An Investigation into the Potential of Faecal sludge for Plant Production

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As the candidate's supervisor I have/have not approved this dissertation for submission.

Signed:_____ Name: _____ Date: _____

"Man is the most extravagant accelerator of waste the world has ever endured. His withering blight has fallen upon every living thing within his reach, himself not excepted; and his besom of destruction in the uncontrolled hands of a generation has swept into the sea soil fertility which only centuries of life could accumulate, and yet this fertility is the substratum of all that is living."

F. H. King (1911)

Abstract

This study was motivated by the need to find an effective and sustainable management option for evacuated faecal sludge from pit latrines. Currently there are approximately 100 000 single vault pit latrines within the eThekwini municipality and it is estimated that as many as 45 000 are full and require servicing. The conventional option of treating faecal sludge at wastewater treatments works is not feasible and other options such as disposal at marine outfalls are increasingly falling out of favour in accordance with international best practise. Working within the framework of ecological sanitation this study examined the potential of faecal sludge as a nutrient source in plant production by conducting two pot experiments. The first experiment investigated growth and photosynthesis characteristics of flooded gum (*Eucalyptus grandis*) and black wattle (*Acacia mearnsii*) above a core of buried faecal sludge (FS) surrounded by sand which had low fertility. This was compared with a group which grew in sand alone and received commercial fertilizer so that the fertility of the sand approximated that of an accompanying field trial. In the second experiment, *Beta vulgaris* (Swiss chard) and *Solanum melongena* (eggplant) were grown in river sand amended with 10%, 20% and 30% (v/v) faecal sludge with a negative control of 0% faecal sludge and a positive control fertilised with commercial fertilizer.

Growth of *E. grandis* and *A.mearnsii* was vigorous above a core of buried FS and greatly exceeded that of the group which received fertilizer. At 26 weeks after planting, when saplings were harvested, mean height, leaf area, root collar diameter and dry biomass of *E. grandis* where FS was applied was (corresponding values where synthetic fertilizer was applied shown in brackets) 244 cm (108 cm), 11.97 m^2 (1.83 m^2), 40 mm (34 mm) and 1.52 kg (0.36 kg), respectively. Corresponding values for *A. mearnsii* were 270 cm (232 cm), 3.73 m^2 (1.95 m^2), 45 mm (39 mm) and 1.6 kg (1.0 kg), respectively. The positive response to FS in both species was reflected at the leaf level by increased rates of maximum CO_2 assimilation (A_{max}) and maximum electron transport (J_{max}) due to increased nutrient availability. Where FS was applied A_{max} and J_{max} of *E.grandis* was 17.3 $\mu mol.m^{-2}.s^{-1}$ (12.7 $\mu mol.m^{-2}.s^{-1}$) and 41.2 $\mu mol.m^{-2}.s^{-1}$ (18.2 $\mu mol.m^{-2}.s^{-1}$), respectively, and corresponding values for *A. mearnsii* were 43.1 $\mu mol.m^{-2}.s^{-1}$ (29.2 $\mu mol.m^{-2}.s^{-1}$) and 16.4 $\mu mol.m^{-2}.s^{-1}$ (11.7 $\mu mol.m^{-2}.s^{-1}$). The comparatively smaller response of *A. mearnsii* to faecal sludge

application, particularly in terms of growth, was possibly due to the nitrogen fixing abilities of this species. Roots of both species grew predominantly laterally which may have been an artefact of early containerised growth. Despite this, root distribution appeared unaffected by the presence of FS and some roots penetrated the sludge core against expectation as the FS was presumed to be anoxic. Water use efficiency (WUE) in *E. grandis* where faecal sludge was applied was 6.2 $\mu mol.mmol^{-1}$ and significantly greater than that of saplings of the same species which received fertilizer (3.2 $\mu mol.mmol^{-1}$) due to the combined effect of increased A and decreased transpiration (E). Though the positive growth and photosynthetic responses to FS application in both species was attributed mainly to increased nutrient supply, the contribution of improved soil properties cannot be underestimated.

Growth of *B. vulgaris* was enhanced in faecal sludge amendments relative to plants in unamended river sand and greatest at the highest amendment rate of 30% but less than that of plants which received synthetic fertilizer. Total dry biomass of *B. vulgaris* at harvest for the 0%, 10%, 20%, 30% and synthetic fertilizer treatments was 0.46 g, 12.02 g, 11.94 g, 33.92 g and 88.78 g, respectively. Only *S. melongena* in the 30% amendment and fertilized treatment could be compared at harvest due to disease in other treatments but indications from early growth of *S. melongena* are that growth followed similar trends to that observed in *B. vulgaris*. Total dry biomass of *S. melongena* at harvest was 42.19 g and 78.60 g for the 30% and fertilized treatments, respectively. A_{max} and J_{max} were strongly and positively related to amendment rate in *B. vulgaris* (R^2=0.71 and R^2=0.84, respectively) and comparable to that of fertilized plants at the highest amendment rate. However, these parameters were reduced in *S. melongena* at 30% amendment rate compared with the fertilized treatment, possibly due to a higher nutrient demand by this species. Based on foliar nutrient concentrations indications are that N and K were major limiting nutrients to growth of both species in amended sand. This was despite greater concentrations of N (and K for *S. melongena*) in the amendments by harvest than that of the fertilized sand. In this respect, it is suggested that sludge heterogeneity could have led to substantial over estimations of nutrients in the amendments and that foliar measurements were a more reliable predictor of specific nutrient limitations to growth. It is probable that, as suggested in the accompanying pot study, the addition of FS improved various soil properties and improvements in growth amongst these treatments were not due to

increased nutrient supply alone. Notwithstanding the risks posed by faecal sludge to human health, faecal sludge showed great potential as a support for plant production and represents a chiefly unexploited source of valuable plant nutrients.

PREFACE

The experimental work described in this dissertation was carried out in the School of Life Sciences, University of KwaZulu-Natal, Durban, from January 2009 to December 2010, under the supervision of Professor N.W. Pammenter.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

DECLARATION 1 – PLAGIARISM

I,……. declare that

1. The research reported in this dissertation, except where otherwise indicated is my original research.

2. This dissertation has not been submitted for any degree or examination at any other university.

3. This dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

4. This dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
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5. This dissertation does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the References sections.

Signed: ……

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1. Introduction

1.1. Plants and nutrients

Plants require at least 13 nutrients for growth and development to proceed normally (Evans and Edwards, 2001). That plants require a range of nutrients to grow was originally established by German botanist Carl Sprengel who asserted: "…*when a plant needs 12 substances to develop, it will not grow if any one of these is not available in a sufficiently large amount as required by the nature of plants*" (Sprengel, 1828, van der Ploeg *et al.*, 1999). In principle, this was the first enunciation of the 'law of the minimum', which would later become popularised by Justus von Liebig (von Liebig, 1855; van der Ploeg *et al.*, 1999). A simple, yet eloquent illustration of this law was provided to German farmers by von Liebig, in which posters of a barrel consisting of staves of varying height were used to express the law and its applicability to potassium addition in their fields with the shortest stave representing actual productivity (Holloway and Paris, 2002). Today, it is well-known that six nutrients, namely N, P, K, Ca, Mg and S – designated as 'macronutrients' – are required by plants in relatively large amounts while Cl, B, Fe, Mn, Zn, Cu and Mo (amongst others) – designated as 'micronutrients' or 'trace elements' – are required by plants in comparatively small amounts (Evans and Edwards, 2001). These 13 nutrients are essential nutrients for plants, together with C, H and O, using the criteria proposed by Arnon and Stout (1939). Those authors proposed three key criteria to establish essentiality of elements: "(a) a deficiency of it makes it impossible for the plant to complete the vegetative or reproductive stage of its life cycle; (b) such deficiency is specific to the element in question, and can be prevented or corrected only by supplying this element; and (c) the element is directly involved in the nutrition of the plant quite apart from its possible effects in correcting some unfavourable microbiological or chemical condition of the soil or other culture medium". Plants acquire all essential nutrients predominantly through their sub-aerial parts (roots) and aerial parts (Le Bot *et al.*, 1998). Insufficient nutrient availability from the soil or uptake by plants manifests as visible deficiency symptoms often specific to a particular elemental deficiency and characterised by abnormal colouration or stunted growth.

In natural habitats nutrients are cycled as plants grow and senesce, returning nutrients to the soil system (Le Bot *et al.*, 1998). However, in agricultural systems nutrients are lost to harvest due to the exportation of plant biomass, and the nutrients contained therein, away from the site of production (Kiley-Worthington, 1980; Cordell *et al.*, 2009). Consequently, nutrients in soil need to be replenished to balance nutrient losses and maintain high yields (Le Bot *et al.*, 1998). Prior to the 1840s this was achieved using recycled natural resources sourced locally including crop residues, manures, bones and fish (Marald, 1998; Neset *et al.*, 2008; Cordell *et al.*, 2009; Dawson and Hilton, 2011). The advent of industrialisation and rapid population growth in the 19th century placed enormous burden on soil fertility to the point that P in particular became primarily limiting (Dawson and Hilton, 2011). The solution to P shortages was initially to make use of guano from afar but as this was a limited resource supplies eventually became scarce (Stewart *et al.*, 2005; Cordell *et al.*, 1995). Phosphate rock became the most widely used source of P in agriculture and remains so to this day (Cordell *et al.*, 2009). Superseding the P shortage was a looming N shortage (Dawson and Hilton, 2011). While the atmosphere consists of 78% N, this is present as diatomic N (N_2) and the strong triple bonds render it biologically unavailable to plants (Galloway *et al.*, 2004). Forms of N that were usable by plants – so-called 'reactive' N – were in critical shortage at the time (Dawson and Hilton, 2011). Concerns were alleviated with the development of the Haber-Bosch process in the early 19th Century which was able to fix atmospheric nitrogen into ammonia, and by the 1920s urea was being produced (Smil, 2001). Approximately 4 billion people born in the previous century are estimated to have been dependant on Haber-Bosch N for food requirements (Erisman *et al.*, 2008). Moreover, the dependence on Haber-Bosch N appears to be growing. Smil (2001) estimated that 40% of the global population was dependant on Haber-N while a more recent estimate calculated by Erisman *et al.* (2008) places this figure at 48%, or approximately half the entire global population.

The practice of mining phosphate rock as a source of P is unsustainable; global phosphate rock reserves are being used at a rapid rate and global phosphorous shortages are anticipated although time-scales are debated (Cordell *et al.*, 2009; van Vuuren *et al.*, 2010; Gilbert, 2009). Generally accepted estimates place available reserves at between 50-100 years (Steen, 1998; Smil, 2000). The production of N

fertilizers has been viewed as less of an environmental concern since atmospheric N is abundant and has a life-cycle of orders of magnitude less than that of P (Dawson and Hilton, 2011). Yet of the 100 Tg of fertilizer N applied each year, 10-40% is returned to the atmosphere through denitrification (Erisman *et al.*, 2008; Galloway *et al.*, 2004). While such levels of denitrification are considered to be environmentally benign, it constitutes a serious waste of energy since a large amount of the global energy supply (1%) is used in the Haber-Bosch process (Erisman *et al.*, 2008).

1.2. Human excreta in agriculture

Human excreta consist of two main components: faeces and urine. Taken together, fresh human excreta can broadly be classified as a mixture consisting of organic matter, nutrients and water (Aalbers, 1999). The generation rate of excreta for each healthy adult is about 500 L of urine and 50 kg of faeces per year representing 10 kg dry matter (Jönsson *et al.*, 2004; Wolgast, 1993). In volumetric terms, approximately 1.5 L per person is excreted daily (SANDEC, 1997). However, the generation rate of excreta has been found to be highly variable. In this respect 15 test subjects from three separate villages in Thailand had generation rates which ranged from 730–1530 g of wet matter per capita per day (cap d^{-1}) and 50-87 g dry matter cap d^{-1} (Schouw *et al.*, 2002). The corresponding production of urine and faeces daily as determined by three test subjects was 0.6-1.2 L and 120–400 g wet matter cap d^{-1}, respectively. Nimpuno (1983) reported a generation rate of excreta (faeces and urine combined) of 1370 g cap d^{-1} in Vietnam while the figure reported by Stoll and Parameswaran (1996) in Thailand was 1000 g cap d^{-1}.

Long predating the unsustainable use of chemical fertilizers human excreta was recognised as a valuable source of nutrients in ancient times. As early as 500 BC human excreta was being treated as a commodity in China, with contractors collecting and trading human excreta with farmers (Brown, 2003). The market value of the annual excreta production in China was estimated by Scott (1952) to be worth £50-80 million at 1924 market prices. The Japanese, too, made prodigious use of human excreta and it is noted in King (1911): *"Among the most common sights on our rides from Yokohama to Tokyo, both within the city and along the roads leading to the fields, starting early in the morning, were the loads of night soil carried on the*

shoulders of men and on the backs of animals, but most commonly on strong carts drawn by men, bearing six to ten tightly covered wooden containers holding forty, sixty or more pounds each". King (1911) estimated that the Japanese returned as much as 385,214 tons of nitrogen, 91,656 tons of phosphorus and 255,778 tons of potassium in human excreta to the soil. In the early 20th century the use of human excreta as a fertilizer was still practised in the United States and Europe although to a far lesser degree than that of Asia (King, 1911; Esrey *et al.*, 2001). Today, the use of human excreta in agriculture remains a common practise in parts of Asia (Phuc *et al.*, 2006; Jensen *et al.*, 2008) but in industrialised western nations this practise is virtually absent (Quitzau, 2007). The widespread shift away from human excreta use in agriculture can be traced to the 11th century, prompted by moral ideologies by social elites perceiving waste and the act of defecation as offensive and repulsive (Horan, 1997; Quitzau, 2007). In the 1800s health issues arose in densely populated cities as a result of the accumulation of human excreta (Quitzau, 2007). These ideas, amongst others, are summarised by Bracken *et al.* (2007) who cite four major contributions to the shift away from human excreta recycling: (1) the practical difficulties of collecting and transporting human excreta from densely populated city centres to relatively distant agricultural areas, (2) the misguided belief of the *miasma* theory, (3) the advent of domestic reticulated water allowed for water-borne sewage systems and (4) the availability of cheap fertilizers which undermined the recycling of human excreta. The flush toilet was in existence by the late 1700s and its ability to accommodate perceptions surrounding human excreta meant that it gained widespread acceptance (Quitzau, 2007). It was this embedding of the flush toilet as the sanitation norm and associated 'lock-in' of sanitation practises that contributed most profoundly to the diminishing use of human excreta as a fertilizer, especially since wastes could no longer easily be recovered (Quitzau, 2007).

