,* 2012; Tambone *et al.,* 2010; Gil *et al.,* 2007 and Garg *et al.,* 2005) have reported that the use of BGS as a soil amendment, improves and sustains soil productivity, thereby increase crop yields. Hao *et al.* (2001) and Chiyoka (2011) noted that composting of organic waste promotes GHG emission via the losses of carbon as CO_2 and CH_4, and nitrogen as ammonia (NH_3) (Eghbill *et al.,* 1997). Yet; when it comes to BGS, majority of N is mineralized and C is converted to CH_4, which results in a material that is more stable and emits less GHGs than composted material. Furthermore, Odlare *et al.* (2008) compared cow and pig manure, compost and a CF with BGS at different rates, results clearly presented that N-mineralization in BGS treated soils was 20% greater than all the other amendments except for cow manure. This then suggests greater ability of the BGS to avail N for plant uptake.

Therefore, BGS could be a more ideal and inexpensive organic amendment to soils in contrast with other organic amendments, such as animal manure, compost and sewage sludge, as well as CF since it is likely to have the least negative environmental effect. This supported by findings reported by Odlare *et al.* (2008) that soils treated with BGS showed the lowest (11%) CO_2 evolution compared to compost and chemical fertilizer, which had over 25% respectively. Biogas slurry could be either directly spread as manures or processed prior to field application, for example, by solid–liquid separation, drying, dilution or filtration which aid in concentration its nutrient composition (Insam *et al.,* 2015).

2.3. Biogas slurry nutrient release patterns

A number of documented efforts indicated that, application of organic amendments in cultivated soils to deviate from landfill disposal, environmental pollution and counteracting the reliance on synthetic fertilizers is being practised throughout Africa and the world (Coban *et al.,* 2016; Chiyoka, 2011; Garg *et al.,* 2005 and Eghbill *et al.,* 1997). This practise has demonstrated that soil quality is improved following treatment with organic amendments including BGS (Abubaker *et al.,* 2012; Tambone *et al.,* 2010; Fuchs *et al.,* 2008; Amon *et al.,* 2007). Now; the understanding of nutrient release potentials succeeding BGS application to cultivated soils remains imprecisely understood (Alburquerque *et al.,* 2012).

So far, very little research work has been conducted within SSA to address this phenomenon (Chiyoka, 2011). In the rural small-scale household digesters of SSA, BGS is applied at rates similar to those of untreated manure (Mwirige *et al*., 2014). Which should not be the case, since the essential plant nutrients contained in BGS are grouped into immediately available, rapidly released and slowly released or unavailable forms in varying proportions in relation to animal manure (Brady and Weil, 2007). Thus, their release characteristics depend on the amount of nutrients held in each of these forms (Smith *et al.,* 2014). Supporting findings, BGS entails more plant available nutrient (i.e. N and P) with respect to the original manure used of AD suggesting that their application rates may not be the same (Abubaker *et al.,* 2012 and Arthurson, 2009). However, their dynamics in BGS treated soils are not well understood due to uncertainties of the material, such as nutrient composition and method of handling amongst others (Alburquerque *et al.,* 2012).

Controlled experiments exclude environmental sources of variation and they are essential for monitoring nutrient mineralization, which becomes useful in providing understanding BGS management and optimal use. The release of nutrient at the time when the plant is not actively assimilating the nutrients may encourage in low nutrient use efficiency subsequently poor crop performance (Loria and Sawyer, 2005). Which then suggest the importance of synchronizing the availability or release with crop nutrient demand (Binder *et al.,* 1996). However, this is may not be easy due to the uncertainties in the mineralization rate of the material (Loria and Sawyer, 2005).

In a study by Chiyoka (2011) two different soils were incubated with BGS from feedlot cattle manure at an application rate of 400 and 800 mg/100g soil sample, which was approximately 100 and 200 kg N/ha respectively for 70 days. The results indicated that separated solids of anaerobic digestion significantly increased the inorganic P concentrations than all the other treatments**.** However, it showed a significant increase compared to other treatments, with a high peak at day 5 followed by a rapid decline, possibly due to P immobilization, and then a gradual P release over time. This suggests high suitability of BGS to quickly release nutrients even under cold period such as early spring. Similarly, Alburquerque *et al.* (2012) assessed soil N dynamics treated with pig slurry and diary slurry over 56 days of incubation. Results showed that for NO_3-N had uniform trend throughout the treatments thus an initial increase followed by a rapid decline and then significant increase within 14 days and after it was steady. However, for the PSB treated soil NO_3-N did not show a rapid increase for 14 days and then became steady. Ammonium-N showed an immediate decreasing trend within 14 days and there after it was constant for all the treatments. The decline in mineral N content may be associated to the induced biological activity following BGS application resulting into partial N immobilisation (Moller and Muller, 2012). It may therefore, be expected that available mineral N and P will be assimilated by crop for better growth.

2.4. Changes of selected soil quality parameters in Biogas Slurry treated soils

Saharan and sub-Saharan regions of Africa remain the only regions in the world where food production per capita is stationary at 0.16% on average per year (Chauvin *at al.,* 2012 and Sanchez, 2002). The continuous practise of nutrient mining without adequate replenishment along with concomitant challenges which include weed, pest and disease infestation are major biophysical factors that induce low food production per capita (Mafongoya *et al.,* 2007). In most instances, nitrogen is the major limiting factor followed by phosphorus (P) and potassium (K) for maize production, maize being a staple crop of southern African region. Traditional counter actions against nutrient depletion is the constant utilization of synthetic fertilizer which are way much expensive for most farmers would afford and which has the potential to contribute to soil acidity consequently influencing nutrient availability (Dong *et al*., 2012). Availability of BGS from biogas technology could ease the pressure on farmers, especially small-scale farmers from investing more on crop production inputs. The utilization of BGS as soil amendment have been studied extensively throughout the world but very little research work done on the sub-Saharan countries (Mshindete and Parawira, 2009).

2.4.1. Soil pH changes following Biogas slurry application

Two soils treated with BGS presented a significant raise from 6.5 to 7.5 and 6.3 to 6.8 in pH for brown and black soil respectively (Chiyoka, 2011) such response is due to the alkaline (8.7) nature of BGS emanating from feedlot cattle manure. Similarly, in an incubation study, soils treated with BGS (mixture of cattle and pig manure) showed increased pH even though the increase was not statistically significant (Yu *et al.,* 2010). The minimal improvement in pH maybe to low temperatures (<160C) that the study was conducted under compared to Chiyoka (2011) findings. In addition, Funch *et al.* (2008) observed 0.5 rise in pH after maize harvest in soils treated with BGS. Gil *et al.* (2007) obtained supporting findings where BGS was compared with compost in an incubation experiment for 80 days. Results displayed a raise in pH of treated soil from 6.6 to 8.0, suggesting it has the potential to ameliorate soil acidity to a range where optimal plant growth is expected. Contrary, in a 4-year field trial conducted by

Odlare *et al.* (2008), pH showed non-significant change in treated soils with respect to the control. Alburquerque *et al.* (2012) suggested that such response is with soils with high buffering capacity. The extent of pH alteration upon BGS application seems to vary with experiments due to natural sources of variation, which include climate difference, soil types and buffering capacity amongst others (Makadi *et al.,* 2012).

2.4.2. Impact of Biogas Slurry on major soil nutrients (NPK)

i) Nitrogen: Nitrogen is one of the primary essential plant nutrient and is general known to be one of the major crop growth limiting factors. It undergoes various processes (following its application) including mineralization, immobilization, nitrification, denitrification as well as leaching and volatilization. During AD mineral nitrogen (N) is significantly conserved (30-50%) with respect to untreated manure (Johansen *et al.,* 2013; Grigatti *et al.,* 2011 and Arthurson, 2009), this then suggests that BGS can provide supplementary N for crop growth (Odlare *et al.,* 2008 and Helgason, 2007). Results reported by Abubaker *et al.* (2012) from a pot experiment displayed that BGS enhanced N-mineralisation in contrast with pig manure, this further suggested improved N availability.

Similarly, BGS emanating from wine waste was used in an incubation study, where results displayed greater N mineralization than compost of the same material (Nkoa, 2014) this is attributed to the low C/N ratio of the slurry (Canali *et al.,* 2011). Also, Chantigny *et al.* (2007) assessed N dynamics in soil incubated with BGS for 112 days, findings showed predominated by NO_3-N (25%). Field experiment displayed congruent trends in the residual NO_3-N following 3 years of amending with BGS. Contrary, Larsen *et al.* (2007) observed N immobilization in soil treated with BGS emanating from bark chip and organic kitchen waste. This may be as a result of lower C/N ratio of the material utilized. Similarly, on a 3 year field study, soil NO_3-N was below the critical range (16-25 mg NO_3-N/kg) (Blackmer *et al*., 1997) was 16 for 2000, 5 for 2001 and 10 for 2002 (Loria *et al*., 2007).

However, even though NO_3-N was below the critical range, an additional N showed no influence on the crop yields suggesting that there was sufficient N for plant uptake throughout the growing season. Vogel (2009) noted that, even though BGS may be applied to meet the maize N demand, in some instances supplementary P and K may be necessary if they are inadequate. In a pot trial, four different BGS from different piggery farms were used under maize production, results displayed enhanced N-mineralization further suggesting improved N availability (Abubaker *et al.,* 2012). This was attributed to reduced P solubility following formation compounds such as struvite (Bachmann *et al.,* 2016), which then reduces P fertilization efficiency (Delin and Nyberg, 2015).

ii) Phosphorus: Phosphorus is another macronutrient that is required in significant quantities by most crops. P deficiency in soils can severely limit crop growth and leads to lower yields. The availability of P in organic waste qualifies them to be utilized as soil amendments. So far, there are few documented efforts on BGS as P source for maize within the SSA (Nkoa, 2014) and the world in general (Alburquerque *et al.,* 2012). Delin and Nyberg (2015) reported a significantly higher P content in plant tissue following BGS application compared to sewage sludge, cattle slurry and the control. This implies that BGS has the ability to improve P availability and uptake. Immediate P availability in BGS treated aids in deviating from early season P deficiencies with potential to reduce yields notably than late season deficiencies (Grant *et al.,* 2001). In addition, Svensson *et al.* (2004) noted that BGS has a low P content as required by most crops and further recommended that mineral supplementary P is necessary to deviate from P deficiencies.

Similarly, three different BGS's originating from dairy slurry, maize silage and wheat corn were compared over three maize growing seasons against their feed-stocks and CF; a significantly increase in available P and uptake was displayed from BGS treated soil than the control (Bachmann *et al.,* 2014) which is consistent with the above findings by Delin and Nyberg (2015). Yet, no differences were observed between BGS's and feedstocks, which suggests that AD does not significantly improve P content during AD (Bachmann et al., 2014). Moreover, pot trial results reported by Bachmann *et al.* (2016) demonstrated no significant P availability in BGS treated soils under four different crops (maize, bean, sorghum and amaranth) compared to undigested slurry.

However, Moller and Muller (2012) noted that the natural supply and availability of P is generally slow. Even though literature highlights that BGS P content is improved following AD, field experiments by Bachmann *et al.* (2014) and Moller and Stinner (2010) showed no significant P improvement on BGS treated soils. Supporting findings were obtained by Batiano and Makwunye (1991) that co-application of BGS and mineral P at 75% and 25% ratio respectively, increase millet yield by 250%. In a 3-year field experiment by Bachmann *et al*. (2014) the P fertilization value of BGS obtained from a dairy farm was tested under maize cultivation. Results showed that at the end of 3 years applying BGS at 30 M^3/ha, did not only increase the plant available P but also the uptake by plants was improve with respect to the control, which is consistent with findings by Delin and Nyberg (2015).

iii) Potassium: Potassium is the third important macronutrient required for plant growth (Brady and Weil, 2002). Yu *et al.* (2010) carried out a study, fertigating tomato plants with 200-800 mL of slurry every 3 days throughout the growing season. The results showed a significant increase in available K content in the order concentrated biogas slurry> biogas slurry> cattle manure> control. Tambone *et al.* (2010) assessed the variation in K concentration levels between BGS and compost treated soils, results indicated that K concentration are generally higher in BGS than compost. In a related study where Haraldsen *et al.* (2010) compared the efficiency of different organic amendments including BGS when applied at 80 kg N/ha. Results showed that BGS (LAD) and nitrified liquid anaerobic digestate (NLAD) supplemented significantly higher amounts of K than any other treatment. There is a general trend that BGS supplements significant quantities of K, which then emphasises more suitability in BGS for supplementing K.

2.4.3. Effect of BGS on Soil organic matter content and physical properties

Chen *et al.* (2012) noted that the incorporation of BGS in soil increased soil microbial communities suggesting that, there is more mineralization occurring thereby improving soil fertility status and nutrient cycling. For the above-mentioned reason, microbial biomass carbon is considered as the most sensitive, reliable and suitable indicative parameter to changes in response to organic fertilizer application within the labile fraction therefore it is used to estimate the effect of amending soils with an organic fertilizer. Seka (2012) noted a 10% increase in soil organic matter in sandy-loam soil treated with BGS obtained from the co-digestion of maize silage and cow manure.

