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Working the food and beverage waste puzzle: extractants and amendments to agricultural soils

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Abstract

Massive amounts of food and beverage wastes are produced worldwide, yet this plethora of resources remain largely untapped. Effective use, both economically and sustainably, of these wastes as alternative resources has become a high priority for a sustainable future. This work investigates potentially efficient ways to use coffee wastes (husks and spent coffee grounds) and by-products of anaerobic digestion with food waste feedstock. These two wastes were chosen due to their lack of research in Australia and potential for valuable reuse.

Coffee is an extremely popular beverage, with approximately 9.1 billion kilograms consumed worldwide in 2015. However, the beverage only accounts for 5-10% of the coffee cherry fruit usage, whereas wastes account for 90-95%. This work investigates potential extractants from coffee husks and Spent Coffee Grounds (SCGs). Additionally, SCGs, with/without urea fertiliser blending and with/without further extractions, were studied as soil amendments in short-term plant growth trials.

This study found that coffee husks contain C18:3 omega-3 fatty acids, which are an important component of the human diet. Use of SCGs in short-term amendment trials had a positive effect on the soil microbial activity and reduced nitrous oxide (N_2O) soil emissions. It was demonstrated that plant growth inhibition from potential phytotoxicity after SCG application may be more complex than the literature suggests as two extractions that removed a substantial amount of potentially phytotoxic components did not increase plant yield.

Anaerobic digestion is internationally utilised as an economical and environmentally viable option for energy production and for waste treatment. Due to the huge amounts of food waste

produced worldwide, its use as a feedstock for anaerobic digestion is growing. Digestates are a by-product of the anaerobic digestion and have been successfully used as an alternative to fertilisers and soil conditioners; however, there is a notable lack of research regarding digestates sourced from food waste. This is particularly evident in Australia where anaerobic digestion is relatively new but could have an important role in environmentally-sustainable practices, however high salinity of the digestates may pose an environmental risk.

Soil column trials were conducted using five food waste-sourced anaerobic digestion by-products (digestates) to preliminary assess the application of these materials. This was completed using digestates collected in the Netherlands and in Australia. Trials using three digestates, collected in the Netherlands, showed high methane (CH_4) soil emissions from one treatment and, generally, high mineral nitrogen leaching. This indicates that the application of digestates must be matched with plant growth in order to manage unwanted nitrogen leaching and soil emissions. Trials with two Australian digestates had high salinity and indicates its potential as a source of plant-essential nutrients in low nutrient sandy soils.

This work provides valuable information on the outcomes for the reuse of selected coffee wastes and digestates, particularly in an Australian context, including value-adding ways to use coffee husks. Variability in crop yield was demonstrated following the soil application of SCGs and digestates indicating complex interactions between the amendment, plant, soil and soil microbes. Further work is warranted into the detailed impact of these complex interactions, including soil microbial experiments using SCGs and studies to increase knowledge of Australian digestates.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

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This thesis is dedicated to Dawn Elizabeth Mercier.

Cherished aunt who was taken early by cancer.

Your smile, love and kindness lit up this world.

Your passion for the environment and its creatures will always inspire me.

Your beautiful presence is profoundly missed.

Rest in peace, beloved.

List of acronyms and abbreviations

Al – aluminium

ANOVA – analysis of variance (statistical test)

As – arsenic

B – boron

Ba – barium

C – carbon

C: N ratio – carbon to nitrogen ratio

Ca – calcium

Ca: Mg ratio – calcium to magnesium ratio

CEC – Cation exchange capacity

CH_4 – methane

CO_2 – carbon dioxide

Cr – chromium

Cu – copper

DI H_2O – deionised water

EC – electrical conductivity

Fe – iron

GAEs – gallic acid equivalents

GC-FID – gas chromatography with flame-ionization detection

GHG – greenhouse gases

K – potassium

LC-MS – liquid chromatography – mass spectrometry

LSD – least significance difference (statistical test)

Mg – magnesium

Mn – manganese

N – nitrogen

N_2O – nitrous oxide

NH_3 – ammonia

NH_4^+ – ammonium

NMR – nuclear magnetic resonance

NO_3^- – nitrate

OM – organic matter

P - phosphorus

Sc-CO_2 – Supercritical carbon dioxide

SCGs – spent coffee grounds

Soil respiration – the carbon dioxide release from the soil due to soil microbes and live root growth

TN – total nitrogen

Zn – zinc

Table of Contents

Chapter 1. Literature review – Valorisation options and land applications of Spent Coffee Grounds and Anaerobic Digestion by-products

1.1. Introduction

Enormous amounts of food and beverage wastes, produced globally, are creating economic, environmental, social and sustainability issues (FAO, 2011, Garcia-Garcia et al., 2017). Waste is a huge international concern, particularly food wastes, where one third of food is wasted worldwide (FAO, 2011). Simply put, the food supply chain is ineffective (Pfaltzgraff et al., 2013), where traditional waste disposal methods, such as landfill, incineration and discharge into water ways, are undesirable. These traditional disposal methods can result in high energy use, toxic methane gas and bad odour production (Arancon et al., 2013), pollution of the environment (Pham et al., 2015) and a loss of potentially valuable resources. Indeed, there is a need to change waste perceptions, to view food wastes as an untapped resource (Pham et al., 2015) rather than a waste – to transition from a food supply chain which uses a "resource stupid" model to a "resource intelligent" model (Clark et al., 2013). The goal to obtain a "zero waste economy" is critical, where wastes are used as raw materials for further applications and products (Mirabella et al., 2014). It is for these reasons that there is an increasing need to find cheap, easy and preferably useful and green options for these materials.

Alternative ways to use wastes have also become increasingly important as landfill, transportation, environmental costs and community objections threaten landfill supply and sustainability (Pickin, 2009). A simple framework and hierarchy to avoid the generation of waste is the three 'R's: Reduce, Recycle and Reuse. The Environment Protection Authority, Victoria (EPAVIC) have used this basic concept to build a framework to assist with decision making regarding waste management (EPAVIC, 2018b) (Figure 1). Alternative waste pathways must be considerate of, and in accordance with, regional regulatory bodies like EPAVIC and their decision-making frameworks.

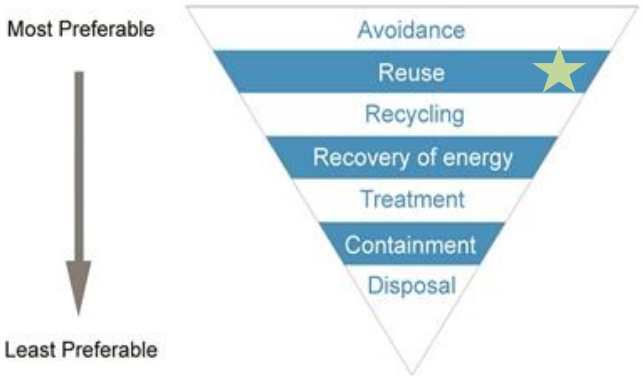


Figure 1 Waste hierarchy, EPAVIC (2018b), where the star represents the aim of this work.

The application of organic amendments, including those sourced from food and beverage wastes, to agricultural soils is a potentially cheap option for landfill diversion. Importantly, the use of organic amendments plays significant role in sustainable agriculture by potentially increasing soil biological functions, soil organic carbon, plant essential nutrients and crop yield (Diacono and Montemurro, 2011b, Thangarajan et al., 2013). Furthermore, organic amendments are important for climate change mitigation in which the applications can influence harmful greenhouse gas (GHG) emissions emitted from soils, particularly carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) (Bouwman, 1996, Le Mer and Roger, 2001, Smith et al., 2008, Thangarajan et al., 2013, Charles et al., 2017). Significantly, the agricultural sector accounts for an approximately one fourth of the global anthropogenic emissions (Bennetzen et al., 2016) and is particularly high for CH_4 and N_2O emissions (Smith et al., 2008). These emissions can be mitigated by effectively managing carbon and nitrogen flows in the soil (Smith et al., 2008, Thangarajan et al., 2013). CH_4 soil emissions are primarily from oxygen deprived soils, e.g. wetlands or rice paddies (Smith et al., 2008), whereas N_2O and CO_2 are often considered more significant in aerobic soil conditions, as discussed in later chapters.

Land applications of waste must strike a balance between the efficient use of wastes, any economic implications and with consideration of the possible impacts to human and environment health,

supported by scientific knowledge. The potential land applications of wastes raise important challenges for the protection of the environment and human health. This is particularly complex due to a huge range of treatments available with corresponding varied impacts on soil, the environment overall and human health.

The use of wastes to land as a soil amendment usually requires approval from the state or territory EPA before the waste can be applied, and the approval and/or regulation process is relatively different between jurisdictions. Focusing on south eastern Australia, EPAVIC (EPA Victoria) have an approval process under 'Research, Development and Demonstration approvals' where the applicant would demonstrate that the proposed amendment does not have adverse impacts and has benefit to land (EPAVIC, 2018a, Innes, 2019). EPANSW (EPA New South Wales) have a number of exemptions which relate to various waste and amendment types, such as compost, manure, mulch, solid and liquid food waste. These EPANSW exemptions set out a thorough list of evidence-based provisions to ensure that application adheres to their regulatory requirements (EPANSW, 2018). EPASA (EPA South Australia) have developed a separate standard for 'waste derived soil enhancers', which includes a comprehensive set of evidence-based risks and requirements that the applier needs to address before and during application (EPASA, 2015).

This review concentrates on two organic wastes: coffee waste and anaerobic digestion residues, both have a wide range of potential uses. Specifically, this will focus on the potentially valuable components that can be extracted from coffee wastes as well as preliminary assessment of land application for both coffee waste and anaerobic digestion residues. Although important, the impact to human health is outside the scope of this project. The aim of this literature review is to understand the research gaps in selected organic wastes and their associated application to select soils.

Coffee husks and Spent Coffee Grounds (SCGs) were chosen because of high production amounts (Caballero-Galván et al., 2018), ease of accessibility and the tremendous potential for a wide range of

valorisation options (Stylianou et al., 2018, Kovalcik et al., 2018). SCGs are approximately 45% of the waste from coffee production (Mussatto et al., 2011b), with hundreds of thousands of tons of SCGs produced per year internationally (Acevedo et al., 2013). Coffee husks are approximately 12% of waste from coffee production (Mussatto et al., 2011b) and these have the benefit of staying at production factories, in comparison to SCGs which have an additional collection challenge.

Coffee wastes, both SCGs and husks, have gained international attention as the valorisation can be created without the need for further extraction or alteration, particularly for land applications (Stylianou et al., 2018). SCGs are often further extracted or altered for use, as there have been over 1000 individual organic components identified (Peshev et al., 2018). Importantly, a variety of components can be economically recovered and commercially used (Kourmentza et al., 2018, Kovalcik et al., 2018).

Anaerobic digestion is an increasingly popular technology for producing biomethane and for the treatment of wastes, typically manure, crop residues and other agricultural wastes. This process produces a high nutrient by-product (digestates) which has been successfully used as fertilisers (Alburquerque et al., 2012, Koszel and Lorencowicz, 2015, Tampio et al., 2016a). Anaerobic digestion is becoming more popular for the treatment of food wastes (FW) (Ren et al., 2017) and this rises new research questions about whether digestates made from these new feedstocks have the same alternative fertiliser potential. The application of digestate to soils in Australia is an emerging practice and therefore requires further study to consider soil impacts from this practice.

1.2. Coffee waste

Coffee is considered the second largest traded commodity (after petroleum) worldwide (Murthy and Naidu, 2012, Mussatto et al., 2011b). It is regarded as a functional food attributed to the high content of antioxidants and other beneficial biological properties (Farah, 2012). In Australia, demands for

cafes and coffee shops is predicted to continue rising (Lin, 2015). Cafes and coffee shops in Australia have an annual growth rate of 2.8%, resulting in a $4.3 billion dollar value industry (Gargano, 2014). New South Wales and Victoria contain the most establishments in the country, with 35.1% and 28.8% of establishments, respectively (Gargano, 2014).

There are two main coffee tree species: *Coffea arabica* (Arabica) which constitutes 75% of world-wide production and *Coffea canephora* (Robusta) with 25% of world-wide production (Mussatto et al., 2011b). Brazil, Vietnam and Colombia hold half of the world's production of coffee (Murthy and Naidu, 2012), where Brazil dominates exportation with 28% of the market (Mussatto et al., 2011b).

Coffee cherries are processed in one of two ways to acquire the coffee bean: dry or wet processing. Dry and wet processing can be broadly distinguished by referring to the processes as unwashed (dry method) and washed (wet method) beans (Knopp et al., 2006). In dry processing, cherries are dried (by sun or machine), cleaned, de-hulled and sorted (Pandey et al., 2000). This method is usually used for Robusta (Mussatto et al., 2011b) and is a relatively simple and cheap process. The wet process is complex in comparison, where 18-36 hour microbial fermentation is used to make sure mucilage is removed from the beans (Oestreich-Janzen, 2010). These different processing methods (and post-harvest treatment) can change the chemical composition of the beans, in particular of sugars in the beans (Knopp et al., 2006).

Spent Coffee Grounds (SCGs) account for the majority of coffee processing waste (Murthy and Naidu, 2012), accounting of 15,600 metric tons of waste per year in the UK (Karmee, 2018) (Figure 2). Landfill is the principal disposal location for coffee residues, which is likely to be harmful to the environment and toxic to plant life due to caffeine, polyphenols and tannins (Fan et al., 2006, Mussatto et al., 2011b). A preliminary survey undertaken by Planet Ark (Cameron and O'Malley, 2016) showed that 93% of SCGs from 921 coffee shops in Sydney go to landfill, whereas only 7% was re-used in composts.

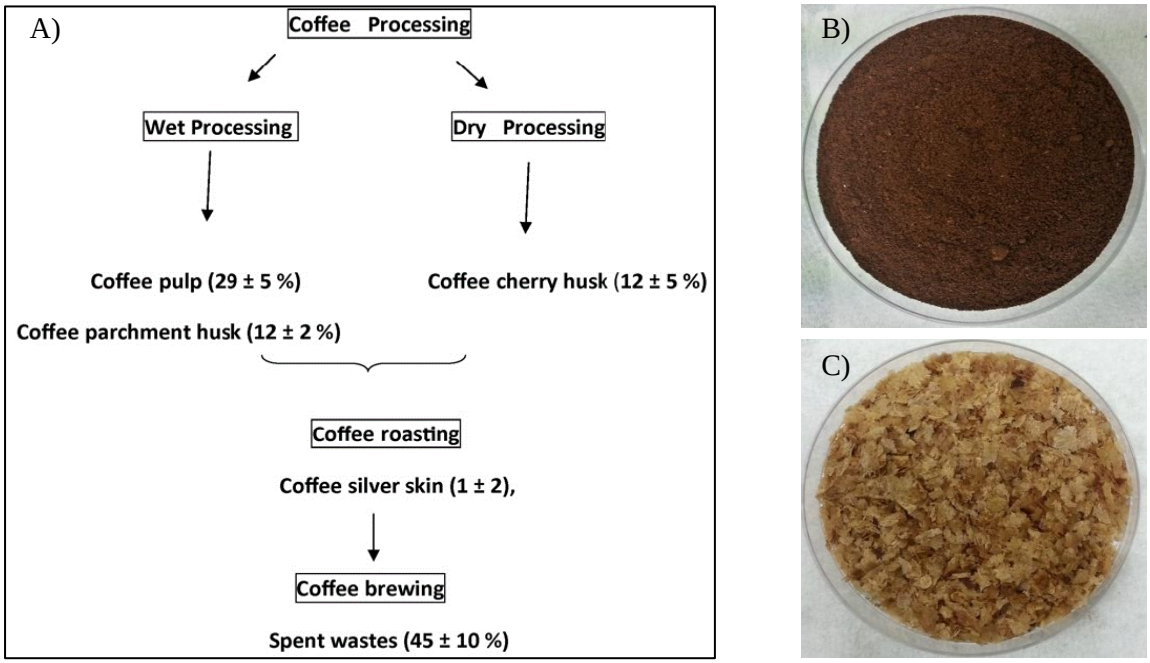


Figure 2 Waste from various stages of coffee processing A) Amounts of coffee waste from dry and wet processing (Murthy and Naidu, 2012). B) Spent Coffee Grounds. C) Coffee Husks.

The major components of SCGs are hemicellulose, proteins cellulose and lipids whereas husks are primarily comprised of cellulose, some lignin, and minor amounts of alkaloids (caffeine) and tannins (Murthy and Naidu, 2012) (Table 1). Coffee waste chemical composition can vary between bean species. For example, arabica beans can have more lipids (Farah, 2012) and hemicelluloses (Mussatto et al., 2011b) in comparison to Robusta beans, which can contain more caffeine (Campos-Vega et al., 2015, Mussatto et al., 2011b).

Coffee waste, both SCGs and husks, has a variety of potentially valuable compounds, including carbohydrates, lipids, proteins, and phenolic compounds (Esquivel and Jiménez, 2012). These components have a wide range of uses, including animal feeds, the production of diesel, bio-syngas, electricity, green composites and soil amendments (Kourmentza et al., 2018). SCGs, sometimes mixed with a variety of oils (e.g. peppermint oil), can be used as a body scrub (Cicillini and Guillen,

2009). Lipids extracted from SCGs have been converted into diesel (Vardon et al., 2013, Kondamudi et al., 2008), polyester production (Obruca et al., 2014), possible fertilizer (Caetano et al., 2014) and cosmetic and sun creams (e.g. Ribeiro et al., 2013, Wagemaker et al., 2011, Chiari et al., 2014). Bioactive compounds (e.g. caffeine, polyphenols, flavonoids, and acids) have been extracted for the health care, medical and pharmaceutical industries (Murthy and Naidu, 2012, Stylianou et al., 2018). A summary of the composition of SCGs is given in Table 1.

Table 1 Chemical composition of spent coffee grounds (SCGs) and coffee husks, averages, various authors.

Component	**SCGs (% of mass)**	**Source**	**Husks (% of mass)**	**Source**
Hemicellulose	36.7 ± 5.0	(Mussatto et al., 2011b, Murthy and Naidu, 2012)	7.0 ± 3.0	(Murthy and Naidu, 2012)
Protein	13.6 ± 3.8	(Murthy and Naidu, 2012)	8.0 ± 5.0	
Cellulose	8.6 ± 1.8		43.0 ± 8.0	
Fat	ND		0.5 ± 5.0	
Total Fibre	ND		24 ± 5.9	
Total polyphenols	1.5 ± 1.0		0.8 ± 5.0	
Total sugars*	8.5 ± 1.2		58.0 ± 20.0	
Lignin	0.05 ± 0.05		9.0 ± 1.6	
Tannins	0.02 ± 0.1		5.0 ± 2.0	
Chlorogenic acid	2.3 ± 1.0		2.5 ± 0.6	
Caffeine	0.02 ± 0.1		1.0 ± 0.5	
Ash	4.5-4.7	(Oestreich-Janzen, 2010)	0.5-1	(Esquivel and Jiménez, 2012)

Component	SCGs (% of mass)	Source	Husks (% of mass)	Source
Carbon	57.2-59.8 48.4 ± 0.7 (total organic carbon) 52.3	(Pujol et al., 2013) (Fenoll et al., 2014b) (Yamane et al., 2014)	44.87 ± 0.50	(Kasongo et al., 2013) (husks & pulp)
Nitrogen	1.2-1.3 7.4 ± 0.5 2.4	(Pujol et al., 2013) (Fenoll et al., 2014b) (Yamane et al., 2014)	1.69 ± 0.03	

*SCG sugars are predominantly: sucrose, glucose, fructose, arabinose, galactose, and mannose. Husks sugars are predominantly: mannose and galactose

ND – not determined

1.2.1. Extractions for oils/lipids

Lipids can be economically extracted from SCGs; for example, lipid manufacturing using supercritical extraction costs approximately US$ 48.60/kg and may earn up to US$ 460/kg (Andrade and Ferreira, 2013). The variation of extraction yields could be attributed to coffee varieties, preparation conditions, raw material pre-treatment and brewing conditions (Andrade et al., 2012). For example, green arabica coffee beans may have 15-17% coffee oil and Robusta 7-10% (Farah, 2012), where Esquivel and Jiménez (2012) reported oils and waxes making up 8-18% of green bean dry mass of most bean types.

Ultrasound-assisted extraction aims to reduce the extraction times, solvent and energy usage required for Soxhlet extractions (Rocha et al., 2014). Additionally, ultrasonic extractions aim to reduce the high pressure and low water content requirements of superfluid critical extractions (Rocha et al., 2014). Yields for ultrasonic extraction of oil using hexane, ethanol, DCM and ethyl acetate were in the range 1.5-3.1% for coffee husks, which is low in comparison to SCG yields of 9.0-12.2% (Andrade et al., 2012).

Recent studies have used supercritical CO_2 (Sc-CO_2) to obtain good yields of lipids (Ahangari and Sargolzaei, 2013, Andrade et al., 2012, Araújo and Sandi, 2007, Couto et al., 2009). Analysis of SCGs extracted using supercritical CO_2 showed that palmitic (C16:0) and linoleic (C18:2) acids make up 35% of the fatty acid content, with other fatty acids including C14, C16, C18 and C20 carbon chains for the remainder (Couto et al., 2009). Ahangari and Sargolzaei (2013) also found a relative composition of linoleic > palmitic > oleic > stearic acids in SCG extraction samples with no differences between using ethanol, *n*-hexane and benzene as solvents.

Supercritical fluid extractions have been extensively researched in the last decade, where CO_2 is the most common fluid used. Sc-CO_2 extraction is attractive due to the use of this method in the coffee industry for decaffeination and separation of essential oils (Ahangari and Sargolzaei, 2013). The use

of Sc-CO_2 is environmentally friendly (non-polluting), relatively safe (non-toxic and non-flammable) and inexpensive.

For Sc-CO_2, extraction times vary between oil separation studies from 25 minutes (Araújo and Sandi, 2007) to 300 minutes (5 hours) (Cruz et al., 2014a). Extraction times involved in Sc-CO_2 vary likely due to apparatus, methods, solvents and the composition variation of SCGs themselves. The majority of studies involving Sc-CO_2 extractions with SCGs use a semi-continuous flow apparatus (Ahangari and Sargolzaei, 2013, Araújo and Sandi, 2007, Couto et al., 2009, Cruz et al., 2014a), occasionally with a co-solvent such as ethanol (Couto et al., 2009). Yields obtained were high, ranging from 11.4 to 15.9%.

1.2.2. Land application

Use of SCGs in domestic and private gardens is a relatively common practice (Hardgrove and Livesley, 2016). There are many positive effects of using SCGs on soils. It is suggested that SCGs could be beneficial because of high water holding capacity, and relatively high P and N compared to some other organic amendments (Kasongo et al., 2011). SCGs may be a source of nutrients that are important for soil and plant health, particularly organic C, K, P and micronutrients (Cruz et al., 2015, Cruz et al., 2014c, Cervera-Mata et al., 2018).

There have also been interesting results regarding the increase of soil respiration, and the stimulation of the soil microbial community, after the application of SCGs (Cervera-Mata et al., 2018). The understanding of the impact of applying SCGs on soil microbiology and functioning is extremely new (Stylianou et al., 2018). Stylianou et al. (2018) hypothesised that the microbial activity would increase with the application of SCGs. This has been supported by a recently reported incubation soil study (Cervera-Mata et al., 2018). Cervera-Mata et al. (2018) found a significant 10-fold increase in soil respiration with SCG application, and after 60 days soil microbes were seen on SCG particles and they were also incorporated into the soil.

The majority of coffee waste applications to soil have involved composting or blending with other amendments. Blending or composting with other materials have included manures (Sathyanarayana and Khan, 2008, Shemekite et al., 2014), seaweed (Velmourougane et al., 2012), cardboard (Liu and Price, 2011), or straw/sawdust and fresh grass (Cruz et al., 2014c). Coffee waste land applications have been found to suppress soil—borne pathogens (Sathyanarayana and Khan, 2008), increased nutrient uptake in Italian ryegrass (Kasongo et al., 2013) as well as nitrogen enrichment and improved C/N ratios in agricultural soils (Yamane et al., 2014). Furthermore, leaching of pesticides and herbicides through the soil profile decreased with coffee waste amended soil (Fenoll et al., 2014a, Fenoll et al., 2014b, Fenoll et al., 2011).

In a review, Stylianou et al. (2018) found that reports have somewhat contradictory results on plant growth when applying SCGs amendments. The contradictory effects of direct application of SCGs on plant growth that have been reported vary in experimental conditions and range from small scale laboratory trials to trials in a controlled environment (such as a glasshouse) to field studies. It is, therefore, important to remember that even if plant inhibition is observed in laboratory experiments or one set of conditions, this does not necessarily translate to a wider range of conditions, plant species or soil types. Furthermore, the variation of results may be due to the complexity of soils (and interactions between the amendment and soil), the use of different plant species (with associated differences in response to amendments) and/or the difference in environmental conditions within studies.

Experiments conducted with direct applications of SCGs have observed beneficial effects to plant growth at low doses (10%) (Cruz et al., 2012a); however, other studies have found higher doses to be detrimental to plant growth (Hardgrove and Livesley, 2016, Cervera-Mata et al., 2018). A few studies have observed little or no effect of higher application rates on plant growth (Cruz et al., 2015). Hardgrove and Livesley (2016) found that direct application of 2.5 to 25% SCG mixtures with soil (v/v) detrimentally affected broccoli, leek, radish, viola and sunflower growth using three Australian

soil types. Cruz et al. (2014b), Cruz et al. (2014c) found in separate experiments, that fresh SCGs increased antioxidant contents, both hydrophilic and lipophilic, and a gradual decrease of lettuce mineral elements with increased applications of SCGs (2.5 to 20%, v/v) in lettuce. Cruz et al. (2014c) also found that SCGs increased concentrations of total Mn, Ca, P, Fe and Zn in soil, and suggested that investigation of plant available nutrients would be beneficial to assess the agronomic value of SCGs.

In cases where plant growth was inhibited phytotoxicity is often the proposed explanation for plant growth inhibition (Cruz et al., 2012a, Ciesielczuk et al., 2017, Cruz et al., 2014c, Yamane et al., 2014, Hardgrove and Livesley, 2016). This phytotoxicity is commonly suggested to be caused by residual caffeine and polyphenols in the SCGs (Cervera-Mata et al., 2018, Ciesielczuk et al., 2017, Hardgrove and Livesley, 2016). Residual caffeine and polyphenols in SCGs may vary depending on the coffee extraction process, but often contain 0.02% caffeine and 1.5% total phenols (Murthy and Naidu, 2012). Phytotoxicity due to caffeine and polyphenols can occur at low concentrations (Wink et al., 1998, Lyu et al., 2018, McCalla and Haskins, 1964), and the mechanism of phytotoxicity has been proposed in literature. The bioactive compounds, such as caffeine, in the SCGs, can cause plant stress (Cruz et al., 2014b) and caffeine may be toxic to soil microorganisms, as a result may decrease N release in soil (Cruz et al., 2012a). Residual caffeine and polyphenols compounds within the SCGs can make the plant protect their membranes from oxidative damage, as a response of a stressed state (Cruz et al., 2014b). However, the exact mechanism of SCGs phytotoxicity has not been well defined (Stylianou et al., 2018), and sometimes the exact reason for plant inhibition remains unclear (Cervera-Mata et al., 2018, Hardgrove and Livesley, 2016).

Composting, or vermicomposting, of SCGs is often proposed to reduce phytotoxicity effects (Stylianou et al., 2018). While composting may reduce phytotoxicity (Santos et al., 2017) and may increase the inherent slow decomposition of SCGs (Kitou and Okuno, 1999, Bollen and Lu, 1961), the success and rate depends on the composting type/protocol and organic waste composition. In general,

composting may increase pre-treatment costs and time for an uncertain outcome and potentially little reproducibility (Polprasert, 2017). Due to these problems, application of composted amendments can be difficult and costly, particularly if there are multiple waste stream feedstocks. For these reasons, it is beneficial to understand the impact of application of these materials to land without lengthy pre-treatments.

There has been very little investigation of greenhouse gas emissions emitted from soil after SCG application (Stylianou et al., 2018). In particular, in a review Stylianou et al. (2018) highlighted that nitrous oxide emissions produced from agricultural soils amended with SCGs have not been well studied. Greenhouse gas emissions from composts containing SCGs have been studied, showing comparable emissions to controls (Santos et al., 2017). As previously mentioned, a large part of anthropogenic nitrous oxide emissions are sourced from agricultural soils receiving synthetic and organic amendment fertilisers which contain nitrogen (Charles et al., 2017). In particular, organic amendments blended with synthetic fertilisers contribute a higher emissions than other practices (Charles et al., 2017). It is for these reasons that investigating nitrous oxide emissions from soils amended with SCGs is an important research gap.

1.3. Anaerobic digestion

Anaerobic digestion is an increasingly popular technology which digests organic waste and produces green energy (methane) from agricultural and industrial wastes. Anaerobic digestion is economically and environmentally viable for energy production and for treatment of wastes (Edwards et al., 2017, Sheets et al., 2015). Granted that methane (CH_4) production and waste treatment is beneficial in its own right, the by-product of this process has a further potential use. An overview of this is observed in Figure 3.

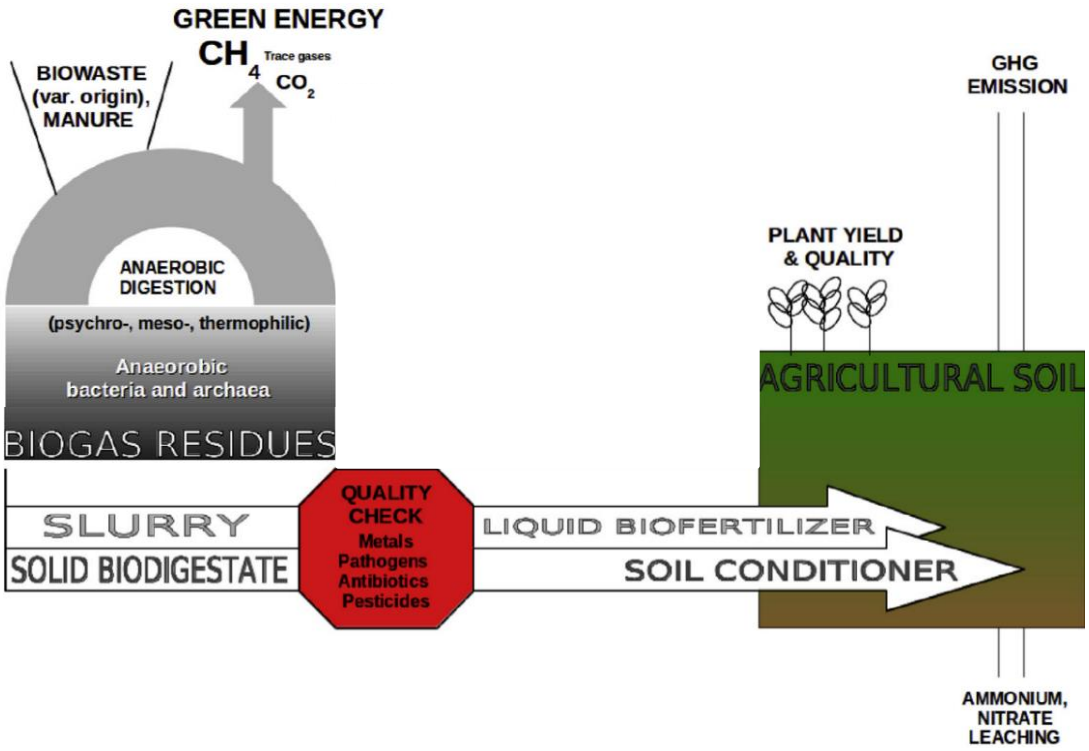


Figure 3 Anaerobic digestate process overview. Modified from Insam et al. (2015)

The feedstock used for anaerobic digestion varies from livestock manure, crop residues, food waste, waste activated sludge, organic fraction of municipal solid waste fruit and vegetable waste, industrial

waste and bio-waste (Akhiar et al., 2017). Although anaerobic digestion is new in Australia and is used primarily for wastewater treatment, it has been growing rapidly since 2010 (Edwards et al., 2015).

Different anaerobic digestion processes are distinguished by the temperature of the digestion process, where psychrophilic digestion ranges from -20 to +10^{o}C, mesophilic digestion ranges from 20 to 45^{o}C and thermophilic digestion ranges from 41 to 122^{o}C. Co-digestion is an increasingly popular way of overcoming single feedstock digestion issues, such as concentrated heavy metal and nutrient loading, and usually involves animal manures in simultaneous digestion of two or more substrates (Mata-Alvarez et al., 2014).

Anaerobic digestion involves multifaceted processes with bacteria and methanogenic archaea which includes the complex breakdown of organic matter (Jang et al., 2015). The rate-limiting step of complex organic degradation in the digestion process is hydrolysis (Ren et al., 2017), also known as hydrolysis fermentation (Braun, 2007). Specifically, the hydrolysis of macromolecules from the feedstock organic matter (e.g. carbohydrates, lipids and proteins) into monomers can form toxic compounds or unwanted volatile fatty acids (Ren et al., 2017, Braun, 2007). Further organic matter decomposition stages are acidogenesis (further decomposes short-chain fatty acids), acetogenesis (digestion of acids) and methanogenesis (CH_4 formation) (Ren et al., 2017).

Anaerobic digestion is one of the most encouraging cost-effective technologies for food waste treatment (Capson-Tojo et al., 2016), anaerobic digestion of food waste has the ability to both obtain better CH_4 yields and manage food waste. Using food waste as a feedstock can also increase substrate hydrolysis and CH_4 yield (Ren et al., 2017), which increases energy production. Food waste as a source of biomass for anaerobic digestion is growing internationally; for example, food waste is one of the primary inputs into anaerobic digestion technologies in the UK (Röder, 2016). Using food waste as a source of anaerobic digestion is a promising treatment pathway for this waste; accordingly,

there is still a large amount of research being produced, particularly on pre-treatment, co-digestion, inhibition and mitigation associated with digestion (Ren et al., 2017). The true benefit of food waste anaerobic digestion most likely relies on the quality of feedstock, the conditions/process of digestion and the effectiveness of the entire process, from waste production to use of by-products.

1.3.1. Anaerobic digestion by-products

The by-products of anaerobic digestion, digestates, are produced in large volumes worldwide (Akhiar et al., 2017). For example, 1,360,000 tons of digestate was produced in Sweden in 2013 with almost 100% returned to the arable land (Risberg et al., 2016). Notably, not all countries allow the application of digestates to land and it depends upon the composition of the biogas residues (Nkoa, 2014, Tiwary et al., 2015b). In these situations, the management of the biogas residues is a problem for the energy industry, who are trying to encourage wide-spread use of anaerobic digestion as a sustainable alternative energy source (Dahlin et al., 2017).

Digestates have a wide range of uses – digestates and extracts have been used as refuse-derived fuels and as biochar or bio-oil after pyrolysis or hydrothermal treatment (Ren et al., 2017). The solid fraction of digestates have even been used as bedding materials and for particleboard (Sheets et al., 2015). The liquid fraction of digestates can be used as supplement nutrient feed for algae production (Xia and Murphy, 2016, Sheets et al., 2015) or can be subjected to extraction processes to remove nitrogen or phosphorus (Sheets et al., 2015). After this algae production, microbial oil can be extracted (Ren et al., 2017) and used as an alternative to vegetable oils. The most common use of digestates and slurries is as an organic fertiliser (Nkoa, 2014).

Digestates have been successfully used as a replacement fertiliser (Koszel and Lorencowicz, 2015, Tampio et al., 2016b, Alburquerque et al., 2012, Tiwary et al., 2015a) or to meet additional nutrient requirements (Kataki et al., 2017). Encouragingly, field applications of digestates have met the EU standards for good agricultural and environmental conditions (GAECs) (Tiwary et al., 2015b). Teglia

et al. (2011a), Teglia et al. (2011b) found that the agronomic value of digestates is not often assessed and these investigations are often superficial. Concerns include odour (Möller and Müller, 2012, Nkoa, 2014), CH_4, N_2O and ammonia emissions, nitrate leaching (Nkoa, 2014), heavy metals (Stefaniuk et al., 2015, Alburquerque et al., 2012) and pathogens (Gómez-Brandón et al., 2016, Fangueiro et al., 2014). Composting is often used to reduce potential pathogens in digestates and can be a cost-effective way to reduce the risk of pathogens (Sheets et al., 2015). Further, it is common practice to separate the solid from the liquid phase (Hupfauf et al., 2016). One reason for this separation is that nitrogen generally stays in the liquid phase and phosphorus is most likely to shift to the solid portion (Insam et al., 2015). Kataki et al. (2017) found that 61-90.5% of the ammonium was in the liquid portions of cattle dung, rice straw and green material mixture digestates. Solid and liquid fractions can often be used without further processing as fertilisers (Al Seadi et al., 2013).

The solid portion of anaerobic digestion residue has gained increasing attention recently (Akhiar et al., 2017) as it is commonly alkaline, has a high organic matter (OM) content and high CEC (Nkoa, 2014). However, the actual structure of the OM content within digestates (humic/fluvic acids, etc) is often not well covered in the literature (Nkoa, 2014), yet, some research has been completed in this area (Barrena et al., 2009). Notably, the OM content of digestates may be an important component to obtain benefits after soil application (Tambone et al., 2010). The liquid fraction has poor biodegradability because of humic substances (Akhiar et al., 2017). Dry matter content is important for land use, but the content is dependent on the digestion process and processing conditions (Teglia et al., 2011a). The large variation in the digestates and slurries from agricultural wastes is partially represented by the results in Table 2.

Table 2 Characteristics of digestates from various authors.

Parameter	Solid fraction	Source	Liquid fraction	Source
Dry matter (% of mass)	1.1-6.6 1.5-45.7	(Risberg et al., 2016) (Various authors in Nkoa, 2014)	13.6 ± 0.2 35.9-58.4	(Chen et al., 2014) (Tambone et al., 2017)
pH	7.3-9	(Nkoa, 2014)	7.6-8.4	(Akhiar et al., 2017)
EC (dS/m)			16-38	
Organic matter (% of mass)	39-75 39-43	(Teglia et al., 2011a) (Drennan and DiStefano, 2010)		
Cation Exchange Capacity (meq/100 g)	20-53	(Teglia et al., 2011a, Teglia et al., 2011b)		
Total Organic Carbon	367-404 (g/kg DM)	(Teglia et al., 2011a, Teglia et al., 2011b)	0.45-3.16 (g/L) 0.18-0.91 (% DM)	(Akhiar et al., 2017) (Kataki et al., 2017)
C: N ratio	3.0-8.5 6.2-24.8	(Nkoa, 2014) (Teglia et al., 2011b)	0.2-1.6 1.5-6.1	(Akhiar et al., 2017) (Tampio et al., 2016a)

1.3.2. Application to land

Digestates can have agronomic value, potentially reducing the need for mineral fertilisers (Tampio et al., 2016b) and they have possible benefits for climate change, the environment and farmers (Insam et al., 2015). Food wastes and organic agricultural wastes as anaerobic digestion feedstocks have higher agronomic value due to potential nutrient recovery and low heavy metal content (Tampio et al., 2016b). The nitrogen (N) content in digestates is likely in the inorganic form (Moraes et al., 2017), which is encouraging for the use of digestates as fertilisers. While digestates are a valuable source of nitrogen, Makádi et al. (2016) suggests that digestates may be a more effective phosphorus and potassium source rather than nitrogen.

Internationally, digestates may be used on agricultural land in certain circumstances, according to specific regulations and quality checks. The United Kingdom Environmental Permitting Regulations allow limited-sourced digestate application to land (including some food waste-sourced material), amounting to 50 t/ha in a 12 month period to provide benefit to the land (SI, 2010). In Australia, wastes intended for land application must adhere to a set of standards, which include a series of quality check tests (pathogens, nutrients, heavy metals etc.). Therefore, in Australia, depending on the state regulations, anaerobic digestate application to land is regulated generally rather than specifically. Similarly, in Europe and America anaerobic digestates and their use on land are commonly regulated with other wastes (Nkoa, 2014), which emphasizes the importance of knowledge of how different materials may affect agricultural soils.

In comparison to other waste-slurries, food wastes and organic wastes as an anaerobic digester feedstock have higher agronomic value (Table 3) as a result of nutrient availabilities and low heavy metal content (Tampio et al., 2016b, Sheets et al., 2015). For example, heavy metals, particularly Cu and Zn, are a particular concern from pig and cattle slurry-sourced material (Alburquerque et al., 2012). Digestates sourced from food waste have lower pathogen risk in comparison to untreated

wastes; however, some pathogens can survive the anaerobic digestion process. Specifically, the pathogens that are particularly concerning in food waste are prions and spore-forming bacteria: *Escherichia coli*, faecal coliforms and *Clostridium perfringens* (Gómez-Brandón et al., 2016, Fangueiro et al., 2014).

Table 3 Summary of the positives and negatives of food waste-sourced digestates compared to digestates sourced from other feedstocks, sourced from Tampio et al. (2016b), Sheets et al. (2015), Gómez-Brandón et al. (2016) and Fangueiro et al. (2014) .

Food waste sourced digestates	Digestates sourced from other feedstocks
Positives - Low heavy metal concentrations - High nutrient availability - Higher CH_4 production during digestion process	Positives - Included in waste to land regulatory approval processes - One to two decades of experiments and industry practice to guide land application
Negatives - Sometimes feedstock is mixed with plastics and other contamination - Relatively unknown effect regarding land application - Can contain pathogens	Negatives - Commonly high metal concentrations - Various nutrient availability - Moderate – high chance of antibiotics and pathogens (especially for manure and biosolid wastes)

1.3.3. Effect on plant growth

There are some studies that record phytotoxicity at high applications (Ramírez et al., 2008) and increased leaching due to digestate application (Schwager et al., 2016). Therefore, application to land must involve considerations to mitigate these potential negative impacts (Tiwary et al., 2015a). In the literature, mixed results have been reported regarding phytotoxicity; showing both no adverse plant growth effects and phytotoxicity (Möller and Müller, 2012). The mixed results in the literature may be due to the variation of digestate compositions, where the feedstock is the major determinant of the associated value and the possible use of the end biogas residue (Nkoa, 2014).

Studies completed on cow manure, pig slurry and biosolids digestate have shown encouraging results, with no adverse effects on lettuce, radish and wheat root and shoot elongation (Gell et al., 2011). Stefaniuk et al. (2015) found that the liquid fraction, with a EC of 21-24 mS/cm^{-1}, had negative impacts on garden cress and white mustard plant growth, while the solid fraction did not. Stefaniuk et al. (2015) used 0.5, 2.5 and 5% (w/w) of biogas residue which was predominantly maize waste with other crop wastes and/or manure. Sánchez et al. (2008) found no phytotoxicity effects of 1, 5 and 10 g to 100 ml DI water with cattle and pig digestate was found on garden cress.

When studies have observed phytotoxicity, like those described above, adverse growth has been attributed to ammonium salt, ammonia and organic acids (Möller and Müller, 2012, Ramírez et al., 2008). Ramírez et al. (2008) found phytotoxicity tests on red clover, oilseed turnip and perennial rye-grass did not correlate with heavy metals or organic pollutants in pig slurry and sewage waste digestate doses ranging from 4.9-1000 g/kg. Stefaniuk et al. (2015) found 0.5, 2.5 and 5 (w/w) of the liquid fraction of crop and manure residue digestate shown garden cress and white willow inhibition at 17.4-100% and 30.5-100% respectively.

1.3.4. Effect of soil emissions and nitrate leaching

Soil emissions, especially CH_4 and N_2O, have been consistent problems with the application of digestate to land (Nkoa, 2014), but the extent of adverse or beneficial impacts depend on the farming system and application (Möller, 2015). Mitigation of soil emissions can be done in various ways; for example, Riva et al. (2016) found that sub-surface injection can help reduce ammonia emissions on maize silage compared to surface application.

Digestate application can increase basal microbial respiration and metabolic quotient with cattle slurry in comparison to untreated slurry and mineral fertilisers on a loamy sand (Hupfauf et al., 2016). In a nine day incubation experiment, Johansen et al. (2013) observed that application of digestate increased N_2O and CO_2 by 30-40% compared to raw cattle slurry.

Other soil application studies have observed some reduction of soil emissions (Schwager et al., 2016, Moraes et al., 2017). Two and a half year field trials with manure and grease water digestate, raw liquid manure and inorganic fertiliser showed that soil N_2O emissions were reduced with digestates materials (Schwager et al., 2016). Application of 100 m^3/ha vinasse digestate suppressed nitrous oxide emissions in comparison to untreated vinasse wastes (Moraes et al., 2017).

On the other hand, leaching, particularly nitrate leaching, is a major problem for digestate application to land (Möller, 2015) which can lead to a loss of nutrients, contamination of groundwater and surface water. Furthermore, while the high nutrient content of digestates can make digestates desirable, it also presents a limitation if local nutrients are already high, particularly phosphorus and nitrate (Dahlin et al., 2017). Nitrate leaching may be reduced if application is matched with nutrient demand by plants; for example, application in spring or preceding crop or plant sowing (Möller, 2015) to ensure that high amounts of nitrogen is taken up by plants.

A two year field experiment with applications of digestate, pig and cattle manure and mineral fertilisers showed similar nitrate leaching between digestate and manure slurries with leaching on maize (Svoboda et al., 2013). However, nitrate leaching was greater from digestate application over two and half years with a comparison between manure and grease water digestate, raw liquid manure and inorganic fertiliser (Schwager et al., 2016). In this study these losses were offset by higher N_2O emissions from raw liquid manure in the second year. These nitrogen losses were attributed to seasonal differences in plant growth and associated nitrogen transformation in the soils.

1.4. Summary

Spent coffee grounds and anaerobic digestates have a potential for land application; however, there are significant concerns regarding soil and environmental health. This is due to the wide range of effects on plant growth, pathogen risk, soil emissions and leachates varying with crops, soils, source of agricultural waste, treatment, time and quantity of application.

1.5. Objectives and aims

The objectives of this work were to:

1. Investigate the potentially valuable components in coffee waste (SCGs and husks) through conventional extractions and using common solvents (Chapter 2.), focusing on extraction of lipids, caffeine and polyphenols

2. Investigate the application of SCGs on short-term glasshouse plant growth trials:

    a. High application rate 20 t/ha with additionally ethanol-extracted and “as is” (air-dried) SCGs in short-term glasshouse plant growth trials using silver beet (Chapter 3.).

b. Phytotoxicity trials using extracted liquid and solids in root elongation and emergence tests (Chapter 4.).

c. Two application rates (5 and 10 t/ha) with additionally water-extracted and air-dried SCGs in short-term glasshouse plant growth trials using silver beet (Chapter 4.).

3. Investigation of digestates in soil column trials and their impact on nitrogen leaching, soil emissions, and, in a separate trial, short-term plant growth (Chapter 5.).

4. Investigation of digestates in soil column trials and their impact on soil emissions, metals and salts in leachates and soils as well as short-term plant growth (Chapter 6.).

The hypothesis of this work is that valuable components can be extracted from coffee waste (Chapter 2.). Furthermore, the extracted SCGs can reduce potential phytotoxicity in short-term glasshouse plant growth trials (Chapter 3. and Chapter 4.). The second part of this work investigated digestates, from the Netherlands, applied to soils. It was theorised that high application rate digestates could increase soil emissions and leaching in the absence of plants and reduce plant growth (Chapter 5.). The investigation of Australian digestates on two contrasting soil textures hypothesised that metals could accumulate in the clay-textured soil and leaching could increase in the sandy-textured soil (Chapter 6). This research is a collaboration with the Environment Protection Authority, Victoria, to contribute to the scientific knowledge relevant to land application of organic wastes.

Chapter 2. Coffee husks and spent coffee grounds: Characterisation and valorisation options using extractions

2.1. Introduction

There is a great potential to valorise coffee waste, due to the high amounts of such material available (Caballero-Galván et al., 2018), the fact that it has many different components (Peshev et al., 2018) and a wide range of valorisation options (Kovalcik et al., 2018). As previously mentioned, this waste comes from Spent Coffee Grounds (SCGs) and coffee husks, with large quantities of SCGs being produced globally. Waste produced from the coffee industry is in the order of hundreds of millions of tons annually world-wide (Caballero-Galván et al., 2018). There have been over 1000 individual organic components identified in SCGs, some of which are in quantities that could be recovered and have thus gained international attraction for waste valorisation (Peshev et al., 2018). Of concern is that this waste often ends up in landfill (Kourmentza et al., 2018, Cameron and O'Malley, 2016) where it can pollute the environment with phytotoxic compounds (Ballesteros et al., 2014).

Extraction of valuable components of coffee waste, particularly from SCGs and coffee husks, is a potentially cheap, sustainable way to valorise these wastes. In particular, lipids and bioactive compounds, such as caffeine and polyphenols, derived from coffee wastes have potential use in the energy, cosmetic, pharmaceutical and food industries (Kourmentza et al., 2018, Mussatto et al., 2011b, Murthy and Naidu, 2012, Kovalcik et al., 2018). Furthermore, the extraction of certain components, such as the caffeine and polyphenols in coffee waste, have an additional outcome: the material can be 'cleaned up' prior to land application. The removal or reduction of certain components could minimise potential environmental, human health and/or plant harm and pollution.

The lipid content of SCGs typically ranges from 10 to 17%, but can sometimes be as high as 20% (Mussatto et al., 2011b, Campos-Vega et al., 2015) and 0.5-3% from husks (Gouvea et al., 2009). Common extraction methods usually involve ultrasonication, Soxhlet, microwave and, more recently, Supercritical Carbon Dioxide (Sc-CO_2) (Ahangari and Sargolzaei, 2013, Kourmentza et al., 2018, Stylianou et al., 2018). Extractions of SCGs are often conducted with a mixture of organic solvents

(e.g. ethanol or hexane), or using Sc-CO_2 with co-solvents, which is commonly the focus of lipid extractions, due to the high lipid content of SCGs. The amount of extractable lipids can depend on processing history, location of the coffee harvest and bean type (Jenkins et al., 2014). For example, Arabica SCGs can have more lipids in comparison to Robusta SCGs (Farah, 2012).

Polyphenols in coffee waste are valuable because they are potent antioxidants. For example, chlorogenic acid and its derivatives are present in SCGs (Kovalcik et al., 2018) and are extremely valuable for their health benefits (Andrade et al., 2012). Antioxidants have gained worldwide attention for their positive effects on human health, such as the potential for prevention of cancer, diabetes and allergies (Caballero-Galván et al., 2018). It is for this reason that polyphenol extracts of coffee wastes are also used in the food industry (Martinez-Saez et al., 2017). Both SCGs and husks can contain 0.8-1.5% total phenols (Murthy and Naidu, 2012) that can be extracted using polar or intermediately polar solvents mixed with deionised water (Kovalcik et al., 2018).

The lipids from both SCGs and coffee husk waste have great potential to be used as cheap diesel (Kourmentza et al., 2018); therefore, reliable, large-scale transesterification processes have gained increasing attention (Stylianou et al., 2018). Lipids have been added to cosmetic oil-in-water creams, showing positive effects on skin fat levels (Ribeiro et al., 2013). Another application is as an addition to sunscreen creams, due to its ultraviolet absorption (Wagemaker et al., 2011). Lipids from coffee waste have also been used in the food industry as additives into bakery goods (Stylianou et al., 2018). Following extraction, solid material can be used for further extractions, as a soil amendment (Vardon et al., 2013), and pesticide capture (Fenoll et al., 2014b, Fenoll et al., 2011).

Caffeine is the most consumed psychoactive substance in the world (Clark and Landolt, 2017) and is commonly used as a co-adjuvant or a pharmaceutical formulation agent (Andrade et al., 2012). However, caffeine has also been identified as an emerging environmental and pharmaceutical pollutant (Stylianou et al., 2018), causing harm to many life processes (Fan et al., 2006). Caffeine

extraction is promising method of preventing contamination and enabling further valorisation. Using caffeine-free coffee waste material is beneficial for animal feed and soil amendments; noting that at certain concentrations they are unpalatable and potentially toxic (Kovalcik et al., 2018).