The implementation of flush toilets in Europe occurred rapidly; in Stockholm alone the number of toilets increased from 127 to 87 817 between the years 1890 to 1925 (Drangert and Hallström, 2002). This was not well received by intellectuals who saw the flush toilet as depriving farmers of manure (Cordell, 2009). Cordell *et al.* (2009) cite Hugo (1862) who wrote in *Les Miserables* at that time: "*Science, after having long groped about, now knows that the most fecundating and the most efficacious of fertilizers is human manure. The Chinese, let us confess it to our shame, knew it*

before us. Not a Chinese peasant – it is Eckberg who says this – goes to town without bringing back with him, at the two extremities of his bamboo pole, two full buckets of what we designate as filth. Thanks to human dung, the earth in China is still as young as in the days of Abraham. Chinese wheat yields hundredfold of the seed. There is no guano comparable in fertility with the detritus of a capital. A great city is the most mighty of dung-makers. Certain success would attend the experiment of employing the city to manure the plain. If our gold is manure, our manure on the other hand, is gold."

1.3. Composition of human excreta

Although for centuries the use of human excreta was based on an empirical rather than a scientific understanding of nutrient fluxes, the value of human excreta primarily as a nutrient source is today well-recognised (Dawson and Hilton, 2011). Guzha et al. (2005) posit that each person can produce sufficient fertilizer to grow enough maize for themselves based on the amounts of N and P contained in excreta. The chemistry of human excreta is subject to variability depending on water consumption, diet and environmental differences but is high in nutrients (Wolgast, 1993; Schouw *et al.*, 2002; Heinonen-Tanski and van Wijk-Sijbesma, 2005). Based on a typical Swedish diet, the annual excretion of N, P and K per person (i.e. in faeces and urine combined) has been reported as 4.5 kg of nitrogen, 0.6 kg of phosphorous and 1.2 kg of potassium (Wolgast, 1993). This equates to daily generation rates of 12.3, 1.6 and 3.3 g N, P and K per capita, respectively. Chemical analysis of human excreta in Thailand showed that the generation rate (cap d^{-1}) of 13 tested elements was as follows: 7.6-7.9 g N, 1.6-1.7 g P, 1.8-2.7 g K, 1.0-1.1 g S, 0.75-1.5 g Ca, 0.25-0.4 g Mg, 9-16 mg Zn, 1.4-1.5 mg Cu, 0.3 mg Ni, 0.02-0.03 mg Cd, 0.07-0.14 mg Pb, 0.01 mg Hg and 0.8-1.1 mg B (Schouw *et al.*, 2002). The corresponding quantities of these elements per kg dry matter were (presently recalculated from the data of those authors): 63.5-159.2 g N, 17.8-31.1 g P, 24.3-51.6 g K, 7.6-20.8 g S, 6.1-17.9 g Ca, 0.9-5.7 g Mg, 1.15-19.05 mg Zn, 13.64-29.07 mg Cu, 90.48-224.24 mg Ni, 1.75-8.33 mg Cd, 0.13-1.00 mg Pb, >0.00-4.26 mg Hg and >0.002-33.94 mg B. In a separate study, raw human excreta was reported to contain 50-70 $g.kg^{-1}$ N, 30-54 $g.kg^{-1}$ P (as P_2O_5) and 10-25 $g.kg^{-1}$ (as K_2O) on a dry mass basis (Cross and Strauss, 1985).

Based on a faecal sludge collection survey in Accra, Ghana, biological oxygen demand (BOD) in human excreta is considerably greater (45 g per capita per day) compared with other forms of excreta which have been stored for some time (SANDEC, 1997). Additionally, mostly fresh excreta from public toilets and bucket toilets which had been stored for durations spanning several days to weeks had a notably high chemical oxygen demand (COD) ranging from 20 000–50 000 $mg.L^{-1}$ (SANDEC, 1997).

Taking account of the nutrients in urine and faeces separately, urine comprises the majority of N, P and K (Drangert, 1998; Schouw *et al.*, 2002; Wolgast, 1993). The physical and chemical composition of human urine normally shows variation amongst individuals but is also dependant on several factors such as diet, water consumption and environmental conditions (Sullivan and Grantham, 1982). For example, Heinonen-Tanski *et al.* (2007) analysed urine samples from two separate households in Finland and found that dry matter alone varied from 4.7 g L^{-1} to 10.4 g L^{-1} between the households. Those authors also found that potassium was 0.59 g L^{-1} and 1.7 g L^{-1} for the respective households – almost three times greater in the latter household than in the former. Total nitrogen in urine has been reported to be as low as 1.795 g L^{-1} and as high as 17.5 g L^{-1} (Vinnerås *et al.*, 2003; Meinzinger and Oldenbrug, 2008). Total phosphorous has also been shown to be highly variable. Ban and Dave (2004) reported a total phosphorous content of 1.8 g L^{-1} while Heinonen-Tanski *et al.* (2007) reported a figure of 0.15 g L^{-1}. When taken on an individual basis the general trend is that N is present in the largest quantity in urine followed by P and K. In this respect, while Pradhan *et al.* (2009) report a K content of urine of 2 g L^{-1} which is greater than the N content of urine of 1.795 g L^{-1} as reported by Vinneras *et al.* (2003), the quantities of N, P and K for each of the urine samples analysed in each study follows the trend of $N>P\geq K$. As reviewed by Karak and Bhattacharyya (2011), total N excreted in urine per person is about 2.5-4.3 kg per year (Feachem *et al.*, 1983; Kirchmann and Pettersson, 1995); Total P is about 0.7-1.0 kg (Fittschen and Hahn, 1998; Kirchmann and Pettersson, 1995; Maurer *et al.*, 2003; Schönning, 2001) and total K is about 0.9-1.0 kg per year (Fittschen and Hahn, 1998; Maurer *et al.*, 2003; Schönning, 2001).

Urine consists of 93-96% water (Polprasert, 1995) and contains nutrients in solution at low concentrations (Lind *et al.*, 2001). Nutrients need to be mineralised before they can be utilized by plants but since most of the nutrients in urine occur in the mineral form they can be readily utilized by plants (Berndtsson, 2006). Of the total N found in urine approximately 80-85% is in the form of urea [$CO(NH_2)_2$] which is a compound derived from ammonium and carbon dioxide which easily dissolves in water and is readily taken up by plants (Drangert, 1998; Jönsson *et al.*, 2004). The other most commonly occurring compound in urine is sodium chloride (NaCl) but urine also contains P as superphosphates and ionic K (Lind *et al.*, 2001). On the other hand, faeces contains P and K in ionic form but N releases slowly since it is organically bound (Kirchmann and Pettersson, 1995). Karak and Bhattacharyya (2011) note that both urine and faeces have excellent potential to improve poor soils. Despite the good fertilizer value of urine and faeces the combination of urine and faeces has several drawbacks in practise. The mixture of urine and faeces has an unpleasant odour which is not conducive to its use as a fertilizer (Drangert, 1998). This odour is created by the breakdown of urea into ammonium gas by *Micrococcus urea* which is present in faeces (Drangert, 1998). In this way N is lost as ammonium gas to the atmosphere which is not desirable from an odour and fertilizer perspective.

1.4. Global sanitation crisis

In 2000, the United Nations (UN) – as represented by its 189 member states – convened in New York City to ratify the UN Millennium Declaration. The Millennium Declaration described the "Role of the United Nations in the twenty-first century" and identified eight core objectives as being integral to international relations in the twenty-first century. These objectives became known as the Millennium Development Goals (MDGs) and would attempt to reduce the gap between rich and poor nations with a deadline of 2015. Millennium Development Goal 7 pertains to environmental sustainability and one of the targets of this goal, target 7.C, is to "Halve, by 2015, the proportion of the population without sustainable access to safe drinking water and basic sanitation" (UN Millennium Development Goals, 2000). The UN recognises access to safe drinking water and sanitation as a basic human right (UN Human Rights Council, 2010). However, in 2006 the UN, 'deeply concerned' by progress in providing sanitation, declared 2008 the International Year of Sanitation in

an effort to raise awareness and to increase efforts towards achieving the MDGs relevant to sanitation and water provision (UN General Assembly, 2006). In the absence of adequate sanitation human waste is a significant cause of disease transmission (Paterson *et al.*, 2007). While urine is relatively sterile, faeces can be high in a range pathogenic bacteria, viruses and enteric microorganisms (Hoglund *et al.*, 2002; Heinonen-Tanski and van Wijk-Sijbesma, 2005). As little as one gram of faeces can contain as many as 100 million bacteria which includes some pathogenic bacteria (Drangert; 1998). Helminth ova are associated with low mortality rates but still have significant social impacts, particularly in children, where helminth infestation can result in learning difficulties and stunted growth (Jensen *et al.*, 2008). The beneficial effects of improved sanitation are numerous and include wide-ranging social and economic benefits resulting from improved quality of life, increased productivity, less health expenditure and improved water management (Hutton and Haller, 2004). Yet, despite the clear benefits of adequate sanitation, it is estimated that 2 billion people worldwide do not have access to adequate sanitation (WHO and UNICEF, 2000).

Numerous sanitation strategies exist but can be broadly classified as either "drop and store" and "flush and forget" depending on their principal mode of operation (Langergraber and Muellegger, 2005). "Drop and store" sanitation systems include pit latrines and other non-water borne systems where excreta are temporarily stored *in situ* until the system is overloaded and requires emptying. On the other hand, "flush and forget" systems refer to water borne sanitation systems in which excreta are carried away, usually to large centralized sewage processing plants. The pit latrine is still the most commonly used sanitation system in developing countries (Esrey *et al*., 2001). The conventional pit latrine is a low technology, cost effective, solution which simply consists of a pit into which excreta accumulates (Paterson *et al.*, 2007). A seat may be present above the pit, resembling that of a flush toilet. Because it is economical, the pit latrine has become ubiquitous in developing countries as a means of meeting basic sanitation needs. There are however some disadvantages to conventional pit latrines and these include groundwater contamination, odour, insect breeding (flies and mosquitoes) and the potential occurrence of pit collapse (Langergraber and Muellegger, 2005). The Ventilated Improved Pit (VIP) toilet has a ventilation pipe covered by a mesh, which allows odours to escape the toilet but also

prevents insect pests from entering the toilet. An inevitable consequence of "drop and store" sanitation systems is that they eventually require emptying once full. Appropriate faecal sludge management strategies in developing countries are largely absent, and each day vast amounts of faecal sludge are indiscriminately disposed of in agriculture, water bodies or public spaces (Ingallinella *et al.*, 2002). Faecal sludge is high in nutrients and consequently improper disposal poses a serious risk of eutrophication to the aquatic environment (Smith *et al.*, 1999).

Although sanitation problems are often thought of as a major issue facing developing countries, it also poses problems – albeit of a different kind – to developed countries. In developing countries facing sanitation crises, the need for proper sanitation facilities is primarily to reduce the spread of disease. However, in developed countries the problems around sanitation have shifted from those of hygienic sanitation to those of environmental impacts associated with sanitation and the reduction thereof (Langergraber and Muellegger, 2005). Apart from the excessive use of water in flush toilets, such systems (including others) are based on the notion that excreta are a waste that needs to be disposed of (Esrey *et al.*, 2001). One of the many problems posed by conventional sanitation systems is the loss of nutrients and trace elements contained in excreta. Implicit with the perception that human excreta are wastes is the belief that excreta are of no use to humans and consequently conventional 'one way' (end-of-pipe) sanitation systems have become the norm (Werner *et al.*, 2009).

1.5. Sanitation crisis in South Africa

South Africa is characterised by inequalities in sanitation provision which can be traced in fairly recent history to the apartheid government under which sanitation provision was made unequally on the basis of race (Tempelhoff, 2009). However, these inequalities in sanitation provision based on racial discrimination have a long history in South Africa and precede apartheid policies, such as in the early 19th Century in colonial Cape Town and Johannesburg (Swanson, 1977; Zangel, 2004; Tempelhoff, 2009). An important policy of the newly elected government in 1994 was to provide clean drinking water and adequate sanitation as per the Reconstruction and Development Programme (RDP) and these became recognised as constitutional rights (Bond, 1999). The policy held by the new government pertaining to water and

sanitation was matched by expectations that sanitation provisions formerly enjoyed by only a few could now be available to all (Beall *et al.*, 2000). In reality, achieving water and sanitation for all presented major practical difficulties for several reasons. First, existing water and sanitation infrastructure which previously supported only about 6 million people was, if the policy was to be successfully implemented, then expected to provide for the entire population - a figure eight-fold the number of people that the infrastructure had previously supported (Tempelhoff, 2009). Second, the financial burden of expanding existing infrastructure was prohibitively expensive (Beall *et al.*, 2000). In this regard, the question of how much South Africa could afford in terms of infrastructure development – including water and sanitation systems – was a highly debated and contentious topic in the late nineties (Bond, 1999). Lastly, aspects of local conditions such as geography, geology and accessibility in general (linked to geography) of areas where infrastructure development was to occur to service end-users was largely overlooked (Bond, 1999). In this regard, it is common for poor, informal communities to be situated on the periphery of urban centres on land which is undulating and geologically unsuitable for urban development. This makes the development of suitable infrastructure in such areas impractical, if not impossible.