Findings obtained by Smith *et al*. (2014) indicated that during AD, the stability of the BGS is improved on the other hand the C/N ratio is lowered, leaving a material with high content of speedily released nutrients which then support the above mentioned reason. Makadi *et al,* (2012) noted that the extent of organic matter breakdown normally varies between 11.1 to 38.4% but may go up to 53% under high load rates and longer retention periods. Anger *et al.* (2012) indicated that the application of BGS improves soil organic matter status which then become beneficial to the soil by facilitating the maintenance of the process of nutrient cycling, availability and storage capacity, biological activity, soil aggregation, drainage and resistance to erosion.

Soil water availability has a direct effect on crop performance and it significantly influenced a number of factors which soil type and climate. Garg *et al.* (2005) conducted a study, assessing the effect of high (15 Mg/ha) and low (4.5 Mg/ha) application rates of fly-ash and BGS under wheat production for two consecutive growing season. Results indicated that soil moisture content was improved from 41% moisture (control) to 44.1 and 43.5% with 15 Mg/ha BGS and fly-ash respectively. This is concomitant with the improve soil OM due to the presence of humic-acids found in BGS (Du *et al.,* 2016). This associated with additional sorption sites for soil water retention (Coban *et al.,* 2016 and Kabede *et al.,* 2016).

2.5. Crop response (growth and yields) to Biogas Slurry fertilization

2.5.1. Growth and Biomass yields

As already established that nutrients in BGS are bioavailable, it can be expected that crop growth and yields will be improved due to the improvement in nutrient availability. Supporting studies conducted by Barbosa *et al.* (2014) that certainly BGS enhanced significantly, maize biomass yields in contrast to the control. Additionally, Rivard *et al.* (1995) observed a direct proportional relation of maize biomass weight and height with BGS application rate. In a similar study, BGS enhanced the root/ shoot ratio in contrast with di-ammonium phosphate as an N source at 80 and 160 kg N i.e. 0.27 and 0.24 for BGS, 0.17 and 0.19 for di-ammonium phosphate respectively (Pino *et al*., 2014).

Wheat, another cereal crop, showed significantly higher aboveground yields following split application of BGS at 70% spring and 30% in autumn (Wulf *et al.,* 2006) and an even split on both seasons (Makadi *et al.,* 2008 and Nyord *et al.,* 2008) than farmyard manure and undigested manure. Maize fodder presented no remarkable difference in biomass yields succeeding CF and BGS when applied at 100% N recommended rate but both were significantly higher than the control (Khan *et al.,* 2015). In the same study, co-application of CF and BGS at 50/50% N recommended rate yielded significantly higher biomass than any other treatment. However, results obtained from a study by Rahman *et al.* (2008) warns that extremely high N application rates may reduce maize fodder yields.

From the literature, there is congruency in the research findings that BGS improves plant biomass. However, it is necessary to point out that understanding of plant nutrient requirement and nutrient composition of the utilized amendments are of paramount importance as they significant influence yields.

2.5.2. Grain yields

Odlare *et al.* (2011) compared the effect of BGS, CF and compost over 8 years under barley cultivation, treatments influence on the yields. Similarly, in a study under wheat production, BGS treated soils recorded considerably higher yields than fly-ash at 15 t/ha and the control (Garg *et al.,* 2005). The increase in wheat yields following the application of BGS is attributed to the improved soil properties such as greater moisture retention and nutrient supply as well as microbial activity.

Nasir *et al.* (2010) compared the effect of BGS and CF on maize grain yields, results indicated that CF had the highest mean grain yield (5.34t/ha) followed by BGS at 20 and 40 t/ha (4.02 and 4.52 t/ha respectively). However, Kocar (2008) obtained contrary findings where BGS was compared with CF and commercial organic fertilizers under safflower cultivation. Notably higher yields were observed under BGS fertilization than both fertilizers. In addition, findings attained by Svensson *et al.* (2004) in comparing the effects of different application rates (50 and 100 kg N/ha) of BGS, compost and CF under barley cultivation over two growing seasons in rotation with oats. The results showed that as the N application rate increased from 50 to 100 kg N/ha, the yields were significantly decreasing for both compost and BGS.

A study conducted by Ahmad and Jabeen (2009) demonstrated improved sunflower (*Helianthus Annuus L*.) growth under saline (irrigating with sea water) conditions after addition of BGS, vermicompost and a combination of both. Results indicated that the number and weight of seeds per floral disc were improved and directly increasing the seed yield from 4.71 - 11.33 g at 0.3% and 1.92 - 5.35 g at 0.6% sea salt concentration. This suggest that BGS has the potential encounter the effect of saline water thus providing conducive growth condition. The oil content per floral disc also increased. Islam *et al.* (2010) conducted a field study to assess the effect of different biogas slurry application rates (0, 60, 70 and 80 kg N/ha) on biomass yields. The results showed a significantly increasing trend from 34.67 MT/ha, 45.23 MT/ha and 54.12 MT/ha for 0, 60 and 70 kgN/ha, respectively, but a significant decrease in biomass yields was observed at 80 kg N/ha (43.67 MT/ha). The decrease in biomass yields with increased application rates suggested that high N content prohibits accumulation of biomass yields. From the results obtained by Ahmad and Jabeen (2009), significantly higher plant biomass yields (dry and wet) were observed under the combined treatment (BGS and vermicompost) followed by vermicompost and then BGS with respect to the control.

2.6. Summary

The utilization and disposal/ treatment of organic waste originating from intensive agricultural production systems is a critical concern worldwide. Use of anaerobic digestion as treatment procedure has been practised in numerous parts of the world but such practise has not been widely spread in South Africa. The AD organic wastes to produce gas has displayed great potential to yield a BGS every day. However, the alterations in chemical composition due to various factors including feedstock and retention time amongst others, which results in uncertainties of the quality of BGS. Therefore, understanding nutrient release patterns and fertilizer value of BGS produced in South Africa is necessary.

CHAPTER 3

NUTRIENT RELEASE POTENTIAL OF BIOGAS SLURRY AND CATTLE MANURE

3.1. Introduction

The use of fertilizers under intensified production systems in sub-Saharan Africa (SSA) is a requirement for improving soil productivity and poverty alleviation. Reliance on chemical fertilizers (CF) in agriculture is attributed to the readily available plant nutrients (NO_3-N, NH_4-N, HPO_4, K^+) (Lazcono *et al.,* 2013). While this practise has improved crop yields, it also has long-term effects of reducing soil organic matter and microbial activity while increasing soil acidity among other effects, leading to poor soil fertility (Brar *et al.,* 2013; Geisseler and Scaw, 2014). Organic amendments, such as animal manure and compost, have been utilized for replenishment of nutrients. Utilization of organic amendments improves soil chemical, physical and biological properties, which improves nutrient cycling and fertility status of the soil (Gil *et al.,* 2007; Odlare *et al.,* 2008; Nkoa, 2014). In addition to their use as soil amendments, the organic wastes have also been used for energy production, as biogas.

The recent rapid adoption of the biogas technology in SSC has resulted in the increase in production of biogas slurry (BGS), which could also be used as an organic soil amendment (Mshandete and Parawira, 2009). The appreciable quantities of readily available plant nutrients in BGS qualifies it as a valuable soil amendment (Arthurson, 2009; Abubaker *et al.,* 2012). For example, during anaerobic digestion (AD) NH_4-N is improved by >25% compared to the feedstock used (Arthurson, 2009). This high proportion of mineral N in BGS suggests its immediate fertilization effect (Vogel, 2009). When BGS is applied as an N source, it subsequently supplies plant available P, even though it could be lower than required by most crops (Svensson *et al*., 2004). Biogas slurry produced from slaughterhouse waste supplies 0.2 kg P for every 2 kg N supplied (Abubaker *et al.,* 2012). Despite the fact that BGS contains readily available plant nutrients, its fertilizer value depends on the quality of the feedstock used (Table 2.1) and the nutrients release patterns. For example, Abubaker *et al.* (2012) noted that, organic household waste has approximately 33% lower total N than slaughterhouse waste.

Plant nutrients contained in BGS are grouped into immediately available, rapidly released and slowly released or unavailable forms (Brady and Weil, 2007). In soils, biochemical (mineralization and immobilization) and physiochemical (precipitation, adsorption and desorption) processes influence the BGS nutrient release. The release of different nutrients from BGS depends on the amount in the different forms, due to feedstock properties and retention time, among other factors, and the properties of the treated soils (Smith *et al.,* 2014).

The uncertainties in the mineralization rate of the material present challenges on synchronizing the availability or release of N and P with crop demand and season-long crop N and P uptake requirements (Binder *et al.,* 1996; Loria *et al.,* 2007). This research used an incubation study to understand nutrient release potential in soil following BGS application, to acquire information that can be useful for field application. The specific objective was to determine the effects of BGS rates on the release of mineral N and P during incubation in two contrasting soils compared to the feedstock, cattle manure (CM). The hypothesis was that BGS would release N and P, more rapidly than CM, upon application in two separate soils.

3.2. Materials and methods

3.2.1. Soil sample collection and preparation

Two soils used in the study were collected from Roodeplaat located at 25°59′ S, 28°35′ E and from Irene located at 25° 53’ S, 28° 11' E of Pretoria in the Gauteng Province of South Africa. The soils were classified as Avalon and Hutton using the South African Classification system (Soil Classification Working Group, 1991). The use of two soils was to test the liming effect of biogas slurry between two contrasting soils since the material in generally alkaline. Thirty samples were randomly collected from the 0 - 30 cm depth at each site with an auger and mixed thoroughly to make a composite sample. The samples were air dried, milled and then sieved (< 2 mm) before use in the incubation study.

3.2.2. Soil Analysis

pH and electrical conductivity

The pH and EC of the samples were both measured at a ratio of 1:2.5 (soil: water) suspension, as defined by Okalebo (2002). Briefly, 10 g of soil was mixed with 25 mL deionised water in a plastic bottle and shaken for 10 minutes with an end-to-end reciprocal shaker. The mixture was allowed to stand for 1 hour. The pH and EC were measured using a glass electrode pH 700 meter, Eutech instruments, Singapore and EC meter multi 9310 IDS, Germany respectively.

Carbon and nitrogen

Total C and N were determined by dry combustion as described by Jimenez and Ladha (1993). Concisely, 10 g of the sample was weighed into tin foil container for each determination. Samples were ignited at high temperatures (1020 ^{o}C) in the presence of oxygen using CHNS-O analyser, Flash 2000, Thermo Scientific.

Available phosphorus

The plant available P was extracted following the Bray 1 extraction method as described by Bray and Kurtz (1945). Briefly, 6.67 g of soil was extracted with 50 mL of extracting solution (0.5M HCL and NH_4F) in a 100 mL inert plastic bottle. The mixture was shaken on a reciprocating shaker for 60 seconds at 120 rpm, followed by filtration with a Whatman No 40 filter paper and the filtrate was analysed for P using a Continuous Flow Auto Analyser 3, *SEAL* Analytical, Australia.

Mineral Nitrogen

Ammonium- and nitrate-N were extracted with 1.0 M KCl as described by Carter and Gregorich (2006). Briefly, 10 g of soil was placed in an extraction tube and 100 ml of 1.0 M KCl added to soil before shaking at 180 rpm for 30 min. The suspension was filtered through Whatman no. 40 filter paper and the filtrate was analysed for NH_4-N and NO_3-N using a Continuous Flow Auto Analyser 3, *SEAL* Analytical, Australia.

Exchangeable bases

The exchangeable bases were extracted with 1M Ammonium acetate solution (NH_4OAc) 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_4OAc solution was added. The mixture was then shaken using a reciprocating shaker at 180 rpm for 30 minutes, and then filtered using Whatman No 40 filter paper. 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. The measurements were done at the beginning and end of the incubation.

Extractible trace elements

Zinc, iron, manganese and copper were extracted following 0.1 M HCl extraction method as described by Baker and Amacher (1982). A 5 g air-dried soil sample was placed in an extraction tube and mixed with 20 ml of 0.1 M HCl. The mixture was shaken horizontally at 180 rpm for 15 minutes. The mixture was filtered using Whatman No. 40 filter paper into a zinc-free silicon stopper container and the extract was analysed for Zn, Fe, Mn and Cu using an ICP. Chemical characteristic of the soils prior to incubation are presented in Table 3.1.

Table 3.1: Initial characterization of soils used in the study

Parameter	Avalon	Hutton
pH (H_2O)	6.88	5.34
Total N (%)	0.081	0.084
Total C (%)	1.22	1.42
N-NH_4 (mg/kg)	2.67	2.57
N-NO_3 (mg/kg)	6.91	1.94
P (mg/kg)	7.99	3.44
K (cmol(+)/kg)	0.249	0.51
Ca (cmol(+)/kg)	5.65	3.02
Mg (cmol(+)/kg)	4.56	1.27
Fe (mg/kg)	5.30	19.7
Mn (mg/kg)	53.8	152
Zn (mg/kg)	4.61	2.61
Cu (mg/kg)	2.97	5.32

3.2.3 Organic amendments sample collection and preparation

Biogas slurry and cattle manure were collected from a farm in Free State located between Harrismith and Qwa-qwa along R712 road (-28^{o}42' S and 28^{o}86' E). The farm has between 40-50 cattle that graze on natural pastures and sometimes on crop (maize and dry-bean) residues. Brief small-scale digester operation: 25 kg of fresh cattle manure is mixed with 25 L of water and fed into the digester, which is operating at temperatures ranging between 35 - 55 degrees. Slurry is collected in the cycles of 24 hrs following the feeding patterns. The samples were transported to Pretoria within 5 hours and were dried in trays in an oven at 60°C for 48 hours. The dried samples were ground, sieved (< 1 mm) and stored in plastic buckets at room temperature.