Coffee husks generally have high levels of caffeine (~1%) (Murthy and Naidu, 2012, Tello et al., 2011), whereas residual caffeine in SCGs can vary from 0.02-0.5% (Murthy and Naidu, 2012, López-Barrera et al., 2016). The level of residual caffeine concentration depends on the coffee extraction process and the coffee species; for example, there is more caffeine in Arabica beans compared to Robusta beans (Campos-Vega et al., 2015).

Ultrasonic extractions can to be more beneficial compared to Soxhlet extractions in recovering valuable chemical components from the coffee waste streams (Rocha et al., 2014). These types of extractions are beneficial for treating SCGs as the ultrasonic bath assists the solute to disperse quickly from solid phase to the solvent (Caballero-Galván et al., 2018). Sc-CO_2 extractions are relatively environmentally-friendly, as Sc-CO_2 equipment is established in the coffee industry and can produce high lipid, caffeine and polyphenol yields (Ahangari and Sargolzaei, 2013, Andrade et al., 2012).

The aim of this work was to compare locally-sourced SCGs and coffee husks, with respect to the lipid, caffeine and polyphenol extraction quantities, using several extraction methods. Ultrasonic and Sc-CO_2 extraction methods were tested with conventional solvents of different polarity (hexane, ethanol, methanol, THF and methanol). While SCGs have been extensively studied world-wide (Kovalcik et al., 2018), little research has been done on Australian materials. In particular, husks lack investigation, mainly due to their low lipid content; however, the advantage of husks is that the waste is produced at the factory making them easily accessible. In comparison, SCGs which are widely spread across cafés and restaurants, which makes collection more difficult.

The research questions were:

1. How different are the results in terms of fatty acid, caffeine and polyphenol content using conventional extraction methods applied to both SCGs and husks from local sources?
2. What is the composition of the extracts, as determined by using ^{13}C and ^{1}H NMR, GC-FID, LC-MS?
3. Is the extraction potentially useful for industry and other valorisation purposes? If so, what industries?

2.2. Materials and methods

2.2.1. Material collection and short collection survey

Spent Coffee Grounds (SCGs) were collected from two cafés in April-May 2015 on the Monash University campus, Clayton, Melbourne, Australia. A short survey of waste amounts found that SCG amounts ranged from 15-21 kg (wet weight) produced per working day per café (n= 4). The initial moisture content after collection was 51-52%. Three of the four lots collected were used in experiments (SCG1, SCG2, SCG3), with one lot discarded due to material contamination. Mixed Arabica and Robusta (MixH), and Arabica only (AraH) coffee husks were sourced from another business, a Mocopan roasting factory in Melbourne, Australia. MixH was used for extraction experiments. SCGs were air dried, to ~10% moisture, before storage. All materials were stored in air-tight drums prior to analysis and then oven dried at 105^{o}C before extraction was conducted.

2.2.2. Initial material characterisation methods and apparatus

Solid State ^{13}Carbon Nuclear Magnetic Resonance spectroscopy (^{13}C NMR), using cross polarization (CP) magic angle spinning (MAS), was used to evaluate the organic nature of the collected SCGs.

Solid state NMR experiments were collected on Bruker Avance I 300 (7.05 Tesla magnet) with a 4mm multinuclear solid state probe at room temperature ca. 24°C. Solid samples were packed into 4mm ZrO_2 rotors with a Kel-F cap and spectra were recorded using CP-MAS techniques. ^{13}C spectra were calibrated against an external sample of glycine. Peaks were integrated into 5 regions: 0–28 ppm; 28–47 ppm, 47–113 ppm, 113–160 ppm and 160–210 ppm following the approach described by Tambone et al. (2010).

Electrical Conductivity (EC) and pH were measured by a sensION+ EC5 portable HACH® and WP-80 TPS electronic probes, using 3A1 method - 1:5 soil to denoised water (DI) water extraction (Rayment and Lyons, 2011b). CHN analysis was done with a Elementar vario MICRO cube analyser equipped with a CHN combustion and reduction tube using a Temperature Programmed Desorption trap column and Thermal conductivity detection.

2.2.3. Lipid extraction

Initially, lipid extractions were conducted using a three by two factorial design, using three extraction times (0.5, 1 and 2 hours) and two solvents (ethanol and hexane). Each treatment was completed in triplicate. Following this, two-hour extractions were conducted with THF and Sc-CO_2. EMSURE® ACS ISO reagent pH Eur ethanol 99.7% absolute grade and methanol as well as EMSURE® ACS *n*-hexane (hexane) and tetrahydrofuran (THF) were purchased new from Sigma-Aldrich for extraction. THF was dried for 24 hours using potassium hydroxide.

2.2.3.1. *Ultrasonic bath extraction method*

Ultrasonic bath extractions were conducted using ethanol, hexane for 0.5, 1 and 2 hours with 10 g coffee waste to 300 ml solvent in 500 ml Schott bottles. Elmasonic S60H ultrasonic bath was used, which operates at 37 kHz, 550 W. Extractions were undertaken without temperature regulation, where

ultrasonication temperature was taken every 30 minutes. The average maximum bath temperatures where 33 ± 1.7ºC, 46 ± 1.8ºC and 51 ± 0.7ºC for 0.5, 1 and 2 hour extraction times, respectively.

Lipid extracts were filtered through a Whatman no.1 filter, transferred to a round bottom flask, and the solvent was recovered using a rotary evaporator under vacuum. The remaining sample was kept overnight in vacuum oven (20ºC, -70 KPa) to ensure all solvent was removed. The extract was then weighed, and yield was calculated as the ratio between mass of extract and mass of raw material.

2.2.3.2. *Supercritical carbon dioxide extractions*

An SFT-110 XW Series SFE System from Supercritical Fluid Technologies, Inc. (Figure 4) was used with a 10 ml capacity vessel using Air Liquide supercritical CO_2 fluid. This was fitted with a Series II SFE systems pump unit and an outflow 0-50 ml/min meter.

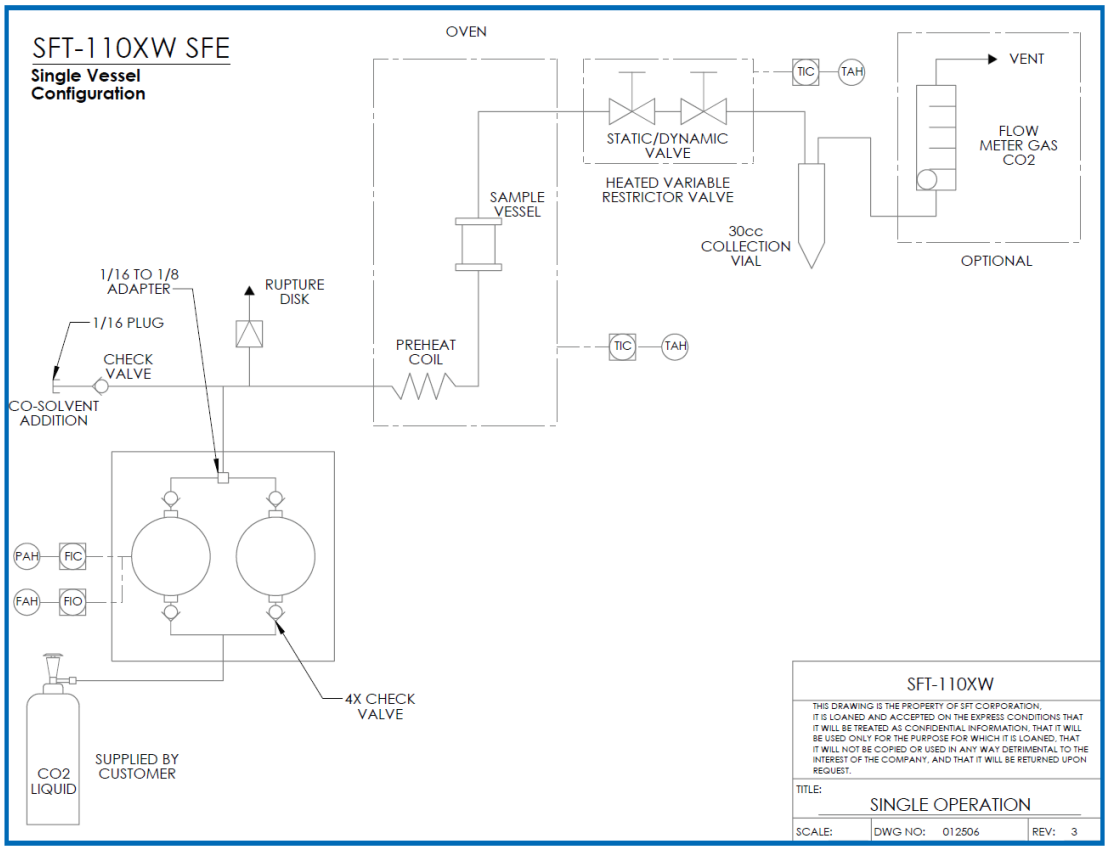


Figure 4 Supercritical CO_2 extraction apparatus (SFT, 2018) .

Pure CO_2 (Sc-CO_2) and 18% of ethanol (Sc-Eth) were used with 5 g of coffee waste (crushed to <0.7 mm) in a fabric extraction bag, following a modified method described by Ahangari and Sargolzaei (2013). The oven temperature was set at 40ºC with a two hour soak at 5080 psi. At the end of the soak, the machine was set to 14 ml/min dynamic flow for 10 minutes, followed by sharp releases of pressure to collect the sample. Each treatment was undertaken in duplicate.

2.2.3.3. *Proton Nuclear Magnetic Resonance analysis*

Extracts from ultrasonication and Sc-CO_2 (~1 mg) were dissolved in deuterated chloroform ($CDCl_3$) for 1D Proton Nuclear Magnetic Resonance (^{1}H NMR) spectroscopy. ^{1}H NMR experiments were collected on Bruker Avance 400 MHz (9.4 Tesla magnet) with a 5mm broadband auto-tunable probe with Z-gradients at 25ºC. ^{1}H spectra were calibrated against residual $CHCl_3$ solvent at δ7.26 chemical

shift. Experiments were collected and processed by a Topspin 3.2 program. Peaks were compared to literature for identification.

2.2.3.4. *Gas Chromatography with Flame-Ionization Detection analysis*

Direct transesterification of the ethanol and hexane extraction lipids was completed as the first step to obtain relative fatty acid composition of the samples. Derivatisation was undertaken by using methanol and acetyl chloride (95:5 v/v) and sodium sulphate following the protocol described by Ahangari and Sargolzaei (2013). The derivatised fatty acids were analysed by Gas Chromatography with Flame-Ionization Detection (GC-FID) to obtain the relative composition of fatty acids using a Sigma-Aldrich Supelco37 Component FAME Mix (100ppm) certified reference material, TraceCERT®, in dichloromethane (varied conc.). The GC analysis used an injection volume of 1µL, split ratio 5:1, HP-5ms (30m×0.25mm×0.25µm) column, oven at 80℃ (0.1m) to 300℃ (15℃/min, hold for 5min), inlet and detector temperature 250℃.

To obtain a comparison of the fatty acid compositions the sum of the most prominent 5-6 peak areas in the chromatogram were calculated, then the individual components were expressed as a relative percent ($\%_{relative}$), uncertainty was obtained by calculating the standard deviation of these percentages. This enabled a relative comparison between samples, while not being a comprehensive quantification. The important distinction of C18:2 compared to C18:3 fatty acids was reliable as C18:3 peaks are resolved and eluted prior to C18:2 fatty acid peaks.

2.2.4. Caffeine and total phenols

Initially, caffeine and total phenol ultrasonic bath extraction was optimised using SCGs in a three by two factorial experimental design, with three solvents and two extraction times (1.5 and 5 hours).

Caffeine and polyphenol extraction was conducted with 70% ethanol (Zuorro and Lavecchia, 2012), 60% methanol (Mussatto et al., 2011a) and 100% DI water. A ratio of 40 ml solvent to 1 g of SCG was used for 1.5 and 2 hours, following Mussatto et al. (2011a).

2.2.4.1. *Liquid Chromatography - Mass Spectrometry*

Polyphenol and caffeine analyses were conducted using an Agilent 1260 Infinity Liquid Chromatography (LC) system coupled with a 6120-series quadrupole Mass Spectrometer (MS). A Kinetex® 5 um C18 100Å Column (250 mm × 4.6 mm I.D; particle size 5 µm) was used. The injection volume was 10 ml with a column oven temperature of 30^{o}C. Gradients elution was 0 min, 0% ACN at 30 min, 20% ACN at 50 min, 60% ACN at 55 min, 100% ACN at 70 min, 100% ACN at 73 min, 0% ACN at 76 min, 0% ACN. UV wavelengths used were 210 nm, 254 nm, 260 nm, 280 nm, 320 nm, 378 nm; with a data acquisition rate of 5 Hz. MS parameters were drying gas flow of 12 L/min, nebuliser pressure of 35 psi, drying gas temperature of 350 ◦C, and capillary voltage of 3000V. A mass scan range of 100–1200 Da with cycle time of 1.49 seconds per cycle.

1,3,7-Trimethylxanthine (caffeine) was quantified by using standards, 0, 50, 150, 250, 350 and 450 mg/L from Sigma-Aldrich analytical ReagentPlus® powder. Identifications were performed using authentic standards where available. Tentative identifications were performed where authentic standards were not available, according to the fragmented ion masses and molecular ions, and also their maximum UV absorbance with reference to literature (Clifford et al., 2003).

2.2.4.2. *Gallic Acid Equivalents (total phenols)*

The total phenols were analysed as Gallic Acid Equivalents (GAEs) using 10% Folin–Ciocalteu reagent and 700mM Na_2CO_3 analysed at 765, 760 and 720 nm (Ainsworth and Gillespie, 2007). Samples were analysed by a Thermo Fisher Scientific™ Multiskan™ GO microplate UV-Vis

spectrophotometer. Standards of 3,4,5-Trihydroxybenzoic acid (gallic acid) at 0, 20, 60, 100, 140 mg/L were made from a Sigma-Aldrich analytical gallic acid, 97.5-102.5% (titration) powder.

2.2.5. Statistical analysis

One-way and two-way ANOVAs were used with a SPSS statistic version 25 package, followed by Tukey's post-hoc test and pairwise comparisons using Least Significance Difference (LSD). Significance for all tests were $p = <0.05$. Solvent and extraction times were fixed factors and yield was the dependent variable. Levene's test of equality of error variances was used to confirm statistical test appropriateness. If significant, then data was log transformed (raw data reported in graphs and tables).

2.3. Results

2.3.1. Characterisation

The two coffee waste streams had a pH of 5.2-5.4 and were composed of 42.9-50% carbon, 6.1-7% hydrogen and 2.4% nitrogen (Table 4). Nitrogen in the SCGs are similar to the average that Murthy and Naidu (2012), Campos-Vega et al. (2015) reported, but is lower than some reports, which can be as high as 7.4% (Fenoll et al., 2014b). Carbon percentage of both SCGs and husks are similar to the literature (Fenoll et al., 2014b, Kasongo et al., 2011).

Table 4 Coffee waste characterisation: pH, carbon, hydrogen and nitrogen content. Deviation represents standard deviation, *n*= 3.

Material	**pH**	**C**	**H**	**N**
		% of mass		
Husks	5.4 ± 0.2	42.9 ± 0.6	6.1 ± 0.1	2.4 ± 0.2
SCGs	5.2 ± 0.1	50.0 ± 0.3	7.0 ± 0.1	2.4 ± <0.1

Solid State ^{13}C NMR spectra showed that the husk and SCG materials were similar in composition (Table 5). All materials had a significant portion of O-alkyl carbons (peaks observed at δ47-113 ppm), such as those found in carbohydrates (e.g. in cellulose and hemicellulose). The dominating signal at δ72 ppm is often found in plant residues, and is also associated with hemicellulose and cellulose. There were also consistent signals in the aliphatic carbon region (δ0-28,δ28-47 ppm) of all materials (Figure 5, Figure 6 and Table 5). Peaks were identified following Kögel-Knabner (2002), unless otherwise stated.

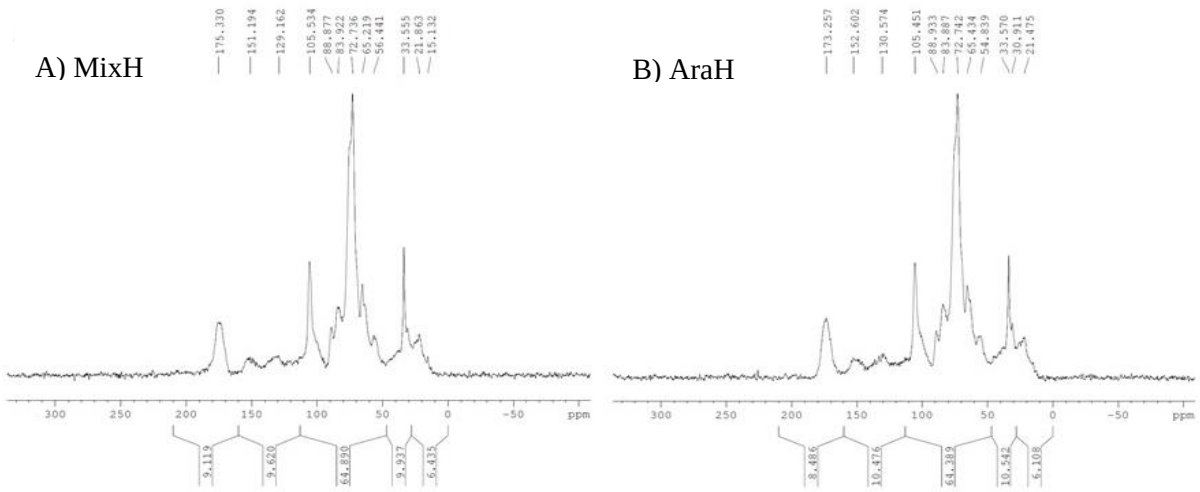


Figure 5 Coffee husks solid state ^{13}C CPMAS NMR spectra. A) Mixed Arabica and Robusta husks, B) Arabica only husks

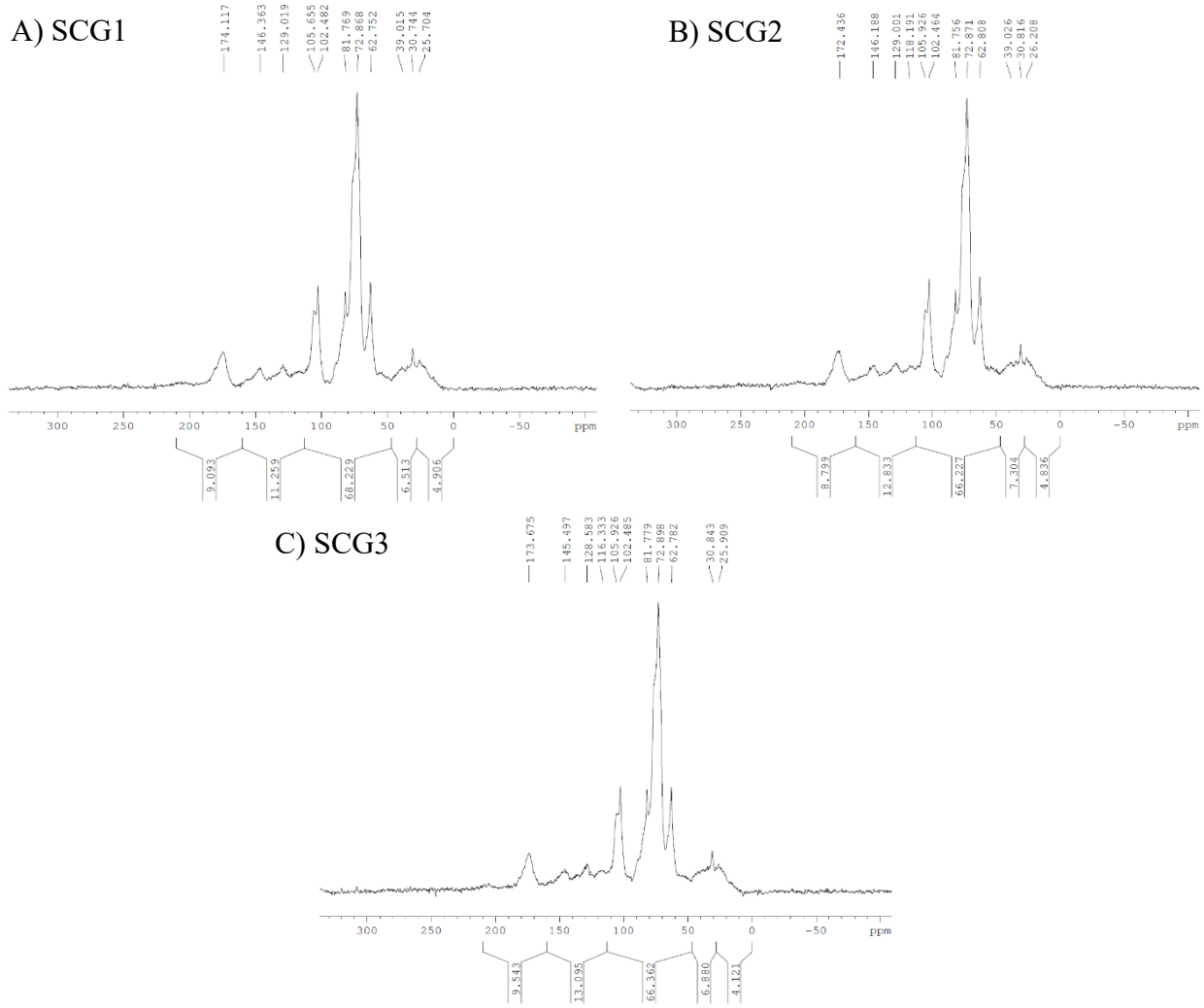


Figure 6 Spent Coffee Grounds solid state ^{13}C CPMAS NMR spectra. A) SCG1, B) SCG2, C) SCG3.

There was also evidence of polysaccarides, lignin and a smaller amount of (potentially) lipids in all materials. A clearly distinguished signal at δ105 ppm is associated with the C1 anomeric carbon of polysaccharides in a glycosidic bond, this peak is more distingushed in the husks compared to the SCGs (Figure 5 and Figure 6). The peak at centred δ175 ppm, observed in the Arabica husks (Figure 5 A)), is associated with the carbonyl component of acetyl groups in hemicelluloses.

The peak at δ30 ppm indicates the presence of long chain alphatic stuctures (e.g. lipids, cutin), this signal is more defined in the husks compared to the SCGs (Figure 5 and Figure 6). Indeed, there are fewer both short and long chain aliphatics in the SCGs compared to the husks (Table 5).

The SCGs spectra are comparable to those of Nogueira et al. (2011) and Panzella et al. (2016), who both observed similar signals in fresh and spent coffee grounds. Nogueira et al. (2011) found polysaccharide signals at δ62 ppm (CH_2-O), δ72 ppm (CH-O) and δ102 ppm (CH-O-CH). The latter signal can be assigned as anomeric carbons. Interestingly, Nogueira et al. (2011) did not observe a chemical shift at ~δ173 ppm with their coffee granule samples, but such a peak is observed in this study. Panzella et al. (2016) found a peaks at ~δ170, 150, 130, 115 and 25 ppm, attributed to aromatic carbons of syringyl and guaiacyl units of lignin and tannin constituents. The chemical shift at ~δ173 ppm may also be associated with the carbonyl in acetyl groups of hemicellulose (Kögel-Knabner, 2002).

All materials produced two small rounded peaks in the 113-160 ppm range associated with aromatic carbon, such as those found in lignin and polyphenol structures (Figure 5 and Figure 6). For example, peaks from δ115-150 ppm have been assoicated with chlorogenic acids and an alkaloid (trigonelline) in ^{13}C NMR of roasted coffee bean extracts (van Duynhoven, 2013). These peaks are slightly more pronouced in the husks compared to the SCGs ^{13}C NMR spectra, with 10.1% vs 12.4% area associated with this region for husks and SCGs, respectively. This is unsurprising, as the lignin content of husks is ~9% (Gouvea et al., 2009) versus ~0.05% in SCGs (Murthy and Naidu, 2012).

Table 5 Integrated carbon distribution (%) of solid state ^{13}C NMR spectra, *n*= 1.

Carbon type		**Short chain aliphatic C**	**Long chain aliphatic C and proteins**	**O-alkyl C**	**Aromatic C (subsitiuted and unsubstituted)**	**Carbonyls in aliphatic esters, carboxyl groups, and amide carboxyl**
Chemical shift (δ)		0-28 ppm	28-47 ppm	47-113 ppm	113-160 ppm	160-210 ppm
Husks	MixH	6.1	10.5	64.4	10.5	8.5
	AraH	6.4	9.9	64.9	9.6	9.1
	Average ± SD	6.3 ± 0.2	10.2 ± 0.4	64.7 ± 0.4	10.1 ± 0.6	8.8 ± 0.4
SCGs	SCG1	4.9	6.5	68.2	11.3	9.1
	SCG2	4.8	7.3	66.2	12.8	8.8
	SCG3	4.1	6.9	66.4	13.1	9.5
	Average ± SD	4.6 ± 0.4	6.9 ± 0.4	66.9 ± 1.1	12.4 ± 1.0	9.1 ± 0.4

2.3.2. Preliminary extraction experiments

Initially, husk experiments (using MixH) were conducted to compare uncrushed to size-homogenised samples (crushed with mortar and pestle) using ethanol with 0.5, 1 and 2 hour extraction times with an ultrasonic bath. The yield did not significantly change with uncrushed/crushed material over extraction time (p= 0.128, two-way ANOVA). However, at 0.5 hour extraction, uncrushed husks obtained a higher yield (p= 0.004), and therefore uncrushed materials were used for all subsequent experiments.

Secondly, different SCG collection sources were compared with ethanol and hexane for 0.5, one and two-hour extractions. Yields did not significantly differ between SCGs sources (p= 0.911, one-way ANOVA); therefore, equal proportions from each SCG source were used.

2.3.3. Lipid extractions

2.3.3.1. ***Yield – ethanol and hexane***

When comparing husk yields from ethanol and hexane at different extraction times, the interaction between solvents and extraction times was significant (p= <0.031, two-way ANOVA). Ethanol produced a higher yield, although there was no difference between ethanol extraction times (p= >0.05) (Figure 7).

When comparing SCGs yields in ethanol and hexane, the interaction between solvents and extraction times was significant (p= <0.001, two-way ANOVA). Ethanol had lower yields in comparison to hexane at 0.5 and 1 hour extraction times (p= <0.001, LSD); however, there were no differences between ethanol and hexane at 2 hour extraction time (p= 0.745, LSD) (Figure 7).

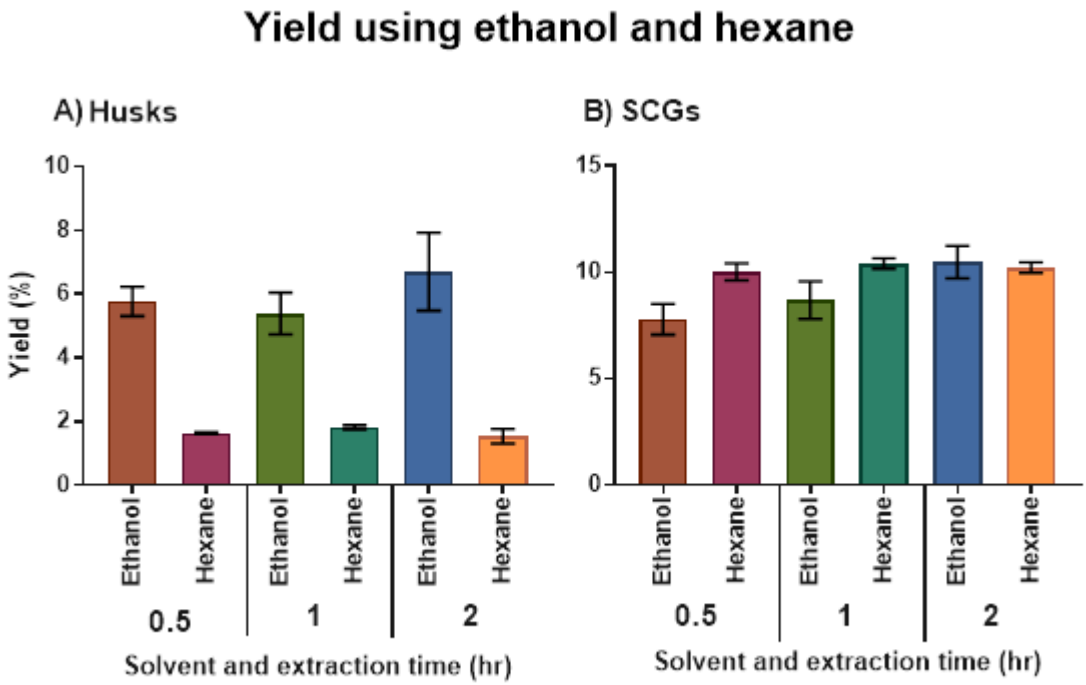


Figure 7 Extractive yield using different extraction times and two solvents. SCGs *n*= 5-6, husks *n*=3-4.

2.3.3.2. ***Two-hour extraction comparison***

When comparing husks with the different solvents using a two-hour extraction time, there were significant differences in extraction yields between solvents (p= <0.001, one way ANOVA) (Figure 8). Ethanol and THF had similarly high yields (p= 0.122, LSD); while hexane and Sc-CO_2 produced low yields (p= 0.954, LSD). The Sc-Eth experiment had a similar yield to Sc-CO_2 extraction yields, with 1.2% (n= 1) and 1.7 ± 0.6% respectively. Replicates of this extraction are required to confirm this finding and were not completed in this study due to machine failure. There was a discrepancy between yield and the weight loss of the raw material. Weight loss in the raw material was 5.1 ± 0.2%, leaving an average of 0.17 g of unaccounted material loss based on the above yield calculations.

When comparing SCGs with different solvents (two hour extraction), there were differences between solvents (p= <0.001, one-way ANOVA) (Figure 8). Hexane and ethanol produced the highest yields (p= 0.745, LSD), THF had a slightly lower yield (~7.6%) and Sc-CO_2 and Sc-Eth had the lowest yields (p= 0.989, LSD). The Sc-CO_2 yield 1.6 ± 0.2% was improved with the addition of 18% ethanol (yield 2.4 ± 0.1%). Again, there was a discrepancy of yield: calculating the mass balance after the extraction, there was a mass of 0.09 g unaccounted for, given that 10 g of SCGs were placed in the extraction vessel.

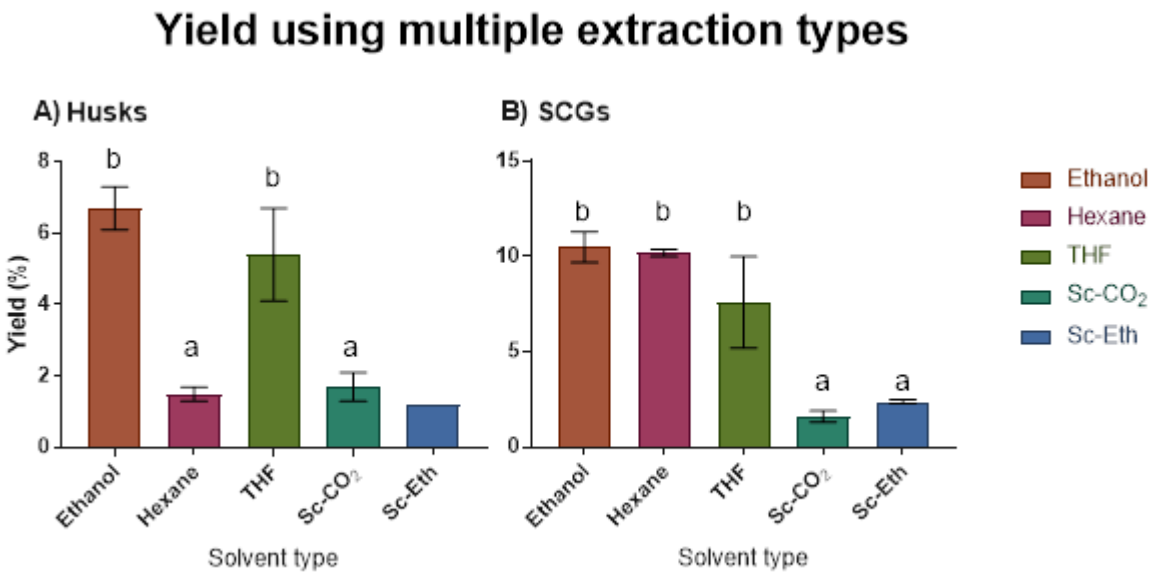


Figure 8 Extractive yield, averages ± SD, from 2 hour extractions using ethanol and hexane, supercritical extraction using pure CO_2 (Sc-CO_2) and 18% ethanol co-solvent (Sc-Eth). Ethanol and hexane: n= 3, THF, Sc-CO2, Sc-Eth(SCGs): n=2, Sc-Eth(husks): n= 1. Letters represent the result of post hoc Tukey's test (significance p= <0.05). NB: Husks and SCGs were statistically analysed separately. Sc-Eth(husks) was excluded from statistical tests due to only one replicate.

2.3.3.3. *^{1}H NMR analysis*

Both the SCGs and husks ^{1}H NMR spectra observed a relatively consistent composition

Figure 9 and Figure 10), with dominant peaks from δ1.22-1.42 ppm, associated with aliphatic –($C\mathbf{H}_2$)$_n$– (Table 6). The ^{1}H NMR spectra in this study are very similar to the spectra of Guillén and Ruiz (2003), who analysed plant-based oils; therefore, assignment of peaks was based on this work (Table 6). In all spectra there was evidence of fatty acid chains at δ1.60 ppm, which is also observed in oil extracted from coffee grounds (Phimsen et al., 2016). The peak at δ0.85 ppm is associated with a terminal methyl group in $CH{=}CHCH_2C\mathbf{H}_3$ in all acids except linolenoyl. The expected methylene and methane of the glycerol component of lipid is evident in the range δ4.10-4.32 ppm (Table 6). Peaks in oils at δ2.31 and δ2.78 ppm have also been associated with triglycerides (Phimsen et al., 2016, de Jesus et al., 2015). There was little evidence of free glycerol, with no major peaks at ~δ3.31, 3.38, 4.48 and 3.45 ppm (BaseChem, 2017).

Table 6 Assignment of ^{1}H NMR peaks following Guillén and Ruiz (2003). **Bold** indicates the hydrogen with which the shift is associated.

Chemical shift (δ)	**Functional group**
0.83-0.93	–C**H**$_3$ (saturated, oleic and linoleic acyl group)
0.93-1.03	–C**H**$_3$ (linolenic acyl group)
1.22-1.42	–(C**H**$_2$)$_n$– (acyl group)
1.94-2.14	–C**H**$_2$–CH=CH– (acyl group)
1.52-1.70	–OCO–CH$_2$–C**H**$_2$– (acyl group)
1.94-2.14	–C**H**$_2$–CH=CH– (acyl group)
2.23-2.36	–OCO–C**H**$_2$– (acyl group)
4.10-4.32	–C**H**$_2$OCOR (glyceryl group)
5.20-5.26	>C**H**OCOR (glyceryl group)
5.26-5.40	–C**H**=C**H**– (acyl group)

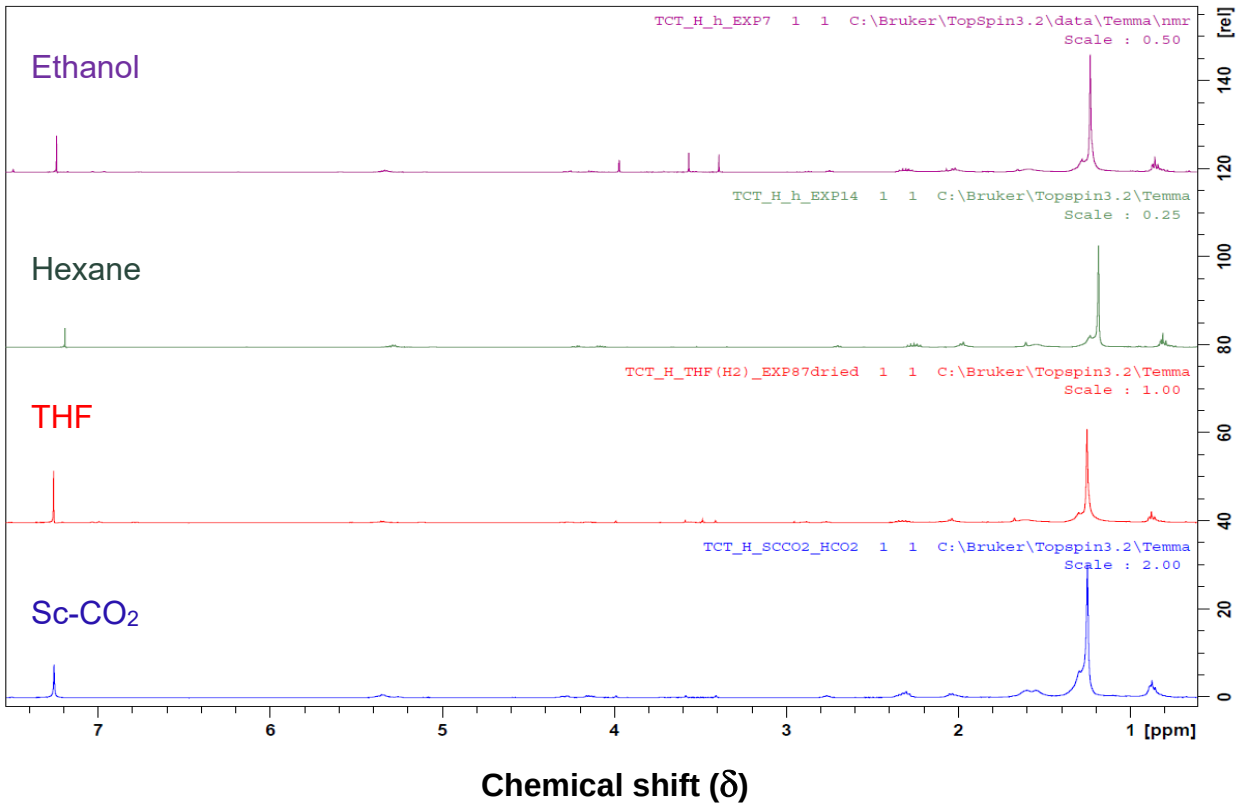


Figure 9 Husk comparison ^{1}H NMR spectra for 2 hour extractions and GC chromatogram showing the relative abundance of fatty acids.

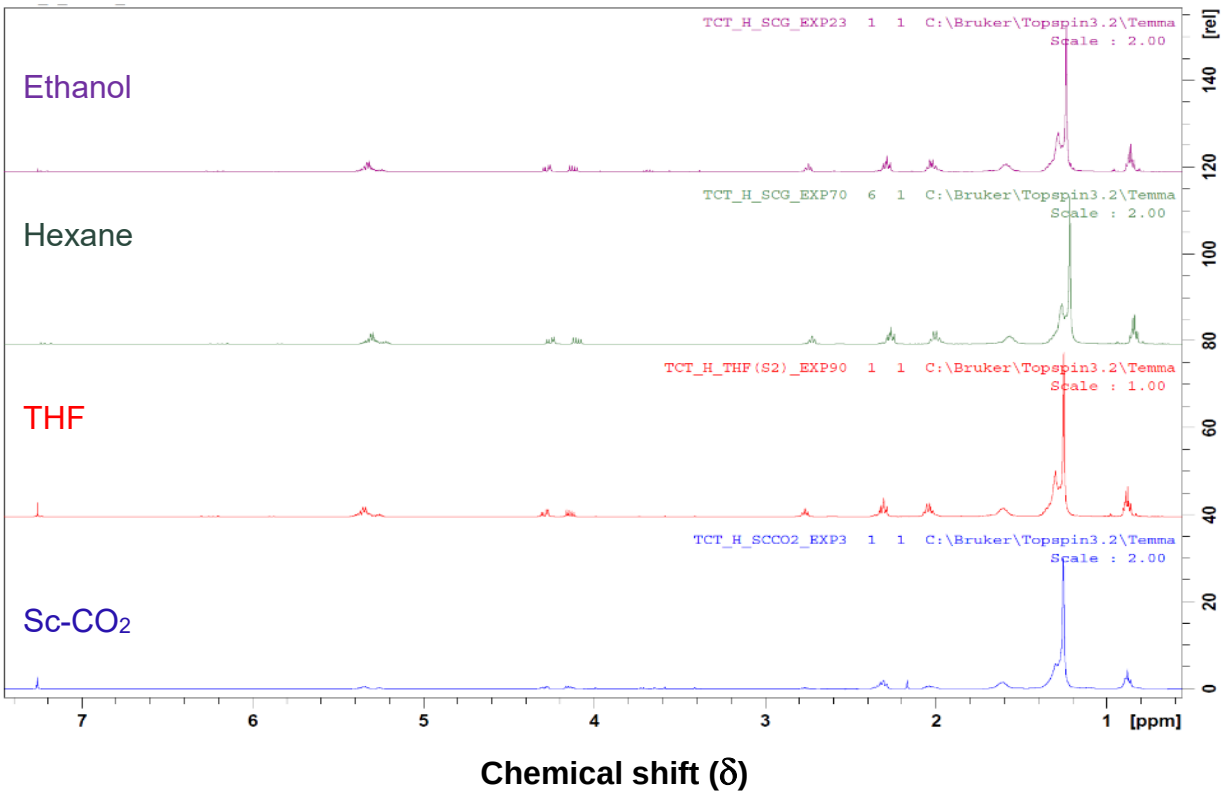


Figure 10 SCGs ^{1}H NMR comparison spectra with 2 hour extraction and GC chromatogram showing the relative abundance of fatty acids.

Residual caffeine, observed at δ7.51, 4.00, 3.59 and 3.41 ppm (HMDB database: Wishart et al., 2009), were detected in the husk ethanol spectra, and, to a smaller extent, in the THF spectra but not in the hexane and Sc-CO_2 spectra (Figure 11). Unsurprisingly, these peaks have been identified in coffee samples by ^{1}H NMR previously (Cagliani et al., 2013, Monakhova et al., 2015).

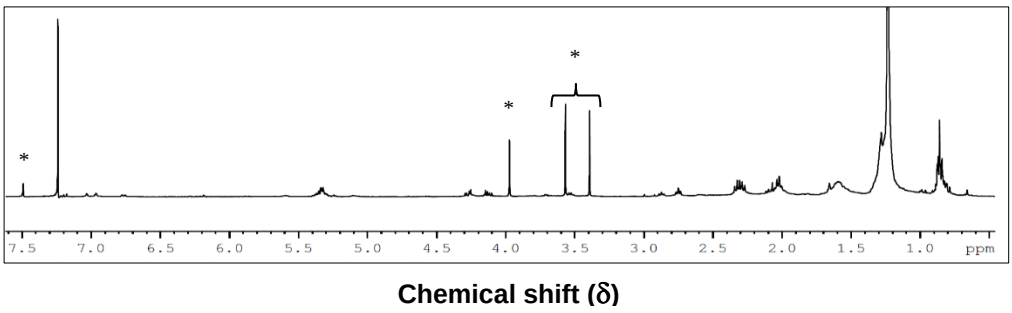


Figure 11 Husk ^{1}H NMR spectra of ethanol 2 hour extractions. *= peaks attributed to caffeine, according to Wishart et al. (2009).

2.3.3.4. *GC chromatograms*

GC chromatograms were completed of the ethanol and hexane extractions, as they were identified as the most economical extractions. When comparing the GC chromatograms, the relative abundance of fatty acids remained similar between solvents for husk extractions (Table 7). Six prominent fatty acids were observed in extract, regardless of solvent. With a relative abundance of C16:0 > C18:3 > C22:0 > C20:0 > C18:0=C18:1 (palmitic, linolenic acids, behenic, arachic, stearic and elaidic acid). Linolenic (C18:3) acids were a significant portion of the extract, with an overall average of 28 ± 12.2 and 30 ± 1.8%$_{relative}$ extracted from ethanol and hexane ultrasonic bath, respectively (Table 7). Linolenic acids are an important omega-3 fatty acid essential for human health (Gramlich et al., 2015, Hennessy et al., 2016) and are lacking in Western diets (Simopoulos, 2002). While the retention times for both C18:2 and C18:3 were very similar (~10.7 minutes), the distinction of these fatty acids are reliable because C18:3 peaks are resolved and eluted prior to C18:2 fatty acid peaks.

Table 7 The 6 most prominent fatty acids in husks detected using GC-FID, expressed as average ± SD %$_{relative}$, *n*= 3. Totals may not add up to 100 because of appearance/disappearance of acids as the most prominent peaks in the chromatogram. Blank squares indicate that these acids were not the most prominent in the chromatograph, but still may be present in the sample.

Solvent	**Extraction time (hrs)**	**C16:0**	**C18:3^**	**C22:0**	**C20:0**	**C18:0**	**C18:1^{+}**	**C18:2$^{/}$**
		%$_{relative}$						
Ethanol	0.5	31 ± 1.0	39 ± 3.8	12 ± 2.7	11 ± 1.7	7 ± 0.4		
Ethanol	1	29 ± 0.6	28 ± 0.8	17 ± 0.9	13 ± 0.5	7 ± 0.2	7 ± 0.2	
Ethanol	2	29 ± 1.3	20 ± 14.8	16 ± 1.5	12 ± 0.6	7 ± 0.4	7 ± 0.0$^{\#}$	29 ± 0.3
Hexane	0.5	30 ± 0.5	31 ± 0.3	13 ± 0.6	11 ± 0.4	8 ± 0.1	8 ± 0.3	
Hexane	1	27 ± 0.3	29 ± 0.7	17 ± 0.9	13 ± 0.4	7 ± 0.2	7 ± 0.2	
Hexane	2	28 ± 1.9	30 ± 2.2	15 ± 3.6	12 ± 1.5	8 ± 0.4	7 ± 0.6	

[#]one extract had C18:1 acid. ^C18:3 was the area sum of c9, c12, c15-C18:3 and c6, c9, c12-C18:3. [+]C18:1 is t9-C18:1. [/]C18:2 is inclusive of t9, t12-C18:2 and c9, c12-C18:2.

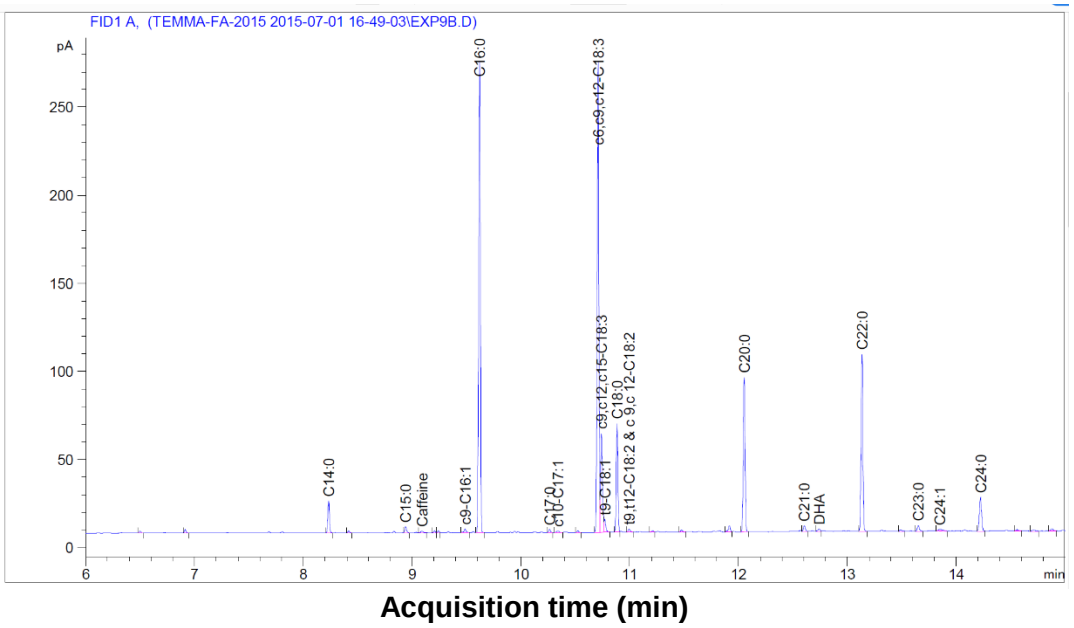


Figure 12 Typical GC chromatogram of husks (this is ethanol extraction at 0.5 hours).

The fatty acid composition of extracts varied slightly; however, there were two prominent fatty acids: C18:2 > C16:0 (linoleic acids and palmitic acid). Consistent portions of C18:0 (stearic acid) and C20:0 (arachic acid) were also detected. A smaller relative percentage of C18:3 was observed than in the husks, C18:3 acids were found consistently in ethanol 0.5 and hexane extractions (Table 8).

Table 8 The 6 most prominent fatty acids in SCGs detected using GC-FID, expressed as average ± SD $\%_{relative}$, *n*= 3. Totals may not add up to 100 because of present or lack thereof of acids as the most prominent peaks in the chromatogram. Blank squares mean that these acids were not the most prominent in the chromatograph, but still may be present in the sample.

Solvent	**Extraction time (hrs)**	**C18:2$^{/}$**	**C16:0**	**C18:1^{+}**	**C18:0**	**C20:0**	**C18:3^**
		$\%_{relative}$					
Ethanol	0.5	44 ± 0.4	35 ± 0.3		7 ± 0.1	3 ± 0.1	10 ± 0.1
Ethanol	1	45 ± 0.4	35 ± 0.2	10 ± 0.1	8 ± 0.2	3 ± 0.1	10 ± 0.0$^{\#}$
Ethanol	2	45 ± 0.1	35 ± 0.3	10 ± 0.1	8 ± 0.2	3 ± 0.1	
Hexane	0.5	44 ± 0.1	35 ± 0.3		8 ± 0.1	3 ± 0.1	10 ± <0.1
Hexane	1	44 ± 0.3	35 ± 0.0		8 ± 0.1	3 ± <0.1	10 ± 0.1
Hexane	2	45 ± 0.2	35 ± 0.2		8 ± 0.1	3 ± <0.1	10 ± 0.1

$^{\#}$one extract had C18:3 acid. ^C18:3 is c9, c12, c15-C18:3. $^{+}$C18:1 is t9-C18:1. $^{/}$C18:2 is inclusive of t9, t12-C18:2 and c9, c12-C18:2.

The fatty acid composition found in this study is consistent with the literature (Al-Hamamre et al., 2012, Jenkins et al., 2014, Ahangari and Sargolzaei, 2013), where Jenkins et al. (2014) found 44-50% C18:2 acids, 35-40% C16:0 acids and 7-8% C18:0 and C18:1 acids. Ahangari and Sargolzaei (2013) observed that palmitic acid was higher (~37%) when Sc-CO_2 extraction was used compared to conventional solvents in ultrasonication, Soxhlet or microwave.

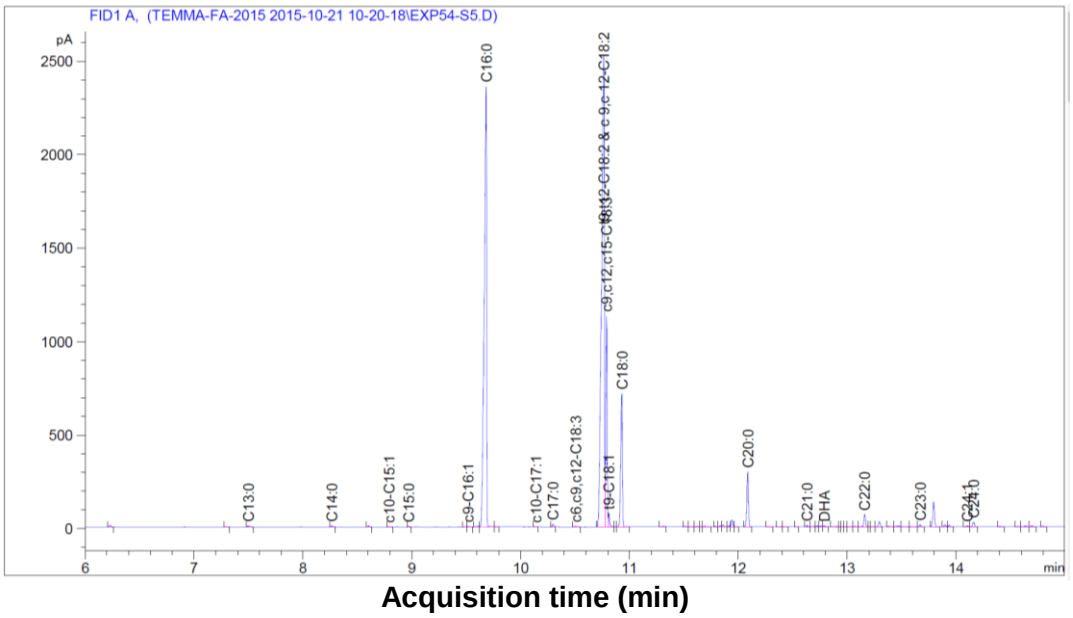


Figure 13 Typical GC chromatogram of SCGs (this is hexane extraction at 0.5 hours).