Recent survey data indicates progress in the provision of sanitation in South Africa (Statistics South Africa, 2007). In this respect the percentage of households in South Africa with access to flush toilets increased from 49.1% to 55.1% in 2001 and 2007, respectively. It is notable that in 2007 as much as 8.2% of households had no access to any form of sanitation although this figure had improved from 13.6% in 2001. Also in 2007, the percentage of households with access to pit latrines and VIP toilets was 20.6% and 6.5%, respectively, indicating that a considerable portion of South Africa's population relied on basic sanitation in that year.

Durban is a coastal city situated in the province of KwaZulu-Natal, which lies on the east coast of South Africa. Of the households in KwaZulu-Natal, 8.8% constitute informal dwellings (Statistics South Africa, 2007) which equates to a figure of over 191 000 informal dwellings (presently recalculated from data, Statistics South Africa, 2007). Durban is the largest city in KwaZulu-Natal, with a population of approximately 3.5 million people (Statistics South Africa, 2007). Durban is

surrounded by suburbs with reticulated water and flush toilets serviced by a sewer line, contrasting with poor, informal communities which have to travel some distance to collect water and are unsewered, having access to only basic sanitation. An estimated backlog of as much as 63 000 households in the eThekwini municipality are yet to be provided with potable water and sanitation (Friedrich *et al.*, 2009). Two basic forms of sanitation are provided in informal settlements depending on settlement density; in dense settlements pit latrines are provided but where the distance between dwellings exceeds 50 m urine diversion (UD) toilets are provided (Friedrich *et al.*, 2009). In 2000 the eThekwini municipality began providing UD toilets since they were considered the most suitable sanitation system for the local undulating geography but also because the UD toilets require no water (Trönnberg *et al.*, 2010). The UD toilet design implemented by the municipality consists of two separate vaults which are used alternately; when one vault is full the toilet pan is placed above the adjacent empty vault. Full vaults are emptied and the composted waste can be used as a fertilizer. The separation of waste streams is achieved by a separate urinal which keeps faeces separate from urine. Often UD toilets are chosen where the separation of urine and faeces is desired based on specific uses for each of these resources (Zurbrügg and Tilley, 2009) although in the case of Durban urine is usually not collected but instead infiltrates through a soak pit (Knight *et al.*, 2007). Over 78 000 UD toilets have been built in the eThekwini municipality since 2003 (Trönnberg *et al.*, 2010).

Construction of VIP toilets began in the early 1990s but has since accelerated from the year 2000 onwards (Still *et al.*, 2009). It is estimated that currently there are 45 000 VIP toilets in the eThekwini municipality that are full and require emptying (Still *et al.*, 2009). Most VIP toilets require emptying every 5 to 9 years but indications are that pits are reaching capacity sooner than expected (Still *et al.*, 2009; Bhagwan *et al.*, 2008). Furthermore, commercial additives which purport to reduce pit filling times have been shown to be ineffective (Foxon *et al.*, 2006). The problem of full pit latrines has caused a considerable backlog in sanitation provision since full pit latrines are effectively removed from service, and households that once had sanitation are left without. Recognising the importance of keeping pit latrines operational (i.e. within usable capacity) the eThekwini municipality currently empties pit latrines every five years at no cost to households, a decision which was also taken with due consideration

of the prohibitively high costs involved in emptying individual pit latrines on request by the user (Still *et al.*, 2009). Apart from the difficulties involved in the actual emptying of pit latrines which are compounded by accessibility issues (caused by hilly terrain and dense unplanned informal settlements), the challenge of disposing of faecal sludge has not been met. Faecal sludge is a generic term for "sludges of variable consistency collected from so-called on-site sanitation systems, such as latrines, non-sewered public toilets, septic tanks and aqua privies. The faecal sludge comprises varying concentrations of settleable or settled solids as well as of other, non-faecal matter" (Heinss *et al.*, 1998). The treatment of faecal sludge at waste water treatment works (WWTWs) is not a feasible option given that 1.5 m^3 of faecal sludge – the volume of a typical pit latrine – represents the equivalent of 210 000 L of waste water in terms of COD and 770 000 L of waste water in terms of total suspended solids (TSS) (Brouckaert, 2001). Therefore the latter treatment option of faecal sludge places an enormous burden on WWTWs. This is especially a problem where WWTW's are operating near, or over, their design capacity. In this regard, 40% of Cape Town's WWTWs are already functioning at or beyond design capacity (Mels *et al.*, 2009). In 2002, the Umhlanga WWTW (in the eThekwini municipality) was processing 8 $ML.d^{-1}$ which exceeded its design capacity of 7 ML/day although this was a relatively small WWTW compared to the Northern WWTW which had a design capacity of 70 $ML.d^{-1}$ and processed 50ML/day in that year (Welgemoed, 2002). Compounding the problem of treating faecal sludge via WWTWs in the eThekwini municipality is the huge number of pit latrines that require emptying and, consequently, high volume of faecal sludge requiring treatment. Furthermore, the sludge generated by WWTWs as a by-product of treatment poses a challenge because of fairly limited reuse options (Fytili and Zabaniotou, 2006).

1.6. Ecological sanitation: A solution?

One possible approach to the problem of dealing with faecal sludge is Ecological Sanitation (Ecosan). Chiefly, Ecosan represents a framework or set of principles in the design and implementation of sanitation systems. Ecosan represents a holistic approach to sanitation which takes into account local environmental and economic sustainability as well as social acceptance in the design and implementation of waste management systems (Werner *et al*., 2009). Ecosan does not impose certain

technologies but aims to achieve sustainability, especially by closing the gap between sanitation and agriculture – the so-called 'nutrient loop' (Esrey *et al.*, 2001; Werner *et al.*, 2009). Ecosan is underpinned by the principle that human excreta is not a waste, although in many societies today it is regarded as such (Langergraber and Muellegger, 2005). In this regard, Ecosan recognises the value of human excreta as a fertilizer since it contains many valuable nutrients required for food production, but which are currently being lost through conventional linear systems i.e. 'Flush and discharge' systems (Esrey *et al.*, 2001). Ecosan is a compelling option given the current faecal management crisis in the eThekwini municipality. By recognising the nutrient value of faecal sludge and its relevance to plant production, Ecosan can represent an efficient solution by realising a number of economic and social benefits. In the eThekwini municipality these benefits could include reduced water pollution and consequently improved health, a cheaper alternative compared with other alternatives, small business promotion involved in the emptying of pits, and otherwise an overall environmentally sustainable solution to the problem (Langergraber and Muellegger, 2005; Werner *et al.*, 2009).

In a strict sense, the use of faecal sludge in the current context does not entirely conform to the ideals of ecological sanitation owing to the design of pit latrines. This is because pit latrines do not represent the most appropriate sanitation system for local conditions (Bond, 1999). Moreover, there are a number of problems often associated with pit latrines such as generally poor composting owing to poor aeration (despite ventilation) and the problem of newer, un-composted waste above older, potentially composted waste, which poses potential health risks (e.g. helminth eggs) when evacuating pits (Heinonen-Tanski and van Wijk-Sijbesma, 2005). The use of faecal sludge to grow plants does however constitute an attempt to close the nutrient loop and this, as discussed, is a core principle of ecosan.

1.7. Characteristics of faecal sludge

Faecal sludge characteristics are dependent on a number of complex processes which occur during storage (Foxon *et al.*, 2006). These processes can be divided into biological and non-biological processes; the former constitutes the breakdown of organic matter by micro-organisms into soluble and gaseous products while the latter

includes fluxes of pit material occurring through physical processes such as the addition of material or loss of gaseous or soluble products (Foxon *et al.*, 2006). Biological activity in pit latrines is thought to be predominantly anaerobic (Mara, 1984; Chaggu, 2004) although aerobic activity in the uppermost layer may occur (Foxon *et al.*, 2006). Using a hypothetical substrate consisting of carbohydrate, protein, lipids and inert material, Batstone *et al.* (2002) have proposed an anaerobic digestion model comprising five fundamental biological processes occurring in succession, *viz.* disintegration, hydrolysis, acidogenesis, acetogenesis and methanogenesis. Disintegration and hydrolysis are primarily mediated by extracellular processes; substrate is disintegrated into polymeric components (i.e. carbohydrates, proteins and lipids) followed by the hydrolysis of polymeric components into their respective monomeric components (i.e. sugars, amino acids and long chain fatty acids (LCFA). Acidogenesis converts monomers into volatile fatty acids (VFAs), with a concomitant decrease in pH arising from the dissociation of VFAs and release of H+ ions into the liquid phase (Foxon *et al.*, 2006). In acetogenesis LCFAs and VFAs are converted to acetic acid which is then converted to methane and carbon dioxide in methanogenesis. The process of methanogenesis is particularly pH sensitive and can become inhibited at pH values of less than 6.5 (Foxon *et al.*, 2006). Consequently, the acid and alkaline generating processes of acidogenesis and acetogenesis, respectively, must remain in fine balance to maintain suitable pH values which preclude inhibition of methanogenesis and a resultant decrease in treatment rate (Foxon *et al.*, 2006).

Anaerobic conversion of faecal sludge in pit latrines will theoretically proceed until no biodegradable material remains at which point the material has fully stabilised (Foxon *et al.*, 2006). Values for chemical oxygen demand (COD), biological oxygen demand (BOD), total solids (TS), organic matter (OM) and N of faecal sludge occur along a continuum depending on the level of stabilisation with highest values present in fresh material and lower values found in material which has been stored for longer periods of time and hence more stable (e.g. SANDEC, 1997). For example, the N content of pit latrine sludge may be only half that of comparatively fresh excreta due to denitrification (SANDEC, 1997; Aalbers, 1999). Thus the degree of stabilisation of pit latrine sludge has important implications for its potential usage in plant production especially given the importance of N as a macronutrient. Faecal sludge which has been stored for long periods of time can exhibit NH_4^+ concentrations of 400 $mg.L^{-1}$

compared with considerably greater concentrations of 5000 $mg.L^{-1}$ in fresher faecal sludge (Heinss *et al.*, 1998). NH_3 or NH_4^+ are valuable sources of N since they are readily available to plants (Artyomov *et al.*, 1994; reviewed by Britto *et al.*, 2002) but can present toxicity to plants in sufficiently high concentrations (van der Eerden, 1982; reviewed by Britto *et al.*, 2002). Kegne *et al.* (2008) observed that *Cyperus papyrus* and *Echinocloa pyramidalis* were sensitive to faecal sludge from public toilets due to the high NH_3 concentration but the same was not observed when more stable forms of sludge from pit latrines and septic tanks was used. Therefore, somewhat paradoxically, some degree of stabilisation and consequent loss of N in the form of NH_3 or NH_4^+ can enhance the value of faecal sludge as a resource for plant production. Faecal sludge is otherwise high in nutrients, as aforementioned, but has the additional benefit of being very low in heavy metals unlike some sewage sludges (Ingallinella *et al.*, 2002).

1.8. Human excreta and plant growth

1.8.1. Faecal sludge

The application of faecal sludge on agricultural land has received little attention, possibly owing to health concerns arising from the handling of faecal sludge, especially regarding the presence of high amounts of helminth ova (Heinonen-Tanski and van Wijk-Sijbesma, 2005). It has been suggested that ideally faecal sludge should be composted before usage to reduce the number of viable helminth ova (Heinonen-Tanski and van Wijk-Sijbesma, 2005; Jensen *et al.*, 2008). Perhaps the closest example of the potential of faecal sludge for plant production is provided by Morgan (2007). That author describes the 'arborloo' which is fundamentally a typical VIP toilet with the notable exception that the superstructure of the toilet can be dismantled and moved over a new pit once the existing pit has reached capacity. The pit walls are not lined and it is intended that once a pit is full that it will be covered with soil and a plant be planted above the buried faecal matter. Leaves, soil and wood-ash are added to the pit on a daily basis (or soil only if the other materials are not available) as this reduces flies and odour. Morgan (2007) has observed that a variety of trees including fruit trees and gum (*Eucalyptus* sp.) trees can grow very well above the buried excreta but notes that it is best to allow the faecal matter to compost for a while before planting occurs.