3.2.4 Organic amendments analysis

pH, electrical conductivity and total C and N

The pH and EC of BGS and CM samples were both measured at a ratio of 1:10 (BGS/CM: water) in the same suspension following the same procedure described for soils (see 3.2.2). Determination of total N and C followed the dry combustion method describe under soil analysis (see 3.2.2).

Total elemental analysis

The BGS and CM were digested with perchloric and nitric acid mixture as described by Zasoski and Burau (1977). Briefly, 0.5 g of dried material was digested with 7 ml HNO_3 (conc. nitric acid, 65%) and 3 ml $HClO_4$ (conc. perchloric acid, 70%) at 170 °C. This was followed by dropwise addition of 0.25 ml H_2O_2 (conc. hydrogen peroxide, 50%), with further heating for 20 minutes and addition of 10 ml 1 M HCl (conc. hydrochloric acid). An aliquot of the digest was analysed for P, K, Ca, Mg, Fe, Mn, Zn, B, and Cu using an Inductively Coupled Plasma Optical Emission Spectrometric (ICP-OES) an Agilent 725 (700 Series). Table 3.2 shows nutrient composition of amendments.

Table 3.2: Initial characterization biogas slurry (BGS) and cattle manure (CM)

Parameter	Biogas slurry	Cattle manure
pH (H_2O)	9.10	8.57
EC (mS/m)	375	357
Total N %	2.55	1.9
Total C %	35.0	28.2
C/N ratio	13.7	14.8
P (g/kg)	5.73	3.37
K (g/kg)	17.7	16.7
Ca (g/kg)	19.1	7.12
Mg (g/kg)	9.12	5.11
Fe (g/kg)	5.18	5.16
Mn (mg/kg)	788	464
Zn (mg/kg)	799	125
Cu (mg/kg)	40.3	24.8
B (mg/kg)	27.7	18.6

BGS = Biogas slurry; CM = Cattle manure

3.2.5. Incubation procedure and sampling

The experiment was laid out in a completely randomised design with each treatment replicated 3 times. Each of the two air-dried soils was thoroughly mixed with oven dried BGS or CM at 0, 1, 2 and 3 % w/w. Enough sample (1 kg), of each treatment was incubated to allow for non-destructive sampling during the 8 weeks of incubation at 23°C. After mixing, the soils were moistened to field capacity using deionised water. The containers had five holes pierced around the rim to allow gaseous exchange without fast drying. The moisture of the samples was adjusted based on weight loss, every 2 - 3 days. A 50 g sample was collected after 0, 1, 2, 4 and 8 weeks, stored in the fridge at 2- 4 °C before analysis within one week. The samples were analysed for pH, EC, NH_4-N, NO_3-N, Bray-1 P and exchangeable bases (Ca, Mg, and K), as described in section 3.2.2.

3.2.5 Data Analysis

The data were subjected to two-way analysis of variance (ANOVA) with GenStat 18th edition software (VSN International, 2016) to determine effects of organic material (BGS and CM) and application rate on concentrations of mineral N (NH_4 and NO_3), phosphorus (P) and exchangeable bases (K, Mg and Ca) for each incubation time.

3.3. Results

3.3.1. The pH of soils incubated with BGS and CM

In general, the pH increased with increasing application rates of both BGS and CM throughout the incubation period for both soils. The soil pH at 1% rate was not significantly different from the control for both BGS and CM, except at weeks 4 and 8 where BGS treatment had higher pH (Figure 3.1a). For each rate at weeks 0 and 1, there were no pH differences between BGS and CM except for 3% where BGS has higher level for the Avalon. The pH values in BGS treatments were higher than corresponding rates of CM for weeks 2, 4 and 8. For Hutton, all treatment levels were higher that the control throughout the incubation period (Figure 3.1b). At 1% rate, there were no difference in soil pH between BGS and CM for all incubation periods except for week 8, where BGS had higher levels. The BGS resulted in higher soil pH than CM at 2 and 3% rate throughout the incubation period except for week 1 at the 2% rate.

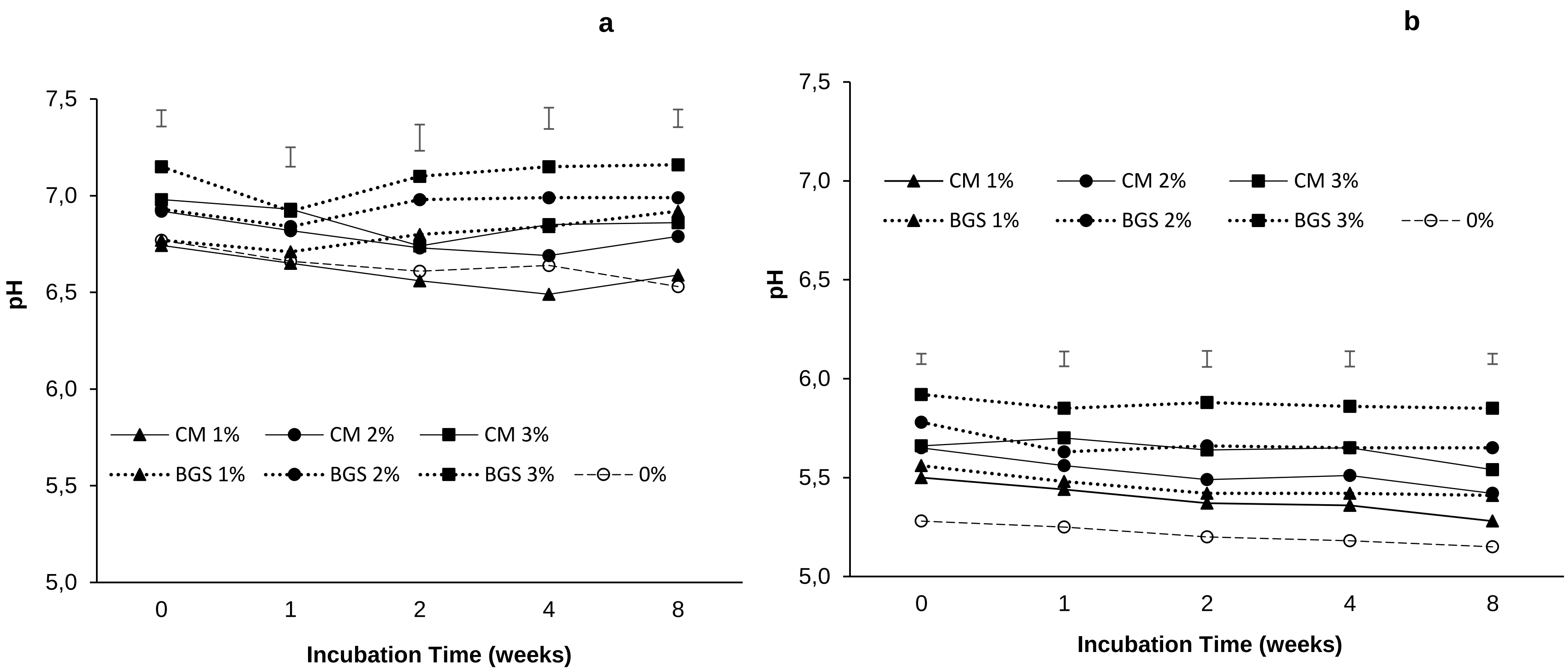

Figure 3.1: Changes in soil pH during incubation following application of biogas slurry (BGS) and Cattle Manure (CM) at 0, 1, 2 and 3% (w/w) under two different soils (A = Avalon and B = Hutton). Error bars within each week indicate least significant differences at the *p* = 0.05. Comparisons in the text are based results of the Tukey test.

3.3.2 Changes in mineral nitrogen (NH_4-N and NO_3-N) concentration

Initial (week 0) concentrations of NH_4-N for Avalon increased with application rates for both amendments except for CM at 1 and 2% rates. There was a general decline from week 0 to week 2 for both soils at all rates. For example, at 3% BGS NH_4-N declined by 73.6% in Avalon compared to the 36.7% in Hutton soil from 0 to 2 weeks. A general increase followed up to week 8 (Figure 3.2a). There were no differences in ammonium-N at 1% rate between BGS and CM for both soils except that in the Hutton, where BGS had higher ammonium-N at week 1 and lower at week 4. Biogas slurry at 2% rate resulted in higher ammonium-N at week 0 and lower at weeks 4 and 8 than the CM in the Avalon. A similar trend occurred in the Hutton soil except for week 1 with higher for BGS and week 4, which was not different between the two materials. At 3% rate, BGS resulted in higher ammonium-N than CM except for week 2 and 8 where there were no differences for Avalon (Figure 3.2a). Similarly, in the Hutton soil ammonium-N was higher in BGS than CM except for week 4 and 8 where there were no differences (Figure 3.2b)

Availability of nitrate-N generally increased with time for CM for both soils (Figure 3.3 a and b). The BGS treatment displayed a similar trend for both soils, where the nitrate-N declined from week 1 to week 2 then followed gradual increase that was not significant for Avalon but notably different for Hutton (Figure 3.3 a and b). At 1% rate, CM had greater nitrate-N than BGS except for weeks 0 and 1, where there were no differences for both soils. Similarly, at 2 and 3% rates, CM had higher nitrate-N than BGS except weeks 0 and 1 for Avalon and week 1 for Hutton, where there were no differences. At each incubation time beyond one week, nitrate-N decreased with increase in BGS rate for both soils.

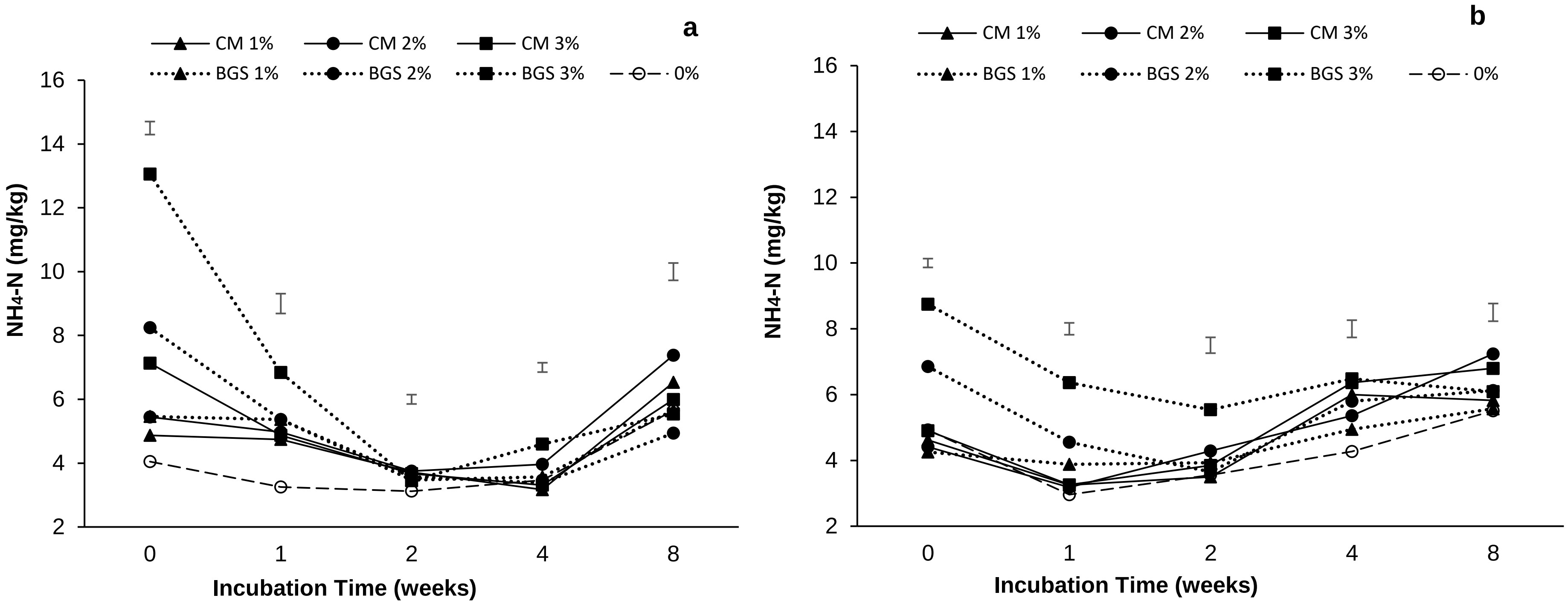

Figure 3.2: Changes in soil NH_4-N during incubation time following different Biogas Slurry (BGS) and Cattle Manure (CM) application rate and the 0, 1, 2 and 3% (w/w) under two different soils (A=Avalon and B=Hutton). Error bars within each week indicate least significant differences at the p = 0.05. Comparisons in the text are based results of the Tukey test.