2.3.4. Caffeine and total phenols

Solvent type had the most effect on caffeine and total phenol concentrations extracted from SCGs (p= <0.001, one way ANOVA). There was no difference for either extractants for the different extraction times (p= >0.4 for both), nor was the interaction between solvents and extraction times significant (p= >0.8 for both). See Table 9 for details. Using 100% DIH_2O had the lowest yield, where 60% MeOH and 70% EtOH were comparable in all extractions (p= >0.5).

Table 9 Caffeine and Gallic Acid Equivalents (GAEs) concentrations from SCGs extractions with different solvents, averages ± SD, in a ultrasonic bath. *n*= 2. Letters represent the Tukey's post-hoc test (*p*= <0.05), caffeine and GAEs were analysed separately. Caffeine was quantified by LC-MS and GAEs by colorimetric analysis.

Solvent	**Extraction time (hrs)**	**Caffeine (mg/g)**	**GAEs (mg/g)**
60% MeOH	1.5	4.9 ± 0.2 [b]	21.8 ± 0.2 [b]
	2	4.9 ± 0.3 [b]	22.6 ± 0.3 [b]
70% EtOH	1.5	4.9 ± 0.1 [b]	23.1 ± 0.8 [b]
	2	4.8 ± 0.1 [b]	22.3 ± 1.7 [b]
100% DIH_2O	1.5	4.1 ± 0.1 [a]	17.0 ± 0.3 [a]
	2	4.1 ± 0.1 [a]	16.8 ± 0.1 [a]

A two-hour extraction for husks (MixH) was conducted to compare SCGs and husks for caffeine and total phenols the results of which are summarised in Table 10 and Figure 14.

Table 10 Caffeine and polyphenols from SCGs and husks, averages ± SD, with a 2 hour ultrasonic extraction using 60% MeOH. *n*= 2.

Material	**Caffeine (mg/g)**	**GAEs (mg/g)**
Husks	10.5 ± 1.2	8.5 ± 1.5
SCGs	4.9 ± 0.2	22.2 ± 0.3

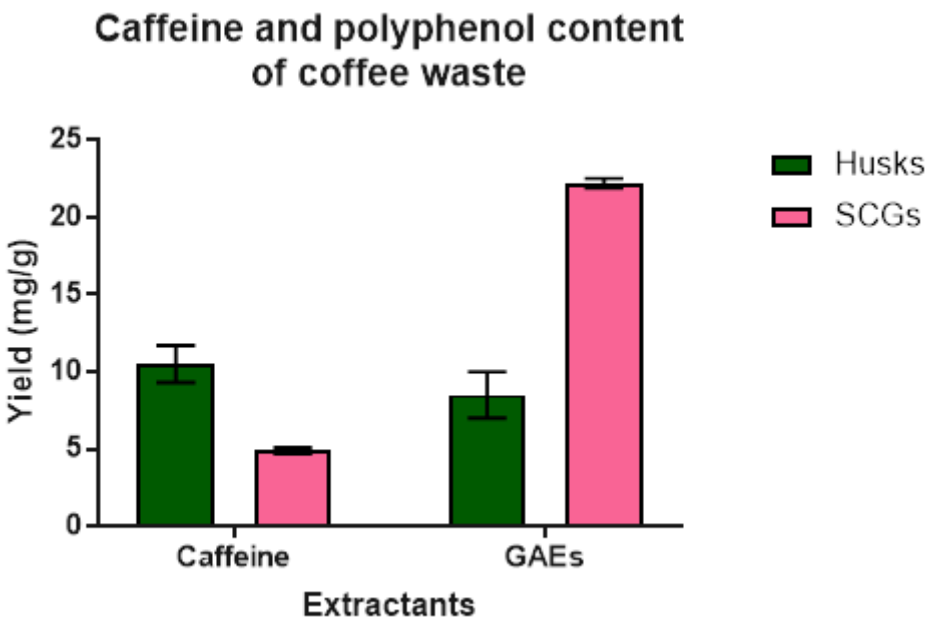


Figure 14 Caffeine and total phenols (GAEs) content, averages ± SD, of coffee waste using ultrasonic bath for 2 hours. *n*= 2.

Identifications in the LC-MS spectra were performed using authentic standards where available; where not available, tentative identifications were performed according to literature (Clifford et al., 2003). Caffeine dominated the spectra of both SCGs and husk material, appearing at 22.7 mins retention time (Figure 16 and Figure 17). The composition remained consistent with solvents in the SCGs (Figure 17). Both the SCGs and husks have comparable MS spectra.

Chlorogenic acids are esters formed between caffeic and quinic acids. They are abundant in plants used in human food and can reduce the risk of some chronic illnesses (Liang and Kitts, 2016). While there are many different subgroups of chlorogenic acids, the major acids associated with coffee are caffeoyl-quinic, *p*-coumaroylquinic, and feruloyquinic acids (Figure 15).

3-,4-,5-*O*-Caffeoyl-quinic acid isomers are observed at 17.9, 23-24 and 3.5 mins. Other chlorogenic acids were identified in the MS (Figure 16): caffeic acid, ferulic acid, cinnamic acid, 3-*O*-*p*-Coumaroylquinic acid, 3-*O*-Feruloylquinic acid and Di-*O*-Caffeoyl-quinic acid. These chlorogenic

acids have previously been identified as abundant in coffee beans and beverages (Liang and Kitts, 2016, Wei et al., 2012) as well as spent coffee (López-Barrera et al., 2016, Andrade et al., 2012). Other components found were protocatechuic and 4-hydroxybenzoic acid, of which both have been previously observed in small amounts within SCGs (Andrade et al., 2012).

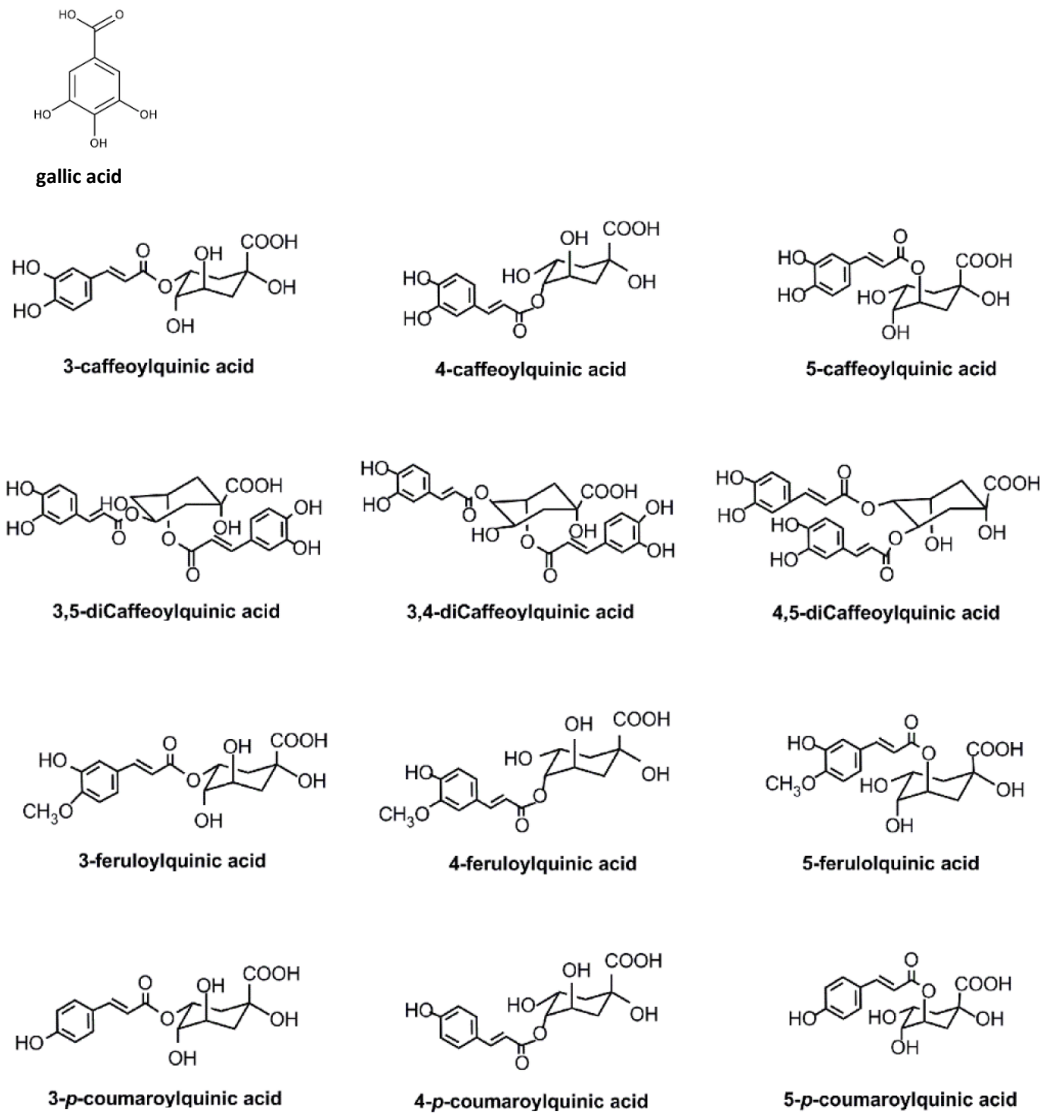
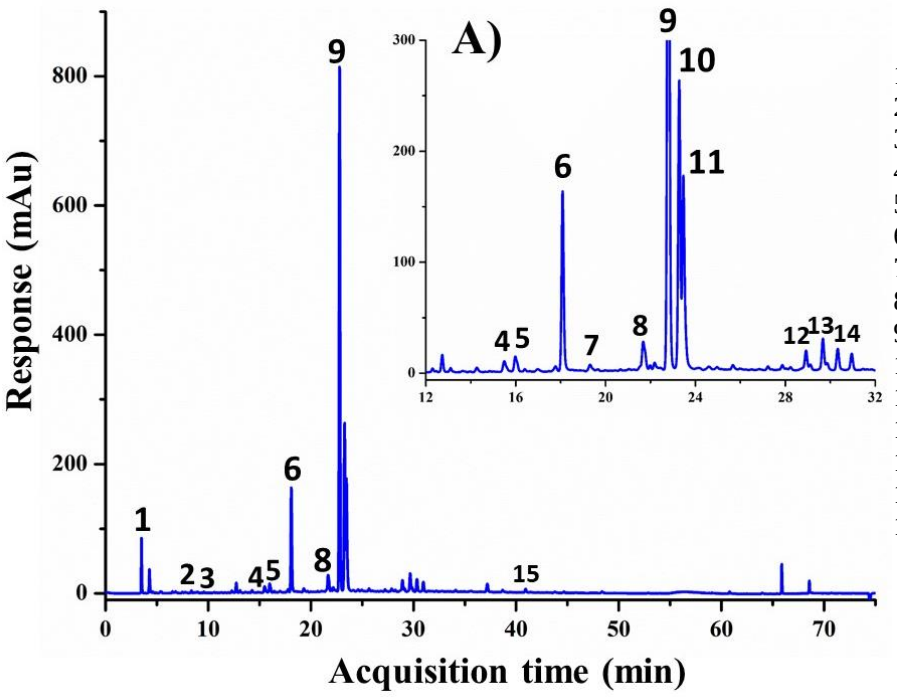


Figure 15 Common chlorogenic acids found in coffee grounds, beverages and spent grounds. Adapted from Liang and Kitts (2016)

Unexpectedly, gallic acid was not observed in these samples, which is contrary to reports in the literature. For example, Andrade et al. (2012), López-Barrera et al. (2016) both found low to relatively high concentrations of gallic acid in their samples (14 µg GAEs/g and 1.3-2.5 mg/g respectively). Gallic acids are reduced depending on the coffee bean roasting process, where a higher roasting intensity decreases gallic acid concentrations (López-Barrera et al., 2016).



1. Caffeic acid
2. Ferulic acid
3. Cinnamic acid
4. Protocatechuic acid
5. *O*-Caffeoyl-quinic acid isomer
6. *O*-Caffeoyl-quinic acid isomer
7. 4-Hydroxybenzoic acid
8. *O*-Caffeoyl-quinic acid isomer
9. Caffeine
10. *O*-Caffeoyl-quinic acid isomer
11. *O*-Caffeoyl-quinic acid isomer
12. 3-*O*-*p*-Coumaroylquinic acid
13. 3-*O*-Feruloylquinic acid
14. 3-*O*-Feruloylquinic acid
15. Di-*O*-caffeoylquinic acid

Figure 16 The typical LC-MS spectra of SCGs, with identified signals and A) enlarged 12-32 min spectra.

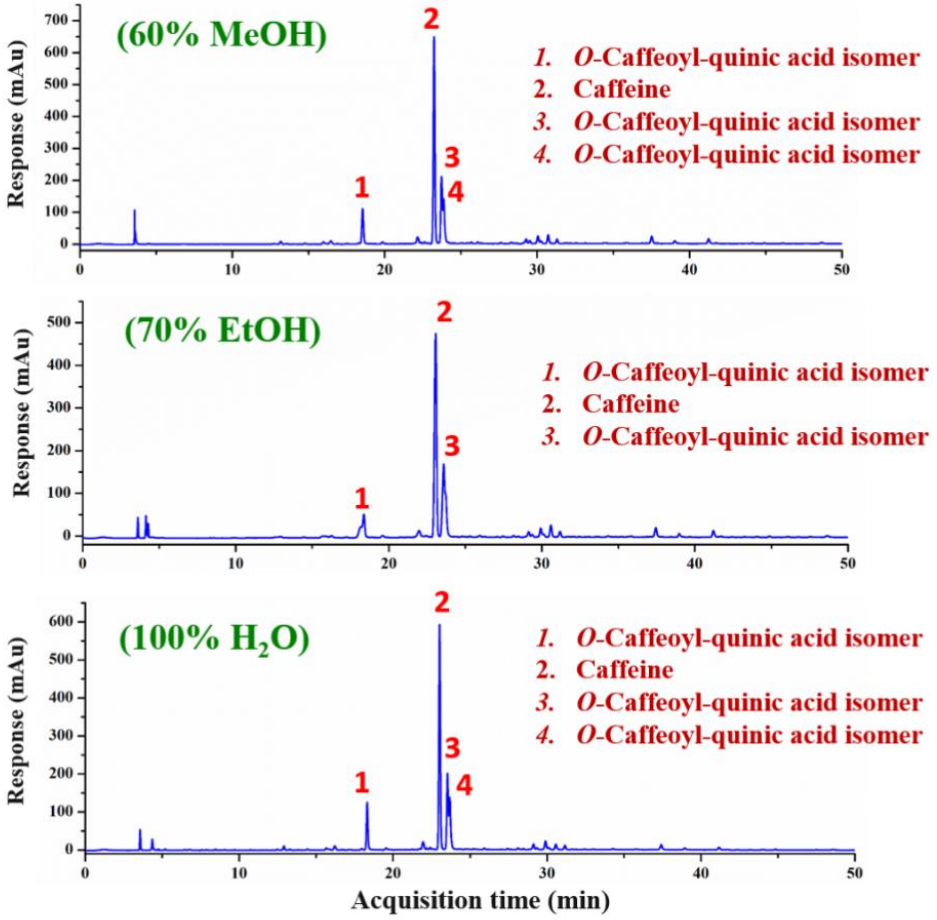


Figure 17 LC-MS spectra of SCGs ultrasonic bath extraction using different solvents for two-hours. The 3-4 major components are identified and it is likely the 4th peak associated with *O*-Caffeoyl-quinic acid isomer in the 70% EtOH spectra was present but not well resolved in the extract.

2.4. Discussion

2.4.1. Husk lipid extractions

Coffee husks are not commonly studied for lipid content, primarily due to a lower lipid content and relatively lower waste amounts produced compared to SCGs. The lipid yields from husks of this study were similar to Andrade et al. (2012), who obtained yields of 1.45-3.1% with ultrasonication. Andrade et al. (2012) also found that hexane gave the lowest yield for husk extraction (1.45%), attributing this

to the low polarity of hexane. Husk extractions with ethanol and THF in this work were substantially higher than Andrade et al. (2012) obtained with Soxhlet, ultrasonication or Sc-CO_2. Husk yield was also substantially higher than the lipid content reported in literature, reported as a maximum lipid content of 3% (Gouvea et al., 2009).

The additional signals observed in the ^{1}H NMR spectra supports that this additional yield is due to caffeine and polyphenols (particularly quinic acid). Quinic acid is abundant in roasted coffee (Wei et al., 2012) and husks (Andrade et al., 2012). This was confirmed by the LC-MS analysis.

2.4.2. SCGs lipid extractions

The highest SCG extraction lipid yield in this work (10.2%, ethanol using ultrasonic bath) is comparable or slightly lower than that reported in the literature using similar extraction processes which often range from 10-14% (Phimsen et al., 2016, Ahangari and Sargolzaei, 2013, Liu et al., 2017, Rocha et al., 2014, Jenkins et al., 2014). In this work, hexane and ethanol extractions at two hours were comparable, which is different to the result reported by Andrade et al. (2012), who found that ethanol produced higher yields. The lipid yield achieved in this study is comparable to solvents that are less environmentally-friendly; for example, heptane, hexane and sulfuric acid (Al-Hamamre et al., 2012, Jenkins et al., 2014, Liu and Price, 2011).

Sc-CO_2 had low yield in comparison to the literature (Andrade et al., 2012, Ahangari and Sargolzaei, 2013) where a semi-continuous system was used with and without a co-solvent. The Sc-CO_2 system used in this study showed encouraging initial results for husks; however, more work must be done to account for apparent sample loss.

2.4.2.1. *Uses of lipids extracted from husks*

Husk extraction may have important value in the health care and food industries, as alpha linolenic is an omega-3 series fatty acid that is essential for human diets (Gramlich et al., 2015). Linolenic acids,

such as the acids extracted by this study, are known for their anti-carcinogenic properties (Hennessy et al., 2016). Western diets are deficient in omega-3 fatty acids (Simopoulos, 2002), and supplements are common; for example, linseed oil is currently used to increase omega-3 fatty acids in human diets (Gómez-Cortés et al., 2016). The ratio of omega-3 to omega-6 fatty acids is also extremely important to human health (Simopoulos, 2002).

It is advantageous, therefore, to limit the amount of omega-6 fatty acids, such as linoleic acid (C18:2), which are common in vegetable oils, and to increase omega-3 fatty oils. To this effect, the high C18:3 and low C18:2 fatty acids (relative abundances) found in the husk extractions are potentially very valuable for human diets. The C18:3 linolenic content in husks has not been investigated yet, to the best of this author's knowledge, and would be an important area to investigate further as husks seem an important, untapped source of omega-3 that is currently being discarded.

Low concentrations of C18:3 linolenic acids have been reported in coffee beans and SCGs (Rocha et al., 2014, Barbosa et al., 2014, Couto et al., 2009, Jenkins et al., 2014). For example, Rocha et al. (2014) found that C18:3 linolenic acids made up 0.4% of fatty acid composition, Couto et al. (2009) found 0.9-5.5% of extract and C18:3 fatty acid was ≤1.5% in Jenkins et al. (2014). Furthermore, the relative abundance of C18:3 as well as C18:2 fatty acids in the SCGs extractions make this oil less appealing for use as a fatty acid in dietary supplements.

2.4.2.2. *Uses of lipid extracted from SCGs*

In a review of SCGs valorisation, Campos-Vega et al. (2015) highlighted SCG oil as the "single most economically viable component" due to easy, cheap and high value extractions. In this study, the primary fatty acids identified were linoleic and palmitic acids from SCGs. These have been used for biodiesel production, also sourced from SCGs (Ahangari and Sargolzaei, 2013, Jenkins et al., 2014, Obruca et al., 2014). Transesterification processes with methanol are critical for biodiesel production, and currently there is extensive research into more efficient and environmental-friendly methods

(Campos-Vega et al., 2015, Stylianou et al., 2018). For example, there are encouraging results for direct transesterification (Campos-Vega et al., 2015) as well as in situ, enzymic and non-catalytic transesterifications (Stylianou et al., 2018).

High amounts of palmitic acid are suitable for application in the soap industry (Ravindranath et al., 1972 cited in Campos-Vega et al., 2015). Additionally, Obruca et al. (2014) found that linoleic, palmitic and oleic acids, extracted from SCGs, were inexpensive and highly suitable and competitive substrates for synthetic polymer production (poly(3-hydroxybutyrate)).

2.4.3. Caffeine and total phenols

Caffeine yields for SCGs (4.9 mg/g) were consistent to other studies and husk caffeine yields (10.5 mg/g) were comparable to the reported content for husks, 10 ± 5 mg/g (Murthy and Naidu, 2012). However, due to differences in cultivars (Esquivel and Jiménez, 2012) and the processing and extraction process (Murthy and Naidu, 2012), the caffeine content of bean and waste products can be extremely variable. A higher yield observed for husks compared to SCGs is consistent with previous reports (Campos-Vega et al., 2015).

López-Barrera et al. (2016) found 4 mg/g caffeine in Mexican SCGs, and Cruz et al. (2012b) observed similar with a 4.5 mg/g average in espresso coffee. Ramalakshmi et al. (2009) found that Arabica SCGs had 5 mg/g 0.5% caffeine and Robusta SCGs had 2 mg/g 0.2%. Samples from coffee bars taken by Panusa et al. (2013) found a range of 6-11.5 mg/g, with Robusta SCGs having higher caffeine content. In another study, ethanol extraction gave a higher yield of 26 and 71 mg/g of extract for SCGs and husks, respectively (Andrade et al., 2012), whereas supercritical extraction obtained substantially yields for husks (684 mg/g of extract). There is higher caffeine content in Arabica beans compared to Robusta (Campos-Vega et al., 2015) which also may affect the results.

The total phenol yield for SCGs (17-23 mg GAEs/g) found in this study is comparable to the 10-36 mg GAEs/g in the literature for similar extraction methods (Mussatto et al., 2011a, Severini et al., 2017, Zuorro and Lavecchia, 2012, Al-Dhabi et al., 2017, Caballero-Galván et al., 2018). Similar to caffeine content, GAEs are highly variable in reported experiments, which can be as high as 273 mg GAEs/g using successive Soxhlet extraction (Acevedo et al., 2013), or 88 mg GAEs/g using subcritical water extraction (Xu et al., 2015). It is encouraging that ultrasonication for a relatively short extraction time (two hours) was comparable to other studies. This work indicates that there is more GAEs in the SCGs in comparison to husks (8.5 mg GAEs/g), using 70% EtOH. Similarly, Andrade et al. (2012) found total chlorogenic acids at four times lower levels from husks compared to SCGs, with 61-133 vs 267-588 mg/g per extract, respectively.

2.4.3.1. *Uses of caffeine and total phenol extract*

Bioactive substances, such as caffeine and polyphenols extracted in this study, have potential value in the pharmaceutical, cosmetic and food industries (Kovalcik et al., 2018, Andrade et al., 2012), and extraction could be highly economical (Cruz et al., 2012b). Martinez-Saez et al. (2017) found that hot water extraction from SCGs could be added to biscuits to provide a natural source of antioxidant, dietary fibre, proteins, essential amino acids and low glycaemic sugars. Chlorogenic acids, in particular, have numerous uses, as medicinal compounds, antioxidants, as well as use for their antiviral, hypoglycemic and antihepatotoxicity functions (Andrade et al., 2012). Interestingly, Panusa et al. (2013) concluded that pure water was the most favourable solvent, as different ethanol/water ratios did not change total yield of chlorogenic acids in extracts. Therefore, the water extraction observed in this study may have a lower yield compared to the ethanol and hexane solvents but may have a higher applicability to utilize these fatty acids. Bioactive compounds extracted from SCGs may also be used as a natural antioxidant in meats (Kim et al., 2016) or as thermo- and photo-oxidative stabilizers (Panzella and Napolitano, 2017). To further understand the value of these extracts,

investigation into the free radical scavenging properties and antioxidant activity is important (Panzella et al., 2016).

2.5. Conclusion

Coffee waste extracts in this study have a wide potential for increased valorisation; for example, in the cosmetics, bioenergy and pharmaceutical industries (Zuorro and Lavecchia, 2012, Ribeiro et al., 2013, Kovalcik et al., 2018). Coffee oil can be used as biodiesel, additive to cosmetic creams and for individual fatty acids. Importantly, the omega 3-fatty acids (linolenic C18:3) extracted from husks in this study, could be of great value to the health and food industries; therefore, further work to investigate the potential use as oil extracted from husks would be highly beneficial.

The chlorogenic acids in the extracts are valuable antioxidants and, with further investigation, may be used in the food and health industry. Cost/benefit analyses, upscale experiments and purification processes must be completed to fully understand commercialisation potential. Cruz et al. (2012b), Andrade and Ferreira (2013) demonstrated that bioactive compounds and lipids can be economically extracted and commercially used. Importantly, the coffee by-products (SCGs and husks) investigated in this study were shown to contain valuable compounds that are currently being discarded. Simple extraction, as demonstrated in this work, would allow recovery of some of these compounds.

Chapter 3. Direct application of spent coffee grounds to a sandy agricultural soil

3.1. Introduction

Spent Coffee Grounds (SCGs) are produced in high amounts, with approximately 8-10 kg dry weight per café produced per working day in this study (Chapter 2). Among other valorisation options, such as lipid extraction, a promising use of SCGs is application to land (Stylianou et al., 2018). Indeed, direct applications of SCGs to soil can provide a source of OM (Ciesielczuk et al., 2017), nitrogen (Stylianou et al., 2018) and has a great potential to increase soil fertility (Cervera-Mata et al., 2018, Yamane et al., 2014). SCGs can also improve plant yield (Cervera-Mata et al., 2018, Gomes et al., 2014) and plant quality (Cruz et al., 2014c, Cruz et al., 2012a). Total nitrogen content in SCGs commonly ranges from 1.2-2.4% (Pujol et al., 2013, Yamane et al., 2014) making these materials a potential supplementary or alternative N source to inorganic nitrogen sources. SCGs may also be a source of nutrients that are important for soil and plant health, particularly organic C, K, P and micronutrients (Cruz et al., 2015, Cruz et al., 2014c, Cervera-Mata et al., 2018).

Organic amendments can be used to increase soil biological health (Quilty and Cattle, 2011, Diacono and Montemurro, 2011a), as soil microbial activity and diversity is vital for maintaining functions associated with soil health (Cardoso et al., 2013). Soil respiration is a one useful bioindicator of soil health (Cardoso et al., 2013) and is a key indicator to assist management practices that are potentially beneficial for maintaining soil health (Bastida et al., 2008). Soil respiration measures the activity of soil microbes; however, it does not indicate whether the particular microbes are active. Preliminary studies have shown that SCGs stimulate the soil biological activity, where SCGs were correlated with a 10-fold increase of soil respiration (Cervera-Mata et al., 2018).

While SCG applications could have important benefits to soil and plants, there are also concerns regarding SCGs causing plant inhibition and nitrogen immobilisation (Stylianou et al., 2018). The most prominent concern is phytotoxicity due to residual caffeine and polyphenols within SCGs (Farah, 2012). As mentioned previously, both caffeine and polyphenols have been known to cause

phytotoxicity (Pandey et al., 2000); however, the exact mechanism is unknown (Cervera-Mata et al., 2018).

SCG decomposition in soil is known to be slow and, therefore, nitrogen immobilisation may be present for a longer period of time in comparison to other organic amendments (Kitou and Okuno, 1999). Fertiliser blending with SCGs might assist decomposition by changing the soil C: N ratio and may assist in reducing nitrogen immobilisation. Currently, there is a lack of knowledge about the impact of direct application of SCGs to soil (Hardgrove and Livesley, 2016); in particular, for high application rates with and without fertiliser blending. Experiments in the literature often limit SCG application rates to 20% or below based on mass (e.g. Cervera-Mata et al., 2018, Ciesielczuk et al., 2017, Gomes et al., 2014, Hardgrove and Livesley, 2016). Loss of gaseous nitrogen, particularly through N_2O from soils, has not been well studied after SCGs application to soils, and has been highlighted as a research gap (Stylianou et al., 2018). N_2O emissions from soils are important because N_2O emissions produced from the agricultural sector contribute the largest (~60%) of all global N_2O sources produced from human activities (Syakila and Kroeze, 2011). Unfortunately, N_2O has 298 times the global warming potential of CO_2 (Charles et al., 2017).

A popular valorisation method for SCGs is the extraction of lipids for use in the energy, cosmetic and pharmaceutical industries (Campos-Vega et al., 2015). However, these extractions still produce a residue, the residue SCGs account for 86-90% volume after extraction, requiring disposal or further use. Kitou and Okuno (1999) found that lipid content of coffee residue caused slower decomposition because a large fraction of the lipids corresponded to structural protein nitrogen; therefore, extracting the lipid content may help SCGs breakdown in soils. Soil amendments using extracted SCGs may increase soil cation exchange capacity (CEC) and increase plant yield (Vardon et al., 2013), and extracted SCGs may be more attractive for land application. The extraction process also removes phytotoxic components, such as caffeine and polyphenols (see Chapter 2 for details), which may also assist to increase plant growth.

The aim of this experiment was to investigate the application of SCGs at a high rate (20 t/ha) of SCGs – locally sourced and with and without urea blending – on silver beet growth, N_2O soil emissions, soil respiration, and soil macro- and micro-nutrients in one soil type (Podosol). An overview is seen in Figure 18. The experimental questions were:

1. How do SCG applications affect silver beet growth?
2. Does N (urea) fertiliser blending help plant growth?
3. Does an ethanol extraction change the effects of the amendment?
4. How do SCG applications impact nitrous oxide soil emissions?
5. Do SCGs increase soil respiration (carbon dioxide emitted from soils)?
6. How do SCGs impact soil nutrients?

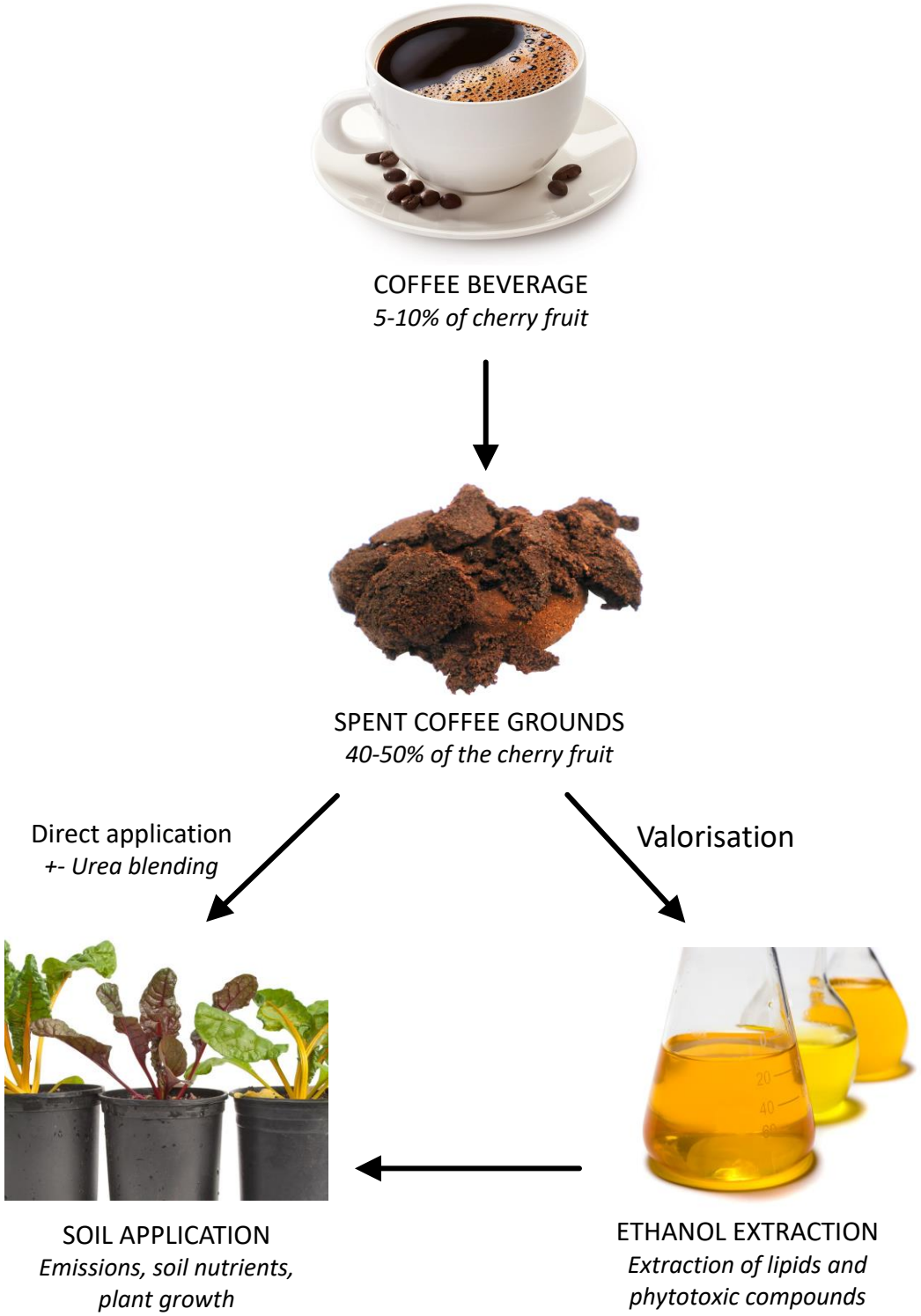


Figure 18 Overview of the aim of the study, waste data from Campos-Vega et al. (2015).

3.2. Materials and methods

3.2.1. Soil and SCG preparation

An acidic Podosol (topsoil) was collected from a vegetable farm that had been converted to pasture in Cranbourne (VIC 3977), the soil was air dried, sieved (<2 mm) and homogenised. SCGs were collected from coffee shops on Monash University, Clayton campus, in 2015. SCGs were air dried to ~10% moisture and stored in sealed plastic drums until used.

Ultrasound extractions were conducted using analysis grade ethanol in a ratio of 1:5 SCGs to ethanol, for two hours without temperature regulation (upscale of the extraction detailed in Chapter 2). Solid-liquid separation was done with a Whatman no.1 filter, solid residue was dried for ~24 hours to allow ethanol evaporation. The yield was 6.0 ± 0.1%, calculated as the ratio between mass of extract and mass of raw material. The soil and SCGs were characterised for a range of physicochemical properties at Environmental Analysis laboratories, at Southern Cross University (Table 11).

Table 11 Soil and Spent Coffee Grounds chemical and nutrient characteristics, *n*= 1. CEC: Effective Cation Exchange Capacity (the sum of exchangeable cations).

Parameter	**Soil**	**Air-dried SCGs**	**Ethanol-extracted SCGs**
pH (H_2O)	5.4	5.2	5.2
EC (dS/m)	0.04	2.0	1.9
Nitrate (mg/kg)[#]	5.4	4.5	2.4
Ammonium (mg/kg)[#]	4.2	57.8	27.9
P (Colwell) (mg/kg)	17	232	238
OM (% of mass)	1.8	82.8	79.8
CEC ($cmol^+/kg$)	2.5	32.7	37.5
Total C (% of mass)	1.0	47.3	48.0

Parameter	Soil	Air-dried SCGs	Ethanol-extracted SCGs
Total N (% of mass)	0.1	2.1	3.0
C: N ratio	16.9	22.6	20.0
Ca (mg/kg)[#]	253	628	683
Na (mg/kg)[#]	23*	13	15
ESP (%)	3.9*	0.2	0.2
Mg (mg/kg)[#]	68	1178	1307
K (mg/kg)[#]	43	4620	5726
Fe (mg/kg)[#]	139	74	9
Zn (mg/kg)[#]	1.4	4.1	7.9
Al (mg/kg)[#]	22	6	5
B (mg/kg)[#]	0.25	2.5	4.3
Mn (mg/kg)[#]	4	18	19
Cu (mg/kg)[#]	0.3	11.5	16.8
S (mg/kg)[#]	17	44	67

[#]Plant available nutrients
*Historical data used

3.2.2. Plant growth trial

A 57-day silver beet pot trial was conducted in a glasshouse during summer (February to March) of 2016; this period was required to achieve full growth of the plants but before flowering. The greenhouse temperature was maintained at 24/17^{o}C day/night cycle. A three by three factorial design, replicated six times, was conducted with randomised pots, giving a total of 54 pots. Three treatments were conducted: controls, air-dried SCGs and ethanol-extracted SCGs. Three urea (cumulative TN kg/ha) application rates were used: zero, low and high (Table 12).

Table 12 Treatment, codes and cumulative Total Nitrogen (TN, urea) application rates in pot trial experiment.

Treatment	**Urea application rate (cumulative kg/ha)**	**Treatment name**
Controls	None (0)	Soil
	Low (122)	L
	High (244)	H
Air-dried SCGs (applied at 20 t/ha)	None (0)	SCGr
	Low (122)	SCGrL
	High (244)	SCGrH
Ethanol-extracted SCGs (applied at 20 t/ha)	None (0)	SCGe
	Low (122)	SCGeL
	High (244)	SCGeH

All SCGs were applied at 20 t/ha – a rate based on Quilty and Cattle (2011) and Kasongo et al. (2013). Treatments had a basal application of phosphate (P) fertiliser at 40 kg/ha and potassium (K) fertiliser at 60 kg/ha to maintain plant growth. Nitrogen fertiliser was applied as urea (46.0% TN), P fertiliser was applied as Superphosphate (9.1% P) and K fertiliser was applied as Potash (41.5% K). SCGs and fertilisers were manually mixed into the top 500 g (~ 5 cm) of soil in 8 kg pots, to mimic topsoil applications. Pots were watered to, and maintained at, 75% water field capacity with deionised water. The 75% was chosen because of a high amount of evaporation and silver beet requires high amounts of water. Silver beet was germinated for 14 days in a Debco sandy seed raising mix (which included fertilisers, wood chips and a high portion of sand). The soil and amendment mixtures were prepared 72 hours before each silver beet seedling was transplanted into a pot (Figure 19).

Figure 19 Pot trial set up

Additional fertiliser was applied weekly for all treatments to support plant growth. This started four weeks after transplanting and included 20 TN kg/ha, 18 P kg/ha and 36 K kg/ha according to DPI NSW for vegetables (Wade, 2009).

At the end of 57 days growth, post-transplant, the plants were cut at the soil surface (above ground biomass) and the fresh weight determined. Roots (below ground biomass) were separated from the soil and washed out. The dry mass was then determined following oven-drying at 60°C for 72 hours and weighed.

3.2.2.1. *Nitrous oxide and soil respiration*

Carbon dioxide (CO_2) and nitrous oxide (N_2O) gas samples were taken post-plant transplant on days 1, 3, 7, 9, 10, 12, 14, 22, 29, 36, 43 and 57. CO_2 emitted from soils is the end product of soil microbial activity, during organic matter decomposition, and live root activity (Schlesinger and Andrews, 2000, Keith et al., 1997). Emissions were sampled using static collection chambers equipped with a rubber septum for gas collection. A chamber was pressed into the soil and after a period of 20 minutes, a glass, air-tight SGE analytical Scientific syringe and needle was used to thoroughly mix the gas in the headspace and then to extract a 20 ml volume aliquot of soil gas. Gas samples were transferred to a

pre-evacuated 12 ml Exetainer® vial with a grey silicone septum (Labco, UK). The 20 minute capping time was determined from a preliminary experiment in which soil gas emissions were collected from replicate chambers at 5 minute intervals from 5-40 minutes (data not shown). This ensured that sampling occurred prior to saturation within the chamber headspace (Van Zwieten et al., 2010).

CO_2 and N_2O samples were analysed by an Agilent technologies Gas Chromatography - Thermal Conductivity Detector and Gas Chromatography - Flame Ionization Detector. Cumulative emissions for each greenhouse gas were calculated, accounting for time past between measurements, following Van Zwieten et al. (2010).

3.2.2.2. *Soil analysis*

Post-harvest of above ground biomass and prior to root separation from soil, the top ~5 cm of soil from each pot was air dried and homogenised; all replicates kept separate. Analysis was conducted only on the 0-5 cm soil layer where the effects of the amendment are likely to be concentrated. All soil analyses were completed by the Environmental Analysis Laboratory, Southern Cross University (EAL, 2018). Soil was analysed for electrical conductivity (EC), pH and organic matter (OM), total nitrogen (TN) and total carbon (TC). Soil was also analysed for plant available calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), manganese (Mn), phosphorus (P), zinc (Zn), iron (Fe), boron (B), copper (Cu), sulfur (S), silicon (Si), aluminium (Al), nitrate (NO_3^-) and ammonium (NH_4^+).. Analyses of the soil was completed using Rayment and Lyons (2011b) and Tucker (1985) standard methods.

3.2.2.3. *Statistical analysis*

Data analysis was completed by using an IBM SPSS Statistics 25 software package. For all tests, the significance value chosen was 5% (p= 0.05), where <0.05 was considered significant. The Levene's

test for homogeneity was used to assess parametric test appropriateness. If failed, a log transformation was undertaken (reported in graphs and tables in the raw data form).

The Pearson Chi-squared test was used to understand the frequency/count of dead or significantly inhibited plant growth with treatment – where <0.5 g of above ground plant biomass and <0.1 g for below ground plant biomass was considered dead or significantly inhibited. This was done by counting the amount of dead and alive plants, and weighed under the different treatments.

One-way and two-way ANOVAs were used to test significance. One-way ANOVAs were used to test the significance between individual treatments, followed by Tukey's HSD test. Two-way ANOVAs were used to test interaction significance between treatments and application rates, followed by pairwise comparisons to test for individual significance between groups using Least Significant Differences (LSD). The treatments and urea application rates were used as the fixed factors and the soil results as the dependent variable. Where *p* values were significant, critical F-values were checked. Graphs in this chapter were produced with GraphPad Prism, version 7.02.

3.3. Results

3.3.1. Yield

Both above and below ground, the biomass of silver beet was severely inhibited with an application of SCGs (p= <0.001). Pearson Chi-squared test revealed that the count of <0.5 gram of plant growth was significantly different between treatments (p= 0.004), with the largest amount of deaths being in ethanol-extracted SCGs (8 dead) and air-dried SCGs (10 dead). For below ground biomass, the death of plants or significantly inhibited growth changed with treatment (p= 0.010), with the largest amount of deaths being in ethanol-extracted SCGs (8 dead) and air-dried SCGs (12 dead). For ANOVA tests, dead or severely inhibited growth was excluded from the analysis. Consequently, the effects were tested on plants that survived.

The interaction of urea application rates with treatment did not significantly affect above ground plant growth (p= 0.057, two-way ANOVA). Further, different treatments and urea application rates had significant effects on above ground biomass (p= <0.001 and 0.004, one-way ANOVA). The sequence of plant yield was control>ethanol-extracted SCGs>air-dried SCGs and high>low>zero (p= <0.05, Tukey's post hoc).

For below ground plant biomass, using the two-way ANOVA, treatment and the interaction between the treatment and urea application rate was significant (p= <0.001 and 0.028, one-way ANOVA). However, the fertiliser application rate alone was insignificant. The interaction significance was at the high urea application rate, where control was higher than both air-dried SCGs (p= 0.001, LSD) and ethanol-extracted SCGs (p= 0.003, LSD). However, there was no difference between the air-dried and ethanol-extracted SCG treatments at any application rate.

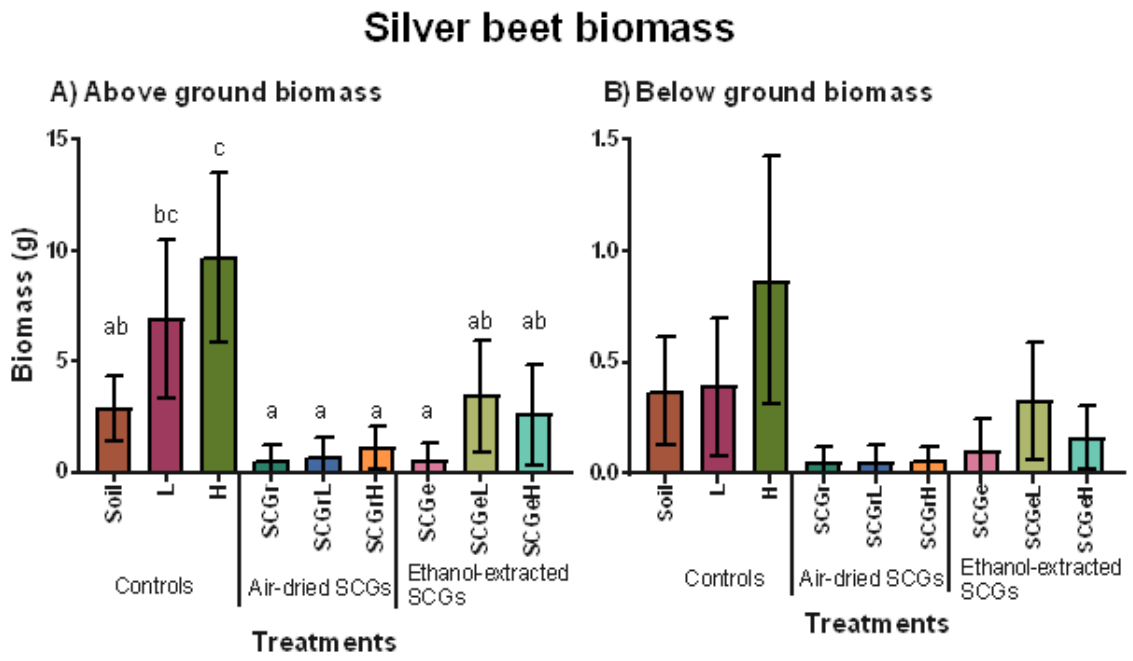


Figure 20 Above and below ground biomass, means ± SEM, of silver beet with 20 t/ha application rate of SCGs. Soil= soil control (no urea), L= 122 kg/ha TN cumulative rate, H= 244 kg/ha TN cumulative rate, SCG= Spent Coffee Grounds, r= air-dried SCGs, e= ethanol-extracted SCGs. *n*= 6. Letters represent Tukey's post hoc test (with plant growth >0.5 g, significance *p*= <0.05).

3.3.2. Soil respiration

Soil respiration (CO_2 emitted from soil) was higher with SCG treatments for all days (*p*= <0.05, one-way ANOVA) (Figure 3). The interaction between treatments and application rate was significant on soil respiration on Day 1, 22 and 29 (*p*= <0.004, two-way ANOVA). Soil respiration was higher within SCG treatments compared to controls at all fertiliser applications rates on all days (*p*= <0.001, LSD). SCGs with the low urea application rate produced the highest emissions at Day 1 (*p*= <0.001, LSD). At Day 22 and 29 SCGs with the highest urea application rate produced the highest emissions (*p*= <0.001), with all controls being lower and no difference between SCG treatments. The trend within SCG treatments from Day 1 to 29, was that the increasing urea application rate correlated with increasing respiration, with air-dried SCGs having lower soil respiration.

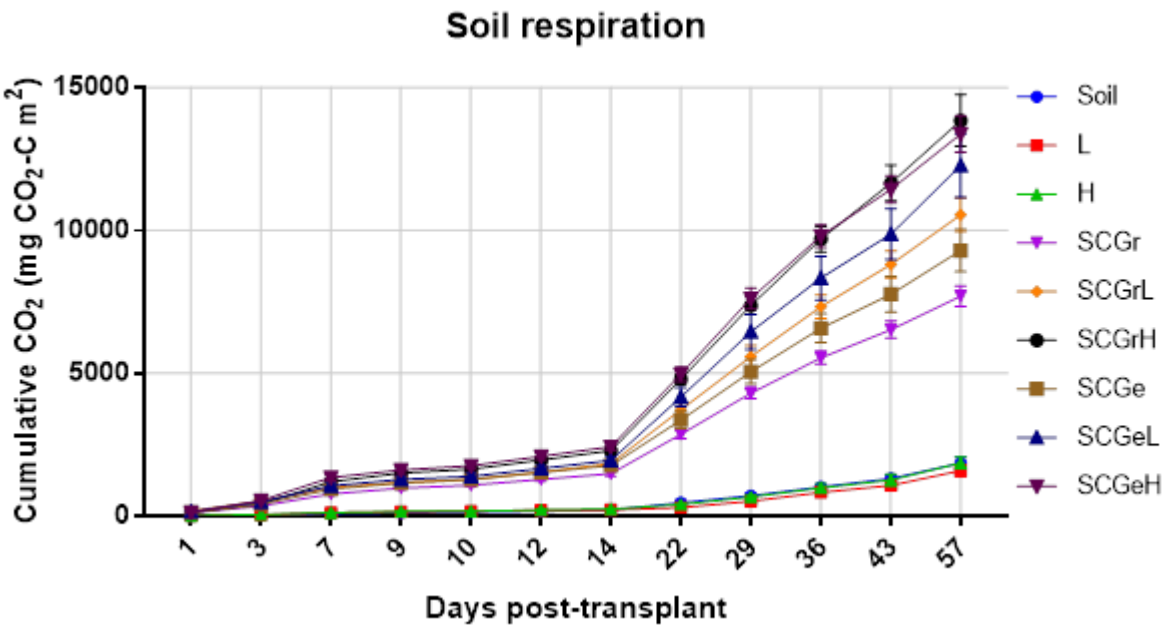


Figure 21 Soil respiration (averages ± SEM). Soil= soil control (no urea) observed behind L and H in graph, L= 122 kg/ha TN cumulative rate, H= 244 kg/ha TN cumulative rate, SCG= Spent Coffee Grounds, r= air-dried SCGs, e= ethanol-extracted SCGs. Error bars denote standard error, *n*= 6.

3.3.3. Soil nitrous oxide emissions

Cumulative N_2O soil emissions, on all days, were all different between treatment and application rate (*p*= <0.005, one way ANOVA), but the interaction of treatment and application rate was not significant. Predictably, in general, SCG treatments had higher N_2O emissions with highest urea application rate. The N_2O emissions from SCG treatments without urea were significantly lower than controls and ethanol-extracted SCGs with high urea application rate from Day 9 to 14 (*p*= <0.02). The high urea application rate in both SCG treatments were similar to the control with the lower urea application rate. From Day 12 to 14 the high urea application rate control was higher than the SCGs with the high urea application rate (*p*= <0.04). From Day 22, the highest rate of urea had the highest emissions.