A few studies have assessed the affect of faecal sludge on plant growth in sludge dewatering beds, also referred to as constructed wetlands (Koottatep *et al.*, 2001; Kegne *et al.*, 2008; Kegne *et al.*, 2009). Sludge dewatering beds have been widely implemented throughout the developed and developing world as an economically viable and simple method for sludge dewatering, stabilisation and humification (Panuvatvanich *et al.*, 2006; Kegne *et al.*, 2008). The sludge dewatering beds are operated by placing sludge onto a matrix which allows the liquid phase to percolate through while retaining solids on the surface of the bed (Kegne *et al.*, 2008). Sludge dewatering beds can be planted with emergent macrophytes (e.g. Koottatep *et al.*, 2005; Kegne *et al.*, 2008; Kegne *et al.*, 2009) or left unplanted (e.g. Cofie *et al.*, 2006; Kuffour *et al.*, 2009). Faecal sludge application to planted dewatering beds has generally been shown to be beneficial to plant growth. Kegne *et al.* (2008) applied faecal sludge from various sources (pit latrines, public toilets and septic tanks) to antelope grass (*Echinochloa pyramidalis*) and papyrus (*Cyperus papyrus*). Those authors observed 3-4 times greater biomass in *E. pyramidalis* following 4 months of faecal sludge application as well as extremely high N and P concentrations in tissues of both species compared with the same species in surrounding wetlands. Using the same comparison, however, *C. papyrus* treated with faecal sludge showed poor aerial growth but higher biomass allocation to roots. Of the cattail (*Typha augustifolia*) planted in a sludge dewatering bed a few plants did not acclimatise well to applications of septic waste resulting in leaf senescence despite root and stem growth (Koottatep *et al.*, 2002). However, following acclimatisation and four months of septic waste addition plants had attained heights of 3.6–4.0 m which was comparatively higher than that of the same species in the surrounding wetland. At one year after planting cattail was well adapted to applications of septic waste and growth was healthy even when applications were frequent (weekly) at one year after planting.

A number of studies have made use of structured interviews or questionnaires with farmers who use faecal sludge in their fields as well as communities in such areas to gain an understanding of perceptions surrounding excreta use in agriculture. However, while such studies lack quantitative components they still prove invaluable in understanding the potential of faecal sludge for agriculture. For example, Jensen *et al.* (2008) conducted interviews with households in two villages in Vietnam who applied human excreta to their land. Farmers who were interviewed noted the benefits of

composted (or regarded as composted) human excreta in their rice fields, not only as a fertilizer, but also to improve soil structure. Furthermore, human excreta was regarded as a better fertilizer than excreta of pigs or cows and better still than synthetic fertilizers which they claimed worked well but only in the short term. It is important to note that in that study Jensen *et al.* (2008) found that the human excreta used in the fields was not yet fully composted; national guidelines (Ministry of Health, 2005) stipulate a minimum composting time of 6 months but actual composting time accorded to the crop cycle which was less than 6 months. Drangert and Nawab (2011) investigated practises and perceptions held by communities in the North West Frontier Province in Pakistan regarding the reuse of human excreta. Farmers recognised the fertilizer value of faecal matter since they encouraged defecation on their land and furthermore would collect and apply a mixture of partially composted excreta and organic household waste to their land from heaps which were several months to a year old. In a broadly similar study Cofie *et al.* (2010) found that users and non-users of excreta in Ghana were in agreement with respect to the beneficial effects of excreta on soil structure. Of the subjects interviewed, 70% claimed to use excreta derived from public toilets, of which 50% are VIP toilets. Farmers who used excreta acknowledged the value of excreta for crop production and had a net income of three times as much as non-users.

1.8.2. Sewage sludge

Sewage sludge (also known as biosolids) is an insoluble residue generated from wastewater treatment works through the treatment of wastewater from a number of sources including domestic and commercial sources as well as street runoff (Singh and Agrawal, 2008). Sewage sludge is generated in vast amounts in developed countries; in the US annual sewage sludge production is 6.2 million dry metric tons (Harrison *et al.*, 2006). The disposal of sewage sludge can be achieved through a number of ways, including incineration, land application or disposal at sea (Fytili and Zabaniotou, 2006). Of these disposal options, disposal at sea is not practised in some parts of the world such as the United States and the United Kingdom where a ban on ocean dumping of sewage sludge was enforced in 1991 (U.S. Environmental Protection Agency, 1999; Council of European Communities, 1991). This has led to a resulting increase in the land application of sewage sludge in such parts of the world

and 60% of the sewage sludge produced in the United States is applied to land (Harrison *et al.*, 2006). In Europe this figure is about 50% (Smith, 2000). In the United Kingdom approximately 500 000 tons of sewage sludge are applied to land annually (Smith, 1996).

Land application of sewage sludge has numerous benefits which include soil conditioning and its potential as a fertilizer (Logan and Harrison, 1995). In this respect sewage sludge is high in organic matter and macronutrients such as N and P as well as essential micro-nutrients (Wang *et al.*, 2008). It should be noted that the chemistry of sewage sludge has been shown to be affected by the treatment methods used at WWTWs (Smith *et al.*, 1998; Petersen *et al.*, 2003). Petersen *et al*. (2008) found that the available N of anaerobically digested sludge was 32% of the total N, compared to that of activated sewage sludge which was 53%. Soil properties which have been shown to be enhanced by the application of sewage sludge include bulk density (Ojeda *et al.*, 2003), water holding capacity (Epstein, 1975; Navas *et al.*, 1998), and humus content (Kulling *et al.*, 2001). Wang *et al.* (2008) found that sewage sludge applications in a field trial increased organic matter from 12.8 to 80.8% as sludge amendment rates increased from 15 to 150 t ha^{-1} and total phosphorous contents increased by 30.2%, 190.5% and 31.8% at sludge application rates of 15, 30 and 90 t ha^{-1} respectively. Total nitrogen was statistically greater compared to the control only at application rates of 150 t ha^{-1}. Perhaps most importantly those authors found that the grasses studied showed an increase in biomass in response to sewage sludge applications due to increased soil nutrient contents and improved soil properties.

A comparison between chemical fertilizer, sewage sludge and anaerobically digested septic waste over a two year period on the growth of corn (*Zea mays*) and grass forage showed that corn yield was comparable between treatments although grass forage yield was greatest in the chemical fertilizer treatment (Warman and Termeer, 2005). However, in particular the availability of N and P of the sludges was far less than an estimated 50%, and the septic waste needed to be mineralized before it could provide sufficient N and P unlike the chemical fertilizer which provided an immediately available source of those nutrients. This was reflected in the relatively low recovery of

applied N and P in crops compared with chemical fertilizer although it was concluded that both sewage sludge and septic waste were effective sources of N, P and K.

Although the land application of sewage sludge can be beneficial, sewage sludge contains a number of human pathogens, mostly derived from human faeces (Sidhu and Toze, 2009). But perhaps the greatest risk posed to the environment by the land application of sewage sludge is the potentially high metal content of sewage sludge (Wang *et al.*, 2005) and presence of organic toxins (Cai *et al.* 2007). In a pot experiment Li *et al.* (2009) found that concentrations of the heavy metals Cu, Zn, Cr, Cd, Pb and Ni in the soil were increased by the addition of sludge as a soil amendment. In that study, the Cu, Zn, Cd and Ni concentrations were found to be above the required standards for sewage sludge for agricultural use. However those authors noted the potential of sewage sludge as a fertilizer since plants generally grew better with increasing application rates in the range of 165 – 495 t ha^{-1}, as evidenced by increasing dry weights of green plant parts. Despite its potential as a fertilizer the often highly heterogeneous nature of sewage sludge creates several practical complications regarding its use as an application to agricultural soils. In their review of land application of sewage sludge to agricultural land, Singh and Agrawal (2008) note the variability of physico-chemico characteristics of sewage sludge based on studies done in Thailand (Parkpain *et al.*, 1998), Spain (Martinez *et al.*, 2002) and India (Nandakumar *et al.*, 1998). In those studies the percentage of organic matter was found to be highly variable, ranging from 19.82% to 43.4%, as was the concentrations of metals such as copper, zinc and manganese. Organic contaminants have also been found to be highly variable in sewage sludge. Cai *et al*. (2007) found that the concentrations of semi-volatile organic compounds (SVOCs) were greater in samples of sewage sludge from selected WWTWs in mainland China compared with Hong Kong. The heterogeneity of sewage sludge (notwithstanding treatment differences as well as variations in metal concentrations, pathogenicity and organic toxins) led Wang *et al.* (2008) to conclude that sewage sludge should be assessed on an individual case basis, taking into account soil type and species, to realise the maximum potential of land application of sewage sludge.

1.8.3. Urine

Only fairly recently has urine received attention regarding its use in agriculture (Karak and Bhattacharyya, 2011). Drangert (1998) attributes this to 'urine blindness' where the 'success' of conventional sewerage systems as well as lack of holistic thinking amongst relevant professionals has masked the potential of urine as a fertiliser. Two trials conducted in Ethiopia investigated the potential of human urine as a fertiliser by applying urine to fields of maize and wheat (Meinzinger *et al.*, 2009). In that study urine was applied to a maize field at application rates varying from 0 to 100 kg N ha^{-1} and a number of growth parameters, including cob size increased with urine application in a linear manner. Similarly, in the wheat field urine was applied at 50 kg N ha^{-1} but this was compared to wheat fertilized with DAP (diammonium phosphate) applied at a rate of 100 kg DAP ha^{-1}. Yields were 1.4 times higher in the wheat field where urine was applied relative to the wheat field where DAP was applied, indicating the efficacy of urine as a fertilizer. Heinonen-Tanski *et al.* (2007) compared the yield of cucumbers fertilized with either human urine or mineral fertilizers and found that while there was no statistical difference between yields at any given harvest date, the cumulative yield of cucumber in plants treated with urine was statistically greater than that of plants treated with mineral fertilizer. Those authors hypothesized that plants grown in the mineral treatment were nitrogen limited in contrast to plants treated with urine. One potential negative regarding the use of urine in agriculture is its tendency to increase soil salinity which may be detrimental to crops which are more sensitive to soil salinity (Mnkeni *et al.*, 2008).

1.8.4. Urine diversion 'waste'

While urine diversion toilets (UD toilets) have been used successfully in developing countries including South Africa as well as a few developed countries, most notably in Nordic countries such as Finland and Sweden (Karak and Bhattacharyya, 2011), few studies have examined the use of urine diversion faecal matter in agriculture. Guzha *et al.* (2005) studied the effects of using human faeces and urine on the yield and water productivity of maize. In that study faeces and urine were sourced from urine diversion toilets and treatments included applications of commercial fertilizer, urine, and faecal matter plus urine. Maize grown with faeces and urine had the

greatest yield, though not statistically greater, as well as greater water use efficiency. Those authors concluded that human waste is at least as good a fertilizer as commercially available synthetic fertilizer. The latter is in contrast with the findings of Mnkeni and Austin (2009) who reported that yield of cabbage provided with UD waste was less than that of inorganic fertilizer at application rates normalised to 100 kg N ha^{-1} for each additive. In a pot experiment spinach and dwarf papaya grown in a relatively nutrient poor soil with a high clay content grew poorly as expected whereas plants of the same species grown above a layer of buried urine diversion faecal matter were significantly taller by week 6 and 8 for the respective species (Rodda, 2006; pers. comm.).

1.9. Present study

In the context of ecological sanitation and the problem of faecal sludge management currently faced by the eThekwini municipality, the present study investigated the potential of applications of evacuated faecal sludge on plant growth in the short term. Apart from the work by Morgan (2007) and various studies using sludge dewatering beds, the effect of faecal sludge on plant growth and particularly photosynthesis has not been sufficiently examined. Accordingly, two separate experiments were conducted in which plants were grown in faecal sludge and the physiological responses of plants to the application of faecal sludge formed the focus of this study. Potential drawbacks arising from faecal sludge application, including groundwater contamination and excessive increases in soil salinity from repeated applications, were beyond the scope of this study.

1.9.1. Sapling growth above buried faecal sludge

Saplings were grown in pots filled with sand containing an arrangement of faecal sludge. This experiment is based on work currently being conducted in Umlazi, Durban, in which faecal sludge from pits in the surrounding area is being buried in trenches. The trenches are covered with topsoil and saplings are planted above the trenches. In this respect the study at Umlazi is similar to work done by Kays *et al.* (2007) in which biosolids (or sewage sludge) was buried in trenches. The present experiment is a sister study to work done in Umlazi, but represents a more controlled

environment since plants are grown in large pots. The species used in this study were *Acacia mearnsii* (black wattle) and *Eucalyptus grandis* (flooded gum). The objectives of this experiment were: 1.) to establish whether faecal sludge has sufficient available nutrients to support healthy plant growth, 2.) to determine how well the saplings grow above a sludge core relative to a fertilized group, 3.) to establish if inter-specific differences in the response to sludge application exist particularly in view of the nitrogen fixing ability of *A. mearnsii* and, 4.) to assess the effect of sludge application on a number of photosynthetic parameters, and 5.) to determine the effects of sludge application on root spatial distribution. It is hypothesised that the faecal sludge used in the study, as sourced from full pit latrines, will have had sufficient time to mineralise during storage and will be able to provide plants with a broad spectrum of readily available macro- and micro-nutrients. Thus saplings grown in sludge should show growth comparable to that of saplings receiving fertiliser and possibly even greater through improved soil properties not related to nutrient content. Any improvements in growth arising from the application of faecal sludge will be less marked in *A. mearnsii* than in *E. grandis* as the nitrogen fixing-ability of the former, which would normally provide an advantage in soils low in N, becomes partly or wholly supressed in the presence of nitrogen-rich faecal sludge. It is expected that photosynthetic parameters will reflect overall growth responses and will not reveal evidence of phytotoxicity arising from possible foreign contaminants contained in the faecal sludge. With regard to root spatial distribution, it is expected that roots will not proliferate within the sludge due to its suspected anoxic nature and instead will proliferate in surrounding substrate.