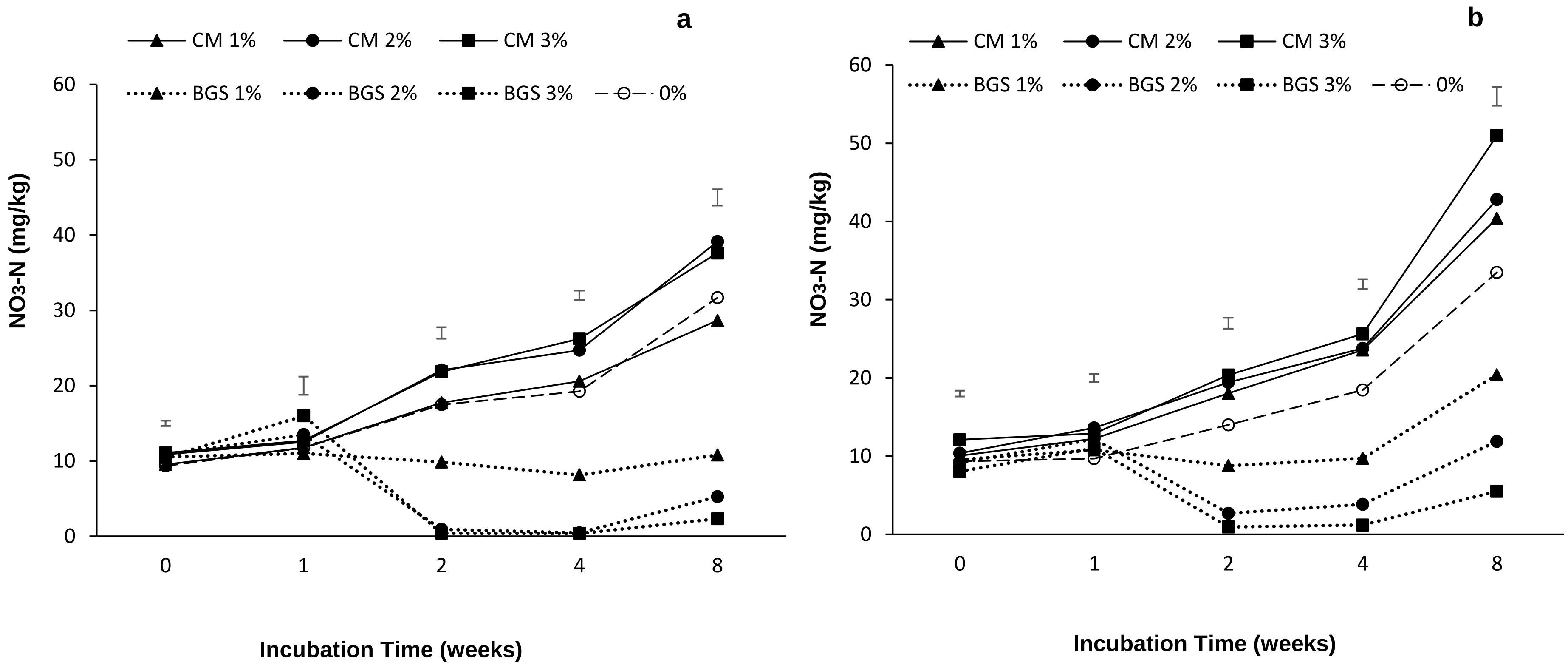

Figure 3.3: Changes in NO_3-N during incubation time following application of biogas slurry (BGS) and cattle manure (CM) at 0, 1, 2 and 3% (w/w) under two different soils (A=Avalon and B=Hutton). Error bars within each week indicate least significant differences at the *p* = 0.05. Comparisons in the text are based results of the Tukey test.

3.3.3. Available P

Available P remained essentially unchanged for the control ranging 1.80 - 3.43 mg P kg^{-1} for Avalon and 4.35 - 8.23 mg P kg^{-1} for the Hutton soil throughout the incubation period (8 weeks). Over the 8 weeks of incubation period, available P for both BGS and CM treatments significantly increased with increasing application rates for both soils (Figure 3.4a and b). However, BGS showed a general decline in available P over the incubation time for both soils (Figure 3.4a and b). At 1 % level, there were no differences in available P between BGS and CM throughout the incubation period except for week 0 for the Avalon and week 1 for the Hutton, where BGS had higher available P (Figure 3.4). The BGS had higher available P than the CM at 2 and 3% rates throughout the incubation period except the 2% rate at week 8 in the Avalon (Figure 3.4a). In the Hutton, the BGS had higher available P than the CM at 2 and 3% rates for weeks 0, 1 and 2 only (Figure 3.4b).

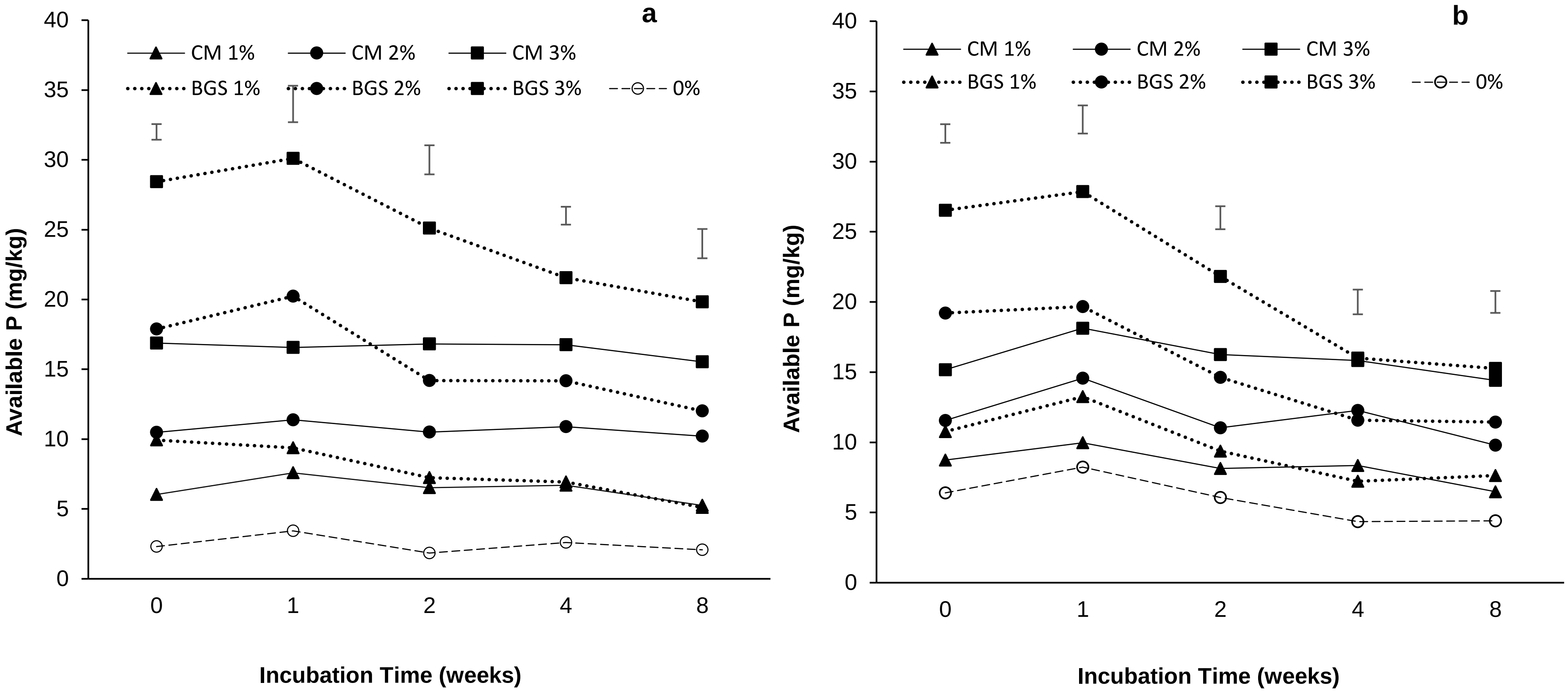

Figure 3.4: Variations in available P concentrations during incubation time following different Biogas Slurry (BGS) and Cattle Manure (CM) application rates at 0, 1, 2 and 3% (w/w) under two different soils (A=Avalon and B=Hutton). Error bars within each week indicate least significant differences at the p = 0.05. Comparisons in the text are based results of the Tukey test.

3.3.4. Electrical conductivity of soils incubated with BGS and CM

On average, EC increased with increasing application rate of both BGS and CM for both soils (Figure 3.5a and b). The EC for CM at 1% was not different from the control except at week 8, where CM had higher EC for Avalon. However, for Hutton soil the two materials were significantly different except at weeks 0 and 1. Unlike for BGS at 1%, the EC was significantly higher in CM than the control for both soils except at weeks 4 and 8 for Avalon and week 8 for Hutton. At 2 and 3% rates, there were no differences in EC between BGS and CM except for weeks 0 and 1, where BGS had higher EC than CM for Avalon soil (Figure 3.5a). For the Hutton soil, BGS had significantly higher EC than CM except for weeks 4 and 8 where there were no difference (Figure 3.5b).

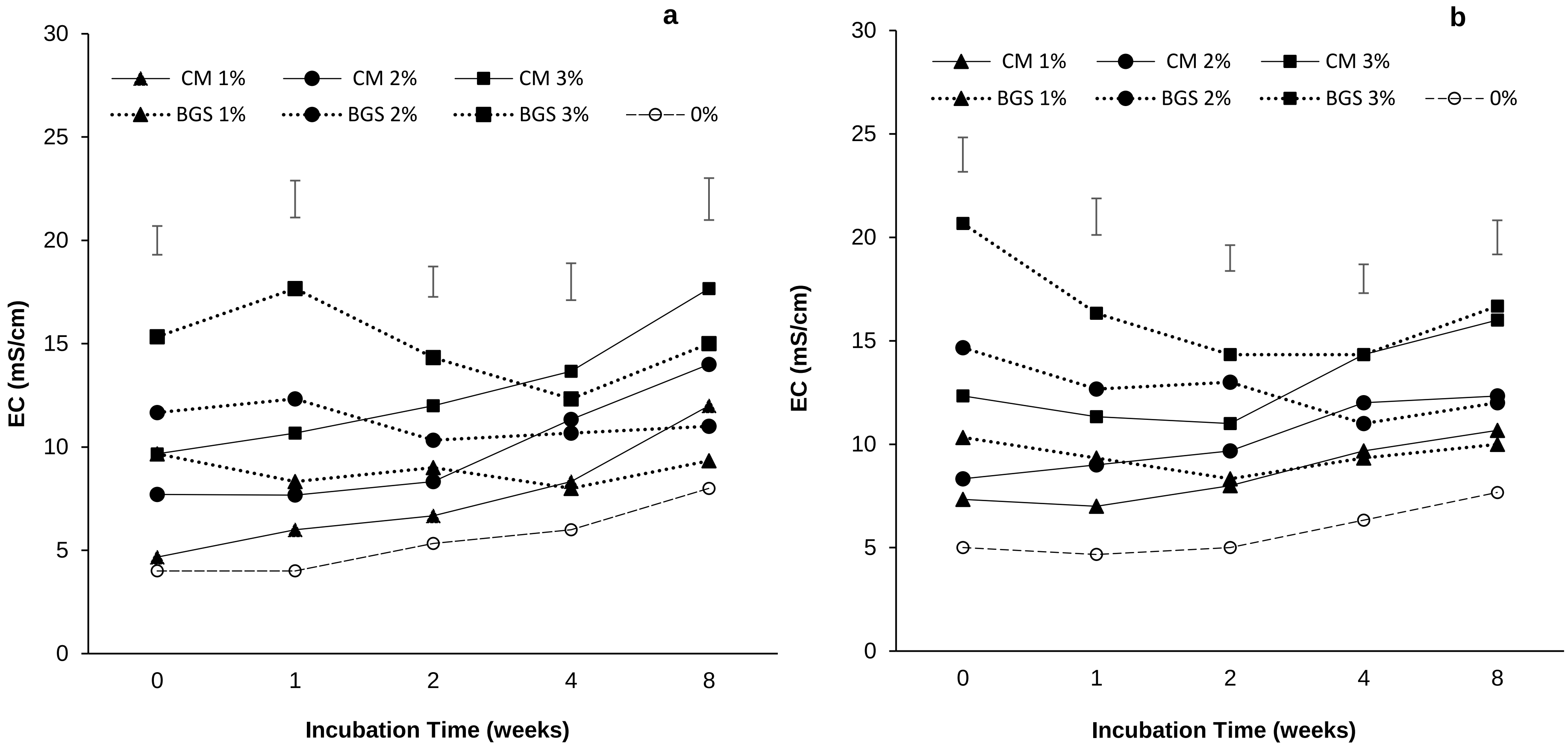

Figure 3.5: Changes in soil EC during incubation after application of biogas slurry (BGS) and cattle manure (CM) at 0, 1, 2 and 3% (w/w) under two different soils (Avalon and Hutton). Error bars within each week indicate statistically significant differences according to the Tukey test at the *P* = 0.05.

3.3.5. Exchangeable K, Ca and Mg

Exchangeable K generally increased with increasing rates of BGS and CM at week 0 for both soils. At each rate, both treatments had comparable exchangeable K. At week 8, a similar trend was observed except at 3% rate, where BGS had lower K than CM (Table 3.3). In addition, for Hutton soil, BGS had higher K than corresponding rate in of CM except at 3% rate, which were not different (Table 3.4). The exchangeable Ca was comparable between BGS and CM at all rates, which were all not different from the control at week 0 for Avalon soil. However, for week 8 all application rates were similar and significantly higher than the control except for BGS at 1%, which was similar to the control for Avalon soil (Table 3.3). For the Hutton soil, exchangeable Ca displayed increasing trend with increasing application rates at both week 0 and 8 for Hutton soil (Table 3.4). In addition, for both week 0 and 8 all corresponding rates were similar between BGS and CM. At week 0, 1 and 2% rates, exchangeable Ca was similar to the control and at week 8 they were both lower than the control except for BGS 2%, which was similar to the control.