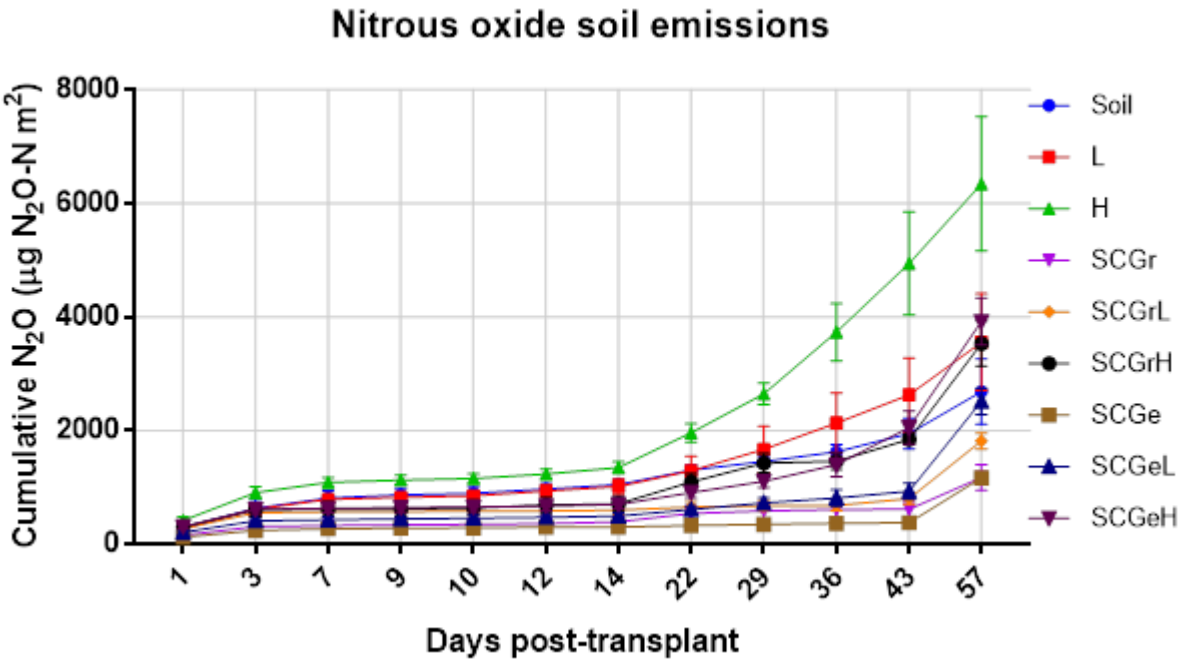


Figure 22 Cumulative N_2O (averages ± SEM). Soil= soil control (no urea), L= 122 kg/ha TN cumulative rate, H= 244 kg/ha TN cumulative rate, SCG= Spent Coffee Grounds, r= air-dried SCGs, e= ethanol-extracted SCGs. Error bars denote standard error, *n*= 6.

3.3.4. Soil analysis

Initially, the effect of the interaction between treatments and urea fertiliser was tested, using two-way ANOVAs. Secondly, the significance of SCG treatments or urea fertiliser application was tested, using one-way ANOVAs. A summary is presented in Table 13, which shows the differences between treatments as independent samples (one-way ANOVAs).

3.3.4.1. ***The effect of urea fertiliser application interaction with treatments on soil parameters***

Soil parameters that significantly differed with the interaction between treatment and urea application rate were: pH (*p*= <0.001), sulphur (*p*= 0.026), ESP (*p*= <0.001), Mg (*p*= 0.037) and K (*p*= 0.05, <0.001) (two-way ANOVAs).

The S concentrations were highest in the soils treated with SCGs without urea (p= <0.001, LSD), and the control was also lower than soils treated with SCGrL (p= 0.043, LSD). ESP was higher in soils treated with SCGs at the highest urea application rate (p= <0.001, LSD).

The pH was increased in the soils treated with SCGs in combination with the highest urea application rate (p= <0.001, LSD), with ethanol-extracted SCGs treated soils having significantly higher pH (p= 0.001, LSD). However, the pH range within the samples are unlikely to have agronomic value, because the range is relatively narrow (5.9-4.6 pH) in an agronomy sense; therefore, caution must be used when drawing conclusions on pH differences.

Mg concentrations increased in the soils treated with SCGs and increasing urea applications (p= <0.001, LSD); however, there was no differences between the soils with different SCG applications. K concentrations in all soils treated with SCGs were higher than the soils treated with L and H (p= <0.012, LSD).

3.3.4.2. *Effect of SCG treatment or urea application rate on soil parameters*

All soil parameters were significantly different between treatments (p= <0.018, one-way ANOVA), excluding Ca. Differences with urea application rates were in S, Si, ESP, pH, K, Mg, P, C: N ratio, Ca: Mg ratio, Zn and Fe (p= <0.03).

SCG treatment significantly decreased the Ca: Mg ratio (p= <0.001, one-way ANOVA, see Table 13), and urea application rate also decreased the Ca: Mg ratio (p= <0.001, LSD). There was a trend within SCG treatments where higher urea application rates resulted in a lower Ca: Mg ratio. This lower Ca: Mg ratio could have been produced as a result of either low Ca or high Mg. SCGs have substantially higher Mg compared to Ca (1176-1307 Mg compared to 628-683 Ca mg/kg); therefore, it is unsurprising that SCGs decreased the Ca: Mg ratio. In that case, where the higher urea rate decreased the Ca: Mg ratio, this could be due to the increased availability of Mg at a higher pH.

Effective Cation Exchange Capacity (CEC) and electrical conductivity were significantly increased with SCG treatments (p= <0.001, one-way ANOVA). Neither CEC nor electrical conductivity were significantly different within SCGs treatments or urea application rate.

Micronutrient concentrations were also increased with SCG treatment compared to urea-only and soil controls: Zn, B, Cu, Si, Mg and Mn with the addition of SCGs (p= <0.001, one-way ANOVA); however, there were no difference within SCG treatments (Table 13). Ammonium and nitrate concentrations were not significantly different between treatments nor was the interaction between treatments and application rates significant (Figure 23).

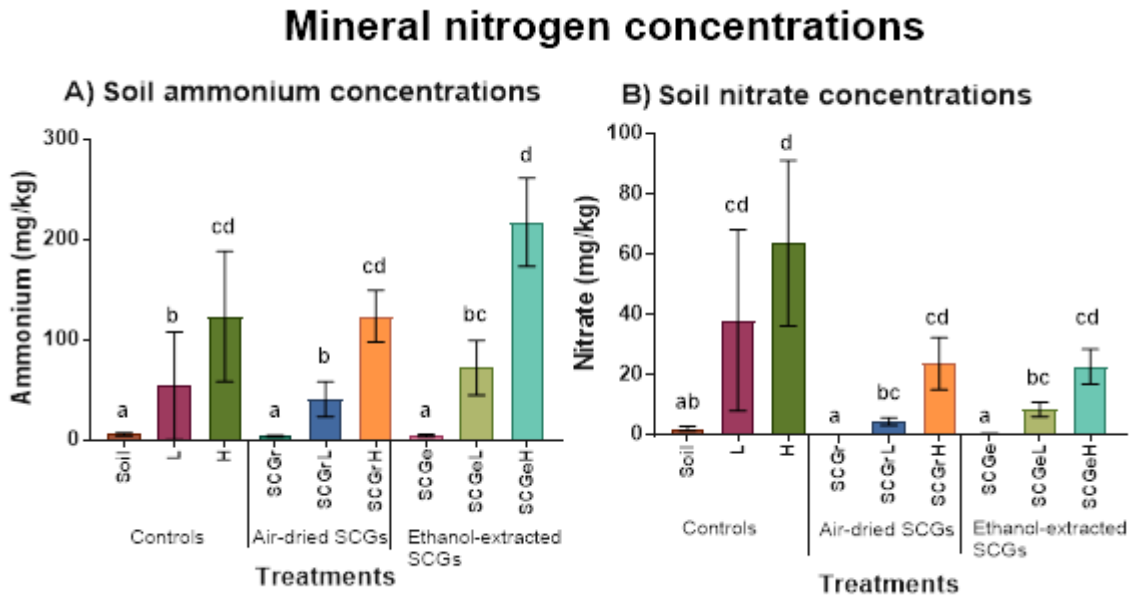


Figure 23 Soil mineral nitrogen concentrations, means ± SEM, for soil ammonium and nitrate. Soil= soil control (no urea), L= 122 kg/ha TN cumulative rate, H= 244 kg/ha TN cumulative rate, SCG= Spent Coffee Grounds, r= air-dried SCGs, e= ethanol-extracted SCGs. n= 6. Letters represent Tukey's post hoc test after a one-way ANOVA.

Total nitrogen values in SCG treatments were significantly higher than controls (p= <0.001, one-way ANOVA) but there was no difference between SCG treatments (Table 13). This was predictable, due to the additional nitrogen contributed from the SCGs which was ~11.7 g TN to each pot.

When considering the retained percent of applied TN in the soil at the trial conclusion, SCG treated soils retained applied nitrogen. With an average 2.7 ± 0.004% applied TN retained in the conclusion soil for both urea controls, and an average 9.5 ± 0.9% applied TN retained in the conclusion soil for all treatments with SCGs.

Table 13 Soil parameter averages ± SEM for all treatments (*n*= 6) at the conclusion of the pot trial. Letters represent the result of Tukey's test (post hoc one-way ANOVA, significance *p*= <0.05).

Parameter	**Method (units)**	**Controls**			**Air-dried SCGs**			**Ethanol-extracted SCGs**		
		Zero urea	**Low urea**	**High urea**	**Zero urea**	**Low urea**	**High urea**	**Zero urea**	**Low urea**	**High urea**
pH	1:5 DI H_2O	4.7 ± 0.06 ^{abc}	4.9 ± 0.06 ^{bcd}	5.0 ± 0.02 ^{d}	4.6 ± 0.02 ^{a}	4.9 ± 0.03 ^{cd}	5.4 ± 0.09 ^{e}	4.7 ± 0.03 ^{ab}	5.1 ± 0.03 ^{de}	5.6 ± 0.06 ^{e}
Electrical conductivity	1:5 DI H_2O (dS/m)	0.8 ± 0.03 ^{a}	0.8 ± 0.13 ^{ab}	0.9 ± 0.11 ^{ab}	1.1 ± 0.06 ^{abc}	1.1 ± 0.06 ^{abc}	1.1 ± 0.05 ^{abc}	1.3 ± 0.07 ^{c}	1.0 ± 0.06 ^{abc}	1.1 ± 0.06 ^{bc}
N	Total (% of mass)	0.07 ± 0.01 ^{a}	0.08 ± 0.01 ^{a}	0.09 ± 0.04 ^{a}	0.29 ± 0.02 ^{b}	0.34 ± 0.03 ^{b}	0.36 ± 0.02 ^{b}	0.26 ± 0.02 ^{b}	0.36 ± 0.02 ^{b}	0.36 ± 0.01 ^{b}
	Soluble ammonium (mg/kg)	6.1 ± 0.7 ^{a}	54.4 ± 21.9 ^{b}	123.3 ± 26.5 ^{cd}	4.2 ± 0.4 ^{a}	41.0 ± 7.1 ^{b}	123.9 ± 10.5 ^{cd}	4.8 ± 0.5 ^{a}	72.3 ± 11.1 ^{bc}	217.2 ± 17.9 ^{d}
	Soluble nitrate (mg/kg)	2.1 ± 0.81 ^{ab}	38.1 ± 30.13 ^{cd}	63.7 ± 27.51 ^{d}	0.5 ± 0.02 ^{a}	4.5 ± 1.29 ^{bc}	23.6 ± 8.63 ^{cd}	0.6 ± 0.04 ^{a}	8.5 ± 2.37 ^{bc}	22.7 ± 5.81 ^{cd}
Total C	(% of mass)	1.1 ± 0.02 ^{a}	1.1 ± 0.04 ^{a}	1.1 ± 0.04 ^{a}	4.2 ± 0.78 ^{b}	4.3 ± 0.39 ^{b}	4.5 ± 0.43 ^{b}	4.3 ± 0.22 ^{b}	4.5 ± 0.32 ^{b}	4.1 ± 0.23 ^{b}
C: N ratio	Mass ratio based on total	16.4 ± 0.20 ^{de}	14.8 ± 0.81 ^{cd}	12.8 ± 0.79 ^{abc}	17.7 ± 0.20 ^{e}	14.1 ± 0.38 ^{bc}	12.4 ± 0.35 ^{ab}	16.5 ± 0.15 ^{de}	12.5 ± 0.07 ^{ab}	11.5 ± 0.17 ^{a}

Parameter	Method (units)	Controls			Air-dried SCGs			Ethanol-extracted SCGs		
		Zero urea	**Low urea**	**High urea**	**Zero urea**	**Low urea**	**High urea**	**Zero urea**	**Low urea**	**High urea**
Soil OM	(% of mass)	1.9 ± 0.03 ^{a}	2.1 ± 0.06 ^{a}	1.9 ± 0.06 ^{a}	8.8 ± 1.37 ^{b}	8.3 ± 0.68 ^{b}	7.8 ± 0.75 ^{b}	7.4 ± 0.39 ^{b}	7.8 ± 0.55 ^{b}	7.2 ± 0.40 ^{b}
Ca: Mg ratio	Mass ratio plant available	8.8 ± 1.3 ^{d}	6.2 ± 0.6 ^{cd}	5.5 ± 0.5 ^{bc}	4.5 ± 0.2 ^{abc}	4.5 ± 0.2 ^{abc}	3.3 ± 0.1 ^{a}	4.2 ± 0.1 ^{ab}	4.7 ± 0.4 ^{abc}	3.7 ± 0.2 ^{a}
Na	Plant available (mg/kg)	43.9 ± 3.7 ^{abc}	33.4 ± 9.4 ^{ab}	24.8 ± 2.5 ^{a}	52.0 ± 2.0 ^{bcd}	54.2 ± 2.6 ^{bcd}	65.6 ± 6.4 ^{d}	65.6 ± 1.9 ^{d}	52.7 ± 4.4 ^{bcd}	55.3 ± 2.8 ^{cd}
	Exchangeable Na (ESP %)	2.7 ± 0.2 ^{c}	1.8 ± 0.3 ^{ab}	1.4 ± 0.2 ^{a}	2.3 ± 0.1 ^{bc}	2.2 ± 0.1 ^{bc}	2.6 ± 0.1 ^{c}	2.6 ± 0.1 ^{c}	2.1 ± 0.1 ^{bc}	2.2 ± 0.1 ^{bc}
K	Soluble (mg/kg)	298.1 ± 21.3 ^{a}	237.3 ± 82.9 ^{a}	102.3 ± 46.9 ^{a}	640.6 ± 22.3 ^{b}	668.1 ± 42.1 ^{b}	704.3 ± 24.3 ^{b}	757.9 ± 41.1 ^{b}	639.6 ± 57.9 ^{b}	702.7 ± 46.8 ^{b}
	Plant available (mg/kg)	383.8 ± 16.3 ^{b}	292.5 ± 97.0 ^{ab}	137.2 ± 62.8 ^{a}	678.3 ± 27.2 ^{c}	741.6 ± 45.5 ^{c}	780.3 ± 28.8 ^{c}	809.6 ± 29.4 ^{c}	737.3 ± 66.2 ^{c}	781.5 ± 50.6 ^{c}
CEC	($cmol^{+}$/kg)	7.3 ± 0.29 ^{a}	7.0 ± 0.72 ^{a}	8.0 ± 0.67 ^{a}	10.0 ± 0.48 ^{b}	10.9 ± 0.46 ^{b}	11.0 ± 0.45 ^{b}	10.7 ± 0.36 ^{b}	10.7 ± 0.43 ^{b}	10.8 ± 0.26 ^{b}

Parameter	Method (units)	Controls			Air-dried SCGs			Ethanol-extracted SCGs		
		Zero urea	Low urea	High urea	Zero urea	Low urea	High urea	Zero urea	Low urea	High urea
P	Plant available Colwell (mg/kg)	272.8± 4.2 [a]	221.70 ± 38.2 [a]	215.8 ± 19.5 [a]	309.3 ± 16.6 [a]	310.2 ± 22.5 [a]	275.3 ± 7.3 [a]	251.8 ± 18.3 [a]	216.6 ± 22.6 [a]	277.0 ± 26.6 [a]
	Plant available Bray 2 (mg/kg)	309.2 ± 13.4 [b]	292.0 ± 33.1 [ab]	275.5 ± 26.4 [ab]	289.4 ± 15.8 [ab]	250.7 ± 15.73 [ab]	217.3 ± 9.8[a]	328.3 ± 14.6 [b]	261.2 ± 17.8 [ab]	271.8 ± 14.6 [ab]
	Soluble (mg/kg)	48.4 ± 2.9 [a]	44.6 ± 9.6 [a]	39.9 ± 6.2 [a]	87.7 ± 3.4 [b]	86.3 ± 6.5 [b]	78.5 ± 1.7 [b]	104.1 ± 6.9 [b]	78.9 ± 5.6 [b]	99.1 ± 10.2 [b]
	Plant available Bray 1 (mg/kg)	262.2 ± 11.3 [c]	238.0 ± 18.4 [bc]	221.9 ± 17.8 [bc]	225.4 ± 11.8 [bc]	193.5 ± 14.4 [ab]	155.0 ± 7.4 [ab]	275.9 ± 10.1 [c]	179.6 ± 14.6 [ab]	192.7 ± 14.2 [ab]
Mg	Soluble (mg/kg)	61.0 ± 5.3 [a]	93.6 ± 13.7 [ab]	107.3 ± 10.4 [bc]	140.7 ± 6.4 [cd]	143.0 ± 6.7 [cd]	180.3 ± 10.6 [d]	162.7 ± 9.0 [d]	139.9 ± 10.7 [cd]	165.7 ± 7.8 [d]
	Plant available (mg/kg)	72.7 ± 6.0 [a]	110.7 ± 16.1 [ab]	130.5 ± 12.0 [bc]	152.8 ± 7.0 [cde]	167.5 ± 9.1 [cde]	211.8 ± 12.6 [e]	175.5 ± 7.5 [cde]	167.2 ± 13.9 [cde]	196.7 ± 8.7 [de]

Parameter	Method (units)	Controls			Air-dried SCGs			Ethanol-extracted SCGs		
		Zero urea	Low urea	High urea	Zero urea	Low urea	High urea	Zero urea	Low urea	High urea
Zn	Plant available (mg/kg)	2.0 ± 0.0 [a]	1.7 ± 0.2 [a]	1.8 ± 0.2 [a]	3.0 ± 0.1 [b]	3.1 ± 0.1 [b]	2.7 ± 0.1 [b]	3.2 ± 0.1 [b]	3.1 ± 0.1 [b]	2.7 ± 0.1 [b]
Cu	Plant available (mg/kg)	0.50 ± 0.02 [a]	0.48 ± 0.02 [a]	0.49 ± 0.02 [a]	1.20 ± 0.05 [b]	1.39 ± 0.10 [b]	1.21 ± 0.04 [b]	1.20 ± 0.05 [b]	1.34 ± 0.07 [b]	1.27 ± 0.06 [b]
Mn	Plant available (mg/kg)	4.2 ± 0.3 [a]	3.6 ± 0.8 [a]	2.8 ± 0.4 [a]	7.2 ± 0.3 [b]	7.3 ± 0.4 [b]	7.2 ± 0.2 [b]	7.6 ± 0.2 [b]	6.9 ± 0.3 [b]	7.0 ± 0.2 [b]
S	Soluble (mg/kg)	648.1 ± 35.9 [a]	699.1 ± 85.0 [a]	732.7 ± 75.3 [ab]	1003.3 ± 76.8 [bc]	886.7 ± 60.8 [abc]	808.1 ± 55.7 [abc]	1086.4 ± 71.1 [c]	767.1 ± 42.5 [ab]	807.6 ± 54.0 [abc]
Fe	Plant available (mg/kg)	50.3 ± 1.2 [a]	52.5 ± 3.4 [ab]	59.6 ± 3.9 [abc]	53.9 ± 1.4 [abc]	65.6 ± 3.3 [abc]	71.5 ± 3.5 [c]	52.8 ± 1.6 [ab]	69.4 ± 5.4 [bc]	69.1 ± 7.4 [bc]
B	Plant available (mg/kg)	0.3 ± 0.03 [a]	0.3 ± 0.03 [a]	0.2 ± 0.02 [a]	0.8 ± 0.04 [b]	0.9 ± 0.11 [b]	0.9 ± 0.02 [b]	0.8 ± 0.04 [b]	0.9 ± 0.02 [b]	0.9 ± 0.05 [b]
Si	Plant available (mg/kg)	18.3 ± 0.8 [a]	17.7 ± 1.5 [a]	17.3 ± 0.6 [a]	41.5 ± 4.6 [c]	31.2 ± 2.4 [bc]	23.8 ± 0.8 [ab]	40.7 ± 5.3 [c]	32.5 ± 3.1 [bc]	28.2 ± 2.5 [bc]

3.4. Discussion

3.4.1. Effects on plant growth

Silver beet growth was inhibited by the application of SCGs in this study. Various studies in the literature, with a range of plant species and conditions, have found that SCG application rates between 2.5-10% of the soil or substrate have deleterious effects on plant yield (Cervera-Mata et al., 2018, Cruz et al., 2015, Hardgrove and Livesley, 2016, Gomes et al., 2014, Cruz et al., 2012a, Cruz et al., 2014c). The application rate in this study was within this range, with 7.3% (by soil to SCG mass) SCGs applied.

Hardgrove and Livesley (2016) suggested that nitrogen immobilisation could partially be a cause for plant growth inhibition, as there was a reduction of nitrate concentrations in SCG treated soils in their study. In this study, there were reduced N_2O emissions from SCG treated soil, indicating a reduction of nitrogen turnover rate. Significantly, in this trial, there were no clear indications of severe nitrogen immobilisation which could have affected plant growth. Moreover, mineral nitrogen concentrations in SCG treatments were similar to controls (Figure 23) and should have been high enough to support plant growth.

Plant growth did not increase with the addition of fertiliser to SCG applications, indicating that additional nitrogen did not play a crucial role in determination of the yield. Similar results were found by Hardgrove and Livesley (2016), where fertiliser blending did not reduce lettuce growth inhibition. This suggests that nitrogen immobilisation with SCG treatment does not fully explain the extent of plant growth inhibition. Although, to fully eliminate nitrogen immobilisation as a factor in plant growth inhibition, further nitrogen cycling studies, plant tissue analysis and microbial experiments should be run. Due to low plant yields, there was a lack of sample to conduct appropriate plant tissue analysis. Stylianou et al. (2018) pointed out in a review that experiments on the effect of SCGs on microorganisms involved in the nitrogen cycle have not been conducted.

When plant inhibition is observed in literature the proposed explanation is often phytotoxicity caused by residual caffeine and polyphenols (Cervera-Mata et al., 2018, Ciesielczuk et al., 2017, Hardgrove and Livesley, 2016). Importantly, both caffeine and polyphenols cause phytotoxicity at low concentrations (Wink et al., 1998, Lyu et al., 2018, McCalla and Haskins, 1964). Indeed, the air-dried SCGs used in this study contained residual compounds of at least 5 mg/g caffeine and 22 mg/g total phenols (see Chapter 2 for details). These caffeine and polyphenol concentrations are comparable to those reported in the literature, ranging 2-11 mg/g for caffeine (Ramalakshmi et al., 2009, López-Barrera et al., 2016, Cruz et al., 2012b) and 10-36 mg/g for polyphenols (Mussatto et al., 2011a, Severini et al., 2017, Zuorro and Lavecchia, 2012, Al-Dhabi et al., 2017, Caballero-Galván et al., 2018). This could be a reason for the plant inhibition observed in this experiment. While ethanol extracted substantial amounts of caffeine and polyphenols, yet ethanol-extracted SCGs did not assist in increasing silver beet growth. Consequently, although the literature suggests the caffeine and polyphenols as the main cause of phytotoxicity, this study suggests that these components in isolation might not be responsible for plant inhibition.

3.4.2. Effects on soil parameters

In this study, there was a substantial increase in soil respiration with the addition of SCGs, which supports Stylianou et al. (2018)'s hypothesis and is similar to Cervera-Mata et al. (2018). This increase in microbial activity may be due to the addition of OM to the soil. It is common to observe an increase of microorganism activity after the addition of fresh OM (Fontaine et al., 2003) and it can continue for days or weeks (Kuzyakov, 2010). While the substantial increase in soil respiration observed in this study is encouraging for soil function, particularly with the associated increase in total soil C and N, further work into the structural change in the microbial community can help to determine the impacts on soil health.

In a review of SCGs use as a valuable resource, Stylianou et al. (2018) highlighted that the production of N_2O emissions after soil application may be particularly important to study because of the climate

change effects. This study found SCGs (with and without urea blending) showed a reduction in emissions compared to the same rate without SCGs. This is a significant finding, as organic amendments blended with synthetic fertilisers often increase N_2O emissions from soils, relative to unfertilised soil (Charles et al., 2017). The application of SCGs might be encouraging as a strategy to reduce N_2O emissions and retain TN in soils; especially considering that fine-textured soils (that is, clay soils) generally have higher N_2O emissions relative to the emissions from coarse-textured soils (sandy soils) (Charles et al., 2017). Therefore, fine-textured soils may have further reductions in or different dynamics of N_2O emissions compared to the results observed in this study. Charles et al. (2017) commented that the effect of N_2O emissions from organic amended soils is based on C: N ratio, rainfall, soil texture and drainage. Rainfall, soil texture and drainage are similar between all treatments in this experiment, and although the C: N ratio did change between treatments there lacked a obvious trend. Another explanation is the reduction of N_2O may be linked to soil microbes, as N_2O production and consumption from soils is predominately due to biotic processes, mainly due to nitrification processes in aerobic soils (Hénault et al., 2012).

This study has suggested that SCGs may be a valuable source of macronutrients, with large increases for total C, N, K, S and Mg concentrations observed in soil. SCGs have previously been suggested as a source of K and Mg (Cruz et al., 2014c), who observed a 13% K and 36% Mg increase with a 10% SCG application. Interestingly, in other trials, there was no significant increase in soil K with a 5% SCG application, potentially due to plant uptake (Cruz et al., 2014c). The greater increase of K observed in this trial (287-220% increase from control) could be due a lower K concentration in the control soils compared to Cruz et al. (2014c), with 290-373 mg/kg available K in this trial vs 2031 mg/kg total K respectively. This is an encouraging result, which supports the potential use of SCGs as a source of macronutrients essential for plants.

Micronutrients and OM are low in Australia due to highly weathered soils (Alloway, 2004) and are particularly deficient in Zn (Holloway et al., 2008). It is, therefore, encouraging that OM as well as

plant-available Zn, B, Cu, Si and Mn were all enhanced from low or lower than preferred ranges for Australian agricultural soils to more desirable concentrations for plant growth (EAL, 2012, Alloway, 2004, Ahmad et al., 2012). In this experiment, the Podosol is deprived of plant-essential macro- and micro-nutrients, which is the main reason for the observed large increase in nutrient concentrations after SCG application. The literature suggests that the most appropriate use of SCGs are applications to low nutrient sandy-textured soils (Cervera-Mata et al., 2018, Kasongo et al., 2011, Kasongo et al., 2013), because that is the best 'fit'. The soil requires higher nutrient concentrations, lower water infiltration and commonly higher OM, which can be offered by the SCGs (Kasongo et al., 2011). Whether the same increase in nutrient concentrations would be observed in a soil that has higher soil nutrient concentrations, such as a Dermosol or Vertosol, is an interesting question. It is suggested that SCGs would not necessarily provide many benefits to these soil types, due to these soils containing good nutrient levels, OM and/or water infiltration status which could not be greatly improved by SCGs. Therefore, it is suggested that the true benefit of SCGs remains to sandy-textured soils because this is where the evidence is based.

Additionally, loss of micronutrients through leaching are particularly a problem in soils with high drainage, such as sandy-textured soils (McKenzie et al., 2004). Consequently, it is advantageous to apply an amendment to hinder leaching of metals to the greater environment. SCGs have been shown to maintain moisture content (Hardgrove and Livesley, 2016, Yamane et al., 2014) and may retain these micronutrients due to SCG's metal-chelating activity (Stylianou et al., 2018). This retention of nutrients and metals doesis probably not appear to have occurred inthe case for this experiment, due to the SCGs adding higher amounts of leachable metals than was found in the original soil, therefore the SCG application will most likely increase leached metals. However, research has suggested that SCGs potentially reduce metal leaching to the greater environment when applied to contaminated soils (Kim et al., 2014, Cervera-Mata et al., 2018).

Prior to soil application ethanol-extracted SCGs, compared to air-dried SCGs, had higher K, Zn, B, Cu and S concentrations and lower Fe, NO_3^- and NH_4^+ concentrations. Interestingly, there was no to little difference between these micronutrients in the soils post application treated with air-dried SCGs compared to soils treated with ethanol-extracted SCGs with. This, in combination with no increased plant growth, suggests that extraction posed no additional soil micronutrient advantage.

Cruz et al. (2014c), in a greenhouse experiment, observed a 50% increase of total soil Cu and a 44% increase in total soil Zn concentrations with SCG application. However, increasing soil micronutrients were correlated with the decrease of these nutrients in lettuce plant tissue. A suggested explanation was that metals were retained due to SCG applications as well as the metal's reduced plant availability, as SCGs are known to have a highly absorbent capacity for metals (Stylianou et al., 2018) – although this did not fully explain plant inhibition (Cruz et al., 2014c). In the Cruz et al. (2014c) study total micronutrients via Atomic Absorption Spectroscopy were analysed. Fortunately, results from this study determined plant available micronutrient concentrations; therefore, the same reduction of micronutrients in plant tissue may not be found.

The OM values reported here are a useful indicator that suggest a potential increase in soil health. These OM values are estimates based on total C, but lack representation of the complexity of soil OM; that is, living and non-living OM, most importantly the humus and microbial biomass. Studies in the literature show encouraging results with regards to soil OM with SCGs application: C contribution of 10 kg/m^{-2} SCGs to a Japanese soil remained significant after 12-24 months (Yamane et al., 2014), and SCGs significantly increased soil organic C (Cervera-Mata et al., 2018). Therefore, the results observed in this study show encouraging preliminary outcomes and prove that further studies into the stability, permanency and form of OM are warranted. These OM parameters are extremely important for assessing the effects of organic amendment on soils (Quilty and Cattle, 2011).

3.5. Conclusions

In general, the treatment of SCGs had the most significant effect on plant growth and soil parameters, with a negative effect on plant growth and positive effect on plant-available nutrients. This study suggests that urea blending with SCGs and ethanol-extraction of SCGs had little effect on the results. The high application (20 t/ha) rate of SCGs caused inhibition of silver beet growth; particularly air-dried SCGs were the most detrimental to plant growth. Despite extracting phytotoxic compounds, ethanol extraction only slightly improved the above ground biomass; however, the yield was significantly lower than urea treatments. Consequently, there is no obvious advantage in using ethanol-extracted SCGs.

The effect of SCGs on soil had some important positive effects observed in this study. SCGs substantially increased soil respiration, indicating that the soil microbial community was stimulated. N_2O emissions from SCGs, with and without urea application, were reduced and retained more of the TN applied, which is an encouraging result for the reduction of this harmful greenhouse gas and nitrogen management. SCGs had a positive effect on soil macro- and micro-nutrients (excluding P) and OM, providing a potentially cheap and consistent source. Furthermore, this study suggests that SCGs may be a source for Zn, B, Cu and Mg, as all these plant available micronutrients were significantly increased (>63%) in the soil.

3.5.1. Future directions

In agreement with Cruz et al. (2014c), the SCGs observed in this study have the potential to provide plant available nutrients. However, detrimental effects to plant growth seen in this trial must be considered, which have also been observed in previous experiments (Cruz et al., 2012a, Ciesielczuk et al., 2017, Cruz et al., 2014c, Yamane et al., 2014, Hardgrove and Livesley, 2016). Phytotoxicity, particularly, should also be investigated in further work, such as experiments with root elongation, application rate limits in more conditions (e.g. soil types). The results of soil respiration in this trial

and previous studies conducted with SCGs (Cervera-Mata et al., 2018) suggest the impact of SCGs on soil microbiology should be studied further. Microbial experiments such as incubation studies, enzymes and DNA experiments would give a more detailed insight to the effect of SCGs on soil microbiology.

These experiments send a message of caution to regulatory bodies for potential future application of this material to land. Although the results here are not extensive and are very focused (one plant species, one soil type, one application rate), they suggest that regulatory bodies and land managers must consider the impact even in the absence of obvious contamination, such as high heavy metal contamination, pathogens, antibiotics etc. Furthermore, steps to reduce potential negative impacts (e.g. ethanol extraction to reduce the phytotoxic components, or fertiliser blending), even if based in good science and demonstrated through a practical experiment, may not necessarily translate into a positive result on plant growth.

Chapter 4. Spent coffee grounds phytotoxicity and effect on silver beet growth in two soil types

4.1. Introduction

Low application rates of directly-applied SCGs have great potential for increasing soil fertility (Cervera-Mata et al., 2018). These rates usually range between 2.5-5% (on volume) SCGs and have the potential to increase plant micronutrient concentrations (Cruz et al., 2014c, Cruz et al., 2012a), improve the soil carbon to nitrogen ratio (Yamane et al., 2014) and stimulate plant growth in some crop types (Gomes et al., 2014). Conversely, some studies have raised concerns of SCG inhibiting crop growth (Hardgrove and Livesley, 2016), which was also noted in Chapter 3. In order to address these varied results, this study undertook further investigation of SCGs application to two soil types. The aim of this study was to investigate two lower application rates than previously used, with two different soil types using silver beet and in similar conditions (see Chapter 3).

Caffeine and polyphenols have been suggested as two of the main culprits causing phytotoxicity when SCGs are directly applied to soil (Hardgrove and Livesley, 2016, Cruz et al., 2014b, Cruz et al., 2014c). Residual caffeine in SCGs is typically 0.02 ± 0.1% (Murthy and Naidu, 2012); however, residual caffeine has been reported as high as 0.2 and 0.5% (Ramalakshmi et al., 2009). Caffeine has been found to be phytotoxic at concentrations as low as 1-5 mM (Wink et al., 1998, Mohanpuria and Yadav, 2009, Batish et al., 2008). Concentrations of <1000 µM were found to drastically reduce successful germination of mung beans (Batish et al., 2008). Another study suggested that 50 ppm was the lowest concentration to cause a toxic effect on ladino clover seedlings in a nutrient culture without inorganic nitrogen (McCalla and Haskins, 1964). Caffeine has also been shown to have an autotoxicity effect on Arabica coffee (*Coffea arabica* L.) seeds (Waller et al., 1986).

Phenols are widely found in products used in the domestic, agricultural and industrial industries; for example, phenols are common in dyes, drugs, polymers and other organic substances (Park et al., 2012). Phenols are also associated with the degradation of pesticides as well as industrial and municipal effluents (Park et al., 2016). Due to phenol chemical stability, environmental mobility and

water solubility, they are an very problematic environmental pollutant (Park et al., 2012). Total polyphenols in SCGs are reported in the range 1.5 ± 1% (Murthy and Naidu, 2012), with concentrations of 17-35 mg/g total phenols (gallic acid equivalents) (Stylianou et al., 2018). Inhibition of plant growth from polyphenols can occur rapidly (Lyu et al., 2018) at concentrations from 20-180 mg/L (Park et al., 2016, Park et al., 2012, Hulzebos et al., 1993).

Importantly, even if caffeine and polyphenols show phytotoxicity in laboratory conditions, this does not necessarily translate to a wider range of crop types or those grown in a growing medium or soil. Ciesielczuk et al. (2017) found that 2.5-10% of a SCG aqueous extraction caused cress root growth inhibition but not in wheat, cucumber or white mustard root growth. When the same species were tested in growth medium (peat-sand mix) with applications of 2.5, 5, 10%, no inhibition was noted.

Previous experiments observed detrimental effects to silver beet growth at a 20 t/ha application rate of SCGs on a Podosol (Chapter 3). While ethanol extraction did not result in an increase in plant growth, a reasonable explanation for plant growth inhibition may still be phytotoxicity (as opposed to nutrient immobilisation). Therefore, in this study, SCGs were air-dried and were extracted using deionised water (DI H_2O), where both the extracted solid material and extraction liquid was used for phytotoxicity tests. Phytotoxicity tests included root elongation tests in petri dishes and an emergence trial in a glasshouse, using lettuce and millet. The application rate-response of plant emergence was conducted with both air-dried and water-extracted SCGs. This emergence trial was used to inform a subsequent glasshouse silver beet growth trial; investigating the effect of two rates (5 and 10 t/ha) of SCGs in two soil types (Podosol and Calcarosol). In Chapter 3, soil respiration increased and N_2O emissions reduced; therefore, soil respiration and N_2O soil emissions were also investigated in this current study. Soil nutrient analysis was focused on N and C concentrations to provide a baseline nutrient understanding.

4.2. Materials and methods

Spent coffee grounds were collected from two cafés on the Monash University, Clayton campus, in 2015. SCGs were air dried to ~10% moisture and stored in sealed plastic drums until used. The caffeine and polyphenol content of the SCGs were investigated. SCGs were extracted (using water) and air-dried, then applied in emergence trials and a silver beet plant growth study (described in sections 2.1 and 2.2). The hemicellulose content of air-dried SCGs was 36.3% (extractive free basis) (Singh, unpublished data), which is typical of SCGs (Murthy and Naidu, 2012, Mussatto et al., 2011b).

An up-scaled two-hour extraction of caffeine and polyphenols was carried out, based on work described in Chapter 2, to generate large volumes of materials for phytotoxicity trials. Initially, this was conducted with two different ratios of solid-solvent (1:5 and 1:3, using 15 g SCGs). It was demonstrated that a 1:5 ratio had twice the yield as 1:3 ratio extractions (7.9 ± 0.2% vs 4.4 ± 0.4% respectively, n=2). A final up-scaled extraction was done with 50 g SCGs to 1000 ml DI H_2O, with a yield of ~6%. Solid and liquid fractions were separated and SCGs were air-dried before use.

4.2.1. Root elongation and emergence tests

Initially, root elongation tests were conducted to investigate the phytotoxicity of the extraction liquid. SCGs extraction liquid (SCGexL) was used from a two-hour water 1:5 extraction, based on previous work detailed in Chapter 2 to extract polyphenols and caffeine. SCGexL (5 mL) was used to moisten filter paper in 90 mm Petri dishes at 6 different concentrations adapted from Mosse et al. (2010). These were replicated six times with 10 seeds per petri dish, using lettuce and millet, measuring the radicle length daily.

The aim of using the extracted liquid was not only to test potential phytotoxicity, but also to confirm that phytotoxic compounds were being extracted from the raw material. Millet has been used in a

variety of phenol toxicity tests (Mosse et al., 2010, Wang, 1991, Wang, 1986), showing increased sensitivity to phenols when compared to lettuce (Wang, 1991).

Caffeine was analysed in this study by liquid chromatography coupled with mass spectrometry (LC-MS). Total phenols were determined by colorimetric analysis using gallic acid as a standard, reported as gallic acid equivalents (GAEs). Methods and equipment for caffeine and GAEs are the same as described in Chapter 2. Total Organic Carbon (TOC) was analysed using a Shimadzu TOC-V CPH/CPN Total Organic Carbon analyser and Shimadzu ASI-5000 Autosampler using standard method 5310B (APHA-AWWA-WEF, 2005).

Table 14 Root elongation treatments and estimated caffeine, gallic acid equivalents (GAE) and total organic carbon (TOC) in treatments, 100% extraction, *n*= 2. Calculated concentrations were based on the 100% concentration extraction.

Treatment code	**Treatment**	**Calculated concentrations**		
		Caffeine (mg/L)	**GAEs (mg/L)**	**TOC (g/L)**
0	Control (DI water)	0	0	0
10	10% SCGexL	15.9	48.4	0.13
25	25% SCGexL	39.7	120.9	0.33
50	50% SCGexL	79.3	241.9	0.65
75	75% SCGexL	118.9	362.8	0.98
100	100% SCGexL	158.6	483.7	1.30

The surface of the seeds were sterilised by immersion in a 1.5% sodium hydrochlorite solution for 10 minutes (Burd et al., 2000). Seeds were then lightly rinsed in DI water. The seeds were kept in the dark using a Memmert Rs 50000 incubator, maintained at 25 ± 1^{o}C.

Within this trial, two measurements were taken: total percent germination and germination index (GI) (Mosse et al., 2010). Where:

Equation 1: Percent germination = (N / 10) x 100

where N is the total number of germinated seeds (≥ 5 mm) and 10 is the total of seeds per petri dish (60 seeds per treatment).

Equation 2: Germination index (GI) = $100 \times \frac{Gs}{Gc} \times \frac{Ls}{Lc}$

where Gs and Gc are the number of seeds germinated in the sample and the control (distilled water), and Ls and Lc are the root lengths in the sample and the control, respectively; the germination index is measured as % of control.

Furthermore, the emergence trial investigated the phytotoxicity of direct application of the SCGs solids (air-dried and water-extracted SCGs) on early stage millet and lettuce growth. The methods followed Gerson and Honma (1978).

Table 15 Spent Coffee Grounds chemical and nutrient characteristics, n= 2.

Parameter	**Air-dried SCGs**	**Water-extracted SCGs**
pH ($CaCl_2$)	4.68	4.58
Electrical conductivity (dS/m)	2.59	1.16
Nitrate (mg/kg)	3.1	1.6
Ammonium (mg/kg)	57.8	ND
Organic matter (%)	83	ND
CEC ($cmol^+/kg$)	32.75	ND
Total Carbon (% of total mass)	47.3	50.0
Total Nitrogen (% of total mass)	2.1	2.1
C: N ratio	22.3	23.4

ND = not determined

Lettuce and millet were used (ten seeds per container), replicated six times, planted in 12.5 x 7 cm containers with 200 g Debco sandy seedling raising mix (which included fertilisers, wood chips and a

high portion of sand). No additional fertiliser was used. Moisture content was maintained at 15% (total moisture content) with tap water. Treatments were covered with transparent plastic to help minimise moisture evaporation.

Rates of SCGs equivalent to 0, 5, 10, 15 and 20 t/ha (2.19, 4.38, 6.56 and 8.75%) were manually mixed into the first ~5 cm of soil, based on recommended application rates for organic amendments (Quilty and Cattle, 2011). The experiment was run for nine days in a greenhouse, during winter (August) 2016, without additional lighting. Emerged seeds, defined as the primary leaves free of soil surface, were counted daily then calculated by mean days to emergence (MDE) as per Gerson and Honma (1978):

MDE = (N1T1 + N2T2 +.....+ NxTx)/total number of seeds emerged,

Where:

N = Number of seeds emerged within consecutive time intervals, and

T= Time (in days) from sowing to end of a particular interval.

4.2.2. Silver beet plant growth trial

Silver beet growth trials were undertaken in greenhouse conditions in late spring – early summer (September to November) 2016, for a total of 72 days post-seeding. Soil type one was a topsoil sourced from a vegetable farm converted to pasture in Cranbourne VIC 3977, south-east of Melbourne (acidic Podosol). Soil type two was a topsoil collected from a wheat property located in Ouyen, north-west VIC (34º 58’0.16’’S; 142º 20’45.85’’E) (alkaline Calcarosol), classified by DEDJTR staff, in Mallee where the soil was located. Soils were collected from the first ~10 cm, air dried, sieved (<2 mm) and homogenised. The soils were characterised by Environmental Analysis laboratories, at Southern Cross University, using Rayment and Lyons (2011b) and Tucker (1985) standard methods.

Table 16 Soil chemical and nutrient characteristics, *n*= 1

Parameter	**Podosol**	**Calcarosol**
pH ($CaCl_2$)	4.22	6.87
Electrical conductivity (dS/m)	0.035	0.041
Nitrate (mg/kg)	25.4	7.0
Ammonium (mg/kg)	7.9	6.5
Organic matter (% of mass)	1.8	0.2
CEC ($cmol^+/kg$)	2.5	4.1
Total Carbon (% of mass)	1.0	0.12
Total Nitrogen (% of mass)	0.1	0.03
C: N ratio	13.4	0.4

The different treatments (Table 17) were replicated six times, with a total of 72 pots.

Table 17 Treatment and application rates in pot trial experiment

Treatment	**Initial N fertiliser application rate + weekly N fertiliser (kg/ha)**	**Spent Grounds application rate (t/ha)**	**Total N fertiliser applied (kg/ha)**
Soil	----	----	----
Urea	80 + 20	----	160
SCGr5	80 + 20	5	160
SCGr10	80 + 20	10	160
SCGex5	80 + 20	5	160
SCGex10	80 + 20	10	160

Every treatment received a basal application of phosphate fertiliser at 40 kg/ha and potassium fertiliser at 60 kg/ha to maintain plant growth. Additional fertiliser was given weekly from Week 4, post-transplant, to maintain plant growth; 20 N kg/ha, 18 P kg/ha and 36 K kg/ha according to DPI NSW for vegetables (Wade, 2009). Nitrogen fertiliser was applied as urea (46.0% N), phosphorus fertiliser was applied as superphosphate (9.1% P) and potassium fertiliser was applied as potash (41.5% K).

Following ultrasound extractions, solid-liquid separation was conducted with a Whatman no.1 filter, and the solid residue was dried for 24 hours to allow water evaporation (applications were based on dried weight). SCGs and fertilisers were manually mixed into the top 500 g (~5 cm) of soil in 8 kg capacity (9.5 cm by 24 cm) pots, to mimic topsoil applications. Masking tape was used to cover drainage holes in the pots to prevent leaching. Pots were watered to, and maintained at, 75% water field capacity with deionised water. Silver beet was grown for 19 days in a Debco sandy seed raising mix (described above). Soil and materials were prepared 2.5 weeks (19 days) beforehand, then a single silver beet seedling was transplanted in the centre of each pot. Pots were randomised and were further randomised at every watering event. Thee glasshouse temperature was maintained at 24/17^{o}C day/night cycle.

Silver beet plants were harvested after 57 days post- transplantation. For above ground biomass, plants were cut at the soil surface. Below ground biomass (roots) was washed out of the soils using tap water and a 2 mm sieve. All plant materials were oven-dried at 60^{o}C for four days, to ensure that all moisture had evaporated, and then the materials were weighed. The roots were separated from the soil and washed out with tap water. All fresh and dried biomass was weighed with a gram scale.

4.2.2.1. *Greenhouse gas emissions*

Soil greenhouse gas (GHG) emissions were collected using static chambers. Following a capping time of 20 minutes, a glass SGE analytical Scientific gas tight syringe was used to remove an aliquot of

soil emissions through a type of rubber septum which were then introduced into an evacuated Labco® exetainer. GHG emissions were collected post-transplant on days 1, 3, 5, 7, 9, 11, 13, 16, 24, 32, 39, 46 and 53. Temperature was recorded each time. Concentrations of CO_2 and N_2O were analysed by using an Agilent technologies Gas Chromatography - Thermal Conductivity Detector (GC-TCD) and GC-Flame Ionization Detector (FID). Cumulative emissions were calculated, accounting for time past between measurements, following the method set out by Van Zwieten et al. (2010).

4.2.2.2. *Basic chemical and nutrient analysis*

Soil from a depth of 0-10 cm was removed for chemical (pH, EC) and nutrient (ammonium, nitrate, total nitrogen and carbon) analysis, in order to analysis the soil with the highest impact of the amendment. The soil pH was determine by a WP-80 TPS electronic probe using 4B3 method - 1:5 0.01M $CaCl_2$ extraction (Rayment and Lyons, 2011b). Electrical Conductivity (EC) was completed by a sensION+ EC5 portable HACH® probe, using 3A1 method - 1:5 soil to DI water extraction (Rayment and Lyons, 2011b). Nitrate and ammonium concentrations in soils were analysed by the sulphanilamide/NED (Miranda et al., 2001) and salicylate/nitroprusside (Foster, 1995) colorimetric methods, respectively. Total carbon, total nitrogen and hydrogen (CHN) was analysed by an Elementar vario MACRO cube CHN analyser, using acetanilide as a standard.

4.2.3. Statistical analysis

Data analysis was completed by IBM SPSS Statistics 25 software package. For all tests, the significance value chosen was 5% (p= 0.05), where <0.05 was considered significant. Data distribution was checked by Levene's test and robust tests of equality of means (Welch and Brown-Forsythe tests). One-way ANOVAs and Tukey's post-hoc and pairwise comparison Least Significant Difference (LSD) tests were used. Critical F-values were checked when p values were significant. Graphs in this chapter were produced with GraphPad Prism, version 7.02.

4.3. Results

4.3.1. Root elongation and emergence tests

The Germination Index (GI, calculated as the percent growth of control) for millet was not statistically different between all treatments (p= 0.24, Figure 24 A)); however, there was a decreasing GI with increasing SCGexL concentration. There was also a significant difference between 0% and 100% treatments (p= 0.014, LSD), with a 45 ± 9.4 GI for 100% SCGexL. The results regarding lettuce GI indicates that there was an increased rate of root growth in the 10% SCGexL, with a 120 ± 9.5 GI. However, from 25-100% SCGexL, a gradual decrease in growth was observed (Figure 24, B).

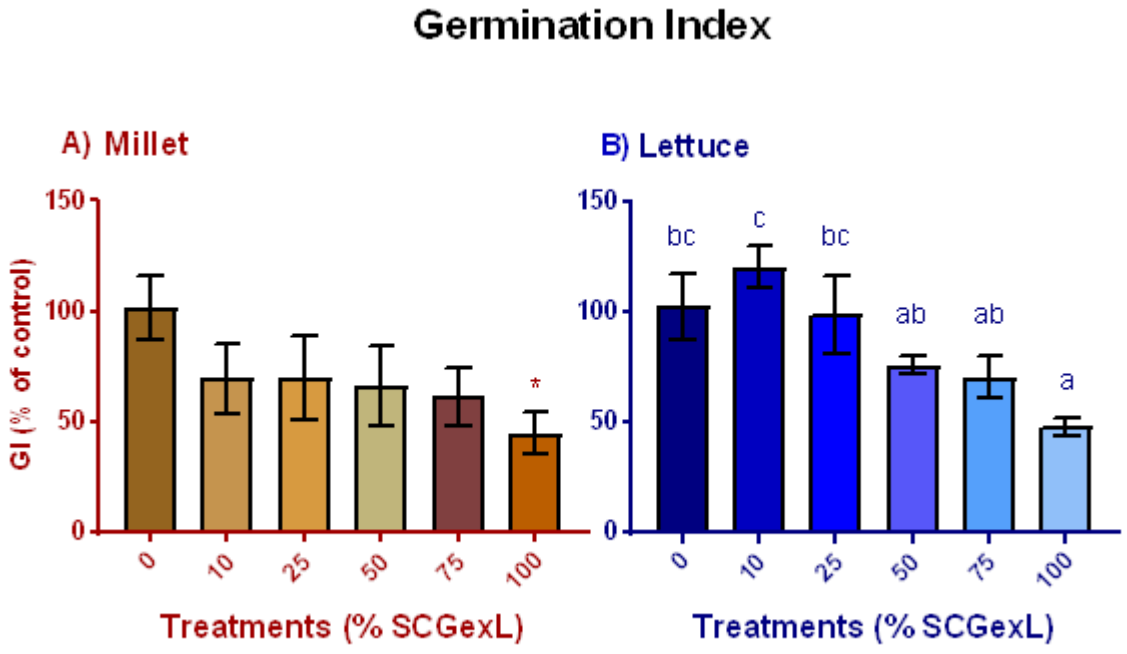


Figure 24 Germination Index for A) millet and B) lettuce root elongation after 120 hours, error bars denote standard error, n= 60. SCGexL = 1:5 SCG to water extraction liquid. *significance from control p= 0.014, using LSD. Letters indicate the results of Tukey's post-hoc test, significance p= <0.05.

Emergence tests showed that mean day to emergence was insignificant for millet (p= 0.055, ANOVA, Figure 25, A) but significantly different for lettuce (p= <0.001, ANOVA Figure 25, B). While there

was no significant effect between millet MDE, a trend showed that MDE decreased at 10 and 15 t/ha SCGs (Figure 25). Lettuce response indicated that there was inhibition with 15 and 20 t/ha of air-dried SCGs. Overall, air-dried SCGs increased growth inhibition, with mean time to germination being 5.6 ± 1.2 days compared to 4.4 ± 0.6 days for extracted and 3.8 ± 0.7 days for control.