1.9.2. Acacia mearnsii and Eucalyptus grandis in South African forestry

Acacia mearnsii is one of many Australian acacias that were brought to South Africa in the 1850s for commercial, horticultural or cultural use (Kull and Rangan, 2008; Richardson and Kluge, 2008). It is a member of the Leguminacae family (Lawrie, 1981) and a pioneer species characterised by fast growth rates (Richardson and Kluge, 2008) and high water consumption (Dye, 2004). *A. mearnsii* found uses as timber for construction, as fuel, and as a valuable source of vegetal tannins; as of the 1950s South Africa had the world's largest land area covered by *A. mearnsii* – over 360 000 ha – although this area had declined to approximately one third of the area it once

occupied by the 1970s and covers approximately 102 000 ha today (Sherry, 1971; Kull and Rangan, 2008; du Toit *et al.*, 2010). This reduction was largely due to the fact that *A. mearnsii* was no longer as economically important due to changes in the timber industry (de Neergard *et al.*, 2005). At present *A. mearnsii* in South Africa is cultivated mainly for pulp and tannins for the export market (Kull and Rangan, 2008).

A. mearnsii is a recognised alien invasive species in South Africa (Le Maitre et al., 2000) and is listed amongst the top ten alien invasive plant species in South Africa. The invasiveness of Austalian acacias is linked to their abundant seed production (Impson *et al.*, 2008). In this regard, rivers have served as a key vector of seed transport of *Acacia mearnsii* and this has allowed it to invade new territory and increase its terrestrial range (Richardson and Kluge, 2008). Despite the general view of *A. mearnsii* as a pest, many South African rural communities rely on black wattle for firewood, medicinal extracts, and timber for construction (de Neergard *et al.*, 2005).

In cultivated plantations *A. mearnsii* has been shown to be responsive to fertilizer applications (du Toit, 2002). For example, wood volume of 8-11 year old stands of *A. mearnsii* increased from 30-50 $m^3.ha^{-1}$ in response to a single application of P or PK at establishment (du Toit, 2002). N application has been shown to be largely ineffective in increasing yields of wattle because it has the ability to fix atmospheric N_2 (Schonau, 1971; du Toit, 2002). Furthermore, the notion that N shortages could occur at establishment before nodulation can occur, or where nodulation is poor, has been refuted by available evidence (du Toit, 2002). In contrast to the poor response of N addition on wattle growth, P addition has been shown to exert the greatest effect on growth in *A. mearnsii* (Herbert, 1984) and superphosphate has been described by Beard (1957) as the "King of wattle fertilizers" (reviewed by du Toit, 2002). The addition of K on wattle growth appears to be varied depending on soil type but in general has been shown to have a positive response on growth (Herbert, 1984; reviewed by du Toit, 2002). However, Schonau (1971) reported no significant response of *A. mearnsii* to K application.

Eucalyptus is a genus which consists of more than 500 hardwood species of which almost all are native to the Australian mainland and Tasmania (Kardell *et al.*, 1986).

Eucalyptus species span a wide variety of habitats, some of which are cultivated commercially in various parts of the world for their wood and are particularly valued for their high productivity (Whitehead and Beadle, 2004). In this respect eucalypt plantations can exhibit increases of wood volume of as much as 70 $m^3.ha^{-1}.yr^{-1}$ (Cossalter and Pye-Smith, 2003). The rotation can be only 6 years in warmer and wetter climates (Forrester *et al.*, 2010) as is the case in Brazil where rotation is approximately 6-7 years (Almeida *et al.*, 2007). The area of *Eucalyptus* plantations globally is over 19 Mha (Iglesias-Trabado and Wilstermann, 2008), consisting mostly of intensively managed mono-specific plantations (Forrester *et al.*, 2010). The water demand by *Eucalyptus* plantations has long been an item of debate and controversy such as in India where local populations feel that eucalypt plantations represent a threat to groundwater supplies (Kallarackal and Somen, 1997) and in South-West Australia where *Eucalyptus* plantations are believed to have reduced the yield from water catchments (Ruprecht and Stoneman, 1993).

The notable qualities of *Eucalyptus grandis* (but not confined to *E. grandis* in the *Eucalyptus* genus) are its straight stem and high growth rate but also its relatively high resistance to disease and pathogens (Kardell, *et al.*, 1986; Turnbull and Pryor, 1984). In good growing conditions *E. grandis* has been reported to reach a height of 20 m in only 3 years and *E. grandis* plantations can achieve increases in volume of over 50 $m^3.ha^{-1}.yr^{-1}$ (Campinhos, 1980). These qualities make E. *grandis* a valuable species in the timber plantation industry and as a result *E. grandis* is grown in many different parts of the world including South Africa, Brazil and India (Burgess, 1988). In South Africa, *Eucalyptus* plantations cover a total of 541 000 ha with *E. grandis* constituting the majority of this area at 311 000 ha (FSA, 2003). The wood of *E. grandis* is mostly used for pulp and for fuel (Kojima *et al.*, 2009).

Extensive research has been conducted into fertilizer application in eucalyptus plantations (Schönau and Herbert, 1989). It has been widely reported that members of the *Eucalyptus* genus typically exhibit considerably increased growth in response to fertilizer application. For example, Leuning *et al.* (1991) reported an increase in photosynthesis and biomass of a 6 month-old plantation of *E. grandis* compared to unfertilized saplings of the same species. Laclau *et al.* (2008) observed that N and K additions improved growth in a stand of *E. grandis* although K had a more marked

effect on overall growth. Similarly, in a pot study Graciano *et al.* (2006) reported increases in growth in response to N and P additions. Cromer and Williams (1982) reported a cumulative increase in above-ground biomass of 3 and 8 $kg.m^{-2}$ in unfertilised and fertilised stands of *E. globulus*, respectively, after 9.5 years (reviewed by Whitehead and Beadle, 2004). Using the same species, Judd *et al.* (1996) observed tree volumes ranging from 0.014 to 0.019 m^3 in control treatments and from 0.031 to 0.055 m^3 in 4 year old trees which received the highest application rate of fertiliser. Irrigated and fertilised stands of *E. grandis* and *E. camaldulensis* achieved growth rates of 60 m^3 in three years, or 20 $m^3.yr^{-1}$ which greatly exceeds typical growth rates of native forests (Hunter, 2001). Accordingly, it is common practice to apply fertilizer at planting, with the amount of fertilizer application varying depending on location and local soil conditions (Graciano *et al.*, 2005; Herbert, 1996; Herbert and Schönau, 1989, 1990). In South Africa application of N and P varies from 30-62 and 10-37 $kg.ha^{-1}$ depending on soil properties (Herbert, 1996).

1.9.3. Plant growth in sand amended with faecal sludge

A second experiment investigated the potential of faecal sludge as an amendment to nutrient-deficient sand to support the growth of food crops commonly grown in the region by local communities. The food crops chosen were *Beta vulgaris cv* Fordhook Giant (*B. vulgaris*) and *Solanum melongena cv* Black Beauty (*S. melongena*). To the author's knowledge no formal study has been conducted into the potential of faecal sludge as applications to grow food crops. This experiment is similar to recent work done by Li *et al.* (2009) (see above). The objectives of this study were to 1.) determine the effect of varying application rates of faecal sludge on plant growth, and 2.) to determine the effects of varying application rates of faecal sludge on various photosynthetic parameters. The purpose of this experiment was not to determine health aspects and food safety of faecal sludge applications. It is hypothesised that a positive correlation will exist between sludge application rate and photosynthetic capacity and plant growth in direct response to increasing nutrient availability.

2. Materials and Methods

2.1. Sapling growth in experimental columns

2.1.1. Site

The experiment was conducted on the western edge of the Howard College campus of the University of KwaZulu-Natal (UKZN; 29º52'5" S 30º58'18" E). The site had been selected in a previous experiment and consisted of 27 concrete structures which had been used to grow plants in (Fig. 2.1).

2.1.2. Selection of Plant Material

Two species were selected for the tree growth trial. These were *Eucalyptus grandis* and *Acacia mearnsii* (commonly known as flooded gum and black wattle, respectively). Both species were produced from seed in composted pine bark in seed trays with 128 divisions. Seedlings were sourced from Sunshine Seedling Services in Pietermaritzburg, South Africa. Seedlings were kept in an open area exposed to full sun and irrigated to field capacity with tap water twice daily.

Figure 2.1: Overview of the experiment site at Howard College campus (UKZN)

2.1.3. Plant growth columns

A total of 24 plant growth columns (hereafter referred to as columns) were used in which to grow the tree species. Each column was constructed from concrete manhole rings with a nominal height and internal diameter of 250 mm and 750 mm, respectively. Four manhole rings were stacked upon one another on a concrete base to form a hollow structure which was 1000 mm high with an internal volume of 0.44 m^3 (Fig. 2.2). A layer of PVC sheeting was partially embedded at the inner base of each column to prevent water penetration into the base. The joints between each manhole ring were sealed with bitumen on the inside and were pointed with cement on the exterior surface of the columns. Each concrete base had a horizontal conduit lined with PVC sheeting which exited the column to allow for drainage of any excess water in the columns.

The columns had been used in a previous experiment and were subsequently emptied and dismantled. The experimental columns were then reconstructed and thoroughly flushed with water to remove any residue remaining on the inside of the columns.

Figure 2.2: A plant growth column

2.1.4. Faecal sludge collection

Faecal sludge was sourced from KwaMashu, in KwaZulu-Natal, South Africa. The faecal sludge was manually extracted from full pit latrines and placed into 100 L bins. The waste was extracted and transported to site on the same day. Upon arrival the sludge was placed into selected columns. Three samples of faecal sludge were collected and air-dried for 48 hours. Samples were refrigerated at 1ºC until analysis.

2.1.5. Application of faecal sludge

Of the 24 columns used in the experiment, 12 columns were treated with faecal sludge estimated to have been in storage in pit latrines for between 5 and 9 years. These columns were filled with river sand and faecal sludge. The river sand was specifically chosen as it closely matched the texture and fertility (i.e. nutrient poor) of the accompanying field trial in Umlazi. The application of faecal sludge was such that it formed a central core with a volume of $0.22m^3$ in the columns surrounded by river sand (Fig. 2.3). Treated columns in the treatment group were first filled to a height of approximately 250 mm with river sand. A cylinder constructed from 1 mm polycarbonate sheeting (Fig. 2.4) with a height of 750 mm and a diameter of 450 mm was partially embedded into the base layer of sand and in the centre of each column. Faecal sludge was applied directly into the cylinder (Fig. 2.5) to an approximate height of 500 mm above the base layer. Consequently the core of faecal sludge was 500mm in height and 450mm in diameter. The remaining space between the polycarbonate cylinder and the wall of each tower was filled with river sand until the sand and the faecal sludge were at the same level. This created an annulus of sand which was approximately 150 mm in width. The polycarbonate cylinder was then removed with the aid of a steel rod which passed through two holes near the top of the cylinder. A covering layer of 250 mm of river sand, which is the minimum advisable amount of covering, was added over the faecal sludge. The reason why this arrangement of faecal sludge was adopted was twofold. First, the arrangement of sludge was chosen to match, as far as was reasonably possible, that of the field study which ran alongside this experiment. In that study faecal sludge was applied into trenches and so there was sand on either side and below the trench. Second, it was decided that a core would allow roots to bypass the sludge through the annulus of

sand if the sludge could not support root growth due to its possibly anoxic nature. This would give the opportunity to learn more about how roots interact with sludge though this was not the focus of the study.

The remaining 12 columns were not treated with any faecal sludge but were filled with river sand to the same height as columns in the treatment group (approximately 1000 mm). Throughout the experiment these columns were treated with fertiliser. Though this treatment is not a true control, it is hereafter referred to as such since it is a convenient term when comparing with the experimental (i.e sludge application) treatment.

Figure 2.3: Central core of sludge surrounded by a ring of river sand.

Figure 2.4: Polycarbonate cylinder used to achieve the desired application of faecal sludge.

Figure 2.5: Application of faecal sludge. Sludge was applied directly into the cylinder until the desired level was reached.

2.1.6. Chemical and physical analyses

Sand samples and plant tissue were analysed by the Soil and Analytical Services Laboratory (KwaZulu-Natal Department of Agriculture and Environmental Affairs, Cedara, South Africa). Sand samples collected at the end of the experiment were taken at a depth of 500 mm between the sludge core and column wall and in the same area in control columns. Faecal sludge samples were analysed by Integral Laboratories (Cape Town, South Africa). As the procedures used to analyse the faecal sludge were proprietary they are not disclosed below.

2.1.6.1. Sand sample analysis

Soil particle size distribution was determined using the pipette method described by Day (1965). Sand samples were air-dried and passed through a 2 mm sieve. A 20 g soil sample was first wetted with de-ionized water and 30ml of 30% hydrogen peroxide to oxidise the organic matter. The sample was made up to a volume of 400 ml with de-ionized water and left overnight. The supernatant was siphoned off and the sample was again made up to a volume of 400 ml, stirred, left overnight and the supernatant siphoned off. Dispersing agents (20 ml sodium hydroxide and 10 ml sodium hexametaphosphate) were added to the sample which was then stirred using magnetic stirrers at high speed. The dispersed sample was washed into a 1 L measuring cylinder and made up to 1 L with de-ionized water. The suspension was agitated with a plunger for 30 s. After 4-5 minutes to allow the sand to settle a 20 ml sample representing the fine silt and clay content was taken at 100 ml below the liquid surface with a pipette. Samples representing clay were taken after 5-6 hours at 75 mm below the liquid surface. Percentage clay and percentage silt were determined gravimetrically by drying the samples at 105ºC overnight. Percentage sand content was determined by difference.