Exchangeable Mg increased with increasing application rates and BGS treatments had similar levels to corresponding rates of CM at week 0 for Hutton soil. Furthermore, 1 and 2% were not different from the control except for BGS 2%, which was higher that the control (Table 3.4). However, for Avalon soil, BGS had higher exchangeable Mg than CM at all rates except at 3% rate where they were similar at week 0 (Table 3.3). At week 8, BGS and CM at all rates had similar exchangeable Mg, which were not different from the control for Hutton soil. For Avalon soil, the control and all rates of BGS had similar exchangeable Mg (Table 3.3). For CM at 2 and 3% rates, exchangeable Mg was higher than the control.

Table 3.3: Selected exchangeable bases at the beginning and end of the incubation period in Avalon soil

Time	Rates (%)	K (cmol+/kg)		Ca (cmol+/kg)		Mg (cmol+/kg)	
		CM	BGS	CM	BGS	CM	BGS
Week 0	0	0.195a	0.195a	5.65ab	5.65ab	4.51a	4.51a
	1	0.285ab	0.316b	5.57a	5.68ab	4.41a	4.67bc
	2	0.365bcd	0.325bc	5.72ab	6.00ab	4.52ab	4.78c
	3	0.438d	0.416cd	5.92ab	6.25b	4.69c	4.81c
Week 8	0	0.249a	0.249a	4.92a	4.92a	3.88a	3.88a
	1	0.339ab	0.209a	5.86b	5.30ab	4.06ab	4.10ab
	2	0.452bc	0.343ab	5.62b	5.40b	4.43c	4.01ab
	3	0.543c	0.211a	5.63b	5.60b	4.27bc	4.06abc

Different lowercase letters within each week indicate statistically significant differences according to the Tukey test at the $P = 0.05$.

Table 3.4: Selected exchangeable bases at the beginning and end of the incubation period in Hutton soil

Time	Rates (%)	K (cmol+/kg)		Ca (cmol+/kg)		Mg (cmol+/kg)	
		CM	BGS	CM	BGS	CM	BGS
Week 0	0	0.349a	0.349a	3.19ab	3.19ab	1.31a	1.31a
	1	0.443b	0.441	2.84a	2.19a	1.33ab	1.39ab
	2	0.544c	0.526c	2.97a	3.15ab	1.45ab	1.54bc
	3	0.700e	0.623d	3.36bc	3.53c	1.68c	1.70c
Week 8	0	0.374de	0.374de	3.33bc	3.33bc	1.20a	1.20a
	1	0.296b	0.402e	2.84a	2.91a	1.28a	1.21a
	2	0.150a	0.465f	2.97a	3.15ab	1.27a	1.25a
	3	0.346cd	0.314bc	3.36bc	3.53c	1.15a	1.28a

Different lowercase letters within each week indicate statistically significant differences according to the Tukey test at the $P = 0.05$.

3.4. Discussion

The increase in pH following application of both amendments was associated to the appreciable quantities of ammonium-N (from ammonia) produced during the incubation, which increase with increasing rates. The results are similar to those obtained by Chiyoka (2011), who reported that soil incubated with feedlot cattle manure displayed a significant pH increase. Gil *et al.* (2007) also showed that soil incubation of BGS raised soil pH from 6.3 to 8.0.

The rapid decline in NH_4-N displayed from week 0 to week 2 of incubation time, indicated that NH_4-N may have been converted into NO_3-N in the CM treated soils (Alburquerque *et al.,* 2011). This is supported by the increasing $N0_3$-N over the incubation period, particularly in CM treatments (Figure 3.4a and b). These findings were in agreement with Grigatti *et al.* (2011), who reported that NH_4-N drastically dropped over incubation time in soils treated with BGS. Although ammonium-N declined with incubation times in BGS treatments, there was no associated increase in nitrate-N. The reason for the decrease of NO_3-N with BGS rate over time is not clear. The results were in agreement with Goberna *et al*., (2011), who reported that NO_3-N declined within 30 days of 100 days of incubation time. A possible explanation is that the ability of BGS to enhance microbial activity due to readily available nutrients may have resulted in the accumulation of N into their cells. This view is supported by the increase of NO_3-N at week 8 for both soils, suggesting that the microbe were dying and the constituent N was mineralising back into soil. The lower nitrate-N that decreased with increase in rate of BGS, than CM, suggest that N availability may be limited in the early stages of crop growth when BGS is used. However, the findings were contrary to those reported by Bruun *et al.* (2011), who showed that NO_3-N from BGS treated soils increased significantly over the incubation time. The high organic matter (≥4 %) and moisture content in the chernozemic soils of cooler origins and utilization of liquid slurry may have induced the differences in the findings.

Both amendments yielded a positive net P mineralization this indicates instant P fertilization effect on both soils (Figure 3.5) and may be attributed to microbial activity, which improves nutrient cycling (Abubaker *et al.,* 2012). The subsequent decline from week 1 to 8 may be due to utilization soil available P by microbes for protein synthesis (Havlin *et al.,* 2005). The optimum P availability is at pH rages of 5.5 to 6.5 such that outside that range fixation occurs. For example, Avalon soil had a soil pH up to 7.2 that coincides with decline in available P due to possible precipitation with Ca to form calcium phosphates. However, at pH below 5.5 such as in Hutton soil, P fixation is associated with Fe, Al and Mn fixation. The possible immobilisation of P followed by later mineralisation by microbes during incubation suggest that this mechanism could moderate fixation and improve uptake by plants. Gichangi et al. (2010) reported that microbial biomass P was closely related to maize yield in a high P fixing soil.

Electrical Conductivity (EC) of both soils increased with increasing application rates for BGS and CM, which coincides with the increase in soil pH. This is due to salts released by the amendments (Yu *et al.,* 2010). It is also necessary to take note that the EC value were all lower than 400 mS/m, which is the threshold to induce soil salinity thereby posing a threat to nutrient imbalance and limited water uptake due to low osmotic water potential (Havlin *et al.,* 2005). For Hutton soil exchangeable K concentrations decrease over the incubation period and yet for Avalon it displays an increasing trend suggesting that Hutton soil may have fixed available K in contrast to Avalon (Havlin et al., 2005). Exchangeable K and NH_4-N have relatively the same ionic radii, which influences the release of the other when one is abundant in soil solution thus explaining the inverse availability trends displayed by K and NH_4-N (Table 3.3). The overall greater K release in CM manure than BGS could be associated the higher K content in CM than in BGS (Table 3.2). The low concentrations of exchangeable Mg in Hutton may be strongly linked to the pH values skewed towards the minimal side of the optimum pH range (5.5 – 7.0) exhibited by the soil (Havlin *et al.,* 2005).

3.5. Conclusion

Both amendments exhibited a liming potential, with greater effects from BGS, when compared with the control. Ammonium-N and extractable P increased with increasing application rates with higher levels in BGS than CM in both soils. Increasing BGS application rate inhibited nitrification but did not result in the accumulation of ammonia. Both BGS and CM displayed significant release of exchangeable base into soil solution. The nutrient release patterns between BGS and CM differed. There is need to determine the fertiliser value of BGS, relative to CM, through glasshouse and field studies. Total carbon and nitrogen were not measured because the LECO was not working at time. Further studies on soil carbon pools following biogas slurry applications would be ideal for wider understanding.

CHAPTER 4

FERTILISER VALUE OF BIOGAS SLURRY ON SPINACH GROWTH AND DRY MATTER YIELDS

4.1. Introduction

Large quantities of biogas slurry, which is a by-product from energy (biogas) production from organic wastes, presents disposal challenges. The high nutrients occurring in available form provides an opportunity for BGS to be utilised as an organic source of nutrients. Rural communities with little or no access to chemical fertilizers (CF) produce crops below their potential and the quality of produce is reduced. According to Moller and Muller (2012), the availability of BGS from domestic household digesters presents benefit for vegetable production where fertilizers are a constraint. The utilization of BGS under vegetable production could be beneficial in the sense that it has readily available plant nutrients and aids in improving soil physical, chemical and biological parameters, thus enhancing soil quality (Gil *et al.,* 2007; Tambone *et al.,* 2010; Abubaker et al., 2013). The effectiveness BGS as a nutrient source is governed by nutrient release patterns.

The higher ammonium-N and extractable P in BGS, and liming effect, than CM (Chapter 3) suggests that the slurry may have greater fertiliser value. Findings by Odlare *et al*. (2011) showed that after 8 years of application, BGS increased barley yield by 12 and 32 % against compost and control, respectively. However, the decline in nitrate-N in BGS treatments, which increased in CM, with incubation time (Chapter 3), complicates the understanding of potential availability of mineral N from BGS, relative to CM, for crops. The decline of nitrate-N may limit its uptake and crop growth particularly during the early stages. This potential limitation of N availability could benefit from co-application with chemical fertilisers. Findings reported by Citak and Sonmez (2010) indicate that spinach produced from organic amendments has better quality (minerals and vitamins) than those fertilized with CF, even though the yield was lower. The specific objectives of this study were to determine the effect of (i) rate of BGS application, as an N source, relative to CM and (ii) co-application of BGS with chemical fertilizers, on spinach dry matter yields and nutrient uptake.

The hypothesis was that (i) application of BGS would enhance dry-matter yields and nutrient uptake more than CM and that (ii) co-application with chemical fertiliser improves the N fertiliser value of BGS.

4.2. Materials and methods

The pot experiment was conducted under glasshouse conditions at Agricultural Research Council – Vegetable and Ornamental Plant Institute (ARC-VOPI), which is located in Roodeplaat (28.58°E; 25.98°S) Gauteng Province, South Africa.

4.2.1. Pot experiments

The air dried and sieved (<2mm) soils (Avalon and Hutton) detailed in Chapter 3 were also used in this study. The biogas slurry (BGS) and cattle manure (CM) (dried and sieved) used in the incubation experiment were also used in this study. While the slurry is normally applied as a liquid to soils, the drying of BGS and CM was done to control consistency in nutrient composition of the amendments for both incubation and pot experiment.

Experiment 1: Application of amendments as nitrogen sources

This experiment was laid in a completely randomised design, with four replicates. The Avalon soil was treated with BGS, CM and chemical fertiliser at a rate equivalent to 100 kg N/ha. The pots used contained 4 kg soil. The fertilizer used in the study was a mixed fertilizer 3:2:1 (28). A negative control (no N applied) was also included. The addition of P or/and K in the treatments through straight fertilizers (single superphosphate and potash) was done so that N becomes the only limiting factor. Phosphorus was the highest in BGS therefore to match what was in BGS; P had to be added in CM and CF treatments. Potassium was the highest in CM, to match what was in CM; K had to be added in BGS and CF treatments. Briefly: BGS (310 g BGS + 1.332g K), CM (408 g CM + 0.401 g P) and CF (5.54 g CF + 1.259 g P + 6.553 g K).
Two-week old spinach seedlings (*Spinacia oleracea L.*) were transplanted to each pot and were thinned to one plant after four weeks. The temperatures in the glasshouse ranged between 18 - 20°C at night and 23 - 25°C during the day, which were regulated with automatic air conditioning system. The plants were irrigated with tap water when required, to ensure that water was not a limiting. The plants were allowed to grow for 8 weeks, and harvested by cutting with a scissors at 1 cm above the soil surface. The plant samples were rinsed with distilled water and oven dried at 65 °C for 48 hrs, and weighed. The plant tissues were ground to <1 mm and analysed for total C, N, P, Ca, Mg, K, Fe, Mn, Zn, Cu and B. The soil from the pots were air dried for a week and sieved (<2mm) before analysis of pH, EC, mineral nitrogen (ammonium and nitrate), available P, exchangeable bases and trace elements.

Experiment 2: Effects of rates of BGS and CM on dry-matter and nutrient uptake

The pot trial was a 2 x 5 factorial experiment in a completely randomised block design with four replications. The Avalon soil was treated with BGS and CM at rates equivalent to 0, 100, 150, 200 and 250 kg N/ha, and homogenised. These rates translated to 0, 310, 465, 620 and 775 g BGS/pot and 0, 408, 612, 816 and 1020 g CM/pot. Two-week old spinach seedlings (*Spinacia oleracea L.*) were transplanted to each pot and were thinned to one plant after four weeks. The glasshouse conditions, management of the trial, sampling and analyses were as detailed for Experiment 1.

Experiment 3:Co-application of biogas slurry with chemical fertilizer

The pot trial was a 2 x 4 factorial experiment in a completely randomised block design with four replications. The two soil (Avalon and Hutton) were amended with BGS co-applied with CF at 0/100 (0 g BGS/ 5.54 g CF); 40/60 (124 g BGS/ 3.32 g CF); 60/40 (186 g BGS/ 2.22 g CF) and 100/0 (310 g BGS/ 0 g CF) kg N/ha (BGS/CF), and homogenised. Two-week old spinach seedlings were transplanted to each pot and were thinned to one plant after four weeks. The glasshouse conditions, management of the trial, sampling and analyses were as detailed for Experiment 1.

4.2.2. Plant analysis

Carbon and N were determined by the dry combustion method describe under soil analysis (see 3.2.2). Tissue P, K, Ca, Mg, Fe, Mn, Zn, B, and Cu were determined after acid digestion as describe under analysis of organic amendments (see 3.2.4).