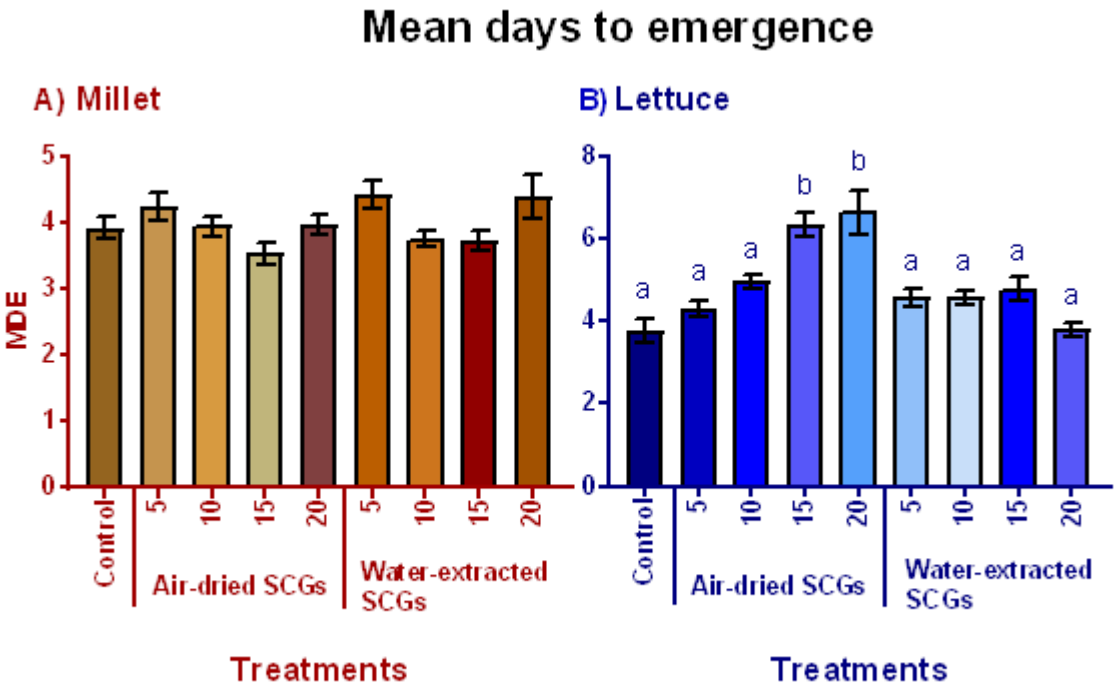


Figure 25 Mean days to emergence (MDE) for A) Millet and B) Lettuce, error bars denote standard error, *n*= 60. Letters represent the result of Tukey's post-hoc test (*p*= <0.05).

4.3.2. Silver beet growth trial

In the Podosol, the silver beet dry yield changed over different treatments (*p*= <0.001, ANOVA), with applications of 5 t/ha air-dried SCGs significantly increased above ground yield (*p*= 0.016, LSD). The application of water-extracted SCGs resulted in similar yields to the urea-only control (*p*= >0.05, Figure 26). Below ground biomass was increased with application of 5 and 10 t/ha air-dried SCGs compared to urea-only control (*p*= 0.003 and 0.005, LSD). In the Calcarosol, air-dried at 5 t/ha had similar yields to urea-only control (*p*= >0.05, ANOVA); however, compared to urea-only control both

10 t/ha SCGs application reduced above ground yields (p= <0.001, LSD, Figure 26). Below ground biomass did not change with different treatments (p= >0.05, ANOVA).

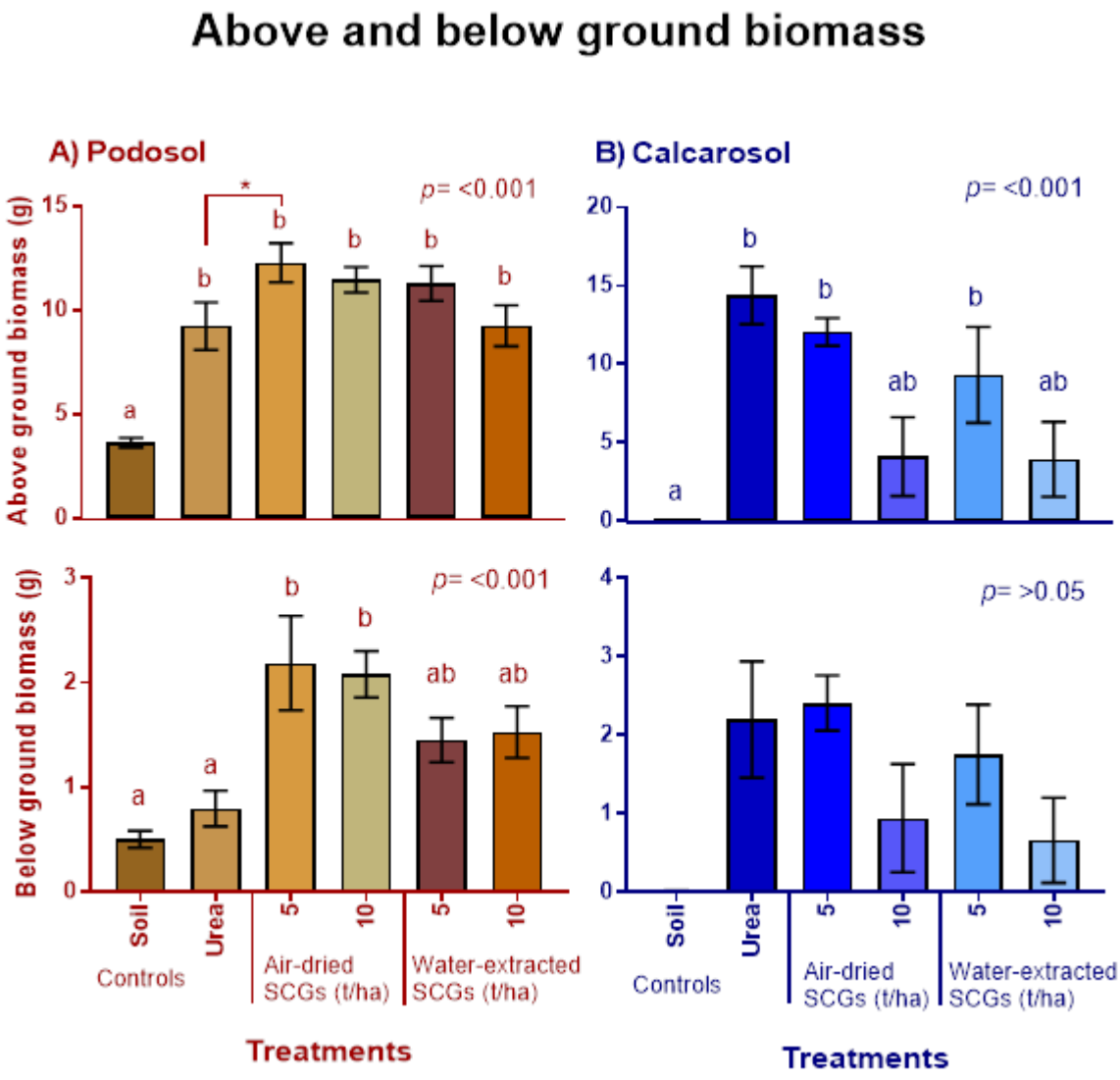


Figure 26 Above and below ground biomass (dry) for silver beet in Podosol and Calcarosol experiment with 5 and 10 t/ha application rate of SCGs. Where SCG= Spent Coffee Grounds. ANOVA significance shown in the top righthand corner of graph. Letters show the result of Tukey's post-hoc test (p= <0.05). *p= 0.016, LSD. Columns represent averages, error bars denote standard error, n=6.

Soil respiration (cumulative CO_2 emissions, mg CO_2-C m^2) was significantly increased with SCG treatments for the Podosol (p= <0.001 for all time points, ANOVA). Soil respiration from 5 t/ha water-extracted SCGs were similar to urea-only control, and all other SCG applications were higher than the urea-only control (p= <0.05, LSD).

Soil respiration from the Calcarosol were also different with treatments from Day 3 post-transplant (p= ≤0.001), with similar results as the Podosol. From Day 16 all SCG applications were higher than the controls (p= <0.05, LSD).

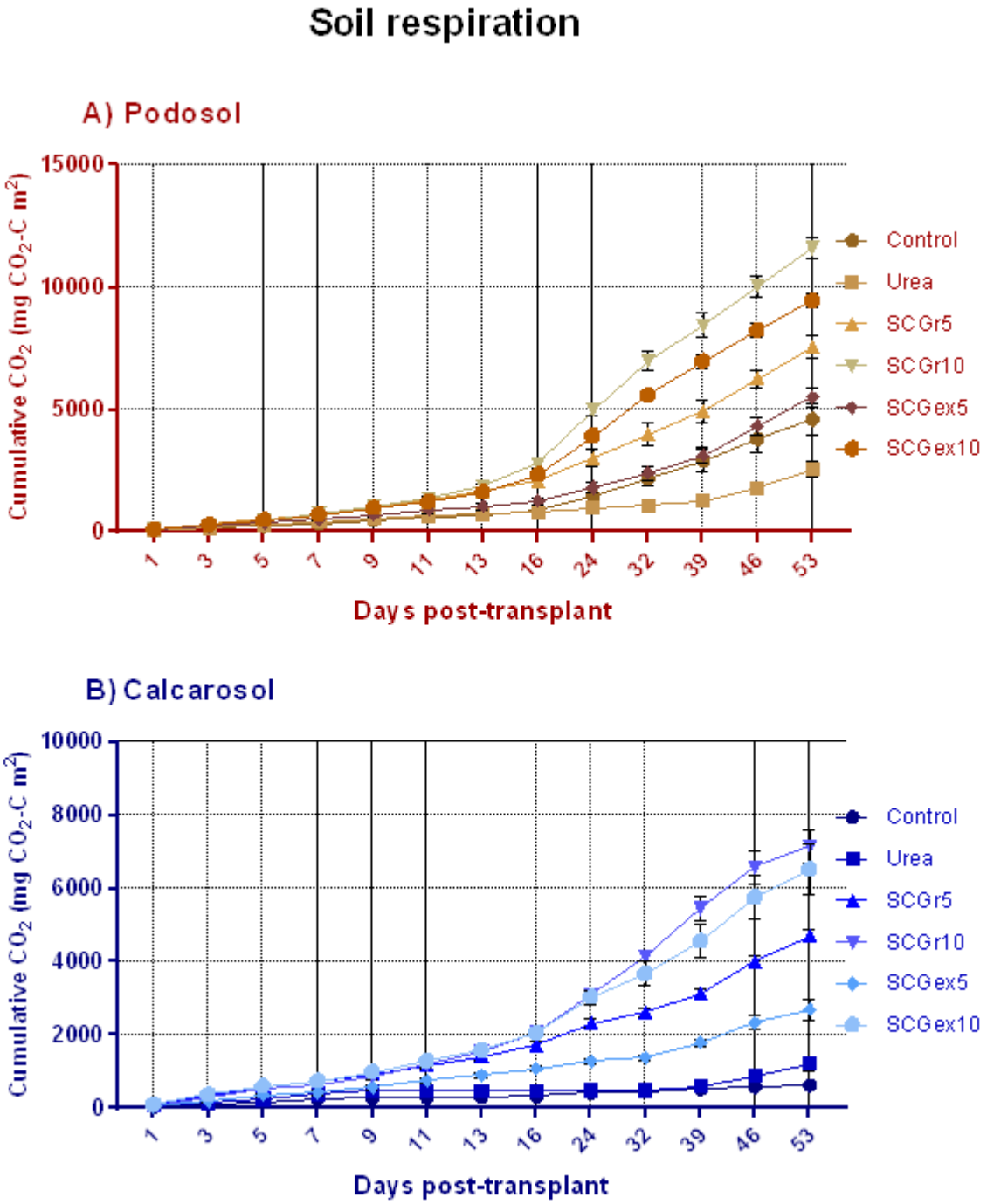


Figure 27 Soil respiration (mg CO_2-C m^2), for A) Podosol and B) Calcarosol. Error bars denote standard error, *n*= 6. Control= soil control, Urea= urea-only control, r5= 5 t/ha air-dried SCGs, r10= 10 t/ha air-dried SCGs, ex5= 5 t/ha water-extracted SCGs, ex10= 10 t/ha water-extracted SCGs.

For the Podosol, N_2O emissions significantly changed with treatments (Days 5 and 7 p= <0.02, from Days 9 to 53, p= ≤0.005, ANOVA). From Days 5 to 9 urea-only control was significantly higher than 5 t/ha water-extracted and 10 t/ha air-dried SCGs. From Days 9 to 53 urea-only control was higher than all SCG treatments (p= <0.03, LSD), excluding 5 t/ha air-dried SCGs.

For the Calcarosol, N_2O emissions were significantly different between treatments from Day 3 post-transplant (for days 5 to 9 p= ≤0.009, from days 11 to 53 p= <0.003), where emissions from soil control were consistently low. SCG treatments had emissions that were similar to the soil control, excluding 5 t/ha air-dried SCG application which was significantly higher than soil control from Day 3 (p= ≤0.004, LSD).

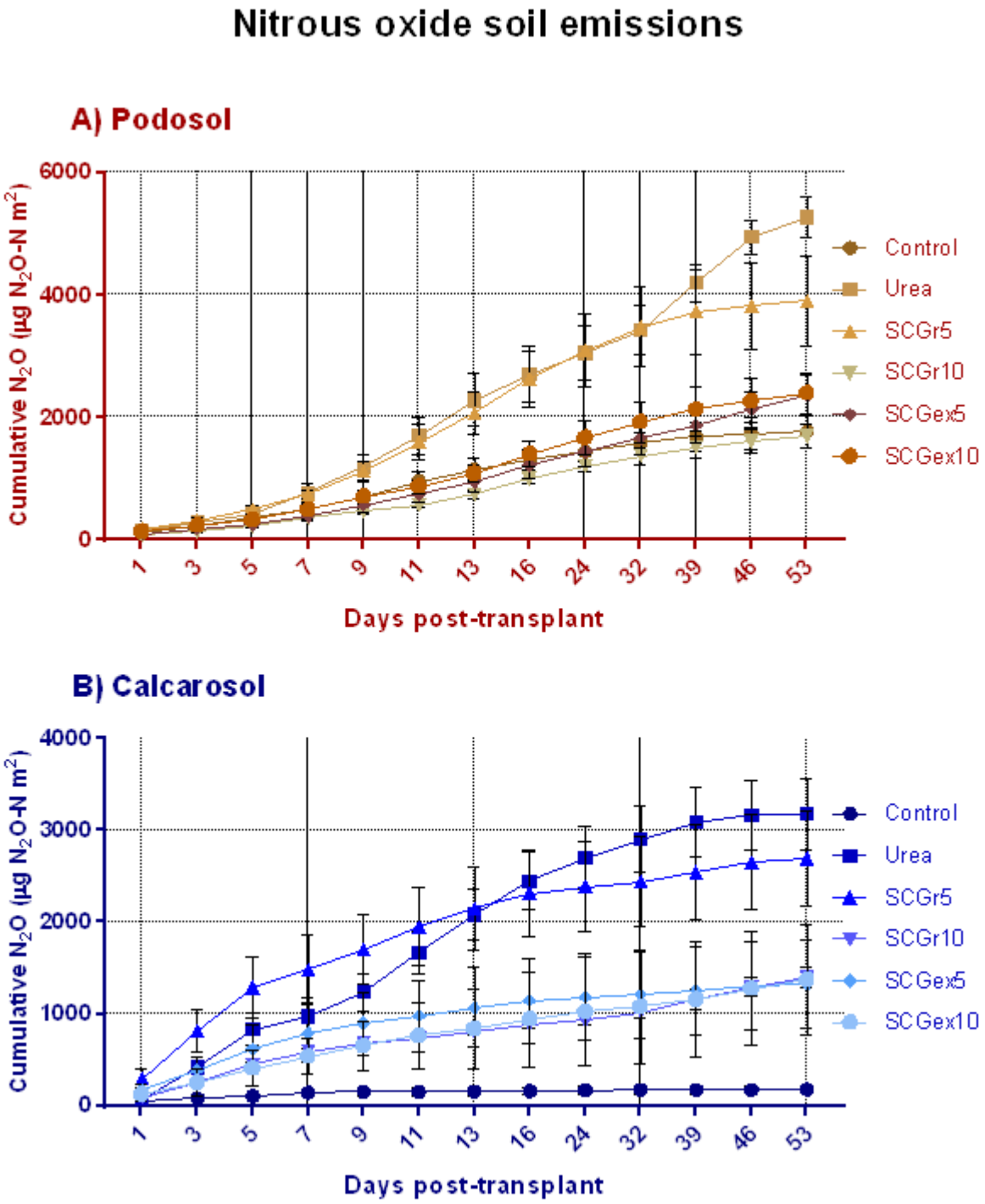


Figure 28 Cumulative N_2O emissions (µg N_2O-N m^2), for A) Podosol and B) Calcarosol. Error bars denote standard error, *n*= 6. Control= soil control, Urea= urea-only control, r5= 5 t/ha air-dried SCGs, r10= 10 t/ha air-dried SCGs, ex5= 5 t/ha water-extracted SCGs, ex10= 10 t/ha water-extracted SCGs

The EC for the Podosol did not change significantly between treatments (p= 0.34) but it did change for the Calcarosol (p= <0.05) (Figure 29). The overall average was 0.88 ± 0.06 dS/m, where urea control had the lowest conductivity, with all other treatments being higher.

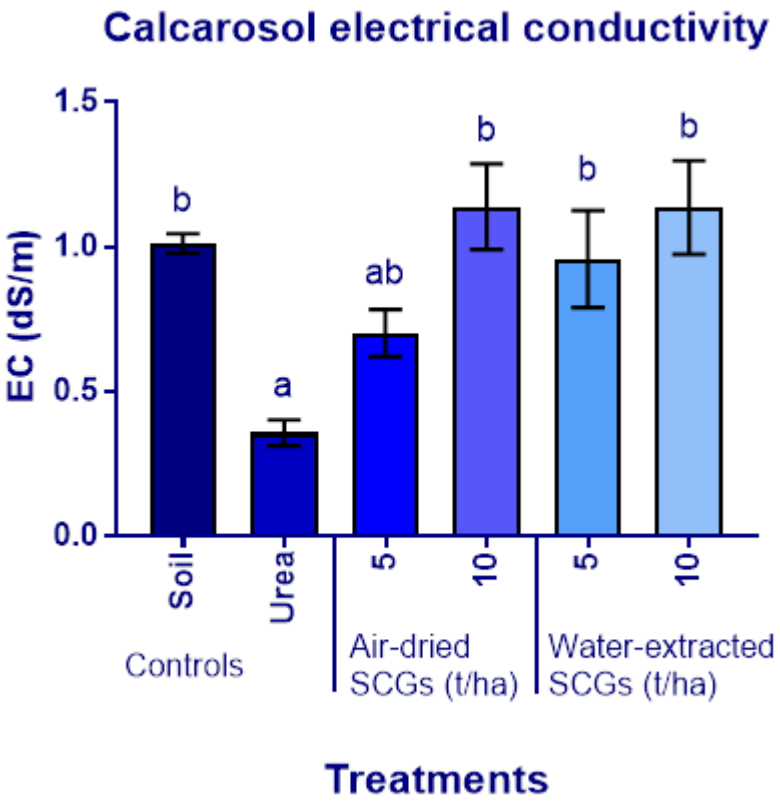


Figure 29 EC for Calcarosol. Columns represent averages, error bars denote standard error, Letters show the result of Tukey's post-hoc test, significance p= <0.05, n= 6.

The pH ($CaCl_2$) was significantly different between treatments for both soil types (p= <0.001 for both, ANOVA). In the Podosol, SCG treatments increased soil pH, with 4.2 for the urea control and a range between 4.6-5.0 for the SCG treatments (Figure 30). The Calcarosol pH was significantly decreased from soil control (excluding one treatment) but was increased by ~0.4 units in comparison to urea control with the application of SCGs (Figure 30).

Predictably, total soil carbon was increased in all SCG treatments (p= <0.001 for Podosol, <0.01 for Calcarosol, ANOVA), due to the high carbon content of the SCGs (Table 15). For the Podosol, the C: N ratio changed with treatments (p= 0.002, ANOVA), and decreased under SCGs treatments (p= ≤0.004, LSD), excluding 5 t/ha of air-dried SCGs (p= 0.07, LSD). For the Calcarosol, there was no overall change in C: N ratio between treatments; however, the C: N ratio in 10 t/ha air-dried SCGs was increased compared to urea-only control (p= 0.031, LSD).

Similarities between the two soil types included an increase in total carbon, nitrogen and ammonium concentrations with increasing SCG application, as expected. Total soil nitrogen was increased in higher rates of SCGs (p= <0.001 for both soils, ANOVA). For the Podosol, there was an average 1.8% applied TN retained in the soil post-harvest for urea-only control, and an average 8.0 ± 1.0% applied TN retained for all SCG treatments. For the Calcarosol, an average of 3.1% applied TN was retained in the soil post-harvest for urea-only control, and an average 9.2 ± 2.3% applied TN retained for all SCG treatments.

In the Podosol, nitrate concentrations changed with treatments (p= <0.001, ANOVA) and were highest in the urea-only control. Ammonium concentrations also changed with treatments (p= <0.001, ANOVA) with both SCG materials applied at 10 t/ha treatments having the highest concentrations (Figure 31). In the Calcarosol, both nitrate and ammonium concentrations were different between treatments (p= <0.001 for both, ANOVA), with SCG treatments having the highest concentrations, increasing nitrate and ammonium concentrations with increasing SCG treatment (Figure 31).

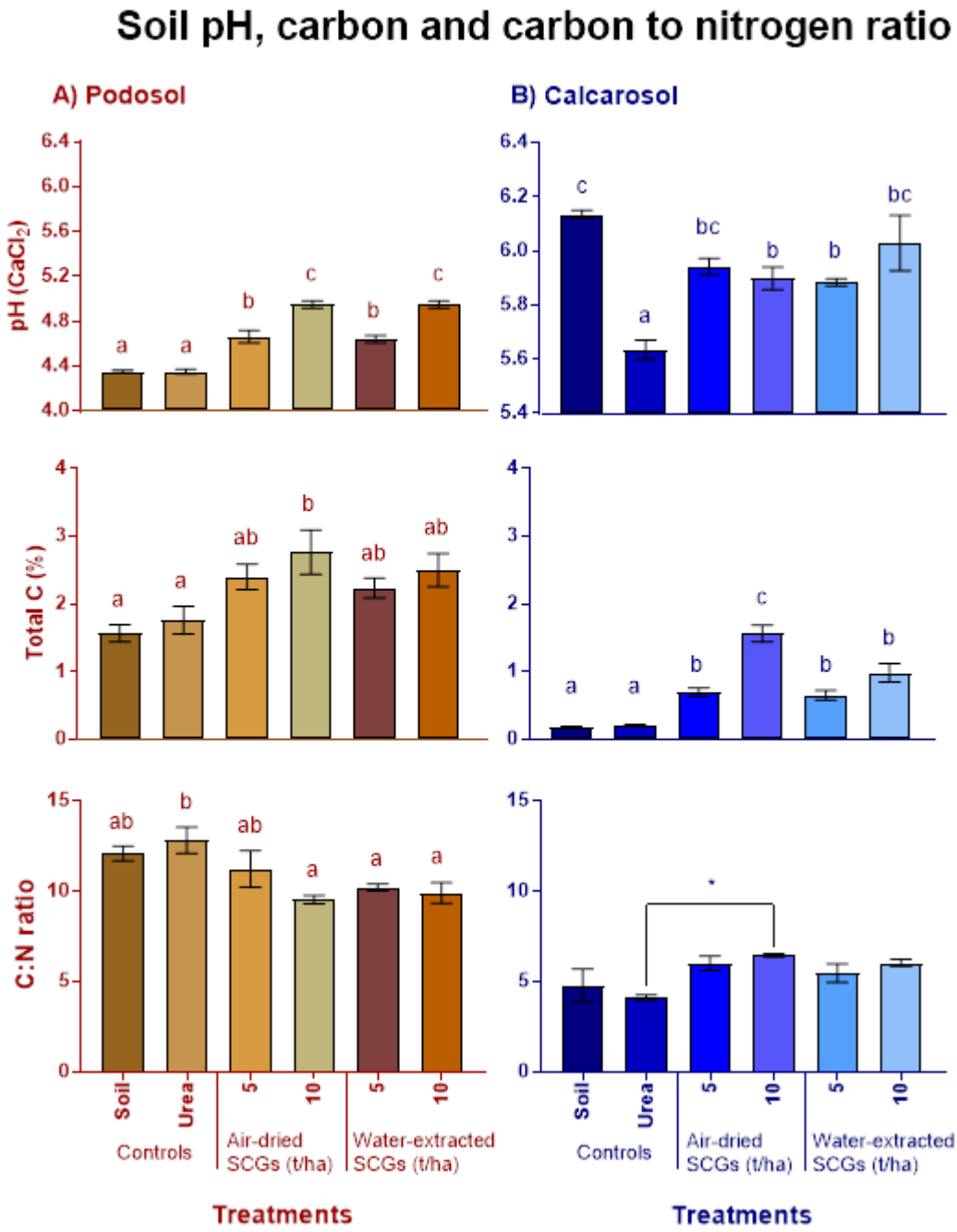


Figure 30 Comparison graphs for pH, total carbon and carbon to nitrogen ratio, for A) Podosol and B) Calcarosol. Columns represent averages, error bars denote standard error, *n*= 6. Letters show the result of Tukey's post-hoc test, significance *p*= <0.05, **p*= 0.031, using LSD test.

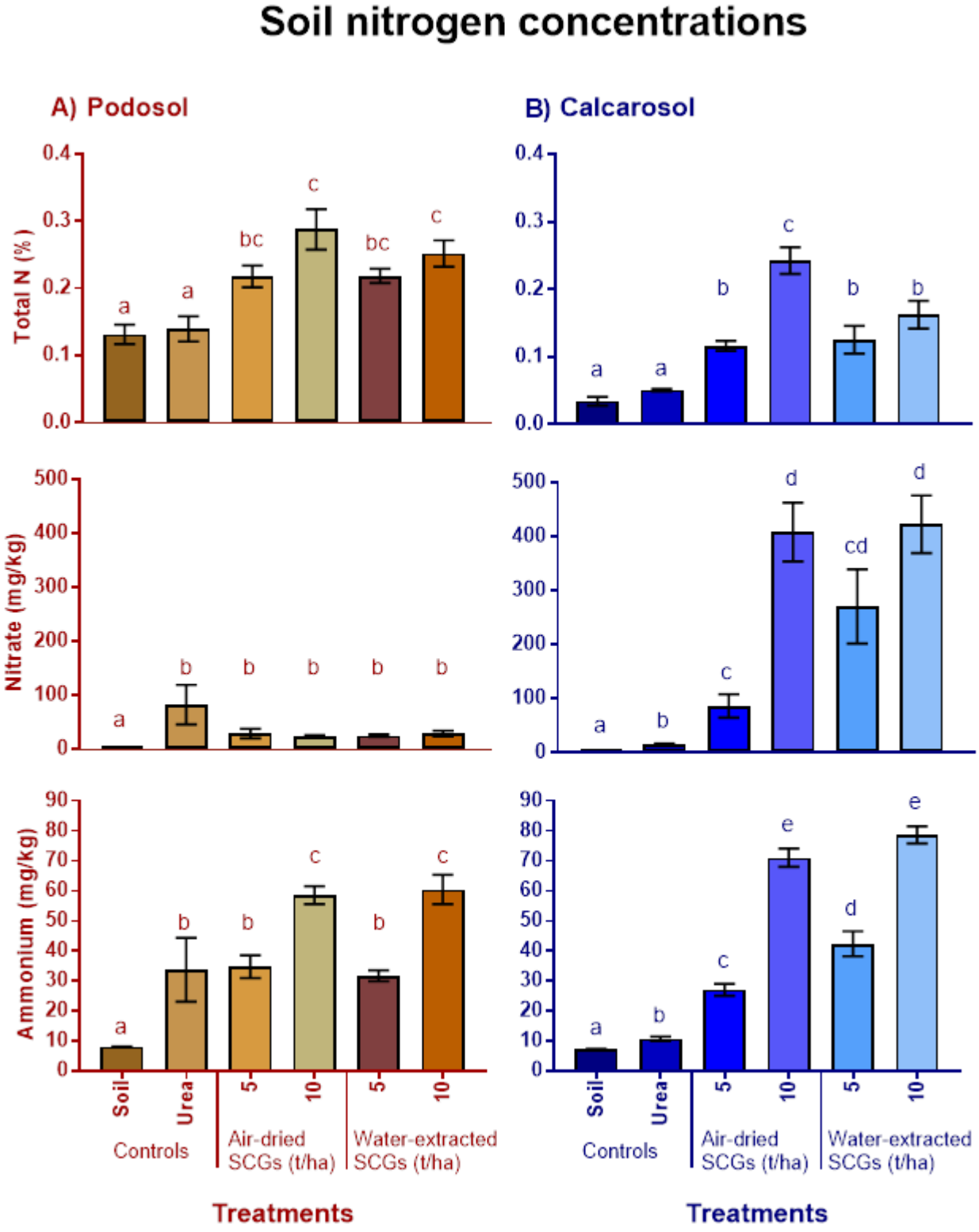

Soil nitrogen concentrations
A) Podosol
B) Calcarosol
Total N (%)
Nitrate (mg/kg)
Ammonium (mg/kg)
Soil
Urea
5
10
5
10
Controls
Air-dried SCGs (t/ha)
Water-extracted SCGs (t/ha)
Treatments

Figure 31 Comparison graphs for total nitrogen, nitrate and ammonium, for A) Podosol and B) Calcarosol. Columns represent averages, error bars denote standard error, *n*= 6. Letters show the result of Tukey's post-hoc test, significance *p*= <0.05.

4.4. Discussion

4.4.1. Root elongation and emergence tests for lettuce and millet

This study indicates that there is a threshold where the SCG extraction liquid could stimulate lettuce growth; however, generally the root elongation trials using the extracted liquid caused a reduction root in growth with increasing percentage of liquid. For lettuce, 10% of the liquid increased plant growth compared to controls, which was equivalent to 15.9 mg/L caffeine and 48.4 mg/L total phenols.

With applications of extracted liquid, millet growth only showed significant growth inhibition at 100% liquid, corresponding with 158.6 mg/L caffeine and 483.7 mg/L total phenols. Applications of 25% and above of liquid showed negative effects on lettuce root elongation, corresponding with 80 mg/L caffeine and 242 mg/L total phenols. These concentrations have been reported in the literature to have phytotoxic effects, with >50 mg/L of caffeine using ladino clover seedlings (McCalla and Haskins, 1964) and >108 mg/L phenols on lettuce roots (Park et al., 2016). Phenol concentration rapidly (within 2 days) inhibits cress root growth (Lyu et al., 2018), where concentrations as low as 20 and 22 mg/L can reduce root elongation by 50% (Park et al., 2012, Hulzebos et al., 1993). While it is important to take into account that the extraction liquid tested is a complex solution and not all of its components were identified or quantified, it is likely that the reduction of growth could be at least partially to due to the caffeine and/or phenol content. This is especially true, given that caffeine and phenols cause plant inhibition at low concentrations (Wink et al., 1998, Lyu et al., 2018).

In the emergence trials, the application rates of air-dried SCGs used here which resulted in lettuce inhibition (at rates of 6.6 and 8.7%) were similar to those that resulted in plant growth inhibition in published literature (Gomes et al., 2014, Cervera-Mata et al., 2018). The application of air-dried

SCGs decreased lettuce germination in comparison to water extracted SCGs. Water-extracted SCGs did reduce the inhibition of lettuce growth, suggesting that extractions could help with early growth inhibition; however, this treatment does not seem economically beneficial enough to use on a large scale. While inhibition was noted in the lettuce growth, these results were not confirmed for millet, with no SCG treatment having a detrimental impact on plant emergence.

4.4.2. Silver beet growth trial

Application rates of 5 and 10 t/ha (1.8 and 3.7% mass) used in this study had neutral or positive effects on silver beet growth in the acidic Podosol. Only the higher application rate resulted in significant growth inhibition in the alkaline Calcarosol soil. Previous studies have also confirmed that low application rates are beneficial for plant growth: Gomes et al. (2014) found that SCGs at application rates of 2.5 and 5% stimulated lettuce growth (although not significantly). Cruz et al. (2012a) also found that 2.5-10% SCGs improved lettuce growth, similarly Cruz et al. (2015) found that up to 10% SCGs had no yield loss of lettuce. However, similar experiments conducted on Australian soils have found that SCG applications as low as 2.5% resulted in growth inhibition of broccoli, viola, sunflower, radish and leek growth (Hardgrove and Livesley, 2016).

While there are encouraging results with the Podosol yield, the question remains as to why the silver beet was inhibited in the Calcarosol. Studies in the literature have suggested SCG causing plant inhibition could be influenced by plant species (Ciesielczuk et al., 2017, Yamane et al., 2014), application rate (Hardgrove and Livesley, 2016, Gomes et al., 2014, Cruz et al., 2012a, Cruz et al., 2015), or the application method and timing (Yamane et al., 2014). Notably, the results of this study suggest that soil type has an important role in the impact of the application of SCGs to silver beet growth. Only one study has highlighted that SCG impact alters with soil types (Cervera-Mata et al., 2018), and no studies have specifically reported that soil type is a major factor in the impact of SCG application on plant growth.

The question remains: why was there a contrasting impact on plant growth in the different soil types? The soil pH may offer an explanation for the impact on plant yields, as silver beet naturally favours slightly acidic to neutral soil pH (Wade, 2009). The pH change associated with the SCG application may have contributed to the increase in yield in the Podosol (the SCGs making the pH more alkaline). In the Calcarosol, the SCGs had a higher pH than the urea-only control treatment (where good plant growth was observed), but lower than the soil-only control treatment (where no plant growth was observed). It is suggested that no plant growth in the soil-only treatment was largely due to the lack of N available for plant growth, rather than the soil pH, as silver beet is known to require large amounts of N to survive (Wade, 2009). The decreased plant yield in the Calcarosol may be due to the SCGs increasing the pH out of preferred growing ranges, compared to the urea-only control treatment.

While the change in pH is unlikely to fully explain the yield differences, as the change is relatively small compared to the urea-only control, the impact on yield may be associated with the pH changes. For example, soil pH correlates directly with nutrient availability and is an important factor in microbial activity (Cardoso et al., 2013). Indeed, as previously noted, there was an increase of soil respiration in this study, which may indicate that the soil microbial community might be involved in these results. The reason for increase in pH maybe be similar to the processes observed with the increase of pH in composts with SCGs. Increases of pH in composted SCGs have been attributed to three main factors: 1) the mineralisation of proteins and peptides into ammonia, 2) the breakdown of acidic compounds, such as phenols or organic acids, and/or 3) humic acid production acting as a buffer (Santos et al., 2017).

The soil type and soil pH could be responsible for the detrimental effect of 10 t/ha SCG application on silver beet growth in the Calcarosol. Nitrogen immobilisation is unlikely to be the cause, because there was little indication of excessive nitrogen immobilisation. Despite extracting substantial caffeine and total phenolics, and emergence tests suggesting potential lower plant inhibition, the water extraction of SCGs did not reduce the negative plant growth impact of SCGs applications. A similar

result was found by Vardon et al. (2013) who observed that a 2% application of defatted SCGs to sorghum did not reduce detrimental plant growth. Yamane et al. (2014) suggested that residence time in the soil is factor in the effect on plant inhibition, where a longer time may assist in reducing plant inhibition. Further studies on the effect of SCGs on time and soil type, particularly with soils having low nutrient and organic matter content, is suggested.

Interestingly, there are a wide variety of published studies, often contradictory, examining the effects on plant growth after application of SCGs (Hardgrove and Livesley, 2016, Cruz et al., 2015, Yamane et al., 2014). Application methods of organic amendments can affect the impact on plant growth and soil health; however, application methods differed between this study and published literature. In the literature, SCGs were applied without fertiliser and incorporated deeper in the soil/growing substrate (Hardgrove and Livesley, 2016, Cruz et al., 2015, Gomes et al., 2014) or applied for longer periods of time (Kasongo et al., 2011, Yamane et al., 2014). The majority of research in the literature was conducted in substantially different environmental conditions; for example, increased humidity, higher temperature, higher rainfall (Yamane et al., 2014, Kasongo et al., 2011, Kasongo et al., 2013). This can significantly affect OM, such as SCGs, and decomposition rates. Hardgrove and Livesley (2016) mixed treatments into the first 10 cm of soil without fertiliser. Kasongo et al. (2011), Kasongo et al. (2013) conducted their experiment in a humid tropical climate and had a longer length of experiment (90 days to 24 months). Yamane et al. (2014) conducted their experiment in Nara, Japan, and found that beneficial effects to soil health (such as C: N ratio and organic matter) were observed 12 months after the SCG application. Therefore, this trial may not have provided enough time for the SCGs to decompose with the soil environment.

N_2O emissions were reduced in both soils in SCG treatments, to a greater extent in the Calcarosol, without significant effect upon soil mineral nitrogen concentrations. N_2O emissions from soil can be affected by many parameters, such as soil moisture content and aeration, pH and C: N ratio. However, reduction of N_2O emissions has been observed in studies using biochar with fertilization (Zhang et al.,

2010, Case et al., 2015). Zhang et al. (2010) attributed reduction N_2O emissions to the biochar influencing the N_2O reductase from denitrifying microorganisms as the pH increased and inhibiting the activity of reductase involved in the conversion of nitrite and nitrate to N_2O. A similar reduction of N_2O emissions and an increase of soil pH is observed in this study. Case et al. (2015) found that denitrification was the dominant source of N_2O production and biochar supressed denitrification and nitrification. Similar to Chapter 3, the results found in this study is encouraging for the management of this harmful greenhouse gas.

Indeed, there is certainly still much to be understood about the effect of SCGs on soil microbiology (Stylianou et al., 2018, Cervera-Mata et al., 2018). Cervera-Mata et al. (2018) hypothesised that the soil microorganisms that degraded the SCG particles could also generate nitrogen deficiency; however, nitrogen deficiency was not seen in the study. Additionally, it has been suggested that SCG influences the microbes within the microbial community that play a role in nitrification and denitrification (Stylianou et al., 2018). This study has shown that the soil microbial community may have an important role in the effect of SCGs to soil; therefore, in-depth biological investigations (which were outside the scope of this study) into which microbes are becoming more active, such as enzymes and DNA sequencing, is warranted.

4.5. Conclusions

Extracted liquid from SCGs caused phytotoxicity, potentially from the high caffeine and polyphenol concentrations. Phytotoxicity was observed in lettuce root growth from ~40 mg/L caffeine and 121 mg/L polyphenols and millet growth at 159 mg/L caffeine and 484 mg/L polyphenols. Early growth of lettuce showed that air-dried SCGs had greater phytotoxicity in comparison to water-extracted SCGs, whereas millet emergence was unaffected by SCG applications. Application rates based on this work suggest ≥15 t/ha (≥6.6%) SCGs could be detrimental for the germination of some plant species.

On the other hand, the positive effects of ≤10 t/ha (≤3.7%) SCGs applications, blended with urea fertiliser, was higher for the acidic Podosol than the alkaline Calcarosol for silver beet growth.

Air-dried SCG applications of 5 t/ha, blended with urea, had neutral or positive effect on silver beet growth in both soil types, stimulating the microbial community and increasing total nitrogen and carbon content. Consequently, the application of SCGs at 5 t/ha might be a beneficial alternative use for SCGs. Further research into the effect of the soil application of SCGs on the structure and function of the soil microbial community, wider range of soil type and soil characteristics is warranted.

4.5.1. Future directions

The suggestions from these experiments is that the 5 t/ha application rates of SCGs was 'safer' than 10 t/ha application rates for plant growth. However, the impact of the application on plant growth was not consistent between soil types at the 10 t/ha SCG application rate. Therefore, there seems to be no specific application rate to predict whether the amendment will exhibit 1) beneficial results for plant growth and 2) impact on different soil types.

If a soil contamination approach is taken such as that in EPA VIC guidelines (e.g. IWRG621), which are based on a set of contaminant concentrations (EPAVIC, 2009a), the complex effects of the application observed in this experiment may lead to unwanted harmful impacts. One suggestion, that may help to avoid negative impacts, is completing a 4-6 week phytotoxicity rate-response experiment with targeted plant species, which is a test that potential appliers may do in order to obtain regulatory approval.

Chapter 5. Preliminary assessment of select food waste-sourced anaerobic digestates on early stage plant growth, soil emissions and mineral nitrogen leaching

5.1. Introduction

Anaerobic digestion is a complex biological process used to treat organic waste and to produce biogas, usually from agricultural and industrial waste materials. While there are many benefits associated with the anaerobic digestion process, a by-product called digestate needs to be disposed of or utilised.

The variability of digestates, due to diverse source material and digestion process, is a constant and recurring issue for alternative uses. Digestates usually have a high water component (>50%), are commonly alkaline (>7 pH), have a high OM content (39-75%) and high CEC (Nkoa, 2014, Risberg et al., 2016, Teglia et al., 2011b, Teglia et al., 2011a, Akhiar et al., 2017). Food waste as a source for anaerobic digestion is growing internationally; food waste is one of the primary inputs into anaerobic digestion technologies in the UK (Röder, 2016). This is also important given that food waste and related organic waste-sourced digestates have high agronomic value, due to nutrient availability and low heavy metal content (Tampio et al., 2016b, Sheets et al., 2015). The uses of digestates within the agricultural sector are diverse. For example, digestates from a range of sources have been successfully used to meet additional nutrient requirements (Kataki et al., 2017) and as a replacement fertiliser (e.g. Koszel and Lorencowicz, 2015, Tampio et al., 2016b, Alburquerque et al., 2012, Tiwary et al., 2015a).

Phytotoxicity trials on digestates have produced contradictory results (Möller and Müller, 2012); possibly due to the variability of digestate composition. The production of greenhouse gases, CH_4, N_2O and ammonia, as well as nitrate leaching are major concerns with the application of digestates to land (Nkoa, 2014, Möller, 2015, Möller and Müller, 2012). Microbial community changes, levels of heavy metals (Stefaniuk et al., 2015, Alburquerque et al., 2012), organic micropollutants (e.g. pharmaceuticals) and pathogens (Gómez-Brandón et al., 2016, Fangueiro et al., 2014) have also been observed.

While digestates have great potential for agricultural application, high nutrient concentrations need to be taken into account. In particular, nitrate leaching from agricultural soils is of great international concern due to its solubility in water (Crolla et al., 2013), leading to the adoption of strategies to reduce nitrogen issues like the Directive 91/676/EEC (the Nitrates Directive) (COM683, 2013). There is still substantial concern about nitrate concentrations in groundwater under sandy soils in the Netherlands (Van Grinsven et al., 2016). Nitrate leaching from digestate-amended soils seem to be impacted primarily by soil management changes (Möller, 2015).

Internationally, digestates may be used on agricultural land in certain circumstances according to particular regulations. For example, the UK Environmental Permitting Regulations allow limited-sourced digestate application (including some food waste sourced material) to land 50 t/ha in a 12 month period to provide benefit to the land (SI, 2010).

In this limited study, we addressed three concerns regarding digestate application to land: potential phytotoxicity, soil greenhouse gas emissions and leaching of nitrate and ammonium. This was conducted in two separate trials. The first trial was a phytotoxicity emergence trial with two digestates using radish and cress under a range of Total Nitrogen (TN) application rates. The second trial was a soil column study analysing GHG soil emissions and leaching of nitrate and ammonium ions using three digestates with a 250 kg/ha TN application rate on one soil type.

5.2. Methods and materials

Three digestates were sourced from waste companies in the Netherlands, collected in February-March 2017, analysed/characterised (Table 18) and stored at 4ºC until used or analysed. Total nitrogen and phosphorus contents of digestates were obtained by using HACH® Dr. Lange test kits. Total solids and moisture were determined by oven drying at 105ºC, and volatile solids were determined by oven drying at 550ºC. Nitrate (NO_3^-), ammonium (NH_4^+) and water reactive phosphorus were analysed by sulphanilamide/NED (Miranda et al., 2001), salicylate/nitroprusside (Foster, 1995) and molybdenum blue (Murphy and Riley, 1962) colorimetric methods, respectively. pH was determined using a MeterLab® Radiometer Analytical PHM210 electronic probe. Electrical Conductivity (EC) was completed by a HQ440d multi HACH probe.

DIG1 and DIG2 originated from the same source – a mesophilic digester operated on a mixture of supermarket food wastes. DIG2 was the thickened fraction (paste) of DIG1 (liquid) after liquid separation and addition of a polymer to promote dewatering. DIG3 (solid) was collected as the thickened fraction from the digestate of a thermophilic digester operated on Vegetable, Fruit and Yard (VFY) waste. DIG3 was distinctly different from DIG1 and DIG2 in that DIG3 had high amounts of woody materials and some visible plastics. In general, total nitrogen, total phosphorus and ammonium concentrations, as well as pH and total solids were consistent with the ranges reported in the literature (Nkoa, 2014). Ammonium concentrations in DIG3 were lower than those reported in the literature, 4.3 ± 1.0 NH_4^+ (% TN) DIG3 was comparable to 35-81 NH_4^+ (% TN) reported in Nkoa (2014). Electrical conductivity was slightly lower than reported in literature (Akhiar et al., 2017), although this may be due to liquid-solid digestate conductivity differences, where EC is expected to be higher in liquid waste due to the water solubility of salts.

Table 18 Characteristics of the digestates, analysis done on wet digestate samples, *n*= 3 for NH_4^+, NO_3^-; *n*= 2 for EC, moisture, Total solids, Volatile Solids; *n*=1 for pH, Total N (TN), Total P (TP).

Parameter	**DIG1**	**DIG2**	**DIG3**
pH	7.36	7.82	8.71
EC (dS/m)	5.69 ± 0.08	3.55 ± 0.10	2.12 ± 0.01
TN (g/kg)	8.96	5.84	12.11
NH_4^+ (mg/kg)	2875 ± 46	2128 ± 46	1427 ± 123
NH_4^+ (% of TN)	32.1 ± 0.5	36.4 ± 0.8	4.3 ± 1.0
NO_3^- (mg/kg)	84.5 ± 15	84.4 ± 10	75.6 ± 4
NO_3^- (% of TN)	0.94 ± 0.17	1.45 ± 0.17	0.62 ± 0.03
TP (g/kg)	2.3	0.8	4.0
Water reactive P (mg/kg)	526.4 ± 5	891.4 ± 12	375.8 ± 2
Moisture (% of mass)	89.9 ± 0.07	82.6 ± 0.11	57.2 ± 1.23
Total Solids (% of mass)	10.2 ± 0.01	17.4 ± <0.01	42.8 ± 0.01
Volatile Solids (% in TS)	75.4 ± <0.01	79.0 ± <0.01	48.6 ± 0.01

In all the analysis and trials, digestates were used without liquid-solid separation and without drying (unless otherwise stated). This was done to reduce amendment pre-treatment time and costs, as well as potentially increase efficiency and practicality of application.

5.2.1. Phytotoxicity trial

Phytotoxicity trials were completed with only DIG1 and DIG2 as early growth stage trials (due to time constraints), similar to Ramírez et al. (2008). The early growth stage trial included a five-day emergence daily count, thinning, then allowing a 14 day growing period, after which the plants were cut and weighed. The substrate used was 535 g of potting mix with 5.7 pH and an EC of 0.8 dS/m,

with components of clay, Baltic peat, garden peat, potting soil, Swedish peat and a mix that contains a 15-10-20 NPK ratio. Due to the high nutrient content of the potting mix no additional fertilisers were used. Pots were maintained at 60% water holding capacity.

DIG1 and DIG2 were applied directly (wet) and mixed roughly through the first ~5 cm of potting mix to mimic harrow/top soil mixing. Pots were randomised. Two plant species, radish (*Raphanus sativus*) and garden cress (*Lepidium sativum*), were used with seven and ten seeds, respectively, in each 14 wide, 12 cm deep plastic pot.

Digestates were manually mixed into the potting material on the basis of total nitrogen (TN) content in six application rates; the equivalent of 0, 50, 90, 180, 250 and 300 kg/ha TN being used. Each application rate was replicated five times. Application rates were based on common TN fertiliser practice for a range of vegetables and conformed to the Netherlands regulations (Schröder and Neeteson, 2008, RVO, 2017). The trial was conducted in a climate-controlled cell with the temperature set at 20 ± 0.1ºC, humidity set at 75% and a photoperiod of 14 hrs/day at 120 mmol/m/sec. Seed emergence was checked daily for five days, after which pots were thinned to five seedlings per pot (Ramírez et al., 2008). At 14 days, shoots were cut at the soil surface, weighed and the length measured; roots were not collected.

5.2.2. Soil column experiment

DIG1, DIG2 and DIG3 were used in a 33-day soil column trial, conducted in a climate-controlled cell with the temperature set at 20 ± 0.1 ºC and humidity 75%. Oven-dried soil was passed through 2-mm and 340 g of soil was mixed with digestate or fertiliser and put into columns (4 by 30 cm width by diameter). The soil used was a slightly acidic (6.43 pH) topsoil (0-25 cm) fine sandy Gooreerd-soil (NL classification) and Mollisol (US taxonomy), sourced locally (Table 19). Mineral nitrogen (ammonium and nitrate) concentrations as well as Colwell-P analysis was done as described above. High Colwell-P concentrations are relatively expected as P concentrations are high in Netherland

agricultural soils (Tóth et al., 2014). Soil total nitrogen was determined by a method used at the Wageningen University with near infrared spectroscopy; this method is accredited by the National Accreditation Body (known as RvA). Phosphorus analysis was done by P-Al using an extraction method using ammonium lactate-acetate (NEN, 2010). EC and pH were determined using 3A1 and 4A1 methods respectively - 1:5 soil to DI water extraction (Rayment and Lyons, 2011b), with the probes described in the Digestates section above.

Table 19 Soil characteristics. *n*= 3 for NH_4^+, NO_3^-, Colwell P and moisture; *n*= 2 for pH and EC; *n*= 1 for CEC, Al-P stock and Total N..

Parameter	Soil
pH	6.43 ± 0.09
EC (dS/m)	0.46 ± 0.02
CEC ($cmol^+/kg$)	6.9
Total N (g/kg)	1.14
NH_4^+ (mg/kg)	3.2 ± 0.5
NO_3^- (mg/kg)	46.4 ± 3.3
Colwell P (mg/kg)	223 ± 0.6
P-Al (mg/kg)	480
Moisture (% of dry mass)	2.9 ± 0.3

Leaching experiments had five different treatments, replicated six times, with treatments applied at 250 kg/ha TN (Figure 32). A high TN application rate was used to investigate intensive conditions based on regulation rates in the Netherlands (Schröder and Neeteson, 2008, RVO, 2017). Different quantities of each digestate were required to apply the same amount of nitrogen to each column, resulting in different levels of applied P. The total phosphorus applied ranged from 4.3 to 10.4 mg/column with a DIG3>DIG1>FCON>DIG2 application trend, as detailed below:

1. SCON – control treatment (no digestates)
2. FCON - fertiliser control (urea at 250 TN kg/ha, 0.09 g/column; potassium phosphate at 40 TP kg/ha 0.02 g/column)
3. DIG1 - digestate #1, 3.52 g/column
4. DIG2 - digestate #2, 5.38 g/column
5. DIG3 - digestate #3, 2.60 g/column

Figure 32 Soil column experiment set up, with gas sampling syringe. PVC caps were removed between greenhouse gas sample collections.

Digestates were manually mixed thoroughly with dry soil to minimise ammonia volatilisation, simulating recommended practices to incorporate and/or harrow digestates into soils (Wulf et al., 2002, Tiwary et al., 2015b, Sommer and Hutchings, 2001). Ammonia volatilisation is dependent on a range of material composition, soil, climate and management factors (Chantigny et al., 2004, Sommer and Hutchings, 2001); therefore, ammonia volatilisation percentage could not be estimated in this study and was unable to be analysed due to time limitations. Preliminary laboratory tests were conducted to determine the soil field capacity, which was 22.5% w/w water content, and subsequent

moisture management was based on the field capacity. Soil columns were brought up to 60% (w/w) water holding capacity before leaching, and the soil between leaching events was maintained at field capacity.

5.2.2.1. *Leaching nutrient concentrations*

160 ml of deionised water was added to each soil column for leaching events. Leaching events were done on days 5, 12, 19 and 26 with a total of four leaching events (L1-L4). After 1.3 hours collection time, leachate sample volumes were recorded and were immediately filtered through a 0.45 µm syringe filter. Samples were stored at -20^{o}C until analysed for nitrate, ammonium and water reactive phosphorus.