Sand pH was measured with a HI 99121 soil pH probe (Hanna Instruments, Rhode Island, USA) in a 1:2.5 soil:1 M KCl suspension. The Walkley-Black wet oxidation procedure (Allison, 1965) was used to determine organic carbon. Total N was determined using the automated Dumas dry combustion method using a LECO CNS 2000 (Leco Corporation, Michigan, USA; Matejovic, 1996). Using this method

samples were weighed into a ceramic crucible and 0.5 g of vanadium pentoxide was added to serve as a combustion catalyst. The sample was burned in a stream of oxygen at 1350ºC in a horizontal furnace and the gases produced were passed through two infrared cells where nitrogen (N_2) was determined in a thermal conductivity cell. Phosphorous, K, Zn, Mn and Cu were extracted with Ambic-2 solution which consists of 0.25 M NH_4CO_3, 0.01 M EDTA, 0.01 M NH_4F and 0.05 $g.L^{-1}$ Superfloc (N100), adjusted to pH 8.0 (Non-Affiliated Soil Analysis Working Committee of South Africa, 1990). A suspension of 25 ml ambic-2 solution and 2.5 ml sand was stirred at 400 r.p.m. for 10 min using a multiple stirrer and the extract was filtered through Whatman No. 1 paper. Phosphorous was determined using a 2 ml aliquot of filtrate using a modification of the molybdenum blue procedure (Hunter, 1974). Zinc, Mn and Cu were determined by atomic absorption on the filtrate while K was determined by atomic absorption on a dilution of a 5 ml aliquot of the filtrate with 20 ml de-ionised water.

2.1.6.2. Leaf tissue analysis

For each replicate a mass of 0.5 g of fresh leaf tissue was dried in an oven at 105ºC for two hours and ashed in a furnace at 450ºC. The ashed samples were wetted with a few drops of de-ionized water to which 2 ml of concentrated HCl was added. The mixture was evaporated to dryness in a water bath and 25 ml of a 1:9 HCl solution was added to each sample. Samples were stirred and filtered through Advantec grade no. 5B filter paper (Advantec MFS, Inc., California, USA). The filtrate was diluted with distilled water to a volume of 1 L using a 5:20 combination dilutor. Calcium, Mg, Zn, Mn, and Cu content was determined using inductively coupled plasma mass spectrometry (ICP-MS).

2.1.7. Planting

A total of 24 one-month-old seedlings were planted in the columns (one seedling per column) one day after the columns were filled. Six seedlings each of *Eucalyptus grandis* and *Acacia mearnsii* were planted into columns where faecal sludge was applied. A further six seedlings of each species were planted in the control columns. Seedlings were randomly selected but due to the fairly marked developmental

differences between seedlings in the same batch only healthy seedlings of a similar height were selected for use in the experiment. Seedlings were carefully removed from seedling trays to avoid damage to the plants. A depression was made in the river sand in the centre of each column and 300 ml of compost was added to the depression. The seedling roots were bedded into the compost and a thin covering of sand was added over the compost. The use of compost was to improve the water retention around the roots of the seedlings and provide nutrients for early seedling establishment (especially in the case for the experimental group as the presence of rising nutrients, if any, may have been insufficient to support healthy plant growth in the early part of the experiment). Longer term growth in experimental columns would however be dependent on nutrients derived from the faecal sludge layer.

2.1.8. Plant maintenance

Unlike the experimental columns where faecal sludge represented a substantial, and ostensibly usable, nutrient source, columns filled with river sand alone could not be expected to support healthy plant growth alone without fertilizer additions. The choice of how much fertilizer to add, if any, was not a trivial one; the variability of sludge made it difficult to evaluate what a suitable comparison would be. Furthermore, an exhaustive fertilizer trial was not feasible. Considerable thought was given to the choice of fertiliser treatment and the possibilities considered, and their likely outcomes, were:

1. No fertilizer application. This was rejected on the basis that it would yield no useful information; it is well known that plants growing on unfertilized washed river sand grow poorly.

2. Apply sufficient fertilizer to maintain growth approximately the same as that of plants growing on sludge. This would actually be a meaningless experiment given the extreme variability of the composition of sludge. The results would only be applicable to plants growing under one set of conditions at one time at one site, using a single source of sludge. It is highly doubtful whether this could be achieved in the first place as the mineralization of sludge over time would mean a constantly changing usable

nutrient pool in experimental columns which would be practically impossible to accurately measure and match accordingly with fertilizer additions.

3. Apply fertiliser at an intermediate rate so that the sand approximates that found at the field site (in terms of fertility). Above all, this makes the results of this experiment more applicable to that of the field study. The level of fertilizer application would be maintained throughout the growth period as changing this would complicate the experiment (especially as it would not be known when, or by how much, to increase the level of fertilizer application). By maintaining a constant amount of nutrient additions to control plants the experimental plants could be compared to a known nutrient loading. This could, at first approximation, lead to plants which would become progressively more nutrient stressed as the plants grow larger and require more nutrients. Nonetheless, this would provide the opportunity to assess the importance of various physiological processes, patterns of growth and biomass partitioning that could contribute to growth reductions under nutrient stress. Although not directly part of this project, the data could contribute significantly to our understanding of stress response at a whole plant level.

The third option seemed the most reasonable as it would certainly provide data that would be more relevant to the project goal and it was chosen as a result.

Plants in the control group were fertilized throughout the experiment as follows: plants received a 2.5 $ml.L^{-1}$ aqueous trace element solution (Trelmix; Hubers, South Africa) administered as a foliar spray each fortnight and plants were administered with 1000 ml of a 1 $g.L^{-1}$ aqueous solution of 3.5 $ml.L^{-1}$ organic fertilizer (Nitrosol; Rural Research Limited, New Zealand) or a 1000 ml aqueous solution of Mondi Orange (as per manufacturer's recommendations) when the foliar spray was administered, alternating weekly. The composition of the fertilizers is given in the Appendix. Care was taken when administering the foliar spray to prevent spray from being carried downwind to any experimental tree and the foliar spray was only administered in the relative cool of the late afternoon to avoid the possibility of burning the leaves. Plants in the experimental group received only water throughout the experiment and were irrigated with the same quantity of water as the control group

when an aqueous fertilizer solution was applied weekly or whenever the plants were irrigated with water.

Over and above the weekly addition of aqueous fertilizer or water to plants in the control and experimental groups, respectively, all plants were irrigated with water between normal weekly fertilizer/water additions. Initially, to ameliorate transplant shock and to aid seedling establishment, seedlings were irrigated with 1000 ml of water every second or third day for three weeks but plants were not irrigated if it was raining or if it had rained the day before. From week 5 to week 17 plants were not irrigated as rainfall during this period was sufficient to prevent water stress. From the 18^{th} week onwards plants were irrigated weekly and every second or third day with 5000 ml of water and from the 19^{th} week onwards plants were irrigated with 10 000 ml of water every day if it was not raining. The increase in irrigation amounts and frequency were to meet the exponential increase in water requirements by the saplings as they grew. The volume of weekly fertilizer/water additions was also increased accordingly to match irrigation volumes during the week. The concentration of aqueous fertilizer was adjusted so that each plant in the control group always received 1g of Mondi Orange and 3.5ml of Nitrosol on a weekly basis. Irrigation water and fertilizer solution was always applied evenly over the entire surface of sand in an attempt to cause even wetting of the sand below the surface.

The presence of pests on leaves, especially aphids during the first few weeks, was remedied by spraying the affected plant with a jet of water. This removed the pests from the plant mechanically which was favoured over chemical control which could have led to contamination of the columns. Columns were kept weed-free by weeding weekly and no herbicides were used throughout the experiment. After only a few weeks of growth a number of black wattle seedlings lodged and began to grow laterally across the sand although this was not accompanied by any visual signs of mechanical damage at the base of the stems. To remedy this, and to prevent further saplings from becoming lodged, all black wattle saplings were securely staked with bamboo stakes at about two months after planting.

2.1.9. Plant growth measurements

Plant height was measured after planting and thereafter every second week. Plant height was defined as the length to the nearest centimetre from the base of each plant to the apical bud. Stem diameter was measured at 2 cm above the ground with vernier callipers every month following planting. For each plant, stem diameter was measured to the nearest 100µm from a 0-180° and 90-270° orientation. Stem diameter was expressed as the mean of the two measured values.

2.1.10. Gas exchange measurements

Photosynthetic measurements were taken on expanding young leaves of both species when plants were approximately six months old. For the eucalypts, leaves which had expanded to about two thirds were used for measurements. Measurements of assimilation (A), intercellular CO_2 concentration (c_i), stomatal conductance (g_s) and transpiration (E) were taken with a Licor 6400 portable photosynthesis system (Licor Inc., Lincoln, Nebraska, USA). The response of A to light (light response curves) was measured in the morning from 8:00 to 13:00 on alternating control and treatment plants. Measurements were taken with the 6400-02B red and blue light source fitted to the sensor head. The CO_2 concentration was held constant at 400 $\mu mol.mol^{-1}$ during measurements. Chamber temperature was set to 27ºC but to suppress high humidity warnings from the Licor 6400 on hot and humid days this was increased accordingly but mean temperature was usually about 29ºC. Recorded air temperature during measurements regularly exceeded 30ºC even by mid-morning which is quite typical for the summer. Vapour pressure deficit (vpd) and relative humidity (rh) were not controlled but generally vpd and rh ranged from about 1.0-1.7 kPa and 60-75%, respectively, during measurement. Tested leaves of the eucalypts always filled the chamber (6 cm^2) but due to the bipinnate leaves of the wattle leaf area had to be determined prior to measurement. This was achieved using a CID-202 leaf area meter (CID, Washington, USA) by placing a representative leaf sample of each plant under a paper mask with a 6 cm^2 window to estimate the effective area in the chamber. The leaf under test was equilibrated to high photosynthetically active radiation (PAR) by maintaining the light level in the chamber at 2000 $\mu mol.m^{-2}.s^{-1}$ for 20 minutes or until photosynthetic parameters had stabilised. The light level was then decreased in a

stepwise manner to 1500, 1000, and thereafter by 200 μmol .$m^{-2}.s^{-1}$ to 0 (but including a measurement at 100 μmol.$m^{-2}.s^{-1}$). Each light level was maintained for a minimum time of two minutes after which parameters were logged automatically if the total coefficient of variance (cv) was <1% but logging occurred irrespectively after three minutes. Stability was usually achieved within three minutes but in instances where the total coefficient of variation was still greater than 1% after three minutes this was often attributed to variability in other parameters which were not of interest.

CO_2 response curves (A-c_i curves) were taken from 8:00 to 13:00 under saturating light conditions. The PAR required to saturate leaves of both species was determined from the light response curves and the PAR used was 1500 and 2000 μmol.$m^2.s^{-1}$ for *E. grandis* and *A. mearnsii*, respectively. The leaf equilibration procedure was the same as with the light curves as was the case with chamber temperature, vpd and rh. The concentration of CO_2 in the chamber was automatically adjusted using the CO_2 mixer according to the following sequence (all values in μmol.mol^{-1}): 400, 300, 200, 100, 50, 100, 200, 300, 400, 400, 600, 800, 1000, 1500 and 2000. At each CO_2 concentration logging occurred after a minimum of one minute or a maximum of two minutes or if total cv was <1%. The sample and reference analyzers of the Licor 6400 were automatically matched before each log occurred.

Raw data for both light and CO_2 response curves were fitted using a monomolecular function (Causton and Dale, 1990):

a(1-exp(b-c*x)

where a, b and c are model constants and x is PAR or c_i at each data point of the light and CO_2 curves, respectively. Photosynthetic parameters derived from light curves were maximum assimilation (A_{max}), light compensation point, dark respiration and photochemical efficiency, determined as follows: A_{max} was determined from *a*, the light compensation point was determined by the ratio *b/c*, dark respiration rate was determined by $a(1-e^b)$ and photochemical efficiency was determined by the product of ace^b. Photosynthetic parameters derived from the A-c_i curve were maximum rates of electron transport (J_{max}), carboxylation of Rubisco (V_{cmax}), and CO_2 compensation point and photorespiration rates, determined as follows: J_{max} was determined from *a*,

V_{cmax} was defined by the product of ace^b, CO_2 compensation point was given by the ratio b/c, and the rate of photorespiration was defined by the term $a(1-e^b)$. Water use efficiency (WUE) was calculated as the ratio of A and E (using raw data from light curves when light was saturating) and expressed as μmol CO_2.$mmol^{-1}$ H_20.

2.1.11. Harvest of saplings

Saplings were harvested at approximately 6 months (26 weeks) after planting. The trunks of the saplings were cut as close to the base of the stems as possible using a hand saw. Twigs were cut from the saplings and leaves were separated from the twigs. The above-ground components were transported to the laboratory.

2.1.12. Evaluation of dry biomass and leaf area determination

It was not practical to determine the dry biomass of entire leaf, twig and trunk components due to the size of saplings at harvest and so total dry biomass for each component was estimated by oven drying samples. Samples were oven dried at 80ºC until constant mass had been reached. A 200 g sample was used for leaves and twigs and trunks were first air dried to constant weight before a 100g sample of each was oven dried. The estimated total dry biomass of each component was calculated as the product of the ratio of fresh mass to dry biomass and the total fresh mass of each component.