4.2.3. Soil analysis

Determination of pH, EC, available P, extractible bases, extractable trace elements and mineral N was done as described under soil analysis (See 3.2.2).

4.2.4 Data Analysis

Statistical analysis was done using GenStat 18th edition software (VSN International, 2016). A one-way analysis of variance (ANOVA) was used to determine effects of BGS CM and CF. A two-way ANOVA was used to determine effects of (i) nutrient source (BGS and CM) and application rate and (ii) soil type and BGS/CF ratios.

4.3. Results

4.3.1. **Spinach yield and nutrient uptake from BGS, CM and CF as a N sources**

4.3.1.1. Dry-matter yield and nutrient uptake

Both the application of CF and organic amendments increased dry matter, with CF having the highest, when compared with the control. There were no differences in spinach dry-matter between BGS (4.037 g/pot) and CM (3.395 g/pot) (Figure 4.1). Biogas slurry yield was 58.10% greater than the control. Nitrogen uptake was significantly higher in CF treatment than all other treatments, which were not different from each other (Table 4.1). Phosphorus uptake differed across all treatment except for BGS and control, which were not different and were lower than both CF and CM. The uptake of K, B and Cu was different for all treatments except for BGS and CM, which were similar and had higher uptake than the control.

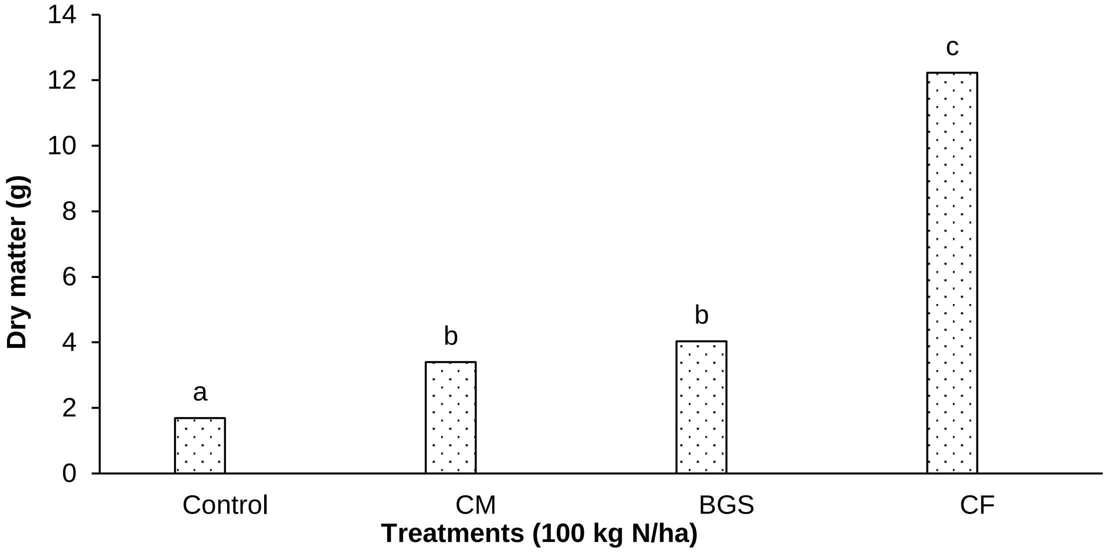

Figure 4.1: Yield response to different treatment effect at 100 kg N/ha on an Avalon soil. Different lowercase letters within each week indicate statistically significant differences according to the Tukey test at the *P* = 0.05.

For Ca and Mg uptake, there were differences across all treatments, with the greater uptake observed under CF> BGS> CM > control (Table 4.1). The uptake of Fe and Mn varied significantly across all treatments, with the highest uptake under CF and least with the control. Uptake of Zn was not different between BGS, CM and control, which were statistically lower than CF.

Table 4.1: Spinach nutrient uptake following different fertiliser treatments at 100 kg N/ha

Parameters	Treatments			
	Control	**BGS**	**CM**	**CF**
Macronutrients (mg/pot)				
N	2.32a	6.88a	5.50a	30.6b
P	5.31a	5.31a	13.8b	84.9c
K	31.9a	146b	111b	585c
Ca	22.1a	61.0c	48.7b	158d
Mg	15.7a	73.1c	36.3b	145d
Micronutrients (mg/pot)				
Fe	0.436a	1.03b	1.91c	8.85d
Mn	0.443a	1.72c	1.07b	3.48d
Zn	0.078a	0.139a	0.149a	0.348b
B	0.102a	0.219b	0.205b	0.675c
Cu	0.005a	0.034b	0.033b	0.125c

Different lowercase letters indicate statistically significant differences according to the Tukey test at the $p = 0.05$.

4.3.1.2. Soil residual plant nutrients after fertilizing with BGS, CM and CF

At harvest, there were no differences in soil pH for all amendments. Soil residual NH_4-N was significantly different between all the treatments, which followed the order of CF > BGS > CM > control (Table 4.2). Nitrate-N was comparable between all the treatments except for BGS, which had higher soil residual NO_3-N but similar to CF. Soil residual P was different between all the treatments except for control and CM, which were similar. Exchangeable K was not different for all the treatments, except for CM, which had higher levels (Table 4.2). There were significant differences in exchangeable Ca between the treatments except for CF and CM, which were similar. Soil exchangeable Mg and extractable Cu were similar in all treatments. Zinc was higher under BGS but not different from CM, which was not different from CF (Table 4.2). Soil residual Mn was significantly different across all treatments.

Table 4.2: Residual soil fertility at harvest following different treatments at 100 kg N/ha

Parameters	Treatments			
	Control	**BGS**	**CM**	**CF**
pH	7.81	7.83	7.92	7.41
Macronutrients (g/kg)				
NH_4-N	4.44c	5.88b	5.04c	7.31a
NO_3-N	2.78b	3.97a	2.96b	3.65ab
P	12.4c	23.1b	14.2c	138a
Exchangeable base (cmol+/kg)				
K	0.353b	0.395b	0.737a	0.387b
Ca	7.81c	11.2a	9.90b	9.89b
Mg	4.72	5.02	4.76	5.12
Micronutrients (mg/kg)				
Fe	6.15ab	7.04a	5.19b	7.23a
Mn	60.8d	99.1b	127a	72.1c
Zn	3.62c	5.06ab	5.26a	4.22bc
Cu	0.978	1.09	0.978	0.981

Different lowercase letters indicate statistically significant differences according to the Tukey test at the $P = 0.05$.

4.3.2. Effects of BGS and CM rates on dry-matter yield and nutrient uptake

4.3.2.1 Dry matter yield and nutrient uptake

The dry-matter accumulated increased from 100 to 150 kg N and remained the same up to 250 kg N for both BGS and CM, which were all significantly higher than the control (Figure 4.2). Uptake of N, P, Ca and Mg for all rates of BGS and CM was higher than the control, except the CM at 100 kg N/ha. Increasing application of both BGS and CM from 100 to 250 kg N/ha did not increase N uptake. At the rates of 100 and 150 kg N/ha BGS had higher N uptake than CM, with the BGS at 150 kg N/ha having higher levels than all the rates of CM (Table 4.3).

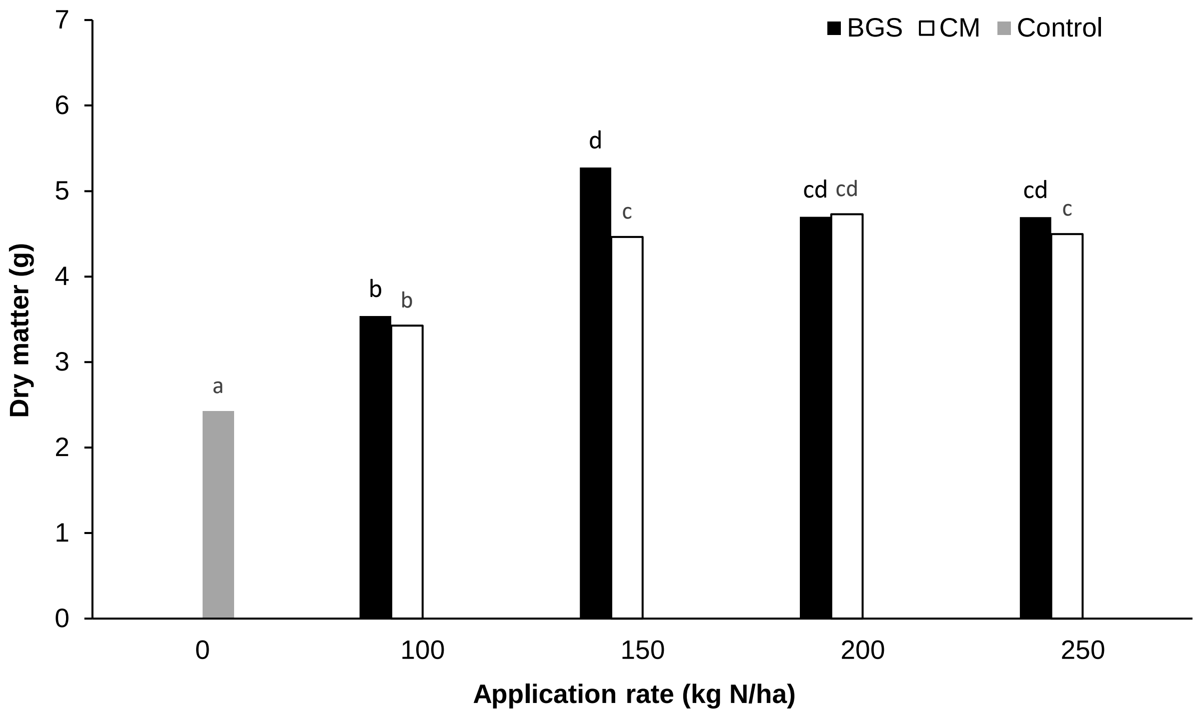

Figure 4.2: Spinach Yield response at increasing BGS and CM application rates. Different lowercase letters within each week indicate statistically significant differences according to the Tukey test at the $p = 0.05$.

Nitrogen, P, K and Ca uptake was similar between BGS and CM at all corresponding rates except at 150 kg N/ha of BGS which had higher levels. At this BGS rate (150 kg N/ha) P uptake was higher than all rates of CM. Increasing rates resulted in increase in P, K and Ca uptake followed by a decline at higher rates, with the higher uptake at 150 kg N/ha for BGS and 200 kg N/ha for CM. Uptake of Mg did not differ between BGS and CM at all corresponding rates, which were all higher than the control.

Increasing application rates for both BGS and CM, increased Mg up 150 kg N and remained unchanged

The Fe uptake significantly increased from 100 to 150 kg N/ha followed by decline higher rates for BGS, whereas for CM, it continued to increase with increasing application rate (Table 4.4). The BGS treatment at 150 followed by the 200 kg N/ ha rate had the highest Fe uptake, which were higher that for all rates of CM. The CM 100 and 150 kg N/ha and BGS at 250 kg N/ha had similar Fe uptake with the control. At 100 and 150 kg N/ha, BGS resulted in significantly lower Mn uptake than corresponding rates of CM, but the two organic waste were similar at 250 kg N/ha and BGS had higher uptake at 200 kg N. Only BGS at 100 kg N/ha similar Mn uptake with the control, which had lower levels than all other treatments (Table 4.4). Uptake of Zn was similar between BGS and CM at all corresponding rates except at 100 kg N/ha, where BGS treatment had lower uptake. Only CM at 200 kg N/ha had higher Zn uptake than the control. At 100 kg N/ha BGS and CM had similar B uptake with the control, while for BGS the 150 kg N/ha rate had higher B uptake than at 100 kg N/ha. The BGS and CM treatments had similar Cu uptake all corresponding rates except at 100 kg N/ha where BGS had lower, and similar to the control (Table 4.4).

Table 4.3: Macronutrient uptake following increasing application rates of BGS and CM

Treatments	Level	N	P	K	Ca	Mg
	kg N/ha	-- mg/pot				--
	100	6.56bc	13.4b	163bc	52.0bc	31.2b
BGS	150	9.93d	27.0e	258e	80.7e	48.5c
	200	8.15cd	23.8de	225de	68.4de	52.1c
	250	7.36cd	22.7cde	219cde	67.7de	48.1bc
	100	4.24ab	12.4b	116ab	46.2b	34.4b
CM	150	6.75bc	19.2cd	181cd	62.0cd	49.6c
	200	6.27abc	19.3cd	207cde	74.2de	48.4c
	250	6.61bc	17.5bc	192cd	65.9d	44.7c
Control	0	3.79a	5.85a	86.7a	32.3a	22.1a

Different lowercase letters indicate statistically significant differences according to the Tukey test at the P = 0.05.

Table 4.4: Trace element uptake following increasing application rates of BGS and CM

Treatments	**Level**	**Fe**	**Mn**	**Zn**	**B**	**Cu**
	kg N/ha	------------------	------------------	mg/pot	------------------	------------------
	100	1.20bc	1.28a	0.12a	0.19a	0.03a
BGS	150	3.09e	2.51b	0.27cd	0.32d	0.06bc
	200	2.37d	3.25c	0.30cde	0.25bc	0.07bcd
	250	0.89abc	2.46b	0.28cd	0.31bc	0.08cd
	100	0.82a	2.12b	0.21b	0.21ab	0.06b
CM	150	0.87ab	3.44c	0.29cde	0.29cd	0.08d
	200	1.24bc	2.61b	0.34e	0.30cd	0.07bcd
	250	1.26c	2.55b	0.31de	0.30cd	0.07bcd
Control	0	0.65a	1.22a	0.25bc	0.17a	0.04a

Different lowercase letters indicate statistically significant differences according to the Tukey test at the $P = 0.05$.