Due to low concentrations, water reactive phosphorus was only analysed in L1 and L2. Colorimetric analysis was used, in a similar manner as described in the Digestates section above. Samples were run in triplicate and standard concentrations were altered to suit the range of the samples.

5.2.2.2. *Recovered soil*

At the end of the trial, the soil in the columns was recovered, air dried, homogenised and analysed for Colwell P- and KCl-extractable nitrate and ammonium. Sodium acetate (NaOAc) extractions were completed on a subset of soil samples for colorimetric analysis of soil-bound ammonium.

5.2.2.3. *Soil emissions*

Soil gas emissions (CH_4, CO_2, and N_2O) were collected from the soil columns on the day of the digestate application (0) and then Days 3, 5, 7, 12, 19, 26 and 33 after application. Gas samples were taken after 10 minutes of capping the individual columns with a fitted plastic cap and rubber septum for air-tight syringe/needle gas collection. Initial greenhouse gas samples were analysed at Wageningen University and Research (WUR) centre to assess the gas saturation point of the soil column headspace, to make sure sampling was taken within the linear increase of gases in soil column

headspace. The gases, CH_4, CO_2, and N_2O were analysed using a Agilent technologies 7890A-G3440A Gas Chromatography system using Thermal Conductivity Detection (TCD) and Flame Ionization Detection (FID) in Monash University laboratories located at Clayton, Australia. Cumulative soil emissions were calculated following the method detailed by Van Zwieten et al. (2010).

5.2.2.4. *Data analysis*

Data analysis was completed by IMB SPSS Statistics 25 software package. For all tests, the significance value chosen was 5% (p= 0.05), where <0.05 was considered significant. If significant, the Levene test was used to check for homogeneity of variances, then the data was analysed using non-parametric Kruskal-Wallis and Mann-Whitney U tests. However, if data was normally distributed, Tukey's test was used. Critical F-values were checked when p values were significant. Graphs in this chapter were produced with GraphPad Prism, version 7.02.

5.3. Results

The primary difference between DIG1 and DIG2 was that DIG1 had higher TN and total phosphorus content (Table 1). DIG2 had a slightly higher relative percent of ammonium, with 32% of TN being ammonium for DIG1 vs 36% of TN being ammonium for DIG2 (n= 1 for TN, n= 3 for NH_4^+). The digestate streams had similar nitrate concentrations (84.5 and 84.4 mg/kg).

5.3.1. Phytotoxicity trials

5.3.1.1. *Early stage growth*

Phytotoxicity trials with DIG1 and DIG2 indicated that cress early stage growth was not significantly affected by a wide range of applications based on TN (p= >0.05). Plant growth was expressed as a percentage of the control weight. The lowest growth rate was observed at 300 TN kg/ha with DIG1

cress having 69 ± 9 % of the control plant weight. Phytotoxicity trials with radish were only significantly affected (p= <0.005, n= 25) with growth at the highest application rate of 300 kg/ha with DIG1 and DIG2 being 72 ± 8 % and 62 ± 9 % of the control plant weight, respectively (Figure 33).

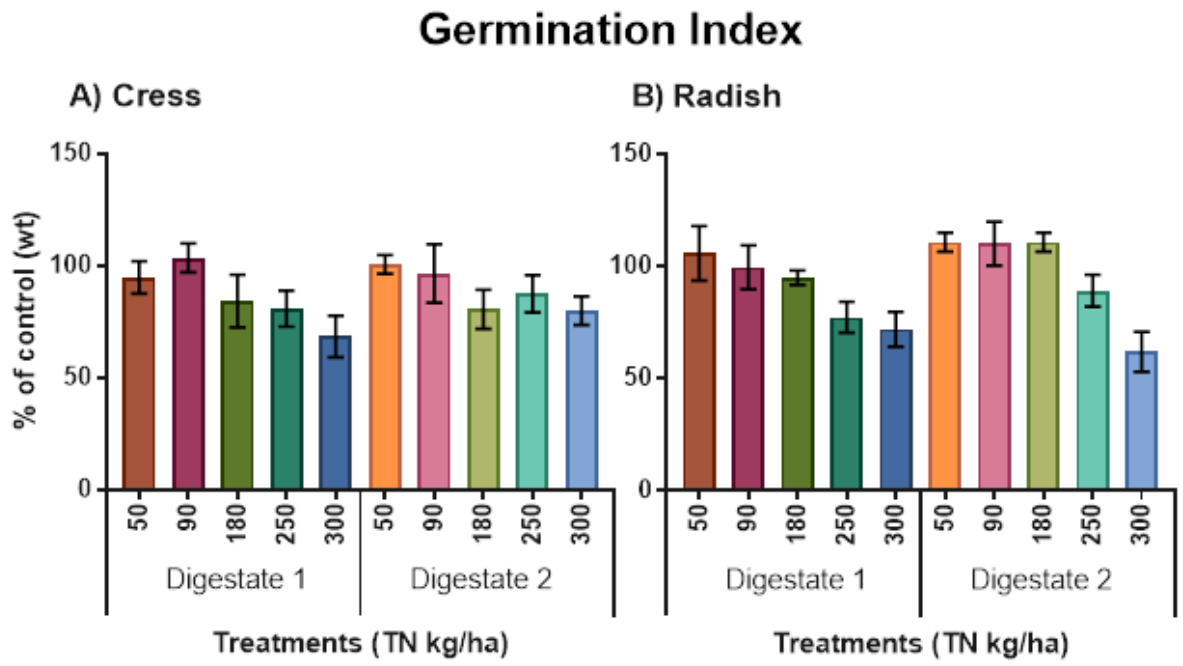


Figure 33 Early stage plant growth trial fresh weight results with A) cress and B) radish. n= 6, error bars denote standard deviation.

5.3.2. Soil column experiment

5.3.2.1. *Leaching nutrients*

All ammonium and nitrate concentrations collected in the leachates over four events were significantly different with treatments (p= <0.001) (Figure 34). When comparing A) and B) in Figure 34, nitrate concentrations exceeded ammonium concentrations in L1 (excluding FCON which leached more ammonium). However, from L2 onwards, ammonium loss exceeded nitrate losses.

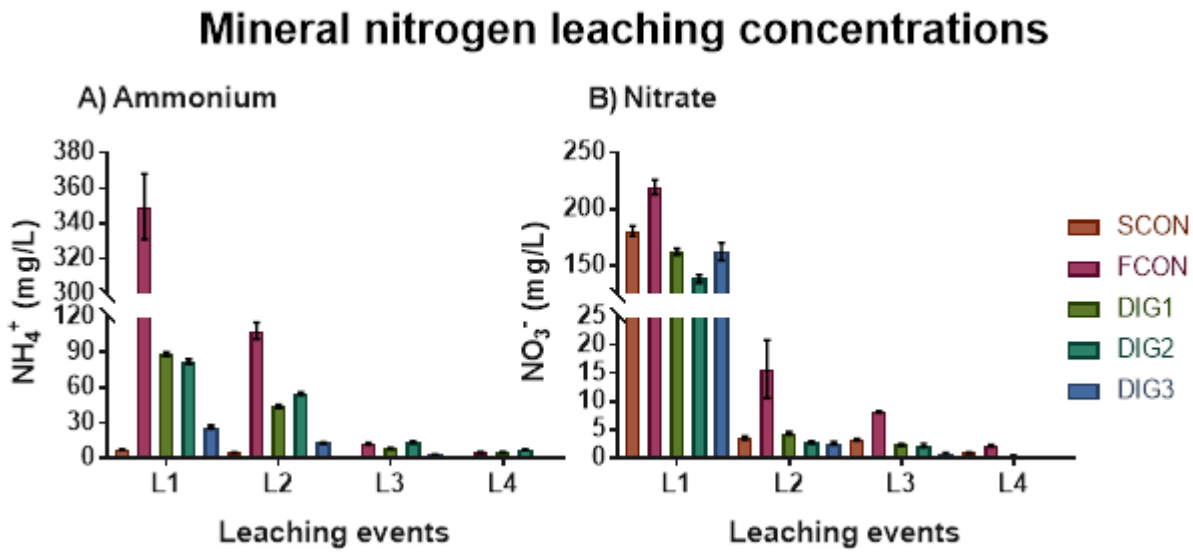


Figure 34 Nutrient leaching, A) ammonium and B) nitrate concentrations in the different leaching events, *n*= 18, error bars denote standard error.

Cumulative leached mineral nitrogen was calculated (Equation 1) and was significantly different with treatments (*p*= <0.001). FCON lost >100%, DIG1 and DIG2 lost 45-46% and DIG3 lost 5% of applied TN through leaching. Leached ammonium concentrations in FCON were substantially higher than the applied total nitrogen, this could be explained by background nitrogen from the soil being leached. Increased CO_2 emissions immediately after application may indicate an increased nitrogen mineralisation/turnover in FCON, although further no studies have been conducted to confirm this theory.

Equation 1: [((NO_3^- + NH_4^+ mg collected per column) – control)/ total nitrogen applied) *100]

Absolute concentrations in L1 and L2 showed that FCON lost the highest ammonium and nitrate concentrations (both *p*= <0.001). Ammonium concentrations in L3 showed that FCON still had the highest loss of ammonium (*p* = <0.001), although overall concentrations were substantially lower in comparison to L1 and L2 (average 108 ± 7 in L2 vs L2 ± 0.9 mg/L in L3). In L4, ammonium concentrations were highest in DIG2 (7 ± 0.3 mg/L NH_4^+).

Coincidentally, the amount of applied ammonium concentrations was similar between DIG1 and DIG2 columns (approx. 11.2 vs 12.5 mg NH_4^+ per column). There were no significant differences in L1 losses (p= 89 ± 1.7 and 82 ± 2.1 mg/L NH_4^+). However, in L2 ammonium loss from DIG2 exceeded DIG1 ammonium concentrations (55 ± 1.1 vs 44 ± 1.2 mg/L NH_4^+), where nitrate loss from DIG1 was higher in L2.

Nitrate concentration losses were highest in FCON throughout the leaching events (p= <0.001). Nitrate concentration losses were lowest in DIG3 in L2 and L3 (p= <0.001 for both events), and similar to DIG2 in the L4 (p= 0.673). SCON had the second highest losses of nitrate in L3 and L4. Nitrate concentrations in DIG1, DIG2 and DIG3 were close to or below detection limits (0.03 NO_3^- mg/L (Miranda et al., 2001)).

In L1, water reactive phosphorus concentrations where generally low (total average 236 ± 38 µg/L PO_4^{3-}) and were significantly different with treatments (p= <0.001). There was an observed FCON> DIG2>DIG1>DIG3=SCON trend, equals indicating similar concentrations (Figure 35). L2 had differences between treatments in P losses (p= 0.015), with an overall water reactive phosphorus average of 90 ± 8 µg/L PO_4^{3-} (Figure 3). SCON had significantly lower and DIG1 had significantly higher losses, whereas FCON, DIG2 and DIG3 had similar concentrations (p= 0.811).

Total water reactive phosphorus leached was 0.01-0.33 % of total phosphorus applied. FCON lost the highest percentage of applied phosphorus (0.33%), followed by DIG2 (0.31%), then DIG1 (0.10%) and then DIG3 (0.01%). The low amounts of lost phosphorus could be a result of the soil effectively retaining phosphorus; however, total phosphorus analysis would be required to understand this further.

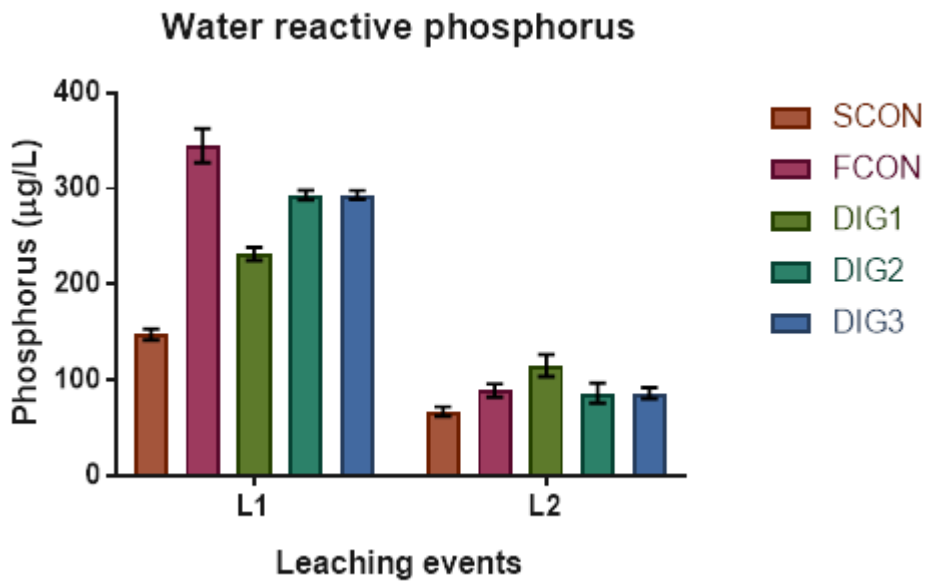


Figure 35 Water reactive phosphorus concentrations in the first two leaching events, columns represent averages, *n*= 12, error bars denote standard error.

5.3.2.2. ***Recovered soil***

Nutrient analysis of the soil to analyse residual concentrations after the leaching events showed statistical differences in KCl and NaOAc extractable ammonium (p= <0.001 in both cases) (Figure 36). DIG2 had the highest residual (KCl and NaOAc extractable) ammonium concentrations. Residual nitrate concentrations changed with treatments (p= <0.001), observing a FCON>SCON=DIG1>DIG2>DIG3 trend, where equals indicate similar concentrations. All treatments had similar pH values, with a total pH range of 6.91 to 7.22 in all treatments.

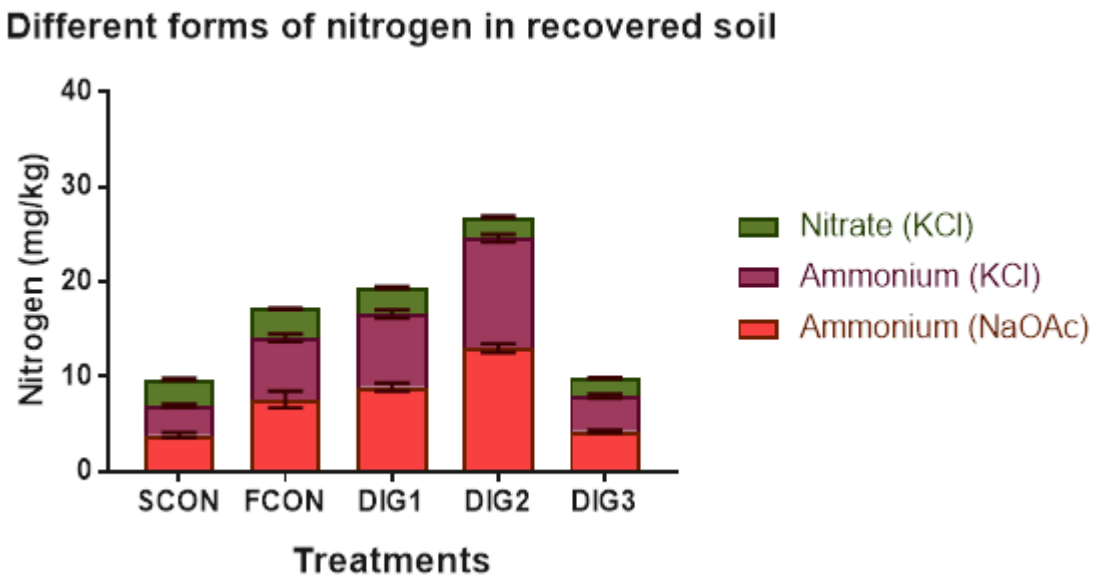


Figure 36 Different forms of nitrogen in recovered soil, represents averages, KCl *n*= 18, NaOAc *n*= 9.

5.3.2.3. *Soil emissions*

Cumulative CH_4 emissions from the soil showed differences at Days 7, 12, 26 and 33 (p= <0.05), where DIG2, in particular, had significantly higher CH_4 emission after Day 12 (Figure 37 A). When considering individual CH_4 fluxes at each time point, days 5 (p= 0.001) and 12-33 (p= <0.03) had significantly different emissions (Figure 38 A).

Cumulative CO_2 emissions emitted from the soil showed differences at all days (p= <0.005) (Figure 37B). CO_2 was higher from DIG2 in comparison to other treatments 5 and 7 days after application. CO_2 emissions trended DIG2>DIG1/DIG3>FCON=SCON, equals indicating similar emissions. When considering individual CO_2 fluxes at each time point, days 0-5, 12 and 19 had significantly different fluxes from treatments (p= <0.001 for days 0-5, p= <0.01 for days 12 and 19) (Figure 37 B).

Cumulative N_2O emissions from treatments showed differences from Day 5 (p= <0.05) (Figure 37 C). These emissions were higher from DIG1 in comparison to FCON (all p= <0.03) and compared to DIG2, DIG3 and SCON (all p= <0.005). Cumulative N_2O emissions from Day 1 trended DIG1>FCON=DIG2>DIG3=SCON, equals indicating similar emissions.

When considering the N_2O individual fluxes at each time point, all days were significantly different (p= <0.02) except Day 7 (Figure 38 C). Immediately after application (Day 0) observed extremely high emissions for DIG1 compared to other treatments, with DIG1 emissions at 2352 N_2O N $\mu g/m^{-2}h^{-1}$ vs other treatments ranging 688-780 N_2O-N $\mu g/m^{-2}h^{-1}$ (p= 0.005). At day 5, DIG2 N_2O flux was substantially higher than other treatments (p= <0.001). Day 12 observed higher N_2O emissions for FCON (p= <0.01).

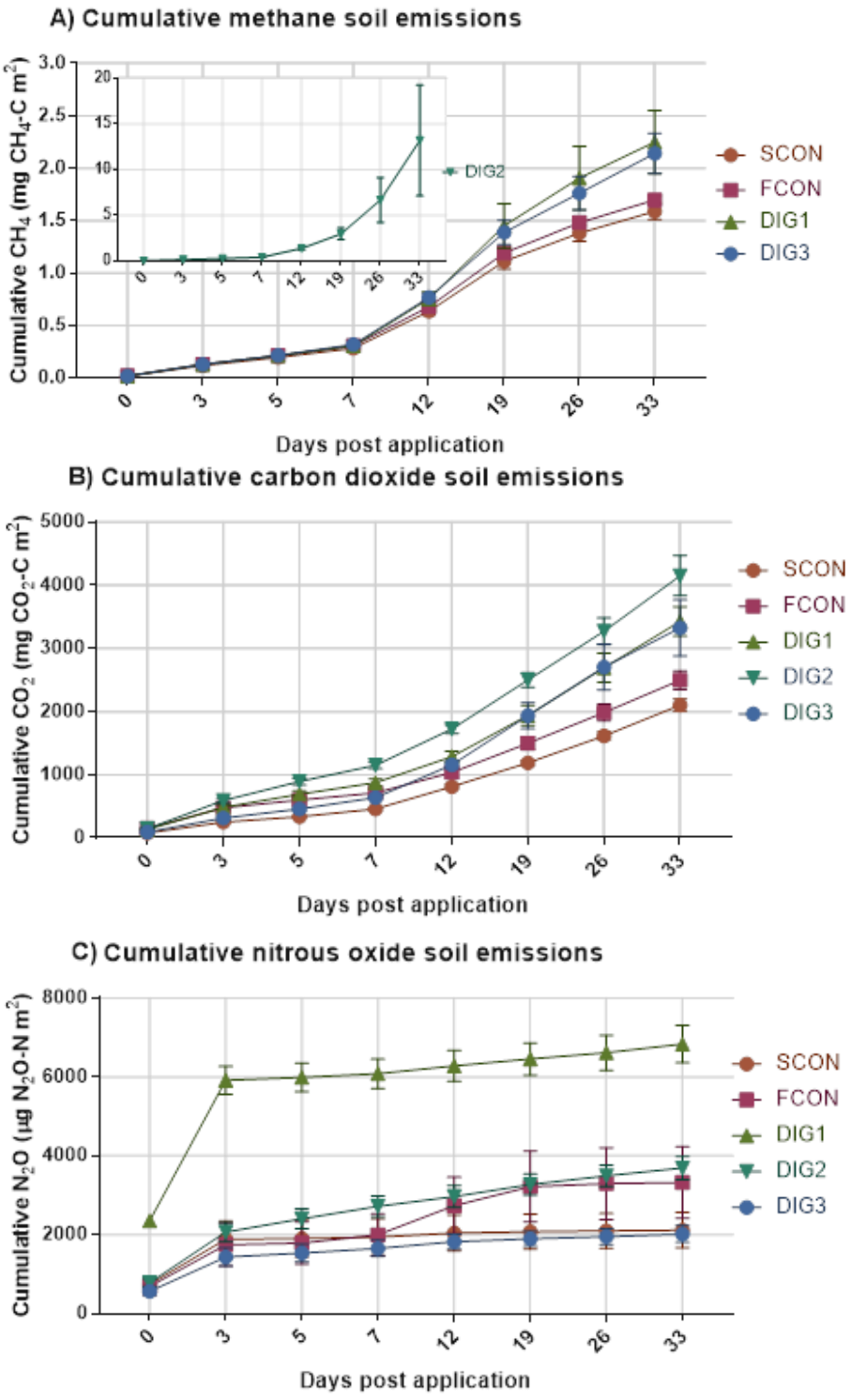


Figure 37 Cumulative emissions emitted from column soils A) Methane, B) CO_2 and C) N_2O. Points represent averages, *n*= 6, error bars denote standard error.

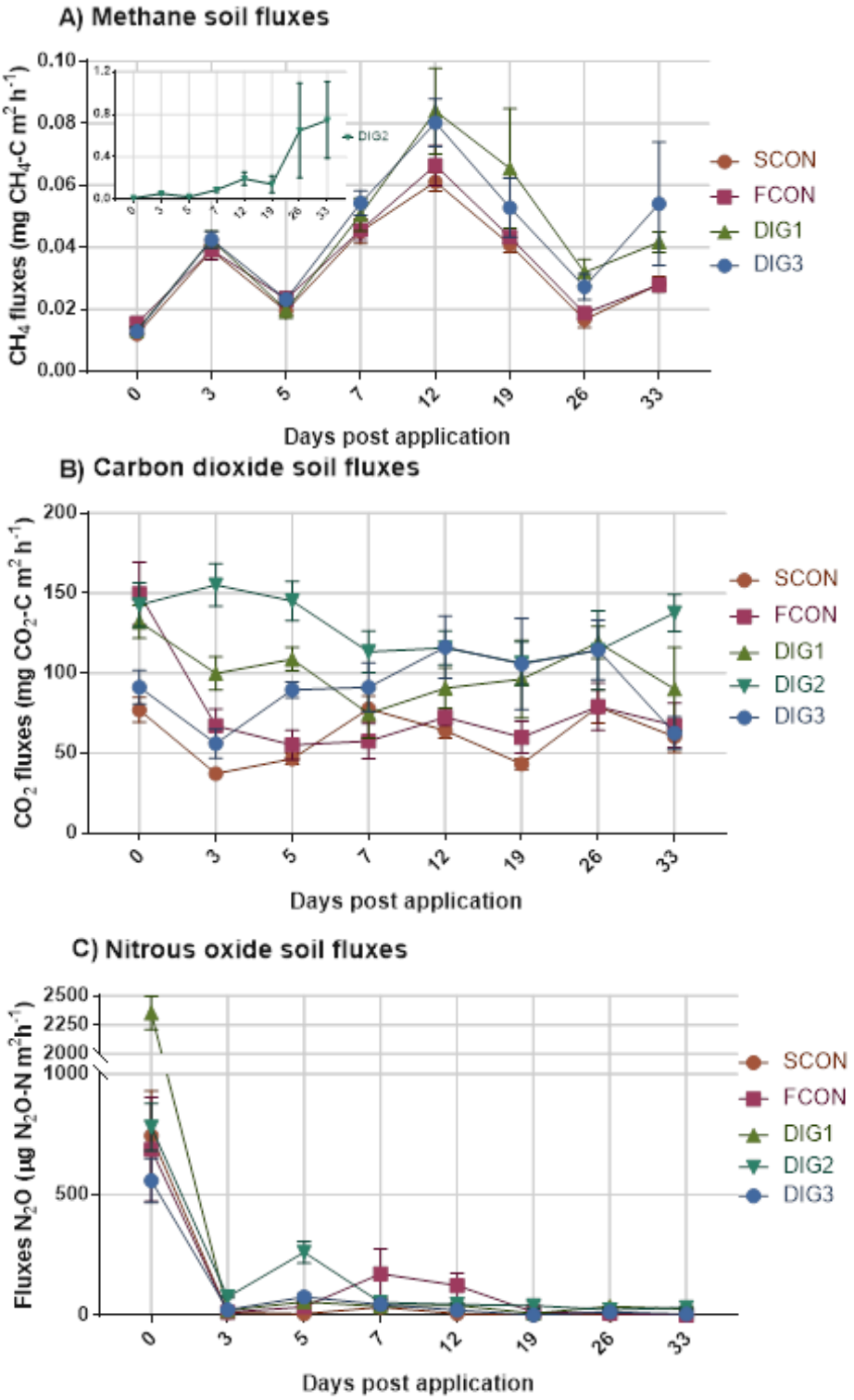


Figure 38 Greenhouse gas fluxes emitted from column soils A) Methane, B) CO_2 and C) N_2O. Points represent averages, *n*= 6, error bars denote standard error.

5.4. Discussion

5.4.1. Phytotoxicity trials

Phytotoxicity trials in this study found little effect on early stage plant growth over a broad range of application rates, with only inhibited plant growth in one treatment (radish, 300 TN kg/ha with DIG1). Phytotoxicity tests with digestates in the literature have been contradictory (Möller and Müller, 2012). Gell et al. (2011) found that 150 TN kg/ha application of cattle manure and human excreta-sourced digestate had no phytotoxicity effects on shoot elongation of lettuce, radish and wheat in a sandy soil. Stefaniuk et al. (2015) found that the solid fraction of six biogas residues were non-toxic; however, liquid fractions and non-separated material showed ecotoxicity. Ramírez et al. (2008), however, found that low (3-100 g/kg) doses of biosolid digestates had moderate phytotoxicity on field mustard, rye grass and red clover shoot weight and length.

Literature reports have suggested that phytotoxicity can be due to easily biodegradable organic material (Abdullahi et al., 2008, Ramírez et al., 2008), such as food and garden waste (Verma, 2002), and total and/or ammonium nitrogen content (Ramírez et al., 2008). The phytotoxicity in this study is possibly due to the high dose of nitrogen, as 300 TN kg/ha is extremely high. The reason for little or no inhibition observed in this trial is likely due to the early growth stage of the plants; however, there might have been more inhibition observed if the plants were fully grown.

5.4.2. Soil column experiment

5.4.2.1. *Soil column leaching*

The peak of ammonium loss was highest for FCON with 350 NH_4^+ mg/L and for digestate treatments at 27-89 NH_4^+ mg/L. These ammonium concentrations are extremely high even compared to similar leaching trials with sandy soils (Li et al., 1997). The peak of nitrate leaching in this study was 220 NO_3^- mg/L in FCON, followed by SCON at 181 NO_3^- mg/L and digestate treatments from 139-163

NO_3^- mg/L. DIG3 had the lowest leached nutrients (after SCON), potentially due to higher total solids, primarily made up of woody material, with reduced leachability.

Li et al. (1997) found a peak of 29 mg/L NH_4^+ and 246 mg/L NO_3^- with 100 t/ha application of compost made from biosolids and municipal solid waste (TN of material ranging from 0.91-2.11%). Goberna et al. (2011) found that in a 100-day lysimeter trial with 40 mg/kg TN biogas digestate and manure treatment, leached concentrations averaged at 0.3 µg/ml NH_4^+ and peaked at ~165 µg/ml NO_3^-. In a 40-week soil column study, a 20 mg/L NO_3^- leaching peak was observed for a 50 t/ha municipal solid waste compost treatment (Kjeldahl-N 1.26 % of municipal waste material) (Burgos et al., 2006).

High leached ammonium and nitrate concentrations found in this study are potentially due to favourable leaching conditions: course-textured soil (Gaines and Gaines, 1994), continued water saturation (Peverill et al., 1999) and the absence of plant for nitrogen uptake. The full leaching of applied nitrogen, as observed in this study in FCON, has been observed in controlled environments previously (Nardi et al., 2017, Wang and Alva, 1996). Importantly, the percentage of applied nitrogen that was leached from all digestate treatments was significantly lower than FCON (100% FCON vs 5-46% for digestates). Additional nitrogen that was leached from FCON can be explained by the leaching conditions being so favourable that background nitrogen from the soil was also leached. Increased CO_2 emissions immediately after application may indicate an increased nitrogen mineralisation/turnover in FCON, although further no studies have been conducted to confirm this theory. Although, this study cannot be compared to field conditions, due to overestimation of leaching potential in soil column studies (Goberna et al., 2011), it highlights possible future study areas. Particularly of concern is the leaching of nitrates from sandy regions to groundwater in the Netherlands, which exceed nitrate levels of 50 mg/L (Van Grinsven et al., 2016).

Ammonium was the main form of cumulative leached mineral nitrogen in all treatments, as has been observed in past studies (Li et al., 1997). This is potentially due to slower nitrogen turnover and/or lack of the soil's ability to retain ammonium ions (lack of negatively charged clay particles for the soil

to hold ammonium) and an alkaline pH (overall average of 7 ± 0.01 pH). Furthermore, that digestates can result in 45-80% of nitrogen in the liquid phase as ammonium (Möller and Müller, 2012) may help to explain the high amount of ammonium losses. However, in this study, it is unknown how much liquid phase ammonium is present because liquid-solid separation was not conducted.

5.4.2.2. *Soil emissions*

The highest CH_4 fluxes from the soil were in DIG2 treatments with a peak of 1.0 ± 0.48 CH_4 mg/$m^{-2}h^{-1}$. These emissions are high for aerobic soils, which are rarely higher than 0.1 CH_4 mg/$m^{-2}h^{-1}$ (Le Mer and Roger, 2001). However, these fluxes are low for anaerobic/wetland soil emissions, which have a median of 10 CH_4 mg/$m^{-2}h^{-1}$ (Le Mer and Roger, 2001). In fact, CH_4 emissions from free draining grassland (or similar) soils are usually considered negligible (Soussana et al., 2010). Indeed, field studies have found negligible CH_4 emissions following digestate application (Eickenscheidt et al., 2014).

Overall, cumulative soil respiration was increased in all digestate treatments in comparison to FCON and SCON treatments; however, FCON had high initial fluxes. High N_2O emissions from digestate treatments suggests that there was an increase in nitrogen turnover in comparison to the other treatments. Studies have suggested that biogas residues have a priming effect, increasing CO_2 and N_2O emissions in incubation and field experiments (Coban et al., 2015, Eickenscheidt et al., 2014).

Initial (Day 0) N_2O emissions from this study are higher than in reported field study experiments (Wulf et al., 2002); however, after Day 0, average fluxes are similar to field studies with similar digestate application rates (Eickenscheidt et al., 2014). Eickenscheidt et al. (2014) found that N_2O peaks in a field study were ~250 µg/$m^{-2}h^{-1}$ and rarely exceeded 50 µg/$m^{-2}h^{-1}$ with 104 and 174 TN kg/ha application rate, observing a clear trend of digestate>slurry>control.

A major factor responsible for the higher relative CH_4 and N_2O seen in this study, could be water saturation. This may account of the high emissions as oxygen-deprived or submerged soils are likely

to produce higher CH_4 emissions (Le Mer and Roger, 2001) and N_2O production (Zhang et al., 2015, Bollmann and Conrad, 1998). Furthermore, residual easily biodegradable organic carbon compounds present in biogas residues (Köster et al., 2011, Möller, 2015, Barrena et al., 2009) could have contributed to increased CH_4, as materials that are easily degraded and organic matter mineralisation impacts CH_4 production (Segers, 1998).

5.5. Conclusions

The application of DIG1 and DIG2 in early stage growth phytotoxicity trials indicated that radish and cress growth were not significantly affected under a wide range of TN application rates. Digestate application only showed negative effect on radish short term growth with DIG1 applied at 300 TN kg/ha.

Mineral nitrogen concentrations that leached from soils were high, but encouragingly, digestates leached lower concentrations than the fertiliser control. Proposed reasons for the high concentrations include: a high nitrogen application rate, soil texture, favourable environmental conditions (water saturated) and lack of plants. FCON had higher losses of ammonium and nitrate, potentially due to higher relative amounts of mineral nitrogen and a high initial nitrogen turnover rate. Peaks in CH_4 emissions were high for aerobic soils (Le Mer and Roger, 2001), and N_2O and CO_2 emissions indicated higher rates of nitrogen and carbon turnover in DIG1 and DIG2 treatments. High CH_4 emissions, with ammonium losses as well as high KCl and NaOAc extractable ammonium concentrations in DIG1 vs DIG2, indicated lower nitrogen turnover, resulting from water-saturated soil conditions.

The benefits of applying these digestates were no negative effect on early stage plant growth, reduced mineral nitrogen leached concentrations compared to fertiliser treatments, and digestates added soil nitrogen for two digestate applications. The risks observed were that soil condition (i.e. water saturation) is important, nitrate concentrations exceeded 50 mg/L set by standards (Van Grinsven et

al., 2016) as well as digestate applications lead to high CH_4 and N_2O emissions. These risks are highlighted in regulations regarding digestate application to land, therefore adhering to these regulatory requirements must be a priority to avoid environmental and human health harm.

Chapter 6. Application of anaerobic digestate to soil columns with two contrasting textures

6.1. Introduction

In Australia, anaerobic digestion is a new, but rapidly growing, industry (Edwards et al., 2015). Australia is one of the world's highest producers per capita of food waste (Zaman and Reynolds, 2015); therefore it is advantageous to utilise anaerobic digestion as a promising sustainable treatment of food waste (Ren et al., 2017).

Currently, there are only four digestion plants in Australia that receive solid organic waste (Clarke, 2018) but these technologies could be applied in an economically beneficial manner on a larger scale (Zaman and Reynolds, 2015, Clarke, 2018). Given an increased need for nutrient recovery, digestates offer a viable sustainable approach to divert food waste from Australian landfills and recycle plant nutrients in agriculture (Clarke, 2018). As a large number of the world's anaerobic digestion plants are located in Europe and America (Sheets et al., 2015) and only a few large-scale plants exist in Australia (Clarke, 2018), it is unsurprising that there has been minimal research of the impact of digestates on Australian soils. Due to a growing anaerobic digestion industry in Australia (Edwards et al., 2015) and the high agronomic value by-product (digestates) (Tampio et al., 2016b), there is a compelling need to investigate Australian digestates in conjunction with soil type, to ensure that digestates are used in an appropriate manner in different agricultural systems.

Australian soils are uniquely biodiverse (Hobday and McDonald, 2014) and organic amendments must adhere to regulations and maintain low levels of contaminants in order to protect this diversity and increase productivity. Anaerobic digestion by-products potentially offer a highly valuable alternative fertiliser, that can have positive effects on plant yield and soil health (Nkoa, 2014, Möller and Müller, 2012). Digestate application must meet relevant regulatory requirements; therefore, the Environment Protection Authority, New South Wales, regulations (EPANSW, 2014b) were considered in this study. The exemption EPANSW (2014a) was used as a guideline for this study, and dictates that food waste application is based on the "agronomic rate for the most limiting factor"

where leaching, run-off and sub-surface flows are of particular concern. Furthermore, according to these regulations, application must minimise environmental harm and provide a net benefit (EPANSW, 2014a).

In the context of potential contaminants, two guidelines were referenced. First, the Western Australian "Ecology Investigation Levels" (EILs) were used for initial screening to investigate if concentrations posed a potential risk to the environment and associated environmental value (DEC, 2010). EILs are based on a wide range of environmental protection and contamination guidelines and indication levels, including (but not limited to) levels set by the US Environmental Protection Agency, Australian and New Zealand Environment and Conservation Council (ANZECC), National Environment Protection Council (NEPC) and other research council protection guidelines (DEC, 2010). Second, the Industrial Waste Resource Guidelines Solid Industrial Waste Hazard Categorisation and Management (IWRG631) (EPAVIC, 2009b) and Soil Hazard Categorisation and Management (IWRG621) (EPAVIC, 2009a) were used to identify potential contamination.

The aim of this work is to investigate low application rates of liquid digestates, blended with conventional fertilisers, for the purpose of understanding the impact on short-term plant growth (using spinach), greenhouse gases emitted from soil, leached concentrations and soil concentrations. Electrical conductivity (EC) is a measurement of soluble salts, including but not limited to the major cations (Ca, Na, Mg, K), which are essential for plant growth and soil health. However, high salt concentrations are well known to be harmful to plant growth and the concentrations at which plant growth is impacted are highly dependent on the select sensitivity of plant species. Furthermore, depending on soil conditions and water table height, salts can leach to the surrounding environment (Rengasamy, 2002). The EC of digestates is generally high (>5 dS/m), where Akhiar et al. (2017) found that the EC ranged from 16-36 dS/m in liquid digestates. The salts Ca and Mg can range from 3-52 g/kg in dry matter from food-sourced digestates (Sheets et al., 2015); whereas, Nkoa (2014) reported that K can range from 0.12-1.15% in fresh matter.

Salinity in Australia is a critical issue that affects 30% of Australian land and 16% of agricultural land (Rengasamy, 2006); therefore, investigation of organic amendments containing high EC must be a key consideration. For this reason, the application rates in this study were determined based on EC, as it was identified as the most limiting agronomic factor. Soil texture is a major consideration regarding leaching and infiltration of potential contaminants. Soil texture is critical factor in salinity and metal accumulation, as clay is strongly correlated with the ability of soil to absorb or desorb chemical ions, by providing more cation exchange sites (Panta et al., 2016).

Background metal and metalloid concentrations due to natural variation, are high in Victorian soils, particularly As, Cr, Cu, Ni, Pb and Zn (Mikkonen et al., 2018). Australian soils also have high background V and Mn concentrations compared to EILs (Reimann and de Caritat, 2017). These high background total metal concentrations in soils have been attributed to different geological processes (Reimann and de Caritat, 2017, Mikkonen et al., 2018). Importantly, previous studies have suggested that Cu, Zn and Mn accumulation in soils after digestate application is an significant issue (Nkoa, 2014). Digestate application to Australian soils requires special consideration as background concentrations of Cu, Zn and Mn in Australian soils might be high already. As previously discussed, N_2O and CH_4 soil emissions are a prominent issue for digestate-amended soils (Nkoa, 2014) and were included in this trial.

It is hypothesised that metals and salts would accumulate in the soil with the higher clay content and that there would be more digestate-associated element leaching from the sandy textured soil. Research questions were:

1. Does digestate application, blended with fertilisers, affect spinach growth?
2. Does digestate application increase greenhouse gas emissions (CH_4, N_2O, CO_2) emitted from the soil?
3. Do the digestates increase soluble metals in leachates and total metals in soils compared to the selected guidelines of EILs and EPAVIC regulations?

4. Is there a distinct difference in the impact of the amendment application (questions 1, 2 and 3) between the two contrasting soil textures of clay and sandy soils after digestate application?

6.2. Materials and methods

6.2.1. Characterisation

Digestates were collected from a small Melbourne-based business, Active Research PTY LTD (http://www.activeresearch.com.au/), from July to September 2017. After liquid (<0.124 µm) and solid (>0.124 µm) separation using a 124 µm sieve, materials were stored at 4^{o}C until analysis. The liquid portion of the material was used for all experiments, with the liquid fraction accounting for more than ~90% of the original material.

PerkinElmer Avio 200 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) was used for analysis of soil and digestate metals. The PerkinElmer multi-mix element standard (Ag, Al, As, B, Ba, Be, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Sn, Sr, Ti, Tl, V, Zn) with 5% HNO_3 were made to the manufacturer's guidelines; diluted to 0.05, 2.5, 12.5, 25 and 50 ppm for all elements with MilliQ water. pH and EC were determined by electronic probes, after liquid and solid separation, by WP-80 TPS and sensION+ EC5 portable HACH® probes, respectively. Nitrate and ammonium concentrations were analysed by the sulphanilamide/NED (Miranda et al., 2001) and salicylate/nitroprusside (Foster, 1995) colorimetric methods, respectively.

The liquid fraction of the digestates were acidified to <2 pH with H_2SO_4, moisture eliminated by freeze-drying, then analysed with a Carbon Hydrogen Nitrogen (CHN) analyser. The CHN analysis was undertaken with an Elementar vario MICRO cube analyser equipped with a CHN combustion and reduction tube using a Temperature Programmed Desorption trap column and Thermal conductivity

detection. In order to obtain a representative sample, liquid digestates were mixed thoroughly manually, and a composite of three samples was used for final analysis (Table 20).

Table 20 Digestate characterisation, pH, EC, total N and C, and soluble forms of elements and metals, *n*= 1. Soluble forms are noted where logical, but left general where the form is more complex, following Wuana and Okieimen (2011).

Parameter (unit)	**Vegetable digestate (Veg)**	**Mushroom digestate (Mush)**	**Limits (mg/L)**
pH	8.97	7.75	6-9^
EC (dS/m)	7.13	9.82	<0.8 - 1[#]
DM (% mass)	1.0	0.9	
TN (%) dry wt	7.09	7.22	
TC (%) dry wt	5.58	5.48	
NH_4^+ (mg/L)	815	1467	
NO_3^- (mg/L)	22	28	2,500*
PO_4^{3+} (mg/L)	3.8	4.9	
Ca^{2+} (mg/L)	72	245	
K^+ (mg/L)	740	696	
Na^+ (mg/L)	175	21	115 - 230[#]
Cl^- (mg/L)	396	348	180-355[#] - 12,500*
Mg^{2+} (mg/L)	650	872	
Al^{3+} (mg/L)	0.53	<0.05	20^
Fe (mg/L)	0.98	0.16	10^
Mn^{2+} (mg/L)	0.20	<0.05	10^
Zn^{2+} (mg/L)	1.05	0.08	5^ - 150*
Ag^+ (mg/L)	<0.05	<0.05	5*
As (mg/L)	<0.05	<0.05	2^ - 0.35*
B (mg/L)	0.31	0.30	0.5^ - 15*

Parameter (unit)	Vegetable digestate (Veg)	Mushroom digestate (Mush)	Limits (mg/L)
Ba^{2+} (mg/L)	<0.05	<0.05	35*
Cd^{2+} (mg/L)	<0.05	<0.05	0.05^ - 0.1*
Co (mg/L)	<0.05	<0.05	0.1^
Cr^{6+} (mg/L)	<0.05	<0.05	1^ - (Cr^{6+}) 2.5*
Cu^{2+} (mg/L)	0.80	<0.05	5^ - 100*
Mo (mg/L)	<0.05	<0.05	0.05^- 2.5*
Ni^{2+} (mg/L)	<0.05	<0.05	1* - 2^
Pb^{2+} (mg/L)	0.10	0.00	0.5* - 5^
Se (mg/L)	<0.05	0.10	0.05^- 0.5*
V (mg/L)	<0.05	<0.05	0.5^

^EILs for short-term irrigation water, Government of Western Australia, DEC (2010).*Industrial waste categorisation, IWRG631 (material meant for landfill), EPAVIC (2009b). #Irrigation water recommendations, Government of Western Australia (DPIRD, 2016), concentrations quoted for sensitive to moderately sensitive plant species.

6.2.2. Soil column trial

A three by two factorial experimental design was conducted. Three treatments were controls, only soil (named soil) and nitrogen, potassium and phosphorus (named NPK-only) fertiliser. Digestate treatments were mushroom-sourced digestate (named Mush) and vegetable sourced digestate (named Veg), with NPK fertiliser. NPK fertiliser was applied to all columns, excluding soil, 40 kg/ha phosphate, 60 kg/ha potassium and 80 kg/ha nitrogen (details below). The liquid fraction of the digestates were applied at two application rates, based on electrical conductivity (EC): 1 and 3 dS/m. This equated to 15-21 ml applied to each column, which this was diluted to 50 ml using DI water.

Treatments were:

A. Soil control (no NPK fertilisers)
B. NPK-only control
C. Veg 1 dS/m (with NPK fertilisers)
D. Veg 3 dS/m (with NPK fertilisers)
E. Mush 1 dS/m (with NPK fertilisers)
F. Mush 3 dS/m (with NPK fertilisers)

Soils were collected from Royal Botanic Gardens, Cranbourne (38°07'46.5"S; 145°16'14.1"E), and Echuca, North central VIC (36°09'18.9"S; 144°38'50.6"E). Soils were air-dried, sieved (2 mm) and homogenised. Soils were classified by soil maps (VRO, 2018) and by according to Isbell (1996) and characterised in Table 21 by Environmental Analysis Laboratory (EAL, 2018).

Table 21 Soil characteristics, *n*= 1.

Parameter (unit)	**Rudosol**	**Vertosol**
Soil texture	Sandy, ~5% clay*	Medium clay, ~45-55% clay*
pH	5.59	6.19
EC (dS/m)	0.005	0.349
OM (% of mass)	0.3	3.0
Na (mg/kg)	<50	174
Ca (mg/kg)	45	2,714
Mg (mg/kg)	51	2,914
K (mg/kg)	49	3,707
P (mg/kg)	129	455
Cl (mg/kg)	6	27

*Clay percentage based on the median for the texture class following Peverill et al. (1999)

Parameter (unit)	Rudosol	Vertosol
CEC ($cmol^+/kg$)	0.8	18.6
- Ca (% of CEC)	50.9	56.1
- Mg (% of CEC)	15.1	32.1
- K (% of CEC)	1.9	9.5
- Na - ESP (% of CEC)	2.0	2.2
- Al (% of CEC)	22.6	0.2
Ca: Mg ratio	3.4	1.7
TC (% of mass)	0.15	1.69
TN (% of mass)	0.01	0.17
C: N ratio	18.4	10.1
B (mg/kg)	0.17	2.50
Ag (mg/kg)	0.1	0.1
As (mg/kg)	0.8	6.1
Pb (mg/kg)	1.6	16.4
Cd (mg/kg)	0.0	0.1
Cr (mg/kg)	4.2	35.4
Cu (mg/kg)	1.3	22.9
Mn (mg/kg)	9.7	609.3
Ni (mg/kg)	1.1	17.6
Se (mg/kg)	<0.5	0.83
Zn (mg/kg)	2.37	32.82
Hg (mg/kg)	0.03	0.04
Fe (%)	0.29	2.66

Parameter (unit)	Rudosol	Vertosol
Al (% of mass)	0.14	2.02
V (mg/kg)	8.88	50.97
Co (mg/kg)	0.41	13.41
Mo (mg/kg)	<0.1	0.60
Ba (mg/kg)	2.4	89.4
S (mg/kg)	17	330

Sieved (<2 mm), air-dried soils (1.2 kg) were gently packed into 9 by 30 cm PVC soil columns. Base fertilisers were manually mixed into the top ~5cm to all columns, excluding the soil control: 40 kg/ha phosphate, 60 kg/ha potassium and 80 kg/ha nitrogen to maintain plant growth. Nitrogen fertiliser was applied as urea (46.0% N), phosphorus fertiliser as superphosphate (9.1% P) and potassium fertiliser as potash (41.5% K). Soil columns were watered to 65% water holding capacity with deionised water.

The soil column trial was conducted in a temperature-controlled greenhouse, without additional lighting, with a 24/17^{o}C day/night cycle. Columns were arranged in randomised blocks and were further randomised every 2-3 days. Digestate application was based on conductivity, identified as the most limiting factor for land application, adapted from these New South Wales regulations: Resource Recovery Exemption under Part 9, Clause 93 of the Protection of the Environment Operations (Waste) Regulation 2014 - The liquid food waste exemption 2014 (EPANSW, 2014a). Applications were injected, at one location, with a 50 ml syringe 5 cm into the soil using a 14G draw-up (blunt ended) needle to apply amendments of 1 and 3 dS/m. Applications differed slightly from the regulations in that digestates were injected to 5 cm, as opposed to the minimum 10 cm recommended in the regulation, because of equipment limitations. Application of the digestate and water mixture amount was based on irrigation water recommendations (VRO, 2018), where 50 ml is equivalent to 7.9 mm of irrigation water applied. This experiment was designed to simulate a farmer using the

digestate-water mixture in an irrigation system for one day. Applications were applied twice (Days 1 and 23) in the 44-day growing period.

Five spinach seeds were planted in each column then emergence was counted for five days, after which the 14 day seedlings were thinned to one per column. After 44 days of growth spinach plants were cut at the soil surface and weighed (fresh weight is reported).

6.2.2.1. *Greenhouse gas emissions*

Carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4) gas samples were taken on Days 1, 3, 5, 7, 9, 16, 25, 27, 38 and 44 post-first digestate application. Emissions were sampled using static collection chambers equipped with a rubber septum for gas collection. A chamber was pressed into the soil and after a period of 20 minutes (based on previous studies, Chapters 3 and 4), a glass, air-tight SGE analytical scientific syringe and needle were used to thoroughly mix the gas in the headspace and to extract a 20 ml volume aliquot of soil gas. Gas samples were transferred to a pre-evacuated 12 ml Exetainer® vial with a grey silicone septum (Labco, UK). The gases CH_4, CO_2, and N_2O were analysed using a Agilent technologies 7890A-G3440A Gas Chromatography system using Thermal Conductivity Detection (TCD) and Flame Ionization Detection (FID). Cumulative emissions for each greenhouse gas was calculated, accounting for time elapsed between measurements, following Van Zwieten et al. (2010).

6.2.2.2. *Leaching samples*

Leaching events were conducted twice over the growth period to collect soluble forms of Ca, Mg, Na, K, Ag, Al, As, B, Ba, Be, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Sn, Sr, Ti, Tl, V, and Zn. Leaching events were completed on Days 16 and 38, where greenhouse gas emission sample collection was completed before leaching events. Drainage problems with the Vertosol occurred, where no leachate was collected for 50% of the soil columns in the last leaching event, due to water not draining through the column. This was most likely as a result of a combination of the medium clay

texture and a high amount of plant roots hindering the infiltration. Therefore, all concentrations were corrected for the amount of leachate collected and reported as total mg/column leached soluble elements; pH and EC were averaged over the two leaching events.

Leachate elements were analysed on a ICP-OES (described in 6.2.1.) using the manufacturer-provided PerkinElmer Ca, Mg, Na, K mix with 5% HNO_3 standards 2.5, 25 and 50 ppm and multi-element standard (described in Chapter 6.2.1.). Chloride (Cl) in leachates was measured by colorimetric analysis, following methods APHA-AWWA-WPCF (2005), Hansen and Ruzicka (1979).

Although total metals were analysed by ICP-OES, the likely forms are Pb^{2+}, Cr^{6+}, Cd^{2+}, Cu^{2+} and Ni^{2+} based on environmental conditions and the element's most common form, described by Wuana and Okieimen (2011). Al^{3+} and Mn^{2+} were considered the most logical species of these elements, based on environmental abundance, pH and other environmental conditions using Rayment and Lyons (2011b), Peverill et al. (1999).

6.2.2.3. *Soil sample analysis*

Total soil metals: Ag, As, Pb, Cd, Cr, Cu, Mn, Ni, Se, Zn, Hg, Fe, Al, B, Si, V, Co, Mo, Ba, Ca, Mg, K, Na, S, P and exchangeable cations: Ca^{2+}, Na^{+}, Mg^{2+}, Al^{3+}, K^{+}, H (and Ca: Mg ratio calculated) were determined by Environmental Analysis laboratories, at Southern Cross University.

The 4B3 method was used to assess soil pH - 1:5 0.01M $CaCl_2$ extraction (Rayment and Lyons, 2011b). Soil Electrical Conductivity (EC) was measured using the 3A1 method - 1:5 soil to DI water extraction (Rayment and Lyons, 2011b). Soil chloride was conducted by initially using the 3A1 method, 1:5 soil to DI water extraction (Rayment and Lyons, 2011b), then measured using a IJ series Ionode© direct chloride ion-selective electrode, following manufacturer's instructions (Ionode, 2014). An ionic strength adjuster of 1M potassium nitrate (5:50 v/v KNO_3 to sample) was used. Standards were made from dried NaCl powder, using DI water, diluted to 2.5, 12.5, 25 and 50 mg/L Cl, range acquired from Rayment and Lyons (2011).