Total leaf area was estimated by determining the ratio of leaf area per unit leaf mass of a 50 g sample for each replicate. Total leaf mass was determined at the time at which samples were taken for each species. Samples were taken once leaves had been thoroughly mixed to account for possibly drier leaves at the top of the storage bags thereby ensuring a representative sample. The leaf area of samples was determined using a CI-202 leaf area meter. The estimated total leaf area was then calculated as the product of the ratio of leaf area to leaf mass and total leaf mass.

2.1.13. Root spatial distribution

Root spatial distribution was determined using a root intersect method broadly similar to that used by Tardieu *et al.* (1998). The method used by Tardieu (1988) involves the enumeration of roots with a grid matrix placed over the soil at vertical planes varying in distance away from the plant. Roots that intersect the vertical plane are classified according to size class and into which cell they intersect. The method used here was simpler with three key distinctions; first, horizontal planes were used instead of vertical planes and second, only a circular ring was used (instead of a grid matrix) with which to classify the spatial component of root intersect and third, only roots above 1.0 mm (i.e. medium and coarse roots) were enumerated. Three depths, each coinciding with the middle of each of the three layers (sand-sludge-sand) were chosen. In other words, columns were emptied to the middle of the covering sand layer, the sludge core and the lowermost sand layer. Horizontal plane depths were corrected according to each tower based on the level of subsidence that had occurred since filling. At each layer the sand/sludge was carefully levelled and a 45 cm (diameter) ring was placed over the layer such that the centre of the ring corresponded with the centre of the growth column. Root enumerations were determined at each plane by counting the number of roots intersecting inside the ring and those that intersected outside the ring i.e. between the ring and the wall of the plant growth column. The same method was used for both control and experimental columns. The purpose of this method was to assess the spatial distribution of roots with respect to the sludge layer to determine if root distribution penetrated the sludge layer or was altered compared to the control. In this regard, the 45cm ring directly corresponded to the diameter of the sludge core (see above) and the control served as a theoretical baseline for root distribution in the absence of a sludge core.

2.1.14. Statistical analyses

Statistical analyses were performed using the PASW 18.0 statistics software (SPSS Inc., Chicago, Illinois, USA). Normal distributions were tested using one sample K-S tests and data which violated the assumption of normality was normalised using log transformations. Independent *t*-tests were employed for intra- and inter-specific comparisons. Differences were considered significant where $p<0.05$.

2.2. Growth of table vegetables in sand amended with faecal sludge

2.2.1. Site

The site selected for the study was an area adjacent to the tree growth columns at the Howard College campus. The plant growth area used in the experiment was open to full sunlight. Crushed stone was spread over the foundation to elevate plants off the concrete surface and facilitate drainage of any leachate.

2.2.2. Experimental design

Beta vulgaris (*cv* Fordhook Giant) and *Solanum melongena* (*cv* Black Beauty) were selected for the experiment. Seedlings which were raised in pine bark and approximately 1 month old were purchased from a nursery and were maintained in a greenhouse before planting. Plants were grown in two completely randomized blocks adjacent to one another, with one block for each species (Fig. 2.6). Each block comprised a 5 x 5 matrix surrounded by a guard row. Plants were grown in black plastic plant bags with a diameter and height of 20cm and 35cm respectively and bags were spaced 35cm apart with 75 cm between each block. Three faecal sludge treatments were used in the experiment with a positive and negative control. The treatments used were 10%, 20% and 30% faecal sludge by volume amended with river sand. Negative controls consisted of 0% faecal sludge (i.e. river sand only) and the positive control received fertiliser. Treatments of 0%, 10%, 20% and 30% faecal sludge and fertilized plants are interchangeably referred to hereafter as T-0, T-1, T-2, T-3 and T-4, respectively. Each treatment including the controls was replicated five times within each block.

2.2.3. Faecal sludge collection and amendment preparations

Faecal sludge was sourced from Umlazi, KwaZulu-Natal, South Africa. Waste was collected as in the previous experiment and transported to the Howard College campus of the University of KwaZulu-Natal. Amendments were prepared on the same day in a disused plant growth column which had been partially dismantled. To achieve the desired mixtures of 10%, 20%, and 30% faecal sludge, river sand and

faecal sludge were added to the column in buckets in the appropriate quantities. The amendments were thoroughly mixed with a spade until a relatively homogenous mixture was achieved.

Figure 2.6: Arrangement of bags in a completely randomized design on site adjacent to the tree growth columns.

2.2.4. Chemical analyses

Leaf tissue analyses were performed Soil and Analytical Services Laboratory (KwaZulu-Natal Department of Agriculture and Environmental Affairs, Cedara, South Africa) using the same procedures described earlier. Faecal sludge samples were analysed by Integral Laboratories (Cape Town, South Africa).

2.2.5. Filling of plant bags and planting

Bags were filled with a 2cm layer of crushed stone and then a 20cm layer of the prepared amendments (or sand only in the T-0 and T-4 treatments) to a level of 22cm. A dowel rod served as a depth gauge for all depth measurements. The contents of the bags were allowed to settle for one week during which 20 mm of rainfall occurred after which some bags were topped up with the corresponding medium to the desired level of 22cm. River sand was added to each bag containing an amendment such that

it formed a 5cm covering layer above the amendment. Bags in treatments T-0 and T-4 were also topped up accordingly with river sand so that all bags were filled to the same level of 27cm. Plastic bottles with a volume of 550 ml which had a single 1mm hole drilled into their base were inserted off-centre into each bag to a depth of about 8cm. Using this method plants are irrigated by adding water via a funnel into each bottle and water is slowly dispensed into the bag. The approximate time for 550 ml of water (or nutrient solution) to dispense from each bottle into the bags was about 15-20 minutes. The purpose of this method was to minimize health risks associated with the application of faecal sludge (despite the covering layer of sand) which can arise from splashing of sand onto edible parts of the plants or onto person during irrigation. An added benefit of this method is that the contents of the bottle are dispensed gradually, allowing water or solution to percolate evenly throughout the bags, particularly those containing sand only.

Bags were watered to field capacity before planting. Seedlings were randomly selected and planted into the bags in the cool of the evening. Special care was taken to minimize root disturbance when removing the seedlings from the trays and when planting to avoid severe transplant shock, especially in the case of *B. vulgaris* which previous observations suggest is particularly sensitive to transplant shock.

2.2.6. Plant maintenance

For the first two weeks after planting temperatures were high and it was observed that water was not conducting towards the surface where the roots of the seedlings were. To aid seedling establishment and to conduct water to the surface 50 ml of water was added by the roots for the first two weeks. Plants were irrigated daily at around noon with 550 ml of water or a hydroponics solution, depending on treatment. All plants grown in treatments T-0 to T-3 were irrigated with water only and T-4 received a hydroponics solution throughout the experiment. The guard row for each block was treated in the same way as T-4 (irrigated each day with the same hydroponics solution). The hydroponics solution was prepared by dissolving hydroponics powder (Chemicult, Kompel, South Africa; Appendix) in water at $2g.L^{-1}$ except for the first week after planting when a half-strength solution ($1g.L^{-1}$) was used as per manufacturer's recommendations for recently germinated seedlings. At seven weeks

all plants were irrigated twice daily for the remainder of the experiment to avoid dehydration stress. No pesticides were used on either species throughout the experiment and aphids and other leaf-eating insects were removed by hand.

2.2.7. Plant growth measurements

Growth measurements were taken weekly. The height of *S. melongena* was defined as the length in millimetres between the base of the plant and the tip of the tallest apical bud. For *B. vulgaris*, the length of the longest leaf in mm was used as a measure of plant height since it represents a more practical measurement for that kind of plant. The stem diameter of *S. melongena* was measured monthly to the nearest 100μm from a 0-180° and 90-270° orientation. Stem diameter was expressed as the mean of the two measured values. The number of leaves on each plant was also enumerated on a weekly basis.

2.2.8. Gas exchange measurements

Light response curves and CO_2 response curves were taken on *S. melongena* in the 8^{th} week and in the 7^{th} and 7^{th}-8^{th} week respectively for *B. vulgaris*. Measurements were taken using a Licor 6400 in the same manner as in the tree growth experiment with several exceptions. For light response curves PAR was decreased from 2000 $\mu mol.m^{-2}.s^{-1}$ at 200 $\mu mol.m^{-2}.s^{-1}$ increments, including a PAR of 100 $\mu mol.m^{-2}.s^{-1}$. For both light curves and CO_2 response curves temperature, vpd and rh were controlled at 28ºC, 1.0 kPa and 70% respectively and deviations in these parameters was generally within 5% of the target value. The PAR required to saturate leaves of both species was 2000 $\mu mol.m^{-2}.s^{-1}$. For the CO_2 response curve logging occurred automatically after a minimum of two minutes at each CO_2 level and a maximum of three minutes or when the total cv was <1%.

Unlike the previous experiment, raw data of A (distinct from A_{max} derived from the light curve), g_s and E were measured using a recently obtained transparent chamber ('sun and sky' attachment for the Licor 6400) rather than the red and blue light source (50% red/blue) used for light curves and A-c_i curves. There are numerous advantages to using such a chamber, most notable of which is the measuring spectrum (i.e.

daylight) which is the same as that under which plants were grown. It is well known that many physiological processes are affected by spectrum (e.g. stomatal conductance, chloroplast movement and other intrinsic aspects of photosynthetic function such as light absorption and excitation balance of the two photosystems). The use of a measuring spectrum which differs from growth spectrum (which plants are acclimated to) can result in measurements which deviate from actual values *in situ*. Additionally, the environmental conditions of the chamber, which are set during light curves, can also differ from ambient. To obtain measurements which closely approximated that occurring *in situ*, environmental conditions of the chamber were not controlled and measurements were purposely brief. The leaf under test was kept in the chamber for only as long as necessary to allow the apparatus to stabilise but generally this was no more than 30 seconds per leaf. Measurements were therefore more or less instantaneous and are referred to here as 'spot measurements'.

Spot measurements were taken 8 days before harvest for *S. melongena* and three days before harvest for *B. vulgaris*. During spot measurements of *S. melongena* mean ambient air temperature was 32.5ºC, mean sample rh was 78.7% and mean PAR inside the chamber was 1506.9 μmol.mol^{-1}. These values were 35.2ºC, 72.3% and 1481.1 μmol.mol^{-1}, respectively, during spot measurements for *B. vulgaris*. Measurements were taken on two sun-exposed leaves per plant which were also the fourth or fifth leaf from an auxiliary bud for *S. melongena* or fourth or fifth leaf from the apical bud for *B. vulgaris* (or the third leaf where plants did not grow well). Care was taken to ensure that the chamber window was directed to face the sun during measurement to expose the entire chamber, and consequently the portion of the leaf being measured, with sunlight. Spot measurements were not taken on two plants from the same treatment in succession and measurements alternated between treatments.

2.2.9. Measurement of chlorophyll fluorescence

Pre-dawn (dark adapted) measurements of F_o and F_v/F_m were taken with a direct drive fluorometer (FP-100, Photon Systems Instruments, Brno, Czech Republic). An initial pulse of insufficient intensity to drive photosynthesis was used to measure F_o followed by a saturating pulse of 2400 μmol .m^{-2}.s^{-1} to determine F_v/F_m. To provide a representative F_o value for each plant, F_o was measured on two leaves from each

plant. These were the third or fourth leaves from the apical or auxiliary bud for each plant and a mature, fully expanded leaf.

2.2.10. Harvest of plants

B. vulgaris and *S. melongena* were harvested at 7 and 9 weeks after planting, respectively. Plants were cut at the base and roots were collected by cutting the plant bags open and gently agitating the roots to remove as much loose sand from the roots as possible. Aboveground growth and roots were transported to the laboratory.

2.2.11. Determination of dry biomass and leaf area

Leaf area was determined using a CI-202 leaf area meter. Roots were carefully rinsed in water to remove sand from the roots. Leaf, stem, root and petiole (*B. vulgaris*) components were oven dried at 80ºC until constant mass was achieved.

2.2.12. Statistical analyses

Statistical analyses were performed using the PASW 18.0 statistics software (SPSS Inc., Chicago, Illinois, USA). Normal distributions were tested using one sample K-S tests and data which violated the assumption of normality was normalised using log transformations. Data which could not be normalised was compared using the Mann-Whitney test with Bonferroni correction applied, where applicable. Comparisons amongst treatments were made using one way ANOVA with Scheffe's post hoc test to determine differences between treatments in instances where differences existed. A two way ANOVA was not used as differences between species were not of interest. Differences were considered significant where $p<0.05$.

3. Results

3.1. Tree growth in experimental columns

3.1.1. Rainfall

Rainfall during the experimental period is shown in Fig. 3.1. A total of 559 mm of rainfall occurred during the experimental period. The peaks in rainfall coincide with the rainy season (summer) but above average rainfall occurred during that period.