4.3.3.2 Soil residual chemical composition

Soil residual pH was not affected by organic nutrient source and rate. All treatments with BGS and CM had similar ammonium-N in soil except at 250 kg N/ha, where BGS had lower than CM, and was similar to the control. Application rate did not affect nitrate-N in BGS treatments but it increased with rate for CM. At 100 and 150 kg N/ha BGS had higher nitrate-N in the soil than CM, which was similar to the control. There were no differences in nitrate N at higher rates (Table 4.5). Only BGS at 100 kg N/ha and CM at 200 kg N/ha had higher exchangeable Mg than the control (Table 4.5). Soil exchangeable K was higher in CM than BGS at all corresponding rates except at 100 kg N/ha, where they were similar. Increasing application rate significantly increased exchangeable K in CM treatments but not BGS, which was similar to the control (Table 4.5). Only BGS at 250 kg N/ha, had higher exchangeable K than the control. At each rate there were no differences between BGS and CM except at 150 kg N/ha, where CM had significantly higher Ca (Table 4.5). All the treatments with BGS and CM had lower extractable Fe than the control. Similar levels of extractable Zn and Cu were also determined in all treatments, including the control (Table 4.6). Mn was not affected increasing application rates for BGS treatments, which were similar to the control. However, for CM, Mn increased with increasing application rate and were significantly higher than corresponding rates of BGS except 100 kg N/ha, where they were similar (Table 4.6).

Table 4.5: Soil residual pH and macronutrients concentration at increasing BGS and CM application rates under Avalon soil

Treatments	Level	pH	NH_4-N	NO_3-N	P	K	Ca	Mg
	kg N/ha		---------------- mg/kg	---------------------		-------------- cmol(+)/kg	-----------	
	100	7.48	4.16bc	4.92bc	14.5c	0.375a	7.91ab	4.77bc
BGS	150	7.59	4.52bc	5.11c	14.8c	0.350a	7.16a	4.43ab
	200	7.56	4.99bc	4.92bc	12.8bc	0.340a	8.35bc	4.67abc
	250	7.57	3.72ab	5.10c	13.6bc	0.331a	9.21c	4.71abc
	100	7.58	4.09bc	2.92a	7.69ab	0.571a	8.45bc	4.65abc
CM	150	7.50	4.08bc	3.32ab	12.0abc	0.883b	8.50bc	4.69abc
	200	7.57	4.09bc	4.38abc	15.7c	1.06bc	8.00ab	4.90c
	250	7.62	5.05c	4.16abc	16.2c	1.31c	8.24abc	4.71abc
Control	0	7.52	2.47a	2.64a	5.99a	0.350a	7.40ab	4.33a

Different lowercase letters indicate statistically significant differences according to the Tukey test at the *p*= 0.05.

Table 4.6: Soil residual micronutrients concentration at increasing BGS and CM application rates under Avalon soil

Treatments	N level	Fe	Mn	Zn	Cu
	kg/ha	---	mg/kg	-------------------------------------	
	100	9.58a	114ab	6.10	1.30
BGS	150	10.3a	112ab	5.73	1.53
	200	10.6a	109ab	5.44	1.29
	250	9.91a	116abc	5.97	1.27
	100	9.26a	125bc	6.08	1.27
CM	150	8.80a	138cd	5.97	1.14
	200	9.53a	158de	7.03	1.25
	250	9.76a	168e	6.36	1.17
Control	0	13.0b	96.5a	4.95	1.47

Different lowercase letters indicate statistically significant differences according to the Tukey test at the *p*= 0.05.

4.3.3. Effects of co-applying BGS and CF on Spinach nutrient uptake and dry-matter yields

4.3.3.1. Dry-matter yield and nutrient uptake

Overall, the Avalon soil had significantly higher dry-matter than the Hutton soil irrespective of application rate. Dry-matter increased significantly with increasing CF proportion (Table 4.7). Similarly, the uptake of N, Mg and Fe increased with increase in the proportion of CF irrespective of the soil. In addition, their uptake was significantly higher in Avalon than Hutton soil (Table 4.7). The uptake of P, K and Ca increased with increase in the proportions of CF for both soils. Phosphorus and B uptake was significantly higher in Avalon soil than Hutton for all treatments except at 100/0 kg N/ha, where they were similar (Table 4.8). Potassium and Ca were higher in Avalon than Hutton soil at all rates. The 60/40 in the Avalon had similar P, K and Ca levels as the 0/100 in the Hutton. The uptake of Mn and Cu between Avalon and Hutton was similar at each rate except for Mn at 40/60 kg N/ha, where it was significantly higher in Avalon (Table 4.8). Zinc was similar between Avalon and Hutton at 40/60 and 60/40 kg N/ha and different at 100/0 and 0/100 kg N/ha.

Table 4.7: Dry-matter yield and uptake of nitrogen, magnesium and iron at different BGS and CF ratios (BGS/CF in kg N/ha) in the soils

Parameters	**Treatments**				**Soils**	
	0/100	**40/60**	**60/40**	**100/0**	**Avalon**	**Hutton**
Dry matter (g/pot)	10.8a	7.21b	4.14c	2.15d	8.36a	3.78b
N (mg/pot)	32.0a	19.7b	10.4c	4.13d	20.44a	12.64b
Mg (mg/pot)	87.4a	69.7b	46.6c	31.4d	86.6a	30.9b
Fe (mg/pot)	11.3a	6.05b	2.12c	0.890d	5.92a	4.28b

Different lowercase letters indicate statistically significant differences according to the Tukey test at the $P = 0.05$

Table 4.8: Macro and micronutrients uptake at different BGS and CF ratios (BGS/CF in kg N/ha) under Avalon and Hutton soils

Parameters	**Soils**							
	Avalon				**Hutton**			
	0/100	**40/60**	**60/40**	**100/0**	**0/100**	**40/60**	**60/40**	**100/0**
Macronutrients (mg/pot)								
P	56.44e	35.60d	15.65bc	7.317ab	21.10c	8.475ab	2.940a	0.834a
K	520.2f	336.4e	200.4d	113.4c	204.2d	72.96bc	19.49ab	4.647a
Ca	152.1e	142.4e	79.07d	41.85bc	70.43d	54.20cd	21.48ab	4.750a
Micronutrients (mg/pot)								
Mn	3.569b	2.556ab	1.838ab	1.933ab	3.865bc	5.767c	2.295ab	0.790a
Zn	0.316d	0.274cd	0.190bc	0.169bc	0.525e	0.239cd	0.074ab	0.019a
B	0.833e	0.579d	0.341c	0.214abc	0.344c	0.250bc	0.090ab	0.025a
Cu	0.110d	0.062c	0.044bc	0.030abc	0.122d	0.044bc	0.018ab	0.005a

Different lowercase letters indicate statistically significant differences according to the Tukey test at the *P* = 0.05.

4.3.3.2. Soil residual chemical composition

Soil residual pH was significantly higher in Avalon soil compared to all equivalent treatments of Hutton soil. Ammonium-N remaining in the soil was higher in Hutton than Avalon for all treatments (Table 4.9). The soil residual nitrate-N was notably higher at 40/60 and 60/40 kg N/ha rates under Hutton than Avalon and the rest were similar. There were similarities in available P at all corresponding treatments for both soils except at 0/100 and 40/60, where Avalon had higher (Table 4.9). Exchangeable Ca and Mg were significantly higher in Avalon than Hutton soil at all equivalent treatments. In addition, there were no variations between treatments within each soil, for both soils except at 100/0 kg N/ha, which was higher than others (Table 4.9). Hutton soil had significantly higher soil residual exchangeable K and extractible Mn and Cu than Avalon soil. Extractible Fe and Zn were higher in Avalon than Hutton soil (Table 4.10).

Table 4.9: Soil residual nutrients and pH at different BGS/CF ratios in Avalon and Hutton soils

Parameters	**Soils**							
	Avalon (BGS/CF; Kg N/ha)				**Hutton** (BGS/CF; Kg N/ha)			
	0/100	**40/60**	**60/40**	**100/0**	**0/100**	**40/60**	**60/40**	**100/0**
pH	6.64b	6.97bc	7.13c	7.50d	5.11a	4.86a	5.01a	5.13a
Primary macronutrients (mg/kg)								
NH_4-N	15.5c	7.24ab	5.73ab	4.91a	71.2e	52.0d	17.6c	8.10b
NO_3-N	8.75c	6.41ab	6.75bc	4.67a	7.99bc	22.2e	18.6d	6.66abc
P	175f	95.0d	52.7bc	18.8a	134e	63.1c	49.6b	16.4a
Exchangeable bases (cmol+/kg)								
Ca	7.29b	7.34b	7.55b	8.41c	3.02a	3.08a	3.10a	3.30a
Mg	4.20b	4.06b	4.09b	4.46c	1.37a	1.46a	1.45a	1.42a

Different lowercase letters indicate statistically significant differences according to the Tukey test at the P = 0.05.

Table 4.10: Soil exchangeable K and micronutrients at different BGS and CF ratios (BGS/CF in kg N/ha) under Avalon and Hutton soils

Parameters (mg/kg**)**	**Treatments**				**Soils**	
	0/100	**40/60**	**60/40**	**100/0**	**Avalon**	**Hutton**
K	0.490	0.517	0.499	0.488	0.347b	0.651a
Fe	12.4	10.7	9.59	7.88	11.5a	8.78b
Zn	87.4	69.7	46.6	31.4	86.6a	30.9b
Mn	195	169	183	170	88.6b	269a
Cu	3.04	3.17	3.20	3.14	1.21b	5.07a

Different lowercase letters indicate statistically significant differences according to the Tukey test at the P = 0.05.

4.4. Discussion

Spinach yield and nutrient uptake response to BGS, CM and CF as a nitrogen source

The higher dry-matter in the CF than organic treatments (BGS and CM) is attributed to the readily available plant nutrients, which resulted in greater uptake of nutrients particularly N, P, K, Ca, Mn, and Zn, and supported biomass accumulation. The results of dry-matter could be explained by uptake of K, Ca, B, Cu and Mn, which followed the same trend (CF > BGS = CM > control), with Ca and Mn uptake being higher for BGS than CM. Uptake of N (no difference between BGS, CM and control) and P (higher in CM than BGS) could not explain the dry-matter results. The ammonium-N in soil after spinach could be explained by the relative concentrations in the original amendments, where mineral N was in the order CF>BGS>CM>control. The higher nitrate-N in soil treated with BGS than CM could be a result of greater nitrification. Similarly, Abubaker *et al.* (2012) reported findings from BGS emanating from organic household waste. This suggests that we could expect that digested materials improves N mineralization than undigested manure of the same origin. The higher soil K in CM treatment than BGS is explained by the higher content in the original CM, considering that uptake of K was the same between the two treatments. The higher soil Ca and Zn in the BGS than the CF treatment was explained by the higher addition in the BGS and higher uptake in the CF treatment. The higher soil Ca in the BGS than CM was explained by the availability of the nutrient after digestion during biogas production. Uptake of Mn (CF>BGS>CM) explained the results of extractable Mn in soil (CM>BGS>CF). The soil nutrients, particularly in BGS and CM could have residual fertility effects on subsequent crops, with minimal residual effects expected from CF. The results of BGS (similar to higher nutrient uptake than CM) suggest that CM could be used as a feedstock for bioenergy production, and the by-product improves or retains the fertiliser value. While CM improved dry-matter by 100%, BGS improved it by 139%, relative to the negative control. In addition to supplying primary nutrients, BGS and CM also supply carbon (not measured), secondary macronutrients and micronutrients, which improve yield and soil quality.

The lower dry-matter and uptake of some nutrients in BGS and CM, than CF indicate that, at least in the short-term the 100 kg N/ha rate used may not be sufficient for spinach, and a higher rate may be required. This view was supported by higher spinach dry-matter where BGS was used at 150 kg N/kg. The higher spinach dry-matter in BGS than CM at the 150 kg N/ha rate was explained by higher uptake of N, P, K, Ca and Fe, which were highest in the same treatment rate. This supported by the positive interaction of N with macronutrients in soils solution, which improves their uptake (Fernandes and Soratto, 2012). The higher uptake of these nutrients could be associated to their occurrence in readily available forms in BGS after the digestion process during biogas production. These results suggest that BGS application for spinach is more optimal at 150 kg N/ha rate and that higher rate do not result in yield benefits but could improve soil organic matter (not measured). The similar and low soil ammonium-N concentrations across all BGS and CM treatments could be a result of uptake and potential loses during the growth stages. Although nitrification could also have occurred, the nitrate N results do not support this view. Which suggests that nitrification may have been inhibited by losses through volatilization (gas emissions were not measured in the study). The increase in soil extractable P and K with increasing CM rate could be associated to higher addition and release during decomposition with no differences in uptake. The lack of differences in soil extractable P in BGS rates could be a result of precipitation as Ca phosphates at pH above 7.5 or due to slow release. Thus, the slow release has the potential to supply P to the crop for longer in contrast to the immediately available P from CF (Loria and Sawyer, 2005). The higher exchangeable Ca with increasing BGS rate is from higher additions in soluble form, further supporting the potential precipitation with P. Although BGS at 150 kg N/ha gave the highest dry-matter of spinach, which was 32.9% higher than for 100 kg N/ha rate, it was still around half of DM realised from the chemical fertiliser in the previous pot trial. Although CF gives high yields, smallholder farmers often apply only 40-60% of the recommended fertiliser rates due to limited financial resources, resulting in a yields gap. Co-application of BGS with CF could result in higher crop yield than when only CF is used at farmer’s rate, and therefore the effects need to be understood.