6.2.2.4. *Statistical analysis*

Data analysis was completed by IBM SPSS Statistics 25 software package. For all tests, the significance value chosen was 5% (p= 0.05), where <0.05 was considered significant. The Levene's test for homogeneity was used to assess parametric test appropriateness; if this failed, a log transformation was done (reported in graphs and tables in the raw data form). Soil types were analysed separately.

The Pearson Chi-squared test was used to understand the frequency/count of <0.2 g plant growth with treatment; where it was assumed that <0.2 g of plant was either dead or significantly inhibited growth. This was done by counting the amount of dead and alive plants, which was then weighed under the different treatments. For the Rudosol, one-way ANOVA was used to test the impact of treatments on plant growth, followed by Tukey's post-hoc tests.

Two-way ANOVAs were run, using treatments and depths as fixed factors and parameters as dependent variables. Pairwise comparisons Least Significance Difference (LSD) were used to test significance between treatments and depths. Critical F-values were checked when p values were significant. Graphs in this chapter were produced with GraphPad Prism, version 7.02.

6.3. Results

6.3.1. Plant growth

There was no difference in Vertosol plant growth with treatments (p= 0.202, Figure 39). However, plant survival was increased with digestate application in the Rudosol (p= 0.002, Chi-squared). The low plant growth observed in the Rudosol is most likely to do with the low nutrient content of the soil, where even applying base fertilisers was not enough to sustain plant growth. Considering only the plants that successfully grew (all the controls were excluded), plant growth increased with corresponding increasing digestate application (p= 0.008, one-way ANOVA, Figure 39).

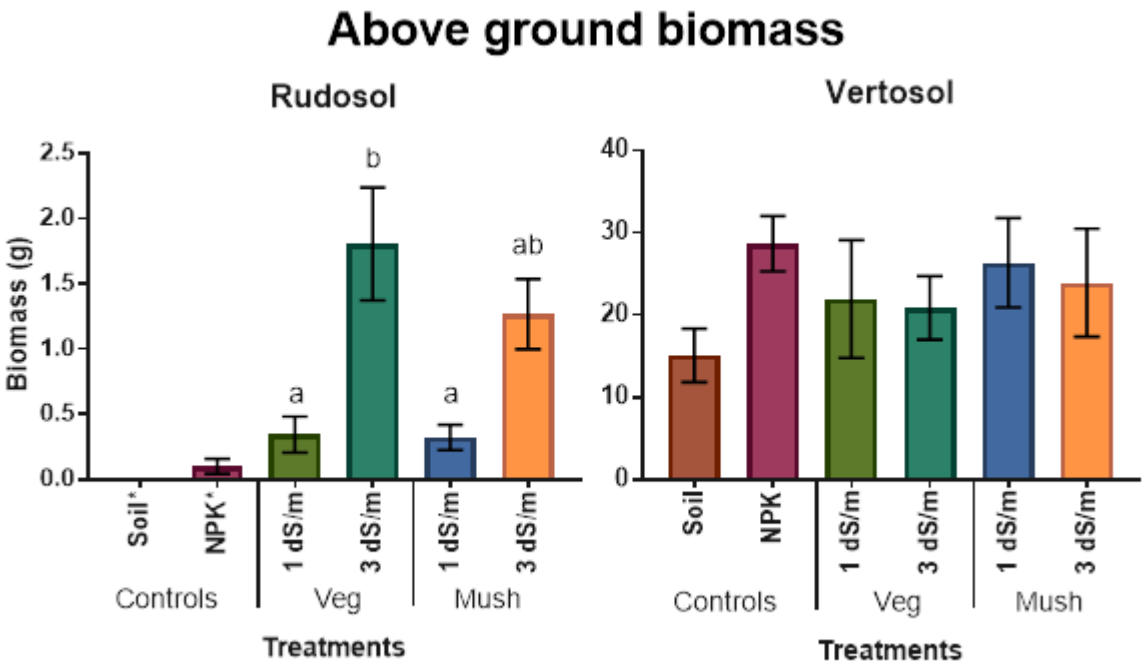


Figure 39 Rudosol and Vertosol above ground biomass of spinach, average ± SEM. For Rudosol *= excluded from analysis as all but one plant was considered dead, letters represent the result of Tukey's post-hoc test. *n*= 6.

6.3.2. Emissions

The Rudosol had higher CH_4 emissions compared to the Vertosol, with a final cumulative value of 0.62-0.71 mg CH_4-C m^2 for the Rudosol and 0.42-0.47 mg CH_4-C m^2 for the Vertosol. The Rudosol had lower CO_2 emissions compared to the Vertosol, with a final cumulative value of 873-1365 mg CO_2-C m^2 for the Vertosol and 3000-668 mg CO_2-C m^2 for the Rudosol. Slightly greater N_2O emissions were released from the Rudosol compared to the Vertosol; however, this was not statistically significant (*p*= 0.267, one-way ANOVA).

6.3.2.1. ***Rudosol emissions***

In the Rudosol, cumulative CH_4 (Figure 40) was different between treatments on Days 1 and 3 post-initial digestate application (*p*= ≤0.005, one-way ANOVA). On Day 1, all digestate treatments had significantly higher emissions than soil control treatment (*p*= ≤0.012, LSD) and both the Veg

digestates treatment had higher emissions than NPK-only control treatment (p= ≤0.012, LSD). On Day 1, Veg 3 dS/m was higher than Mush 3 dS/m (p= 0.026, LSD). On Day 3, Mush 1 dS/m and both Veg treatments were also significantly higher than soil (p= ≤0.017, LSD). On day 3, Veg 3 dS/m treatment remained higher than NPK-only treatment (p= 0.005, LSD) and Mush 3 dS/m treatment (p= 0.022, LSD). Although there were no overall significant differences in CH_4 emissions between treatments, Veg 3 dS/m treatment was higher than soil control (p= ≤0.037, LSD) on Days 7, 9 and 16. On Days 38 and 44, Mush 3 dS/m treatment was higher than soil control treatment (p= ≤0.029, LSD). On Days 30, 38 and 44, Mush 1 dS/m treatment was higher than Mush 3 dS/m treatment (p= ≤0.026).

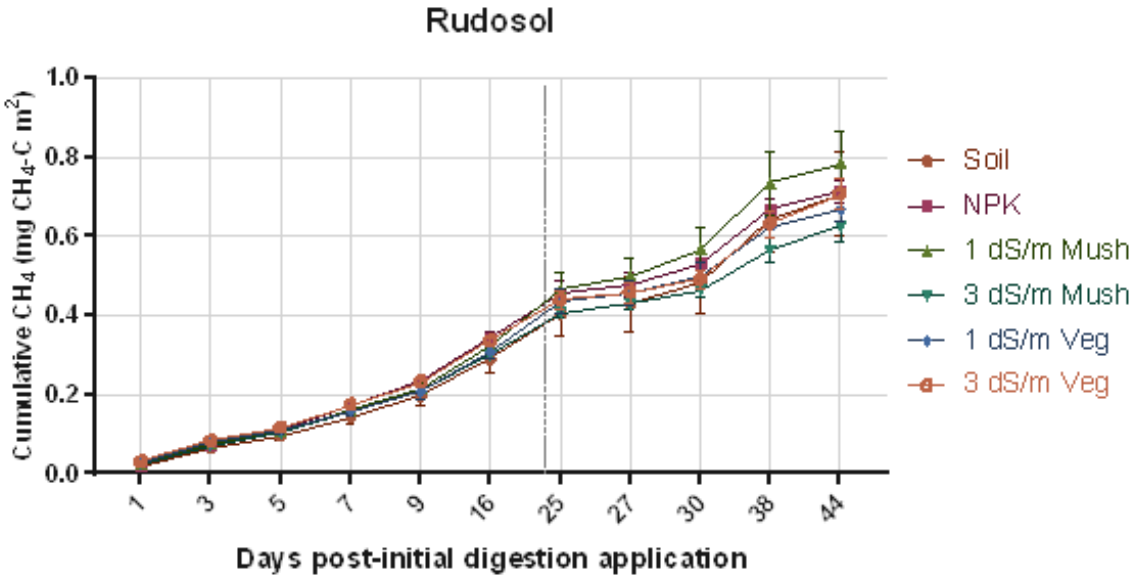


Figure 40 Cumulative methane soil emissions emitted from Rudosol. Data presented as averages ± SEM, n= 6. Dotted line represents the second digestate application at Day 23.

In the Rudosol, cumulative CO_2 emission (Figure 41) differences were observed from Day 5 post-initial digestate application (p= ≤0.038, one-way ANOVA). From Day 5, Mush 3 dS/m treatment was higher than NPK-only control treatment (p= 0.002). From Day 30, Veg 3 dS/m treatment had higher emissions compared to NPK-only control treatment (p= ≤0.044). On Days 5, 7 9 and 16, Mush 3 dS/m

treatment had higher emissions than all other treatments (p= ≤0.050, LSD), excluding soil on Day 16. On Days 25, 27, 30, 38 and 44, soil and both Mush 3 dS/m and Veg 3 dS/m treatments had the highest CO_2 emissions (p= ≤0.050, LSD). NPK-only control treatments had low emissions.

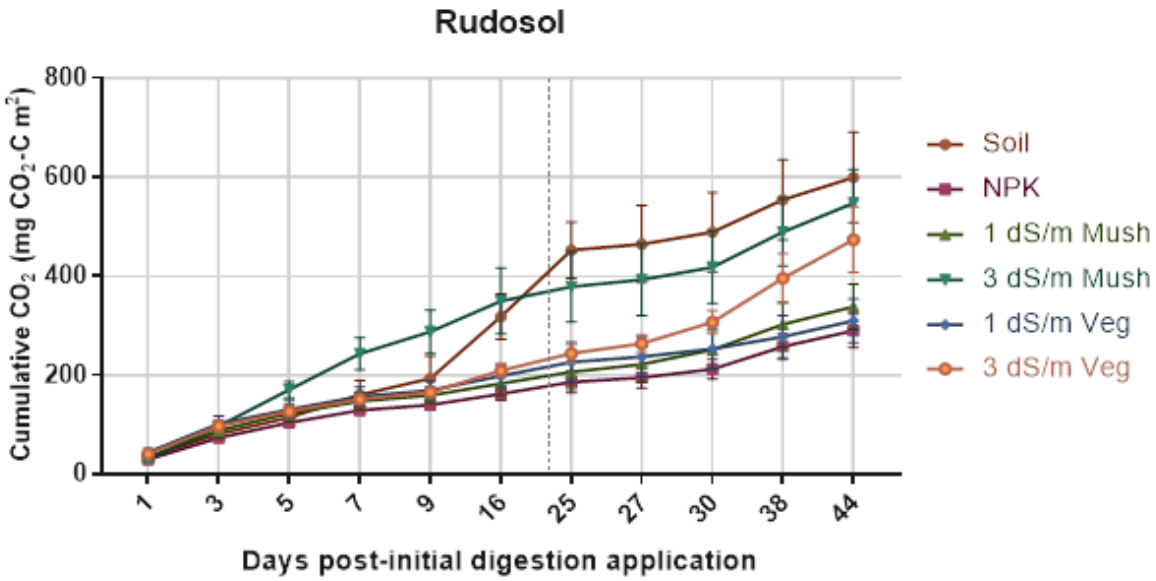


Figure 41 Cumulative carbon dioxide emissions emitted from Rudosol. Data presented as averages ± SEM, n= 6. Dotted line represents the second digestate application at Day 23.

In the Rudosol, cumulative N_2O soil emissions differed between treatments from Day 7 post-initial digestate application (p= ≤0.007, one-way ANOVA) (Figure 42). On Days 7, 9, 16, 25, 27, 38 and 44, Mush 3 dS/m treatment had higher emissions than NPK-only control treatment (p= ≤0.011, LSD). On Days 27, 30, 38 and 44, Veg 3 dS/m treatment had higher emissions than NPK-only control treatment (p= 0.015, LSD). On Days 7 and 9, Mush 3 dS/m treatment had higher emissions than all other treatments (p= ≤0.029, LSD). On Days 16, 25, 27, 30, 38 and 44, both Mush 3 dS/m and Veg 3 dS/m treatments had higher emissions than all other treatments (p= ≤0.011, LSD).

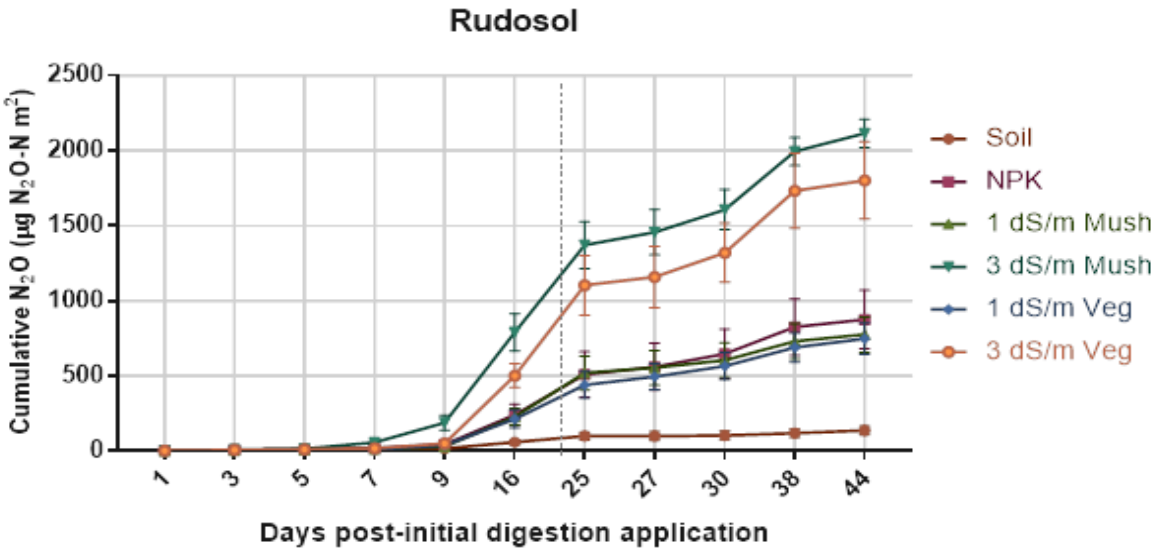


Figure 42 Cumulative nitrous oxide soil emissions emitted from Rudosol. Data presented as averages ± SEM, *n*= 6. Dotted line represents the second digestate application at Day 23.

6.3.2.2. ***Vertosol emissions***

In the Vertosol, cumulative CH_4 (Figure 43) was not significantly different between treatments (p= >0.434, one-way ANOVA).

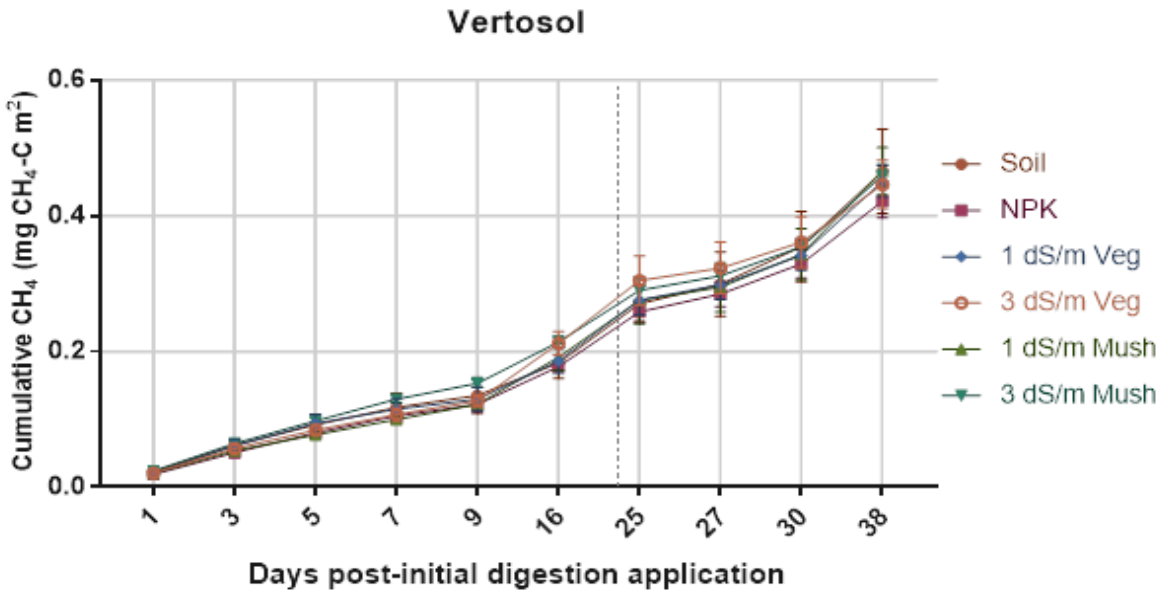


Figure 43 Cumulative methane soil emissions emitted from Vertosol. Data presented as averages ± SEM, *n*= 6. Dotted line represents the second digestate application at Day 23.

In the Vertosol, cumulative CO_2 (Figure 44) was significantly different between treatments from Day 7 post-initial digestate application (*p*= <0.045, one-way ANOVA). Day 7, NPK-only treatment had higher emissions than Mush 1 dS/m treatment (*p*= 0.012, LSD). On days 9, 25, 27, 30 and 38, the NPK-only control treatment had higher emissions than all digestate treatments (*p*= ≤0.033, LSD), excluding Veg 3 dS/m on Days 30 and 38. There was no significant differences between digestate treatments. Importantly, this is the opposite of the Rudosol.

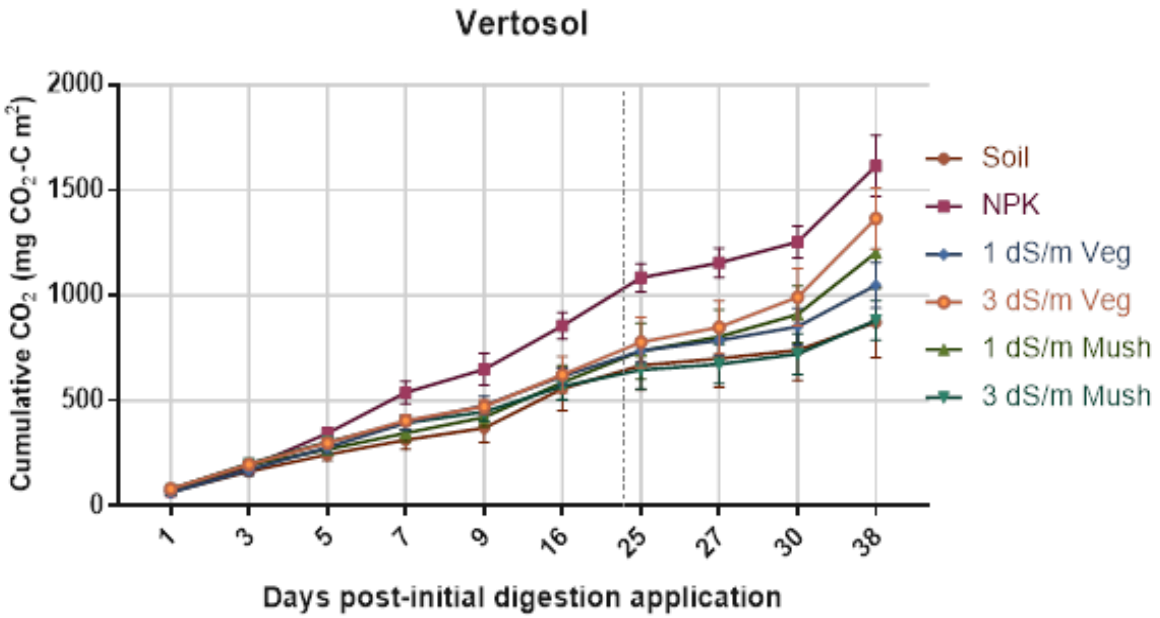


Figure 44 Cumulative carbon dioxide emissions emitted from Vertosol. Data presented as averages ± SEM, *n*= 6. Dotted line represents the second digestate application at Day 23.

In the Vertosol, cumulative N_2O (Figure 45) observed differences all days post-initial digestate application (*p*= ≤0.025, one-way ANOVA), excluding Day 5 (*p*= 0.155, one-way ANOVA). On Day 1, both Veg 3 dS/m and Mush 3 dS/m treatments had higher emissions than NPK-only treatment (*p*= ≤0.028, LSD), and Veg 1 dS/m treatment (*p*= ≤0.002, LSD). On Day 3, Mush 3 dS/m treatment N_2O emissions remained higher than NPK-only treatment (*p*= 0.016, LSD). On Days 7, 9, 16, 25, 27, 30 and 38, both Mush 3 dS/m and Veg 3 dS/m treatments had higher emissions than soil control (*p*= <0.001, LSD). On Day 7, Mush 3 dS/m treatment had higher emissions than Mush 1 dS/m and Veg 1 dS/m treatments (*p*= ≤0.042, LSD). On Days 9, 16, 25, 27, 30 and 38, Mush 3 dS/m and Veg 3 dS/m treatments had higher emissions than all other treatments (*p*= ≤0.002, LSD).

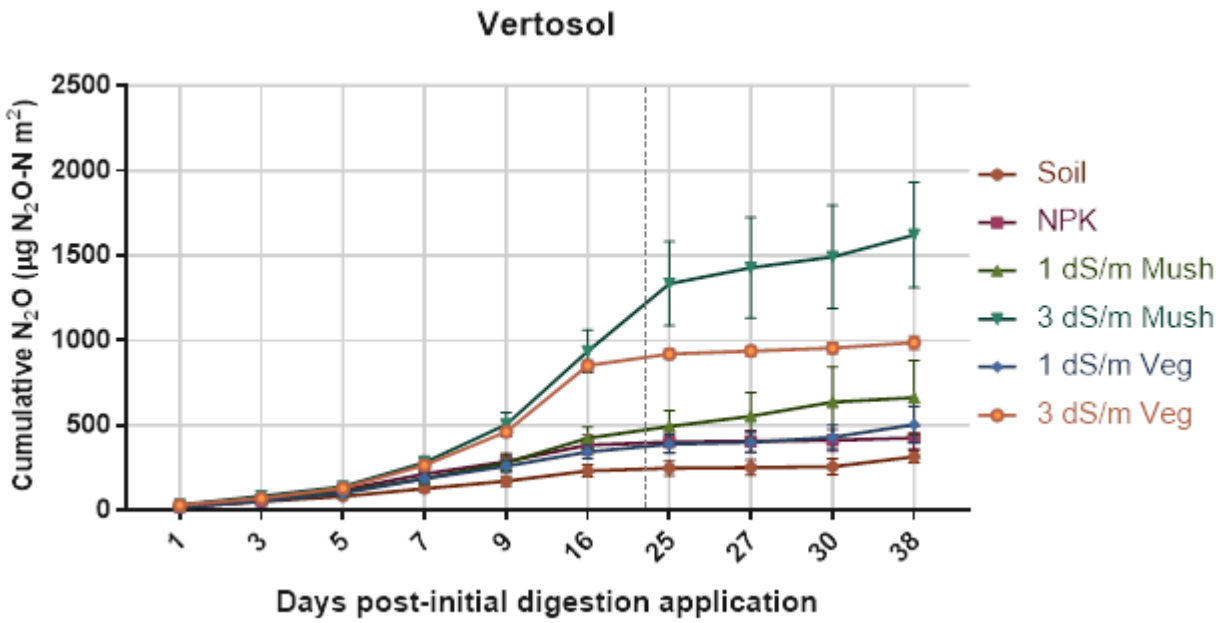


Figure 45 Cumulative nitrous oxide soil emissions emitted from Vertosol. Data presented as averages ± SEM, *n*= 6. Dotted line represents the second digestate application at Day 23.

6.3.3. Soluble concentrations in leachates

In both the Rudosol and Vertosol leached soluble elements Ag^{+}, As, Cd^{2+}, Co, Cr^{6+}, Cu, Mn^{2+}, Mo, Ni^{2+}, Pb^{2+}, Se and V concentrations were below detection limits (<0.05 mg/L). For the Vertosol, Al^{3+} concentrations were below detection limits (<0.05 mg/L). The EC measurements for both soils were high due to the measurement being taken on unfiltered samples. Concentrations in leachates in mg/L are presented in Table 22 for comparison to contamination guidelines.

In the Rudosol, EC and soluble forms of Ca^{2+}, Cl^{-}, K^{+}, Na^{+}, Mg^{2+}, Al^{3+}, soluble Fe, Mn^{2+}, Zn^{2+}, and Ba^{2+} concentrations were all significantly different with treatments (p= ≤0.029, one-way ANOVA). Figure 46 and Figure 47 show the difference observed with leached elements. The leachate pH was not affected by the digestate treatments (p= >0.05, one-way ANOVA). The EC value and leached Na^{+}, K^{+}, Cl^{-}, soluble Fe and Al^{3+} concentrations from Veg 3 dS/m treatment were significantly higher

than NPK-only control and Veg 1 dS/m treatments (p= <0.05, LSD). There were no differences within digestate treatments observed for leached Ca^{2+}, Mn^{2+} and Ba^{2+} concentrations (p= >0.050, LSD).

Unsurprisingly, the leachate EC increased with increasing digestate application, where there was a trend of soil control < NPK-only control < Veg 1 dS/m=Mush 1 dS/m < Veg 3dS/m=Mush 3 dS/m treatments, equals indicated same EC. Mg^{2+} concentrations in leachates increased with increasing Mush digestate application (p= 0.018, LSD). Soluble Fe concentrations in leachates increased with increasing Veg digestate application (p= 0.032, LSD). NPK-only treatment had lower Cl^- concentrations leached compared to Mush 3 dS/m and Veg 3 dS/m treatments (p= ≤0.002, LSD). Maximum Cl^- leached was 69 mg/L, with an average of 36 mg/L. The control had the highest Cl leached concentrations. This is due to substantially high Cl concentrations in the soil compared to other treatments in the second leaching event (p= <0.001, LSD). In contrast, in the first leaching event, Mush 3 dS/m and Veg 3 dS/m treatments had the highest Cl^- leached (p= <0.050, LSD). The soil control in the Rudosol had high Cl^-, soluble Fe and Al^{3+} concentrations in leachates (Figure 46 and Figure 47). While the exact reason for this is unknown, the soil control had high C turnover (high CO_2 soil emissions, see Chapter 6.3.2.1.) which may indicate soil conditions favourable to leaching.

In the Vertosol, there were no significant differences between treatments for leached element concentrations or pH (p= >0.050, one-way ANOVA, Figure 48). Overall, there were no significant differences in leachates; however, the leachate EC for NPK-only treatment had higher EC compared to Mush 3 dS/m treatment (p= 0.015, LSD). Salts were predictably high in the Vertosol leachates compared to the Rudosol; particularly Na^{2+}, Ca^{+}, K^{+}, Mg^{2+} and Cl^- concentrations. Cl^- concentrations leached were high in Vertosol leachate, with a peak of 634 mg/L and average of 424 mg/L Cl^-.

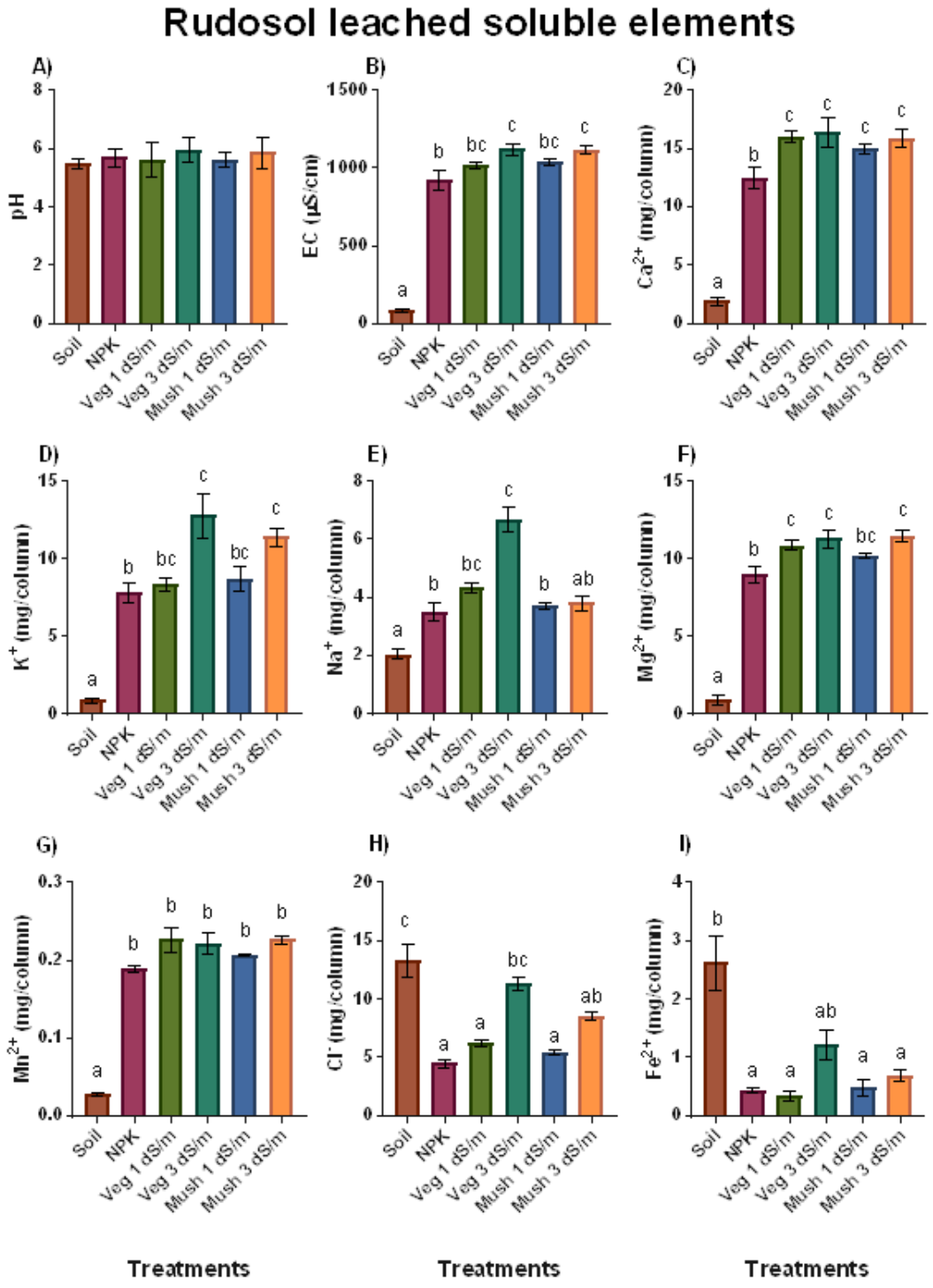


Figure 46 Leached soluble elements from Rudosol, averages mg/column (of both leaching events) ± SEM, A) pH, B) EC, C) Ca, D) K, E) Na, F) Mg, G) Mn, H) Cl, I) Fe. Where pH and EC *n*= 12, for everything else *n*= 6. Letters represent Tukey's post-hoc test (*p*= <0.05).

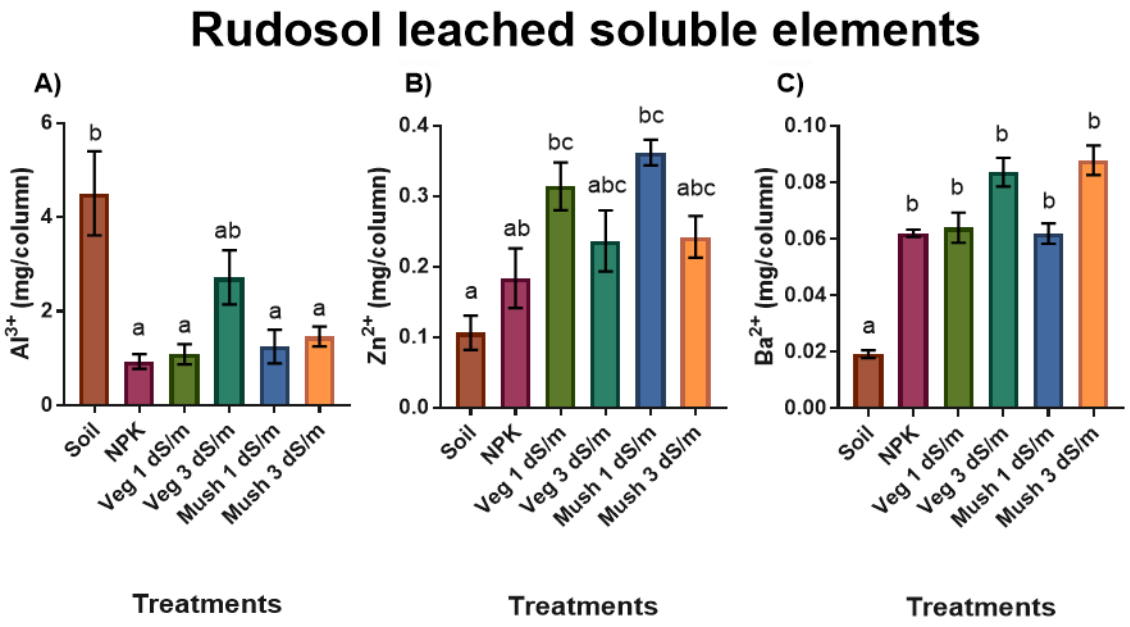


Figure 47 Total leached soluble elements from the Rudosol, averages mg/column (of both leaching events) ± SEM. A) Al, B) Zn, C) Ba. *n*= 6. Letters represent Tukey's post-hoc test (*p*= <0.05).

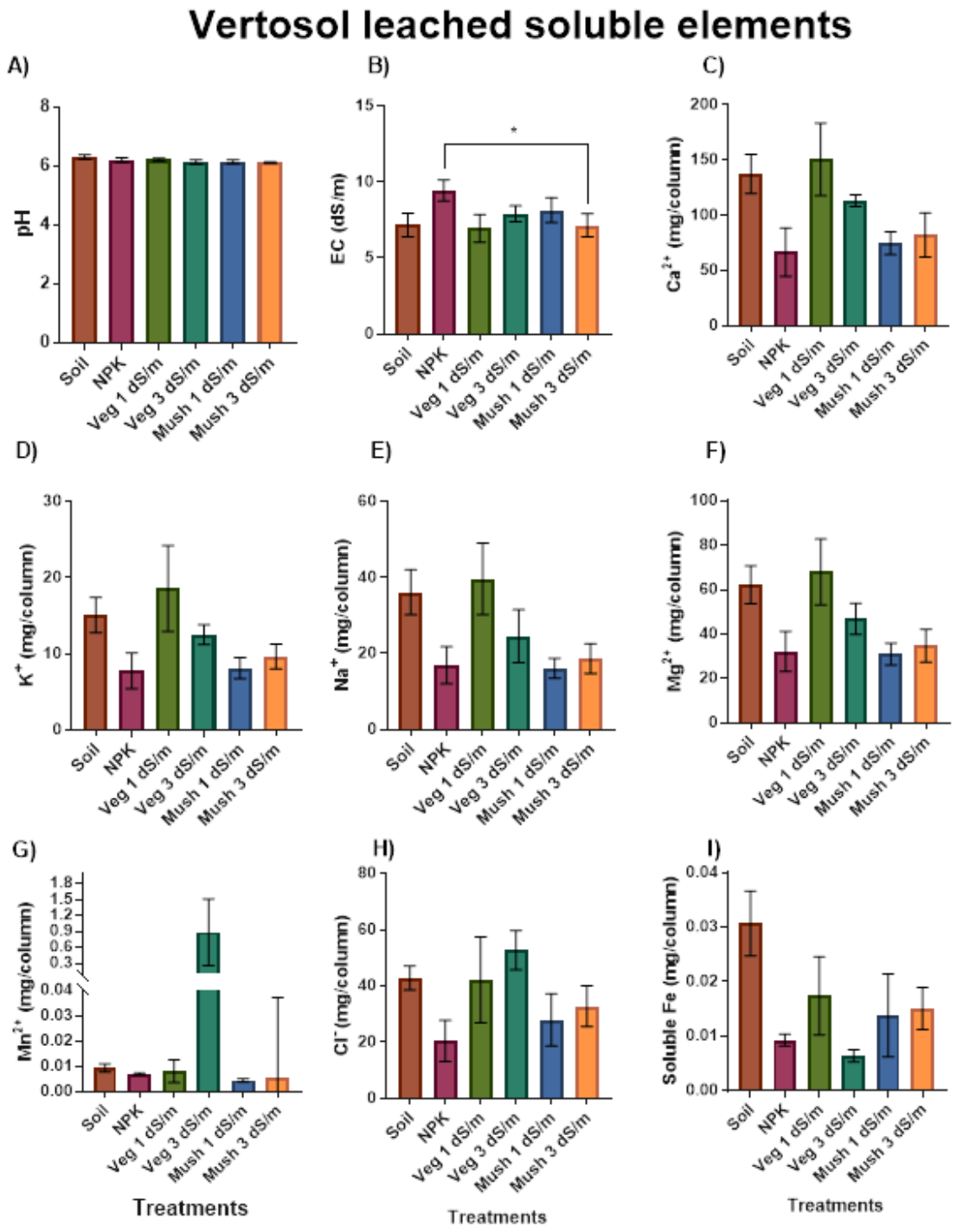


Figure 48 Total leached soluble elements from Vertosol columns, averages mg/column (of both leaching events) ± SEM. A) pH, B) EC, C) Ca, D) K, E) Na, F) Mg, G) Mn, H) Cl, I) Fe. Where pH and EC *n*= 12, everything else *n*= 4 to 6. *= significant differences between treatments (*p*= 0.015), all other differences between leachate concentrations were not significant (*p*= >0.05).

Table 22 Soluble element concentrations in leachates, average mg/L SD and range (min, max), compared to EILs for short-term irrigation water (DEC, 2010).

Element (mg/L)	Rudosol				Vertosol				EILs (mg/L)
	Average	SD	Range (min, max)		Average	SD	Range (min, max)		
Ca	28	23	2	64	874	407	20	1612	
K	18	15	1	56	101	36	8	158	
Na	9	5	2	18	222	73	7	381	115 – 230*
Mg	19	16	1	42	389	148	6	628	
Al	4.50	5.08	0.29	26.10	<0.05	0.0	<0.05	<0.05	20
Fe	2.18	2.91	0.06	14.27	0.12	0.18	<0.05	0.87	10
Mn	0.39	0.39	0.03	1.08	0.62	2.57	<0.05	13.91	10
Zn	0.52	0.48	0.01	1.55	<0.05	0.09	<0.05	0.38	5
As	<0.05		<0.05	<0.05	0.05		<0.05	0.07	2
Cu	<0.05		<0.05	<0.05	<0.05		<0.05	0.03	5
Pb	<0.05		<0.05	<0.05	<0.05		<0.05	0.04	5
Ag	<0.05		<0.05	<0.05	<0.05		<0.05	0.02	
B	<0.05		<0.05	<0.05	0.93	0.31	<0.05	1.71	0.5

Element (mg/L)	Rudosol				Vertosol				EILs (mg/L)
	Average	SD	Range (min, max)		Average	SD	Range (min, max)		
Ba	0.14	0.07	<0.05	0.27	0.15	0.06	0.05	0.28	
Cd	<0.05		<0.05	<0.05	<0.05		<0.05	<0.05	0.05
Co	<0.05		<0.05	<0.05	<0.05		<0.05	<0.05	0.1
Cr	<0.05		<0.05	<0.05	<0.05		<0.05	<0.05	1
Mo	<0.05		<0.05	<0.05	<0.05		<0.05	<0.05	0.05
Ni	<0.05		<0.05	<0.05	<0.05		<0.05	<0.05	2
Se	<0.05		<0.05	<0.05	<0.05		<0.05	<0.05	0.05
V	<0.05		<0.05	0.10	<0.05		<0.05	0.15	0.5

*WA irrigation water guidelines

6.3.4. Total concentrations in soil samples

6.3.4.1. *Rudosol total soil concentrations*

Ag, Cd, Hg concentrations were below detection limits. There were no significant differences found for Cu, Mn, Fe, Cl concentrations (p= >0.05), and overall concentrations were low (Table 23). Total soil Al, Ba, Cr, Co, pH, Pb, Ni, Zn, Mg, S, V and Ca concentrations were significantly different with the interaction of treatment and depth (p= ≤0.029, two-way ANOVA). EC, Cr, Ni, Zn, Mg, Co and Ba concentrations significantly different with treatment (p= ≤0.020, one-way ANOVA). Zn, Ba, S, Ca and As concentrations changed significantly with depth, (p= ≤0.049), discussed in more detail in the following paragraphs. See Figure 49 and Figure 51.

Soil pH decreased with depth and the higher digestate level treatments resulted in decreases in pH compared to controls (p= <0.001, for both, LSD). ECs in all treatments were higher in comparison to the soil control treatment (p= <0.001, one-way ANOVA), and at 0-5 cm had the lowest EC (p= <0.001, one-way ANOVA).

As concentrations increased with depth (p= 0.038, one-way ANOVA); however, Veg 3 dS/m treatment had higher As than control (p= 0.035, LSD). In 0-5 cm, Pb concentrations were higher in both Veg treatments compared to both Mush treatments (p= ≤0.023, LSD), although Veg treatments were not significant from soil or NPK-only treatments (p= ≥0.340, LSD). In 0-5 cm, Cr and Ni concentrations were highest in Veg 3 dS/m treatment (p= ≤0.014, LSD) and both element concentrations were higher in Veg 1 dS/m than Mush digestate treatments (p= ≤0.037, LSD). In 0-5 cm, Co concentrations were higher in Veg 3 dS/m compared to both Mush treatments (p= ≤0.001, LSD), and in all depths Veg 1 dS/m had higher Co concentrations than Mush 1 dS/m treatment (p= ≤0.030, LSD). In 5-15 cm, Zn concentrations increased with increasing digestate treatments, both Mush 3 dS/m and Veg 3 dS/m treatments had higher Zn concentrations compared to NPK-only treatment (p= ≤0.010, LSD).

For the Mg concentrations at 0-5 cm, soil control treatment had significantly higher Mg concentrations than both Mush treatments (p= <0.001, LSD), Veg 1 dS/m treatment had lower Mg concentrations than Mush 1 dS/m treatment (p= 0.029, LSD), and Veg 3 dS/m treatment had lower Mg concentrations than both Mush treatments (p= ≤0.004, LSD). In 5-15 cm, NPK-only control treatment had lower Mg concentrations than Veg 3 dS/m and Mush 3 dS/m treatments (p= ≤0.021, LSD). Mush 3 dS/m treatment had higher Mg concentrations than Veg 1 dS/m treatment (p= 0.008, LSD). Veg 1 dS/m treatment had higher Mg concentrations than Veg 3 dS/m treatment (p= 0.012, LSD). In 15-30 cm, soil control treatment had higher Mg concentrations than Mush 3 dS/m treatment (p= 0.005, LSD), Mush 3 dS/m treatment had lower Mg concentrations than NPK-only control treatment (p= 0.005, LSD). In 0-5 cm, Al concentrations were lower in both Mush treatments compared with both control treatments (p= ≤0.029, LSD).

V concentrations were higher in Veg 3 dS/m treatment compared to NPK-only control treatment and both Mush treatments in the 0-5 cm (p= ≤0.020, LSD), and both Mush treatments had lower V concentrations than NPK-only control treatment in 15-30 cm (p= ≤0.017, LSD). Ba concentrations generally increased with depth, where 0-5 cm had lower Ba concentrations than 15-30 cm (p= 0.015, LSD). Ca concentrations were higher in soil control treatment compared to all digestate treatments in 0-5 and 15-30 cm (p= ≤0.033, LSD).

CEC decreased with depth. The percentage of Ca and Mg within CEC were higher in 0-5 cm concentrations compared to 15-30 cm (p= <0.001, LSD). In 0-5 cm, the Ca percentage in soil had lower Ca percentage than all treatments. In 0-5 and 5-15 cm, Mg percentage were higher in all digestate treatments compared to NPK-only treatment (p= ≤0.022, LSD), excluding Mush 1 dS/m treatment in 5-15 cm. At these soil depths, the Mg percentage increased with increasing digestate application.

The Ca: Mg ratio was significantly different with the interaction between factors (p= <0.001), where the 0-5 cm layer had a higher Ca: Mg ratio in all digestate treatments than soil and lower than NPK-

only treatment (p= <0.05, LSD). The Ca: Mg ratio in both 0-5 and 5-15 cm decreased with increasing digestate application (p= ≤0.044, LSD). In 5-15 cm, soil control treatment had lower Ca: Mg ratio than Mush 1 dS/m and Veg treatments (p= ≤0.035, LSD).

6.3.4.2. *Vertosol total soil concentrations*

Al, K, Mg, ESP, exchangeable Mg and Ca: Mg ratio were significant with respect to the interaction between depth and treatment (p= <0.031, two-way ANOVA). See Figure 50 and Figure 52.

There was accumulation of S, exchangeable Ca, Mg and Na, CEC, ESP at 15-30 cm. The Ca: Mg ratio was highest at 0-5 cm. The EC was higher at the lowest depth (p= <0.001, LSD) and higher in Veg 3 dS/m and Mush 3 dS/m treatments compared to both control treatments (p= <0.02, LSD). Cl concentrations increased with depth (p= <0.001, one-way ANOVA). For Al concentrations at 5-15 cm, NPK-only control had higher Al concentrations than Mush 1 dS/m treatment (p= 0.019, LSD). At 15-30 cm, both controls (soil and NPK-only) were lower than all treatments (p= <0.040, LSD) and Mush 3 dS/m treatments had higher Al concentrations than Veg 1 dS/m treatments (p= 0.009, LSD).

For Mg concentrations at 5-15 cm, NPK-only control treatment had higher Mg concentrations than all treatments (p= <0.040, LSD), excluding Veg 1 dS/m treatment. At 15-30 cm, both controls (soil and NPK-only) had lower Mg concentrations than Mush 3 dS/m treatment (p= ≤0.007, LSD). For K concentrations at 5-15 cm, soil was higher than all the treatments (p= <0.040, LSD), excluding Veg 3 dS/m treatment. NPK-only control treatment was higher than Veg 3 dS/m treatment (p= 0.046, LSD). At 15-30 cm, NPK-only control treatment was lower than all treatments (p= <0.050, LSD), excluding soil control and Mush 1 dS/m treatments. Concentrations of As at 0-5 cm observed that Veg 1 dS/m treatment had higher As concentrations than Veg 3 dS/m treatment (p= 0.010, LSD), potentially due to lack of water infiltration issues in Veg 1 dS/m treatments. At 15-30 cm, Veg 1 dS/m treatment had higher As concentrations compared to all treatments (p= ≤0.035, LSD), excluding Mush 3 dS/m treatment.

Table 23 Total element concentrations in soil samples post-digestate application, averages, SD and range for both soil types. *n*= 9. EPAVIC (2009a) and DEC (2010) soil limits for comparison.

Element (unit)	**Rudosol**				**Vertosol**				**Limits (mg/kg)**
	Average	**SD**	**Range (min, max)**		**Average**	**SD**	**Range (min, max)**		
Ag (mg/kg)	0.03	0.01	0.00	0.15	0.13	0.01	0.10	0.26	10*
As (mg/kg)	0.81	0.05	0.60	1.18	6.30	0.05	5.85	6.78	20^*
Pb (mg/kg)	1.02	0.05	0.71	1.42	16.02	0.08	15.47	16.58	300*-600^
Cd (mg/kg)	0.006	0.001	0.001	0.016	0.082	0.003	0.059	0.099	3^*
Cr (mg/kg)	3.1	0.1	2.1	4.7	34.4	0.2	32.3	36.1	Cr^{6+} 1^*
Cu (mg/kg)	0.8	0.1	0.5	1.6	21.3	0.1	20.4	22.5	100^*
Mn (mg/kg)	5	1	3	10	584	4	552	634	500^
Ni (mg/kg)	0.91	0.05	0.67	1.48	17.06	0.09	16.32	17.69	60^*
Se (mg/kg)	<0.5	---	---	<0.5	0.73	0.02	0.57	0.87	10*
Zn (mg/kg)	2.2	0.1	1.3	3.3	30.4	0.2	28.2	31.8	200^*
Hg (mg/kg)	0.01	0.00	0.00	0.01	0.03	0.00	0.02	0.03	1^*
Fe (% of mass)	0.20	0.02	0.14	0.49	2.51	0.01	2.39	2.62	
Al (% of mass)	0.13	0.00	0.10	0.16	1.88	0.02	1.71	2.01	

Element (unit)	Rudosol				Vertosol				Limits (mg/kg)
	Average	SD	Range (min, max)		Average	SD	Range (min, max)		
B (mg/kg)	<2	---	---	<2	6.3	0.1	5.8	7.2	50^
Si (mg/kg)	515	5	466	555	749	20	611	909	
V (mg/kg)	7.0	0.2	5.4	9.1	50.9	0.3	48.8	52	50^
Co (mg/kg)	0.3	0.0	0.2	0.4	13.6	0.1	13.0	14.2	50^
Mo (mg/kg)	<0.1	---	---	<0.1	0.5	0.0	0.4	0.6	40^*
Ba (mg/kg)	2	0	1	2	90	3	82	146	300^
Ca (mg/kg)	62	7	31	131	2526	17	2423	2702	
Mg (mg/kg)	49	4	30	103	2655	14	2566	2762	
K (mg/kg)	49	2	38	64	3356	38	3037	3578	
Na (mg/kg)	<50	---	---	<50	138	7	104	191	
S (mg/kg)	19	2	8	37	288	26	189	500	600^
P (mg/kg)	<50	---	---	<50	446	5	415	481	2,000^

^EILs for soil, Government of Western Australia, DEC (2010).*Soil fill material categorisation, IWRG621, EPAVIC (2009a).