The dry-matter results where BGS was co-applied with CF were explained by the uptake of N, Mg and Fe, which followed the same trend. These elements are associated with chlorophyll production and growth (Kalaji *et al.,* 2014). Higher proportion of CF resulted in more readily available N, which supported growth and uptake of other nutrients, including Mg and Fe. The results appeared to be additive based on the proportion of BGS/CF. For example, the 40/60 (BGS/CF) treatment yielded 7.2 mg/pot, which was close to the expected 7.3 mg/pot based on the proportions ((0.4x2.15) + (0.6x10.8) = 7.34). This dry-matter was higher than measured for BGS at 150 kg N/ha in the previous pot trial. The dry-matter when BGS was co-applied with CF was averaged across soils such that if only the Avalon was considered (Hutton not used like in the previous pot trial), the difference with that of BGS at 150 kg N/ha could have been higher. Although this dry-matter is still lower than 100% CF, it suggests that farmers can apply CF at 60 kg N/ha (60% of recommended rate) supplemented with BGS at 40 kg N/ha and realise 11% yield increase. This constitutes deriving value from BGS, which would otherwise present disposal challenges. The higher dry-matter, nutrient uptake and soil nutrients after spinach in the Avalon than the Hutton are a result of the fertility status of the original soils. The decline in soil ammonium- and nitrate-N and extractable P with reduction in the proportion of CF is attributed to lower availability of the nutrients in the amendments. The higher available nutrients in treatments with higher proportion of CF could benefit subsequent crops, while nitrate-N is susceptible to leaching. The higher ammonium-N, K, Mn and Cu in the Hutton than the Avalon soil could be explained by great plant growth and uptake of these nutrients in the Avalon. The residual nutrients in the Hutton can benefit subsequent crops.

4.5. Conclusion

Application of BGS and CM as N sources improve dry matter yield and nutrient uptake of spinach. The increasing application of BGS and CM from 100 to 150 kg N/ha also displayed to improve dry-matter yields but remain steady beyond 150 kg N rate. Co-application of BGS with CF increased dry-matter yield and nutrient uptake than BGS alone at the same N rate. The results indicate that BGS can be an important nutrient source for crops and the best effects are when co-applied with chemical fertiliser, because co-application of BGS with CF affected crop response. Therefore, there is need for field experiments to be conducted in assess if similar response will be observed.

CHAPTER 5

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1. General discussion

Use of untreated animal manure on arable land has been practised, studies indicate enhanced N loss as ammonia, spreading of pathogens and weeds amongst others (Oenema *et al.,* 2006; Steinfeld *et al.,* 2006; Helm-Nielsen *et al.*, 2009). Anaerobic digestion of organic waste to produce biogas produces a stable by-product (BGS) and sterile of pathogens (Abubaker *et al.,* 2012; Alburquerque *et al*., 2012). The quality of BGS is influenced strongly by the feedstock used, retention time, and temperature amongst other factors (Khalid *et al.,* 2011). Therefore, the potential of BGS as soil amendment is determined by its nutrient composition, ability to release those nutrients as well as the impact on plant growth and development. The overall objective of the study was to assess BGS nutrient release patterns and its fertilizer value as an N source through comparison with CM and CF, increasing application rate and co-application with CF on dry-matter and nutrient uptake by spinach.

The overall increase in ammonium-N availability was due to mineralization of N from the organic amendments in the incubation study (Alburquerque *et al*., 2012). However, nitrate-N decline during incubation under BGS treated soils and dropped further with increasing application rates was a result of N utilization by microbes (Abubaker *et al.,* 2012). The loss of C during AD could explain such a response, which proposes that spinach would have access to nitrate-N within a week after BGS application. Chiyoka (2011) reported initial N immobilisation followed by mineralization, which is similar to findings observed in this study. Yet for CM treated soil, nitrate-N increase over the incubation period and with increasing application rate, which could be explained by the greater N loading proportion that was nitrified.

The effect of higher N application rates is evident from 100 to 150 kg N in the dry-matter yield; beyond 150 kg N dry-matter accumulation does not change (Figure 4.2). This suggests that N could be lost in form of NH_3, which was enhanced by alkaline pH (Alburquerque et al., 2012). In addition, these results could be explained by the results from Chapter 3, where N immobilisation increased with increasing application rates of BGS could explain. The increase in pH and availability of Ca could have formed phosphate precipitates, which explains the decline in available P in the incubation study for BGS than CM treated soils (Havlin *et al*., 2005). This observation was supported by the pH values that were greater in BGS than CM throughout incubation period. This could also explain the non-significant differences in P uptake between BGS and control (Table 4.1).

During co-application of BGS with CF, dry matter yields notably dropped (Figure 4.4) with increasing BGS ratio, which suggests that there was no synergistic effect between the two amendments and that spinach was just assimilating the available nutrients for both Hutton and Avalon soil. These trends were similar for macronutrient uptake. The increase in exchangeable K, Mg and C with increasing application rates presented in the incubation study, which was similar over the incubation period was not evident in the uptake of K, suggesting that availability these bases was affected by BGS and CM loading rates. Studies by Heraldsen *et al.,* (2010) and Tambone *et al.,* (2010) obtained similar findings, where BGS display to enhance exchangeable K availability against compost and control. Although BGS and CM displayed relative similar trends in nutrient availability under the incubation study, the soils residual nutrient showed variations. This is evident in the dry matter yield obtained under effect of the materials as N sources and increasing application rates. In addition, soil residual exchangeable K, Mg and Ca reflected the trend observed under the incubation study. However, for mineral N, there no differences at increasing application rates. The results also indicate that, there is potential for long-term benefit.

5.2. Conclusion and Recommendation

From the incubation study, BGS showed greater ammonium-N and available P with increasing application rates than CM. in addition, improved spinach yields and soil residual fertility that has the potential to benefit following crop. Application of both organic amendments were optimal at 150 kg N/ha. Co-application of BGS with CF at 40/60 kg N/ha (BGS/CF) would be an ideal for farmers who are under applying. BGS as a by-product of biogas production displayed to improve nutrient replenishment for better crop growth. Biogas slurry utilization as soil amendment should be practised and further research should be conducted on the fields to assess the response.

Future Research Needs:

1) Therefore, there is need for field experiments to be conducted in assess if similar responses in the pot trial will be observed under field conditions.

2) Use of different feedstock and co-digestion of various feedstock to produce biogas slurry may also need to be studied to get the best combination of biogas and biogas slurry for use as a soil amendment.

3) Long-term studies to assess effect of different types of BGS on soil physical properties should be conducted.

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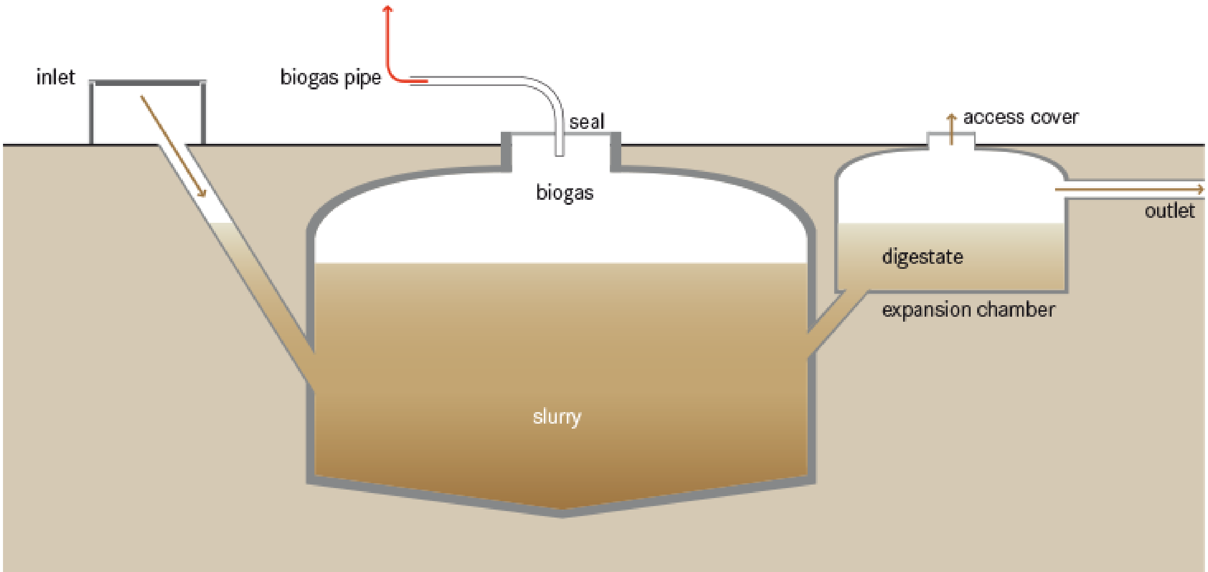
APPENDICES



Appendix 1: Typical representation of a below ground small-scale biogas reactor known as the *Anaerobic Contact* (complete-mix digesters) systems (Mes *et al.*, 2003).

Appendix 2: Spinach tissue accumulated nutrients in response to different treatments

Parameters	**Treatments**			
	Control	**BGS**	**CM**	**CF**
Macronutrients (g/kg)				
N	1.380a	1.692a	1.619a	2.499b
P	3.143a	4.915c	4.066b	6.929d
K	18.92a	36.01c	32.68b	47.86d
Ca	13.06a	15.03b	14.33ab	12.93a
Mg	92.85a	18.11c	10.68ab	11.89b
Micronutrients (mg/kg)				
Fe	256.2a	254.6a	560.8b	723.5c
Mn	260.5a	429.8b	314.9a	284.1a
Zn	45.94c	34.12ab	43.98bc	28.34a
B	60.12a	54.53a	60.63a	55.12a
Cu	2.930a	8.426b	9.667bc	10.17c

Appendix 3: Spinach tissue Mg, Ca and trace elements concentration at increasing BGS and CM application rates under Avalon soil.

Treatments	Level	N	P	K	Ca	Mg	Fe	Mn	Zn	B	Cu
		------------------------------ g/kg			------------------------		-------------------------- mg/kg		------------------------		
BGS	100	1.856c	3.787b	46.08cd	14.69ab	8.812ab	340.9b	361.6a	33.95a	51.13a	8.200a
	150	1.878c	5.127d	48.84d	15.33b	9.195abc	586.6c	476.4b	51.43b	61.43bc	11.31b
	200	1.736bc	5.076d	47.78cd	14.55ab	11.13d	504.9c	692.2e	64.43cd	53.66ab	15.42cd
	250	1.563abc	4.809cd	46.68cd	14.42ab	10.27cd	189.1a	523.0bc	59.10c	66.11c	16.45cde
CM	100	1.240a	3.624b	33.85ab	13.50ab	10.05bcd	237.9a	617.3d	60.10c	60.96bc	16.94de
	150	1.513abc	4.305bcd	40.63bc	13.88ab	11.09d	195.3a	773.7f	64.55cd	65.50c	18.22e
	200	1.324ab	4.076bc	43.81cd	15.73b	10.27cd	264.7ab	551.7cd	72.39e	64.10c	14.80cd
	250	1.478abc	3.897b	42.77cd	14.81ab	9.959bcd	278.9ab	566.9cd	68.67de	67.19c	14.68cd
Control	0	1.414abc	2.227a	32.84a	12.12a	8.388a	249.5a	461.7b	92.950f	65.91c	14.090c

Appendix 4: Spinach tissue macro and trace elements concentrations at different BGS and CF combination under Avalon and Hutton soils

Soil	Treatment (BGS/CF)	N	P	K	Ca	Mg	Fe	Mn	Zn	B	Cu
		----------------------- g/kg				-----------------------	--------------------------- mg/kg				-----------------------------
Avalon	0/100	2.814ab	4.131e	38.32e	11.17b	8.799b	879.8bc	263.1a	23.14a	60.94b	7.985ab
	40/60	2.338ab	3.729d	35.27de	14.91c	9.820b	767.0b	267.2a	28.69ab	60.48b	6.464a
	60/40	2.336ab	2.427c	31.36d	12.27b	11.85c	442.8a	285.9a	29.72ab	53.18ab	6.849a
	100/0	1.785a	1.962b	30.14cd	11.24b	15.24d	398.6a	511.5a	44.70de	57.96b	7.895ab
Hutton	0/100	3.298b	2.667c	25.91c	8.905a	7.026a	1337d	490.1a	66.27f	43.31a	15.50c
	40/60	3.548b	1.743ab	14.97b	11.38b	9.400b	988.5c	1184b	49.62e	49.45ab	8.893b
	60/40	3.326b	1.594ab	10.80ab	11.81b	9.648b	779.8b	1291bc	41.57cd	49.36ab	9.874b
	100/0	3.166b	1.513a	8.420a	8.657a	9.602b	510.3a	1435c	34.99bc	44.72a	8.408ab