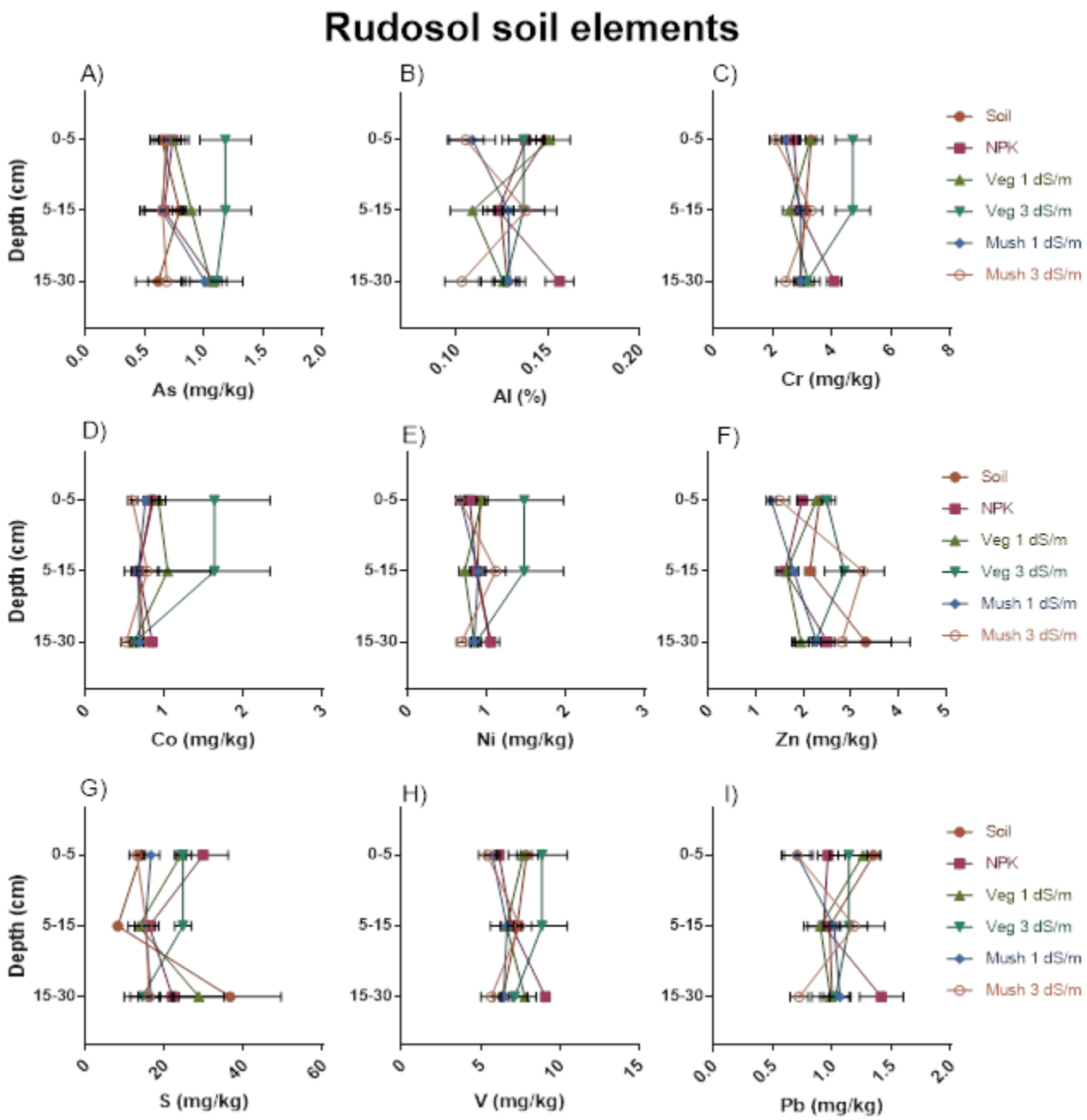


Figure 49 Rudosol total soil elements, averages ± SEM. A) As, B) Al, C) Cr, D) Co, E) Ni, F) Zn, G) S, H) V, I) Pb., *n*= 3

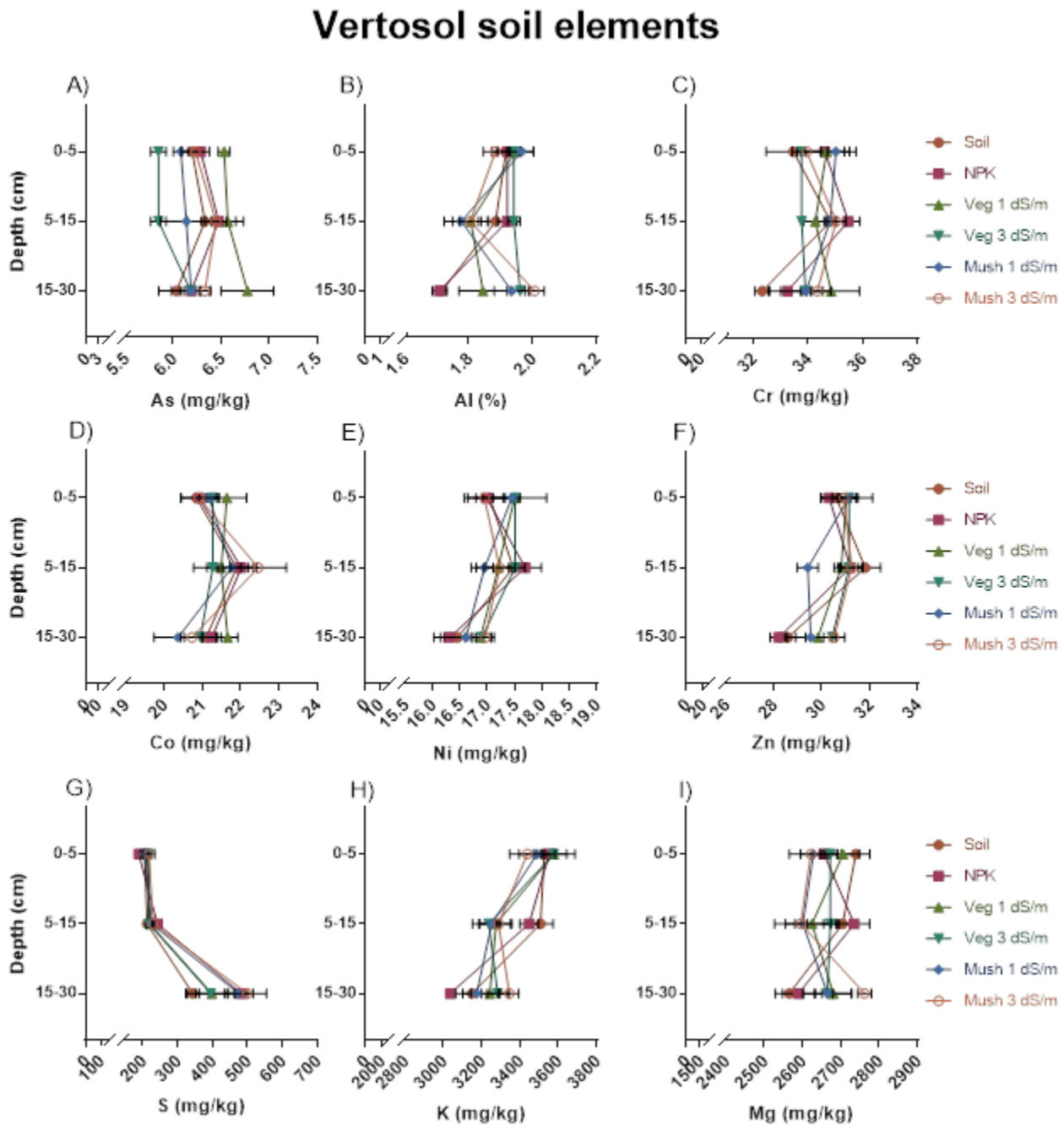


Figure 50 Vertosol total soil elements, averages ± SEM. A) As, B) Al, C) Cr, D) Co, E) Ni, F) Zn, G) S, H) K, I) Mg. *n*= 3

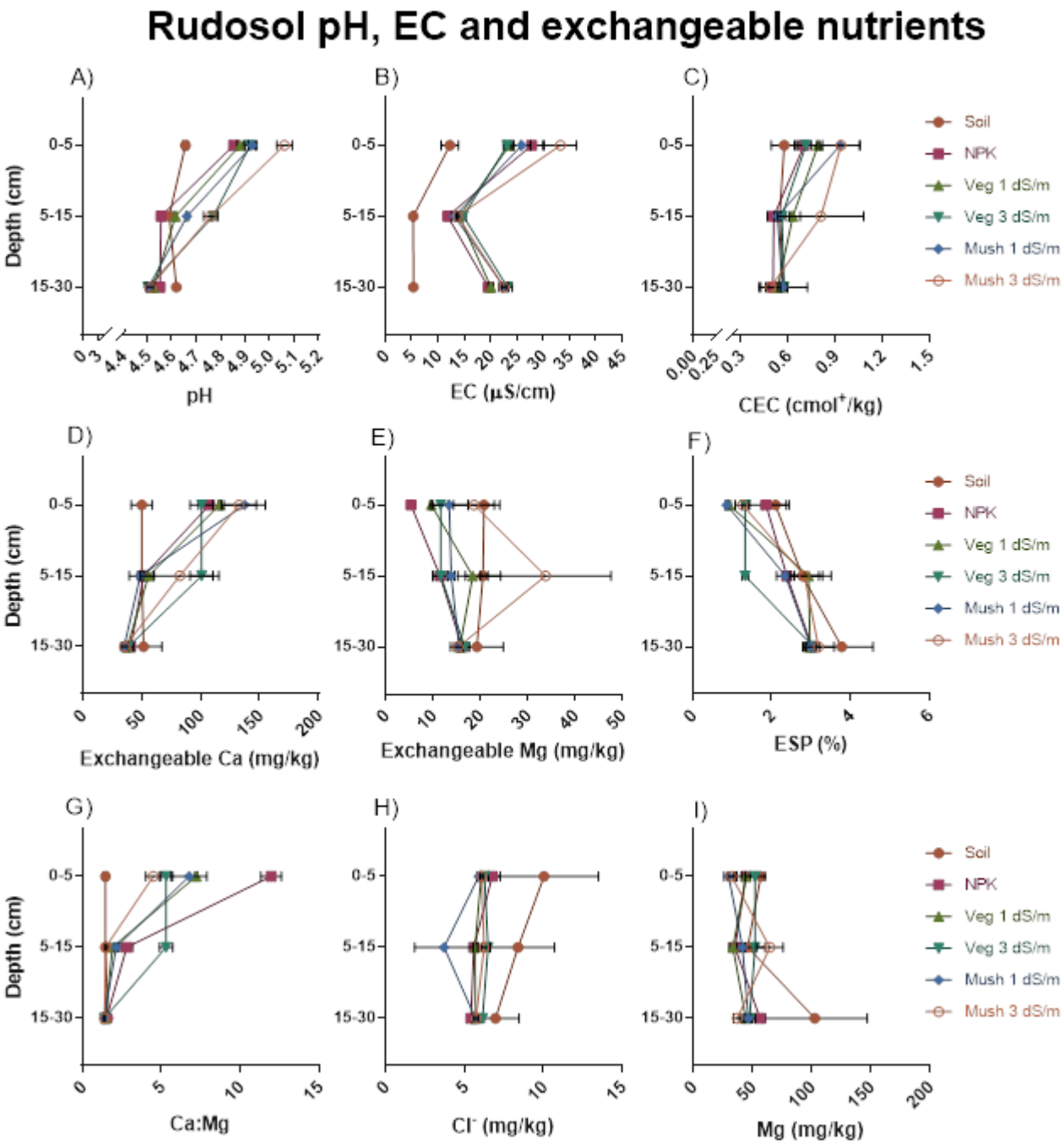


Figure 51 Rudosol pH, EC and exchangeable elements in soil samples, averages ± SEM. A) pH, B) EC, C) CEC, D) Exchangeable Ca, E) Exchangeable Mg, F) ESP, G) Ca: Mg, H) Cl, I) Mg. *n*= 3

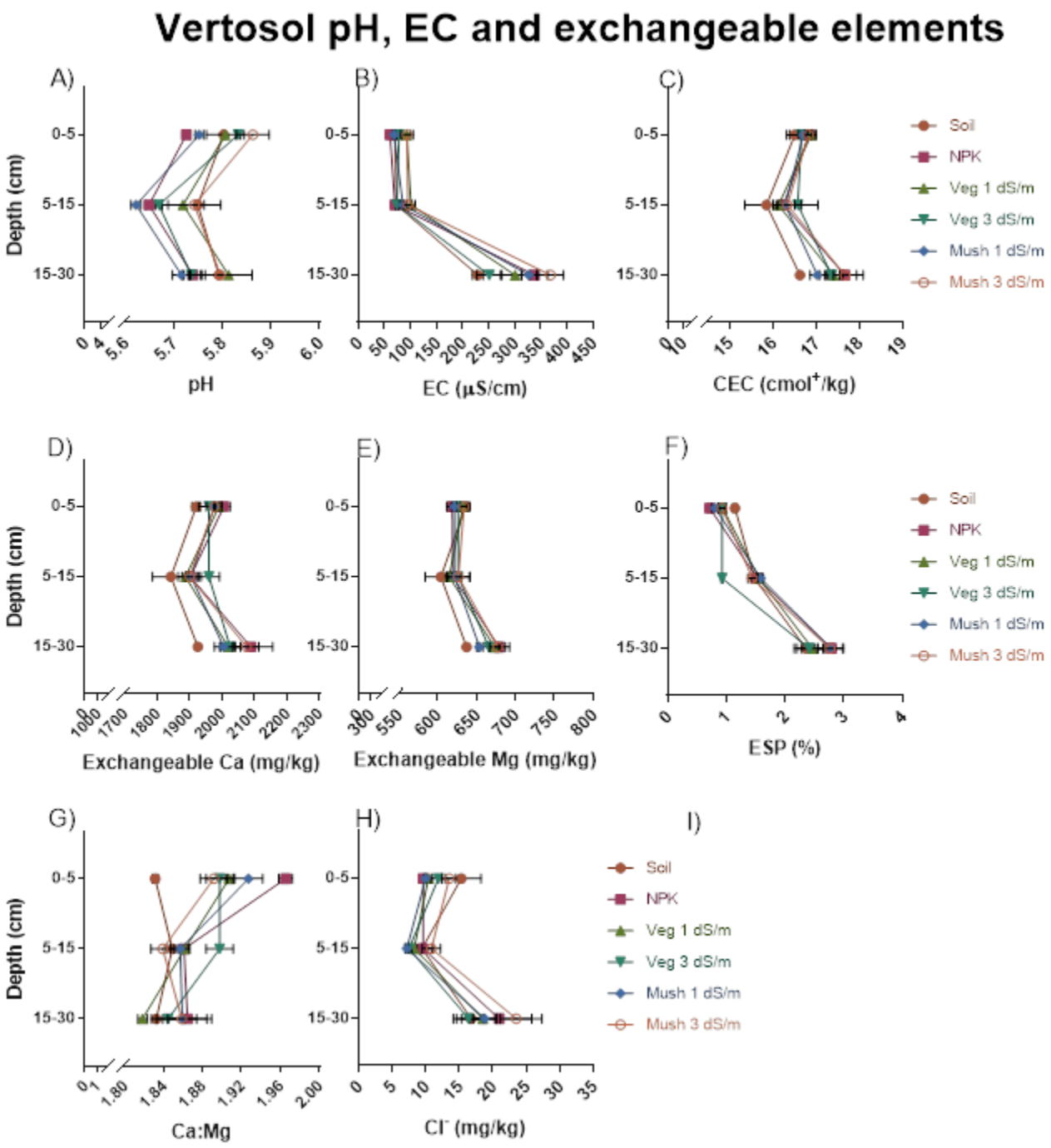


Figure 52 Vertosol pH, EC and exchangeable elements in soil samples, averages ± SEM. A) pH, B) EC, C) CEC, D) Exchangeable Ca, E) Exchangeable Mg, F) ESP, G) Ca: Mg, H) Cl. *n*= 3

6.4. Discussion

6.4.1. Plant growth

When considering the Rudosol, the application of digestate had a positive effect on plant growth, where increasing digestate application corresponded with increasing plant growth. Indeed, it is known that digestates can provide plant essential nutrients (Alburquerque et al., 2012, Tampio et al., 2016b) which was demonstrated in this study. On the other hand, there was no effect on plant growth with digestate applications in the Vertosol. This may have been as a result of a relatively low application rates of the digestates, where digestate treatments were applied twice, corresponding to 7.9 mm using a solution of maximum 30.5-42% (15-21 ml) digestate. This low application rate was necessary due to spinach being moderately sensitive to salts (DEDJTR, 2017) and to provide baseline knowledge of this material.

The difference in plant-essential nutrients in the soil prior to application could be a factor in the different plant growth response in the soil types. In a nutrient poor soil (Rudosol) plant growth was increased, but in a relatively nutrient rich soil (Vertosol) there was no effect to plant growth using the same application rates. In this regard, future applications could be increased (with considerations of negative effects). For example, Ould Ahmed et al. (2010) found positive effects to wheat yield using saline farmyard manures at 0.11 and 2 dS/m irrigation, with 59 mm applied during crop establishment and ~150-250 mm over the growing season. Panta et al. (2016) used 0.04, 8 and 16 dS/m wastewater irrigation equivalent to ~2 and 3.8 mm/day, with positive effects to salt-tolerant plant growth.

6.4.2. Soil emissions

Initially, in the Rudosol, CH_4 emissions were high from digestate-treated columns, particularly from the Veg 3 dS/m treatment. Throughout the trial, the CH_4 emissions from the high digestate applications were occasionally higher than soil control treatment. Interestingly, there was greater CH_4 emissions released from the Rudosol in comparison to the Vertosol, with 0.62-0.71 vs 0.42-0.47 mg CH_4-C m^2, respectively. This differs from the general trend where coarse-textured soils are commonly found to have lower CH_4 emissions compared to fine-textured soils (Boeckx et al., 1997, Wagner et al., 1999). The reason for may be due to

nitrogen dynamics, which affect CH_4 emissions (Wagner et al., 1999), where higher application of ammonium and nitrate can enhance CH_4 emissions (Bodelier and Laanbroek, 2004). Potentially, the lack of nitrogen uptake by plants in the Rudosol has led to sufficient mineral nitrogen concentrations remaining that could have enhanced the CH_4 emissions.

Significant CH_4 soil emissions in the first few days after digestate application is consistent with the application of other organic amendments to soil (Eickenscheidt et al., 2014). Chadwick et al. (2000) found that the first 24 hours after pig and dairy cow slurry application can account for up to 90% of CH_4 emissions. In this trial, the first time point accounted for 14% and 16% of the Rudosol and Vertosol CH_4 emissions. Unexpectedly, the peak in CH_4 emissions may not have been measured, as Wulf et al. (2002) found that the majority of the CH_4 emissions were released immediately after digestate application, and continued for 24-48 hours depending on the soil type (arable or grassland soil). Tiwary et al. (2015b) tested different methods of digestate application and found that for mixed waste digestate and surface applied digestates, CH_4 emissions peaked before 24 hours, whereas digestate that was incorporated into soil took two days to peak. However, in this trial, the CH_4 soil emissions from both soil types are low, with maximum fluxes of 0.05 CH_4 $mg/m^{-2}h^{-1}$, in agreement with the finding that aerobic soils rarely exceed 0.1 CH_4 $mg/m^{-2}h^{-1}$ (Le Mer and Roger, 2001).

In the Rudosol, the soil microbial community was stimulated (compared to NPK-only) with Mush 3 dS/m treatment from day 5 and Veg 3 dS/m treatment from Day 16. However, CO_2 soil emissions were comparable to the control. In contrast, the Vertosol CO_2 soil emissions were lower from NPK-only compared to digestate treatments. There were greater CO_2 emissions released from the Vertosol in comparison to the Rudosol, with 873-1365 vs 3000-668 mg CO_2-C m^2, respectively. This is likely due to the low nutrient content of the Rudosol compared to the Vertosol; therefore, the Vertosol provided a more favourable habitat for microorganisms.

Chen et al. (2012) found that biogas residues reduced C turnover (CO_2) and increased soil C content compared to maize straw. This was attributed to the lower labile C (hemicellulose, cellulose and lignin content) in the biogas residues compared to maize straw (Chen et al., 2012). In agreement, Möller (2015) found that most authors reported a short-term reduction in CO_2 soil emissions after digestate application.

While the reason for this is unclear, it could be the digestate application or land use management, such as crop system changes. Johansen et al. (2013) found that digestated materials in an incubation experiment increased CO_2 emissions, but only to a minor extent and it was attributed to the available organic material in the soil. Soil respiration can be dependent on the availability of carbon in the soil (Eberwein et al., 2015), on soil moisture content and water retention (Bouma and Bryla, 2000, Cable et al., 2008). The effect of soil texture on soil respiration is not consistent and commonly insignificant as a factor effecting soil respiration changes in the literature (Bouma and Bryla, 2000, Cable et al., 2008). However, at times, the interaction of organic carbon and clay minerals are found to be significant (Wang et al., 2003) and indirect effects of soil texture's impact on moisture content are important. Wang et al, 2003 suggested that clay minerals have a protective effect on organic carbon decomposition, where the carbon mineralisation rate decreases with increasing clay content. Although Wang et al, 2003 highlighted that there are many factors that impact soil respiration and some processes may interact and increase complexity. These results suggest that digestate application and the conditions of the Rudosol, with NPK fertiliser blending, was favourable to the soil microbes. Whereas, the same application in the Vertosol reduced microbial activity and may be effected by the clay mineral's protective nature.

In both soils, there was a distinct effect of digestate treatment on N_2O soil emissions. As the digestate application increased, the nitrous oxide soil emissions increased correspondingly. The N_2O soil fluxes in this study, 100 and 115 $\mu g/m^{-2}h^{-1}$ from Rudosol and Vertosol, respectively, are comparable to the emissions observed in other field studies. The results in this study are comparable to Wulf et al. (2002) who found a peak of 128 $\mu g/m^{-2}h^{-1}$ and lower than Eickenscheidt et al. (2014) who found largest peaks ~250 and ~150 $\mu g/m^{-2}h^{-1}$, depending on the time of year. Importantly, Veg 3 dS/m and both Mush applications had the highest N_2O emissions in the first day after digestate application in the Vertosol.

The amount of nitrogen volatilisation can depend nitrogen fertiliser application, as well as application methods, digestate pH and environmental conditions (Crolla et al., 2013). The application of additional nitrogen may provide an explanation for increased N_2O emissions from digestate-applied treatments. In combination with the base NPK fertiliser rate, additional amounts of nitrogen were applied, predominantly in

NH_4^+ form (Table 20). Furthermore, high NH_4^+ concentrations and easily degradable organic C, which may be present in these digestates, can enhance N_2O soil emissions (Wulf et al., 2002).

Indeed, application methods may be a contributing factor to the N_2O emissions produced from soils. Although digestate injection or soil incorporation are a common strategy to reduce NH_3 emissions (Riva et al., 2016, Nkoa, 2014), there are some reports suggesting that N_2O emissions can be high from digestates that are injected into the soil (Wulf et al., 2002, Möller and Stinner, 2009). Wulf et al. (2002) found that injected digestates had higher cumulative N_2O emissions in comparison to harrowed, splash plate and trail hose methods. Möller and Stinner (2009) found that emissions from injected cattle slurry digestate was higher than controls but lower than undigested cattle slurry.

Furthermore, the salinity of the water may have contributed to increased N_2O emissions. Zhang et al. (2016) found that using 0.35, 4.61 and 8.04 dS/m waste water in drip irrigation increased N_2O emissions significantly, increasing with increasing salinity content. This was attributed to salts inhibiting nitrification, resulting in incomplete nitrification and accumulation of NO_2 in the soil, increasing N_2O production (Zhang et al., 2016). The higher amount of nitrogen in the digestate with the NPK fertiliser is most likely the cause for the higher N_2O emissions; however, the application method and the salinity of the digestates may also be contributing factors.

6.4.3. Soluble metals in leachates and total metals in soils

The majority of research into digestate application to land has focused on N and/or P (Crolla et al., 2013, Nkoa, 2014, Möller and Müller, 2012, Edwards et al., 2015, Monfet et al., 2017). This is mainly due to high concentrations of N and P in the digestates; unlike in this trial. While salts are commonly high in digestates, they have rarely been the focus of research into digestate application to land. It must be noted that the high salt content and low N and P observed in this study could be due to the choice of sampling technique. Samples were deliberately taken from the top of the digester without extensive mixing of the digestate tank; where the highest concentrations of water-soluble elements (particularly salts) would be located. This enables useful findings, as it this material is distinctly different from the majority of digestate characteristics reported in the literature. The digestates in this study have more similarity to saline wastewaters, and using

saline wastewaters for irrigation has an important role in the sustainability of food production (Niu and Cabrera, 2010). Using saline wastewater for irrigation requires caution due to potential environmental harm with released salts that could affect plant growth and soil stability (Panta et al., 2016).

Leached salt concentrations from the Vertosol were high, although there were no significant differences between treatments. The Cl^- concentrations peaked at 18 mmol/L, average 12 mmol/L, where Rengasamy (2002) observed a peak at ~14 mmol/L in restricted drainage soils. Similarly, leached Na^+, Ca^{2+}, K^+ and Mg^{2+} concentrations are high, with averages of 874 Ca^{2+} mg/L, 222 Na^+ mg/L, 101 K^+ mg/L and 389 Mg^{2+} mg/L for Vertosol. However, this was probably due to the soil type rather than digestate treatments as the Vertosol prior to application already had high soil cations (Table 21). Regardless of this, high salt concentrations in leached water may contribute to salinity issues in underlying water tables; especially in areas with shallow soil horizons and no plants that will further increase the likelihood of saline water leaching (Rengasamy, 2002). Furthermore, high concentrations of Ca^{2+}, Na^+ and Mg^{2+} can increase the sodium absorption ratio, which is important for the binding of Ca^{2+} and Mg^{2+} to organic ligands and soil hydraulic conductivity (Halliwell et al., 2001).

It was hypothesised in this study that the Rudosol would leach higher amounts of digestate-associated elements due to a sandy soil texture that is free-draining. This was proven, as the Rudosol leachates had increasing Na^+, K^+, Cl^-, soluble Fe and Al^{3+} concentrations in leachates with increasing Veg digestate application, and Mg^{2+} concentrations in leachates increased with Mush digestate application. Encouragingly, the soluble metal concentrations in the Vertosol leachates did not increase with increasing digestate application. This is likely due to the high metal and nutrient concentrations (in comparison to the Rudosol and the dilute digestate treatments) in the pre-experiment Vertosol (Table 20). Furthermore, both Rudosol and Vertosol leachates were much lower than EILs for short-term irrigation water (DEC, 2010), regardless of treatment. When EILs are used as a guideline, the increase in potentially harmful element concentrations in the Rudosol leachates were not at concentrations that may be harmful to the environment. However, it should be noted that EILs are only meant for initial screening (DEC, 2010); therefore, future work should include additional assessments to test application appropriateness for other soils, plants and/or environments. This study suggests that sandy soils may pose potential problems in leaching, particularly with the Veg digestate.

This study researched the hypothesis that the Vertosol would accumulate higher metals and salts within the soil, due to the higher clay content and associated high CEC (18 $cmol^+/kg$). Significantly, there was no obvious increase of metals and salts due to digestate treatment. Depth seemed to have the most influence on the metals and salts for the Vertosol, where 15-30 cm had the highest EC, CEC and ESP values, as well as the highest Cl, S, exchangeable Ca, Mg and Na concentrations. In the Vertosol, Mn concentrations were above EILs for soil; however, Reimann and de Caritat (2017) commented that the EILs for Mn are unrealistically low for Australian soils. Importantly, the Mn concentration in the original soil, prior to digestate application, was higher than the EILs. Therefore, these results suggest that depth and soil type are the most influential factors for the Vertosol, as opposed to digestate treatments.

In the Rudosol, Veg digestate applied at 3 dS/m had high total metals in the soil (As, Cr, Mg, Co, Zn, Ni and V), particularly in 0-5 and 5-15 cm. The increase of Zn, Co and Mg concentrations in this study can be considered in a positive light due to their observed concentrations, their importance in plant growth and soil health and/or their deficiency in Australian soils (Armour and Brennan, 1999, Aitken and Scott, 1999, Peverill and Judson, 1999). Ni is essential at low concentrations (as observed in this study) and does not biomagnify up the food chain (Wuana and Okieimen, 2011). V concentrations in the Rudosol were below the common range for soils of 20-120 mg/kg (Baken et al., 2012), where the highest was 9.1 mg/kg. However, elevated soil V concentrations in soils can negatively affect a wide range of biota, including humans, plants and micro-organisms (Baken et al., 2012). Additionally, both As and Cr are common pollutants that require careful management; therefore, the increase of V, As and Cr metals in this study is potentially concerning. Nkoa (2014)'s concern that accumulation of Cu, Zn and Mn in digestate-amended soils in the long-term may occur does not seem likely for Cu but may be an issue for Mn and Zn. This may be a particular problem if the application rate or frequency is increased. Promisingly, the majority of total metal concentrations were lower than EILs for soil (DEC, 2010), regardless of treatment or soil type.

In the Vertosol, there was an increase of ESP with depth (2.6%) and Cl concentrations at 15-30 cm. An ESP of >15% can indicate sodicity (Walker and Bernal, 2008); however, an ESP as low as 5% can create issues with soil dispersion (Rengasamy and Churchman, 1999). Furthermore, the soil EC of both the Rudosol and Vertosol are <0.3 dS/m. Based on these values, the Rudosol and Vertosol both are not sodic. However,

consideration and future research of long-term effects is required, as research on saline wastewaters and their application to soil have shown that effects of Na in irrigation water can be latent (Halliwell et al., 2001).

6.5. Conclusions and future directions

The NSW EPA regulation outlined two main goals: 1) that applications provide a net benefit and 2) that there was minimal contamination, particularly from leaching and run-off (DEC, 2010). While "benefit" is a vague provision that warrants definition, it is noteworthy that the results in this trial suggest that digestate applications, blended with NPK fertilisers, may assist with plant growth in low nutrient soils, such as the Rudosol used in this trial. In contrast, digestate applications did not affect plant growth in soils with higher nutrient content such as the Vertosol.

Of concern is that the high N_2O emissions from both soils with digestate applications is significant, given the low application rate. The higher N_2O emissions are possibly because of the higher amount of applied nitrogen to digestate treatments, blended with NPK fertilisers. Speculatively, it may be important that injection of the digestates into the soil could be contributing to high emissions, as this has been observed in previous soil trials (Wulf et al., 2002). The salinity of the treatments may have also contributed to the higher N_2O emissions, as reported in previous saline wastewater irrigation trials (Zhang et al., 2016).

The application of digestates did not increase soluble and soil metal concentrations above potentially harmful concentrations, using EILs as a guideline; in terms of the salts and metal concentrations found in the leachates and soil. Considering that the digestates in this study showed a benefit to plant growth in the low nutrient soil, these digestates might be applied either more frequently or with a higher loading, potentially with a plant species of higher salt tolerance. However, special consideration must be maintained for sandy soils such as Rudosol and digestates similar to the Vegetable digestate, as this digestate increased potentially undesirable salts and metals in leachates (Na^+, K^+, Cl^-, soluble Fe and Al^{3+}) and soil (As, Cr, Zn, and V). This trial provides a necessary baseline for the application of these digestates, particularly concerning Australian digestates; however, further investigation and consideration is required into the effects on soil metals and salts. Considering the contrasting effects of the impacts with the Rudosol versus the Vertosol, a soil with an

intermediate soil texture (e.g. loam) could allow more insights into the effect of digestate on soils. Furthermore, for genuinely sustainable and safe application to land practices with digestates, a holistic assessment of the effects regarding the entire chain, not just the final product, must be completed (Möller, 2015).

While this experiment raises a number of additional questions, this assists assist in asking the *right* questions. Such as: is the salinity and sodicity noted in these digestates consistent with digestates from other Australian digestate plants? If so, is the high dilution necessary to fulfil regulatory requirements and avoid negative impacts worth the effort? It is possible that if the digestates were mixed in with a fertigation or irrigation system, the addition of the material may be relatively easy and may provide beneficial micronutrients (e.g. Zn, Co and Mg). Another important question is: How much can soil with low nutrient concentrations benefit from digestate application? There is inadequate data from this work to provide a definitive answer this question, but this experiment highlights an important research gap for future investigations.

Chapter 7. Conclusions

7.1. Introduction

Massive amounts of food and beverage "waste" are produced globally and are recognised as a resource that remains largely untapped (Pham et al., 2015). As previously stated, organic amendments, such as those sourced from food and beverage waste, can increase soil biological functions, soil organic carbon and crop yields (Diacono and Montemurro, 2011a). Organic amendments are vital for climate change mitigation to sequester carbon (Diacono and Montemurro, 2011a) and manage land use for N_2O and CH_4 gases released from soils (Thangarajan et al., 2013).

Valorisation options of two select waste streams presented in this thesis have shown significant results that add to existing knowledge on waste reuse and soil organic amendment options.

The objectives of this work, with the relevant outcomes, were to:

1. Investigate the potentially valuable components in coffee waste (SCGs and husks) through conventional extractions and using common solvents (Chapter 2.), focusing on extraction of lipids, caffeine and polyphenols

   Outcome: Characterisation of the SCGs and husks showed that the composition was very similar, in terms of total nitrogen, hemicellulose and cellulose. A comparison of the caffeine and polyphenol content of coffee wastes is useful to understand the valorisation options that may be beneficial.

   Outcome: Coffee husks are a potentially valuable source of C18:3 fatty acids.

2. Investigate the application of SCGs on short-term glasshouse plant growth trials:

   a. High application rate 20 t/ha with additionally ethanol-extracted and "as is" (air-dried) SCGs in short-term glasshouse plant growth trials using silver beet
      (Chapter 3.).

   b. Phytotoxicity trials using extracted liquid and solids in root elongation and emergence tests
      (Chapter 4.).

c. Two application rates (5 and 10 t/ha) with additionally water-extracted and air-dried SCGs in short-term glasshouse plant growth trials using silver beet (Chapter 4.).

Outcome: Application of Spent Coffee Grounds (SCGs) may reduce N_2O soil emissions and increase soil respiration.

Outcome: There were varied effects on silver beet growth with application SCGs in two short-term trials.

3. Investigation of digestates in soil column trials and their impact on nitrogen leaching, soil emissions, and, in a separate trial, short-term plant growth (Chapter 5.).

   Outcome: Food-waste digestate sourced in the Netherlands had high mineral nitrogen leaching and CH_4 soil emissions, with the only effect to early stage plant growth occurring at the highest application rate.

4. Investigation of digestates in soil column trials and their impact on soil emissions, metals and salts in leachates and soils as well as short-term plant growth (Chapter 6.).

   Outcome: Australian digestates, sourced from food waste treated in an anaerobic digestates, are comparable to saline wastewater. These could provide plant-essential nutrients in low fertility soils, provided the salinity is managed through dilution, but increased N_2O soil emissions.

The hypothesis of this work is that valuable components can be extracted from coffee waste (Chapter 2.). This was true.

It was further hypothesized, the extracted SCGs can reduce potential phytotoxicity in short-term glasshouse plant growth trials (Chapter 3. and Chapter 4.). The extractions used did not significantly reduce plant growth inhibition.

The second part of this work investigated digestates, from the Netherlands, applied to soils. It was theorised that high application rate digestates could increase soil emissions and nitrogen leaching in the absence of plants and reduce plant growth (Chapter 5.). It was true that digestates increased soil emissions. Digestates

did not increase nitrogen leaching compared to the fertiliser control, however, the concentrations of nitrogen leached were still concerningly high. The digestates did not reduce plant growth, however, only early stage plant growth was tested.

The investigation of Australian digestates on two contrasting soil textures hypothesised that metals would accumulate in the clay-textured soil and leaching would increase in the sandy-textured soil (Chapter 6). It was not true that digestates accumulated metals in the clay-textured soil, however, this may be due to high metals in the original soil. It was true that digestates increased leaching in the sandy-textured soil.

7.2. Coffee residues

SCGs are often the focus of valorisation of coffee waste (Stylianou et al., 2018, Kondamudi et al., 2008, Campos-Vega et al., 2015), in which comparisons of SCGs to other coffee wastes, such as husks, are not commonly reported other than land applications. The results found in this work are useful to broaden an understanding of the composition of other coffee wastes. SCGs and coffee husks collected from Melbourne, Australia, were characterised, used in extractions and applied in a series of plant growth and soil trials. Notably, the SCGs and husks collected were similar in terms of total nitrogen (2.4%), cellulose and hemicellulose, where solid state ^{13}C NMR showed a similar spectrum between three different SCGs and two husk types. Husks were a higher and potentially more economical source of caffeine (due to waste production at manufacturing rather than in disseminated cafes) in comparison to SCGs, with 9.7-11.3 mg/g for husks vs 4.1-4.9 mg/g for SCGs. In contrast, SCGs were a substantially higher source of polyphenols compared to husks, with 17-23 vs 7.5-9.6 mg gallic acid equivalents/g.

The presence of C18:3 omega-3 fatty acids was 20-39%$_{relative}$ in the husk extractions, which revealed a potentially cheap source of these human-essential fatty acids that are currently being discarded. Notably, the presence of fatty acids in husks has not been reported in literature previously. The ratios of the omega-3 to omega-6 fatty acids are also encouraging for untapped valorisation in the health industry; particularly as supplements in Western diets (Simopoulos, 2002).

The cumulative N_2O soil emissions from both plant growth trials using SCGs, with and without urea blending, were significantly lower than the urea controls. Significantly, this has not previously been reported in the literature (Stylianou et al., 2018). This finding is also noteworthy because organic amendments with high nitrogen contents, particularly blended with fertilisers, have generally been found to increase emissions (Charles et al., 2017). The work reported here contradicts this where the 20 t/ha SCGs, including those blended with urea, substantially reduced N_2O emissions compared to the fertilised and unfertilised control. The reduction of N_2O emissions remained significant with reduced application rates of 5 and 10 t/ha SCGs, also blended with urea, when compared to the unfertilised control. As a result, the total nitrogen (TN) applied was retained in the soil with the application of SCGs. This is an very informative finding for the management of these harmful greenhouse gas emissions in order to mitigate climate change (Stylianou et al., 2018), as well as retaining applied TN in soils.

Only one other study, by Cervera-Mata et al. (2018) has previously reported the effect of SCGs on the soil microbial community and in different soil types (and associated pH) on plant growth. That study was an incubation experiment which found a significant increase of soil microbial activity (Cervera-Mata et al., 2018), unlike this work. The work reported here has confirmed that the soil microbial community was stimulated in glasshouse experiments and in Australian soils, in contrast to the other study that focussed on Mediterranean soils in an growth chamber. This results in this study observed a substantial increase of soil respiration after application of 20 t/ha SCGs, which remained significant even with a reduced application rates of 5 and 10 t/ha SCGs. The increase of soil respiration is a useful bioindicator for increased soil health, particularly with no observed reduction in plant available N and P (Cardoso et al., 2013). In a wider context, soil respiration contributes the largest portion of overall ecosystem respiration (Barba et al., 2018). This study also found that a commonly reported application rate (5 and 10 t/ha) of SCGs had contradictory results in two sandy agricultural soils with contrasting pH. This validates the hypothesis that organic amendments and their impact on plant growth is contextual, where soil type and pH may play an important role in the context of the impact of SCGs as soil amendments. Therefore, the findings in this study could help inform the use of SCGs as soil amendments and their impact on soils, plants and soil emissions. Importantly, this

research shows that it is complex to predict the likely agronomic and environmental effects of SCGs applied to land.

The phytotoxicity of caffeine and polyphenols within SCGs have been reported to be the main culprit of plant growth inhibition after SCG application (Cruz et al., 2012a, Cruz et al., 2014b, Cruz et al., 2014c, Hardgrove and Livesley, 2016). This work showed that liquid extractions from SCGs caused root elongation inhibition in lettuce, which has a high concentration of caffeine and polyphenol. However, despite ethanol and water extractions and emergence tests, the plant inhibition did not seem to be a direct result of caffeine and polyphenol concentrations. Consequently, these two additional extractions (ethanol and water) of SCGs did not increase the agronomic value and, in one case (the Calcarosol), the additionally extracted SCGs were less favourable (amplified plant growth inhibition) than the air-dried "as is" SCGs. While an increase of soil respiration and reduction of N_2O emissions could indicate a immobilisation of plant-essential nutrients, which was observed in the fresh application of organic matter to soils (Fontaine et al., 2003), this was not observed in the soil sample analysis. Therefore, this study demonstrates that direct phytotoxicity due to caffeine and polyphenols or immobilisation of plant-essential nutrients is possibly not the reason for plant inhibition. To further test this, a future direction could be to apply polyphenols and caffeine separately in experiments, however, polyphenol standards are expensive and there is a large variety of polyphenols.

Could this be a microbial story? It is probable, considering the significant increase in soil respiration and a reduction in N_2O emissions relate to microbial activity. Organic amendments promote changes in the microbial community physiology (Bastida et al., 2015) and soil microbes are known to be important regulators of plant growth promotion (Van Der Heijden et al., 2008). Indeed, it is difficult to separate the direct and indirect effects of an amendment on soil microorganism behaviour (Diacono and Montemurro, 2011b). The results reported in this study indicate a complex plant response to the application of SCGs, where soil type may be important; further in-depth studies on the soil microbial response to SCGs may assist in understanding this complexity.

7.3. Digestates

Digestates, from food waste feedstocks, application on a Dutch sandy soil reported significant positive findings (Chapter 5.), observing that mineral nitrogen leaching was significantly lower in digestate treatments compared to a nitrogen fertiliser control. However, all concentrations in leachates were higher compared to similar experiments conducted by Li et al. (1997), Goberna et al. (2011), Burgos et al. (2006), and higher than limits of the nitrate directive on leaching concentrations (Van Grinsven et al., 2016). Furthermore, digestate treatments had higher losses of nitrogen through N_2O soil emissions, which is undesirable. Therefore, these results support the recommendation that nitrogen application should be matched with plant demands (Nkoa, 2014); allowing for consideration of soil texture on leaching potentials. Another potentially problematic finding found in this study (Chapter 5.) was that CH_4 soil emissions were significantly increased in one digestate treatment, suggesting that an anaerobic zone was generated. Factors such as digestate composition, soil texture and the water saturation of the soil could influence these findings. This highlights the fact that soil conditions require monitoring to avoid higher than desired CH_4 soil emissions.

In a separate trial with the same Dutch food waste-sourced digestates, an emergence test using lettuce and radish showed encouraging results, observing no short-term plant growth inhibition from an application rate range of 50-250 kg N/ha equivalent. The results are significant because the reported effects of digestates on plant growth varies greatly in the literature (Möller and Müller, 2012) and feedstock largely determines the composition, therefore the use, of digestates (Nkoa, 2014). A shortage of research on food-waste sourced digestates means that these results are a useful addition to the evidence for the use and regulation formulation of digestates.

This work provides an important preliminary assessment for future use of Australian digestates, sourced from food waste, as previous studies have not adequately reported on this area. The assessment of digestates in Australia is valuable because of the increasing popularity of anaerobic digestion in Australia (Edwards et al., 2015) and an increasing worldwide demand for the reuse of potentially valuable nutrients for agriculture

(Tampio et al., 2016b). Regarding the material observed in this study, comparisons and guidance can also be taken from saline wastewater research, due to the high salt content.

Positive results in this study suggests that digestates could provide a source of nutrients in low fertility soils. However, in both clay- and sandy-textured soils there was a substantial increase in N_2O soil emissions that must be addressed. Furthermore, in the sandy soil, higher concentrations of metals in the leachates (Na^+, K^+, Cl^-, soluble Fe and Al^{3+}) and soil (particularly As, Cr, Mg, Co, Zn, Ni, V) were measured in one digestate treatment when compared to the controls. The increase of As, Cr and V concentrations in the soil could be potentially problematic for contamination. Encouragingly, plant growth was not inhibited in both soils and digestate treatments did not increase the soil metals beyond Ecology Investigation Levels, suggesting that there was no observed contamination of both soils due to digestate treatment.

7.4. Summary

This work advocates the proposition that coffee husks could be a valuable source of C18:3 fatty acids for human-essential dietary supplements. Regarding SCGs as organic soil amendments, the results indicate that SCGs can be used, without modification or further extractions, to reduce N_2O emissions emitted from the soil and to increase the soil microbial activity. The inhibition of plant growth due to the application of SCGs may be more complex than the literature suggests, as these experiments indicate that direct phytotoxicity due to caffeine and polyphenol concentrations does not seem likely. However, this does require further testing.

It is important that nitrogen supply should be matched with plant growth to avoid nitrogen leaching and emissions when considering the food waste-sourced digestates, in both experiments. In addition, the soil conditions require monitoring to avoid unwanted CH_4 emissions, even from typically aerobic soils. In the Australian digestate trials, baseline research was completed using soil column trials, indicating that control of the salinity in the digestates may be a primary consideration when using this material on crops. The N_2O soil emissions were high in both sandy and clay soil types requiring monitoring and further investigation to mitigate the production of this harmful greenhouse gas. Positive results for plant growth were observed in the sandy soil with low fertility, and no soil contamination was observed in both soil types.

Informed, science-based and environmentally-friendly use of food and beverage wastes, such as the wastes reported in this study, is critical for a sustainable future. This work adds to research in order to assist the use of these materials. The results suggest alternative, value-adding ways to use coffee husks, guidance in the use of SCGs as soil amendments and reports preliminary experiments on food waste-sourced digestates.

7.5. Discussion regarding impact on waste to land regulations and future research

The constant struggle between waste diversion from landfill, protection of environmental and human health, maintaining food security and economic efficiency is a continuing balancing act. This balancing act involves many people, sectors of government, industry and academia as well as those with related expertise and viewpoints. It is for these reasons that continued investigations are paramount to support the successful regulation of regulate organic amendments sourced from wastes to protect humans, the environment, soils and plants from negative impacts. The questions raised by these investigations and experiments are important to ask and consider in future regulatory considerations and best practice implementation.

One such question which is raised by this work is: when there is seemingly no benefit or negative impacts to plant growth by the organic amendment (and no observed soil contamination) should the amendment be used? Consider the SCG trials with the Podosol at 5 and 10 t/ha application rates as well as the experiments of anaerobic digestates on the Vertosol. In both of these experiments there were no significant benefit to plant growth. Is it still worthwhile applying a treatment that will have little agronomic benefit on plant yield? Crossing the line of using our land as a dumping ground has been a topic of discussion in Australia and New Zealand for decades. For example, Cameron et al. (1997) concluded that wastes are suitable fertilisers and soil conditioners, but harmful effects are not well understood or investigated. Is the risk of discovering another emerging contaminant, like per- and poly-fluoroalkyl substances (PFAS), in these wastes too high to risk potential future effects on humans, land, plants and soil health?

Importantly, the EPA regulations (such as those in Victoria, New South Wales and South Australia) require soil amendments to have a benefit to land in order to satisfy the provisions of land application of waste derived amendments. The EPASA standard for waste derived amendments specifically state that land must not become an alternative, more convenient, disposal method. It is suggested that considerations of amendments should extend beyond plant growth effect and be inclusive of soil attributes. In the cases of the SCG trials with the Podosol at 5 and 10 t/ha application rates as well as the experiments of anaerobic

digestates on the Vertosol there were increases of soil micronutrient concentrations, increases in soil respiration and reduction in nitrous oxide soil emissions. In fact, both SCGs and digestates provided micronutrients which are generally lacking in Australian soils (Holloway et al., 2008). It is therefore suggested that the applications of the amendments in these cases mentioned are still potentially beneficial.

The majority of the soils used in this thesis were nutrient impoverished sandy-textured soils (Chapters 3-5), where both the SCGs and digestates provided plant-essential nutrients. However, even though the nutrient concentrations in the soils were increased, the plant growth did not always follow the same trend, especially with the SCG applications. Low nutrient soils with sandy textures remain the best 'fit' for these materials. This 'fit' was generally proved to be favourable where the application of the amendments did increase the soil's nutrient concentrations and benefits were observed in increased soil respiration. However, the effect on plant growth was harder to predict than the benefit to the soil, mainly for SCGs. It may be prudent to be inclusive of both effect on soils and plant growth to obtain a greater understanding of the amendment application effect.

When considering the impact of SCG and digestate amendments to soils, the important attributes were: plant species, soil conditions (e.g. water saturation) and soil characteristics (e.g. soil texture). The continual monitoring of soil emissions and soil conditions were particularly important in these experiments, to ensure that emissions and leaching do not exceed environmental risk levels. While these aspects have previously been identified as important in regulation of wastes to land, such as in the EPASA regulations and EPANSW exemptions, it is critical to continue evaluation of these factors when using new materials.

7.5.1. Spent Coffee Grounds

This thesis suggests the main considerations when applying SCGs to soil are: plant species, application rate and soil type. It is suggested, based on silver beet trials as well as millet and lettuce rate response experiments, the effects of SCG application on plant species is complex. The positive effect of SCG application on soil nutrient concentrations, reduction of nitrous oxide soil emissions and increase of soil respiration was more consistent. It is recommended that SCGs are applied at low application rates to sandy-textured soil, where plant species which are sensitive to phytotoxic components are avoided (e.g. lettuce).

It is also suggested, as previously mentioned, that sandy-textured soils with low fertility would best benefit from SCG application, due to these soils having low nutrient concentrations, low water retention, which can positively affected by the application of SCGs. For the same reasons Kasongo et al. (2011), Kasongo et al. (2013) and Cervera-Mata et al. (2018) also theorised that sandy-textured soils with low nutrient concentrations would benefit most from SCG application. However, caution still must be used, as noted in the Podosol versus Calcarosol experiment, and due process, like those set up by EPA regulations, are necessary to test individual soil types and situations.

It is suggested that SCGs are used without extraction of potential phytotoxic components, as both the ethanol and water extractions did little to reduce plant growth inhibition. Furthermore, the extractions increased the time and cost before application, even though both extractions were reasonably environmentally friendly, time and cost-effective extractions. A more thorough extraction method, such as supercritical carbon dioxide extraction, may increase the success of removing the phytotoxic components, however, this would also increase the costs, time, and expertise required. It is suggested that these extraction methods would only be considered if the components extracted are valuable for further use (such as lipids, caffeine or polyphenols), rather than aiming to 'clean up' the material. This thesis and the literature demonstrates (Campos-Vega et al., 2015, Kovalcik et al., 2018, Stylianou et al., 2018) that there are an abundance of valuable components within SCGs that can be extracted for further use. Although this is outside the scope of this thesis, extractions of valuable components must also consider circular economy, lifecycle analysis, and perspectives to ensure sustainability.

It is recommended that SCGs are potentially appropriate for application on small to medium scale horticulture farms and urban gardens, but likely not appropriate for large-scale farms. Small to medium scale horticulture farms and urban gardens could more easily monitor and manage the application rate and short- and long-term effects of application in comparison to large farms. This recommendation is based on that fact that the majority of experiments, including the experiments in this thesis, predominantly use horticulture plant species on small scales (Cruz et al., 2012a, Ciesielczuk et al., 2017, Cruz et al., 2014c, Yamane et al., 2014, Hardgrove and Livesley, 2016). Furthermore, from a sustainability point of view, such as transportation, SCGs application to large-scale farms is unattractive and perhaps not realistic. Therefore, the

translation from experiments to implementation could be easier because experiment conditions and management strategies mimics horticulture conditions in comparison to large-scale agricultural farming systems.

7.5.2. Digestates

The digestates investigated in this thesis show that the Netherlands digestates have different chemical characteristics compared to the Australian sourced digestates. The total nitrogen, total phosphorus and ammonium concentrations, as well as pH and total solids characteristics in the three Netherland digestates were consistent with the ranges reported in the literature (Nkoa, 2014), and electrical conductivity was slightly lower than reported in literature (Akhiar et al., 2017). Importantly, the two Australian digestates had substantially higher EC, lower nitrogen concentrations (total and mineral), and higher moisture content in comparison to the Netherlands digestates. Although these samples are undoubtedly inadequate to make conclusions and lack representation of digestates as a whole, the difference in chemical characteristics is large. Additionally, while differences in digestate chemical characteristics are somewhat expected due to the extensive variety of feedstocks found in different countries as well as in varying operation conditions between anaerobic digestion plants, it highlights that Australian regulatory bodies must use caution when adopting or modifying overseas regulations and guidelines.

Based on these digestate characteristic differences, it would be ideal for the regulation of digestates to soil in Australia be based on Australian research using Australian digestates, conditions and soils. Furthermore, in Australia, digestates intended for land application should have salinity and sodicity checked as a priority, especially considering the prominent salinity and sodicity issues in Australia (Rengasamy, 2002). Other aspects of digestate application to land, such as nitrate leaching, emissions and soil contamination, are considered in regulations and the experiments here show that these regulations should be continued. The heavy metal concentrations in the food waste sourced digestate samples are low, which is consistent with the literature (Tampio et al., 2016b, Sheets et al., 2015). It is recommended that this thesis provides a future direction for investigation, but an expansion of the data set is certainly required for digestate characteristics in Australia and their effect on soil health and plant growth.

7.5.3. Summary

The organic amendments in this thesis and their effect on soil, plant growth and soil emissions are important, a waste material can have a wide range of impacts which can depend on application rate, soil type, soil condition and plant species. There are potential benefits of SCGs to sandy-textured horticultural soils using low application rates, where soil nutrient concentrations and soil respiration was increased, and nitrous oxide emissions were reduced. The potential risk of SCG application is a reduction of plant growth if either application is on sensitive plant species or moderate to high application rates are used. Digestates also have a potential benefit to soils with low nutrient concentrations, where digestate application may assist plant growth. However, leaching in sandy-textured soils and increased harmful soil emissions remain a concern.

Furthermore, regulations requiring amendment testing, particularly for soil emissions, leaching and soil contamination, remain paramount to maintain protection of the environment. Considerations for regulatory bodies are: 1) the applicability of SCGs for use in small to medium horticultural farms and 2) the chemical characteristic differences of Australian anaerobic digestates to the Netherland (and literature) digestates chemical characteristics.

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