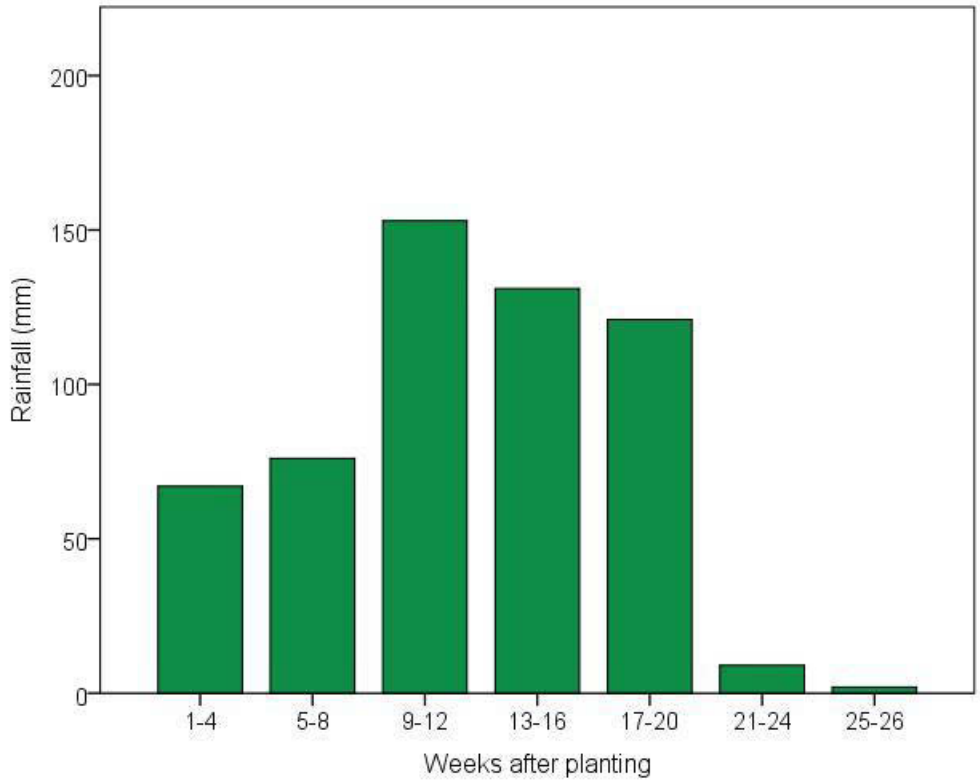

Figure 3.1: Rainfall during the experimental period

3.1.2. Physical and chemical properties of sand and faecal sludge

Physical and chemical properties of the faecal sludge and river sand, as applied and at the time of harvest, are shown in Tables 3.1 and 3.2. The river sand was notably poor in nutrients in comparison with the faecal sludge which exhibited greater concentrations of all measured nutrients with the exception of Al. Of the

macronutrient concentrations of the faecal sludge, however, mean total N concentration was only 2.2 $mg.kg^{-1}$ and considerably lower than expected given the typically high concentrations of that nutrient in faecal sludge. Total P and Bray P occurred in notably high concentrations compared N and K.

N concentrations of faecal sludge where *E. grandis* had been grown were slightly reduced compared with the applied faecal sludge, although this difference was significant only at the 0.1 level (p=0.07). The corresponding difference for *A. mearnsii* was negligible and not of statistical significance. Interestingly, P and Bray P concentrations both showed sizeable increases at harvest compared with the applied faecal sludge suggesting that mineralisation had occurred. However, the variability in concentrations of P and Bray P where *E. grandis* had been grown was considerable and no significant difference could be established. Where *A. mearnsii* had been grown, on the other hand, both differences in P and Bray P were significant (p=0.009 and p=0.008, respectively). K concentrations were approximately three and five times less than applied faecal sludge where *E. grandis* and *A. mearnsii* had been grown, respectively, and these differences were significant (p=0.002 and p<0.005, respectively). Ca concentrations of applied faecal sludge were approximately twice that of faecal sludge where the species had been grown but variability in the applied faecal sludge was considerable and these differences were not statistically significant. Concentrations of Mg of faecal sludge post-harvest was similar for both species and greater than that of applied faecal sludge by a factor of approximately 2.5 (p<0.005). Of the micronutrients measured, Na and Fe concentrations had declined significantly in the faecal sludge where both species had been grown (p<0.005) while Mn decreased and Al increased where *E. grandis* and *A. mearnsii* had been grown, respectively (p=0.04 and p=0.01, respectively). Interspecific comparisons for all measured nutrients established that only the difference in Al, which was greater in faecal sludge where *A. mearnsii* had been grown, was of statistical significance (p=0.02).

Fertilizer additions generally had little effect on nutrient concentrations of river sand as determined from a depth of 500 mm. N concentrations had only marginally increased at harvest in comparison with the applied river sand where *E. grandis* and *A. mearnsii* had been grown, although the latter difference was only marginally non-

significant (p=0.06). K concentrations showed similarly small increases at harvest and was statistically significant only in sand where *E. grandis* had been grown (p=0.04). Of the remaining macronutrients, none of P, Bray P, Ca and Mg had increased by harvest where either species had grown but these differences were not of statistical significance. Micronutrient concentrations of the sand had generally increased at harvest with statisitically significant increases in the concentrations of Zn, Cu, Fe and Al where *A. mearnsii* had been grown and the difference in Al was significant where *E. grandis* had been grown. Only the concentration of Mn had declined significantly by harvest in both species. Interspecific comparisons showed that, of the nutrients measured, Zn and Fe were occurred in significantly greater concentrations where *A. mearnsii* had been grown while the converse was true of Ca where *E. grandis* had been grown.

Table 3.1: Chemical properties of faecal sludge applied to columns and of sludge collected at the time of harvest from a depth of 500 mm where *E. grandis* and *A. mearnsii* had been grown.

		At harvest	
Parameter	At application	*E. grandis*	*A. mearnsii*
N ($mg.kg^{-1}$)	2.2 ±0.1a	1.8 ±0.4a	2.3 ±0.4a
Total P ($mg.kg^{-1}$)	4500.7 ±140.0a	5398.32 ±1380.0a	5111.8 ±335.4b
Bray P ($mg.kg^{-1}$)	3774.6 ±109.4a	4474.1 ±1151.5a	4235.3 ±235.8b
K ($mg.kg^{-1}$)	462.4 ±26.8a	156.7 ±109.8b	82.2 ±65.3b
Ca ($mg.kg^{-1}$)	753.9 ±1023.2a	382.8 ±48.2a	336.5 ±44.6a
Mg ($mg.kg^{-1}$)	123.7 ±18.9a	327.5 ±63.8b	347.6 ±36.4b
Zn ($mg.kg^{-1}$)	154.4 ±87.0a	111.2 ±33.4	132.3 ±32.8
Cu ($mg.kg^{-1}$)	12.9 ±7.3a	19.6 ±6.9a	13.0 ±0.1a
Fe ($mg.kg^{-1}$)	186.5 ±116.1a	87.1 ±21.4b	91.7 ±11.1b
Al ($mg.kg^{-1}$)	8.1 ±5.0a	9.7 ±1.7a	14.4 ±3.2b
Mn ($mg.kg^{-1}$)	67.3 ±3.9a	33.9 ±17.5b	51.5 ±19.9a
Na ($mg.kg^{-1}$)	412.9 ±19.8a	124.4 ±70.7b	64.5 ±41.1b

Values in the same row for the respective species followed by the same letter are not significantly different (p>0.05). Variations shown are ±SD around the mean (n=5).

Table 3.2: Physical properties and nutrient concentrations of river sand applied to columns and the nutrient concentrations of sand collected at the time of harvest from the annular ring at a depth of 500 mm where *E. grandis* and *A. mearnsii* had been grown.

		At harvest	
Parameter	At application	*E. grandis*	*A. mearnsii*
Sand (0.05–2.0mm) (%)	95.7 ±0.6		
Silt (0.002mm–0.05) (%)	>0.00		
Clay (<0.002mm) (%)	4.3 ±0.6		
N ($mg.kg^{-1}$)	0.04 ±0.00a	0.04 ±0.00a	0.05 ±0.00a
Total P ($mg.kg^{-1}$)	2.6 ±0.7a	2.1 ±0.7a	2.3 ±1.5a
Bray P ($mg.kg^{-1}$)	2.1 ±0.6a	1.7 ±0.6a	1.9 ±1.2a
K ($mg.kg^{-1}$)	2.8 ±0.1a	3.5 ±0.2b	3.4 ±0.9a
Ca ($mg.kg^{-1}$)	54.7 ±71.2a	15.4 ±1.4a	13.5 ±0.9a
Mg ($mg.kg^{-1}$)	4.6 ±0.2a	4.0 ±0.4a	3.6 ±0.4a
Zn ($mg.kg^{-1}$)	0.3 ±0.1a	0.5 ±0.04a	0.6 ±0.1b
Cu ($mg.kg^{-1}$)	0.4 ±0.1a	1.7 ±2.4a	0.58 ±0.1b
Fe ($mg.kg^{-1}$)	58.1 ±3.3a	68.5 ±10.0a	82.8 ±8.3b
Al ($mg.kg^{-1}$)	25.1 ±4.5a	36.2 ±6.8b	37.3 ±5.8b
Mn ($mg.kg^{-1}$)	9.4 ±0.8a	1.9 ±2.2b	2.94 ±1.5b
Na ($mg.kg^{-1}$)	1.1 ±0.1a	1.3 ±0.3a	1.5 ±0.6a

Values in the same row for the respective species followed by the same letter are not significantly different (p>0.05). Variations shown are ±SD around the mean (n=3).

3.1.3. Sapling growth measurements

3.1.3.1. Sapling height

For the first 9 weeks (63 days) after planting no significant difference was observed in the height of *E. grandis* between the experimental and control groups (Figs. 3.2, 3.3). However, at 10 weeks (70 days) the mean height of *E. grandis* in the control and experimental groups was 0.84 and 1.10 m, respectively, and this difference in height was significant (p=0.007). Beyond 10 weeks the eucalypts grown in faecal sludge showed a continuous increase in growth, while those grown in the control showed only very marginal growth for the rest of the experimental period. These contrasting patterns of tree height over time for the respective groups can be described as approximately exponential increases in height in eucalypts grown in faecal sludge compared with a distinct sigmoidal response in tree height for eucalypts grown in the control. The continuous growth of the eucalypts in the treatment group throughout the experiment together with the restricted growth of eucalypts in the control group beyond 10 weeks resulted in a large difference in height between the groups by the

end of the experiment (26 weeks). At that point the mean height of the treatment and control groups was 244 cm and 108 cm, respectively, or about 2.3 times greater in the experimental group, and this difference was highly significant (p<0.005). Variation about the mean at the end of the experiment was ± 21.7 SD for the control group and ± 10.9 SD for the treatment group. The relatively small variation about the mean for both groups does reveal two clear responses little affected by genotypic differences which could have been expected to be quite high since the saplings were grown from seed.

In contrast with the marked differences in tree height observed between the treatment and control groups for *E. grandis*, the corresponding differences in height of the wattle were small throughout the experiment (Figs. 3.3, 3.4). Differences in height were significant at one week before harvest (week 25; p=0.041) but unlike the difference observed for the eucalypts, the difference in height between groups was relatively small (26 cm). From week 8 to 10 the wattle in the control group made greater gains in height than the corresponding treatment group although these differences were not significant (p>0.05). At harvest the mean height of wattle was 270 cm and 232 cm for the treatment and control groups, respectively, and this difference was significant (p=0.014). In particular, differences in height and overall growth between control and experimental groups were clearly visible in *E. grandis* (Figs. 3.5a and b) but less evident in *A. mearnsii* (Figs. 3.6a and b). Differences in height between species in control groups were significant (p<0.005) while the corresponding difference in experimental groups was significant at the p=0.1 level (p=0.07). However, the direction of these differences differed with species.

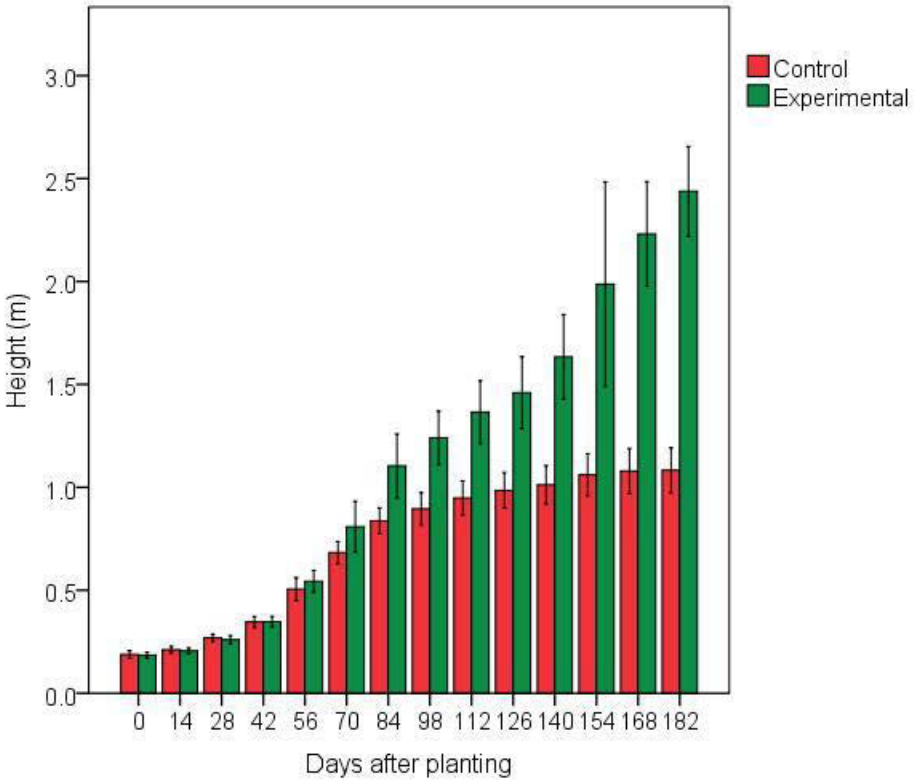

Figure 3.2: Mean height of *E. grandis* measured at two week intervals over the 26 week experimental period (*n*=6).

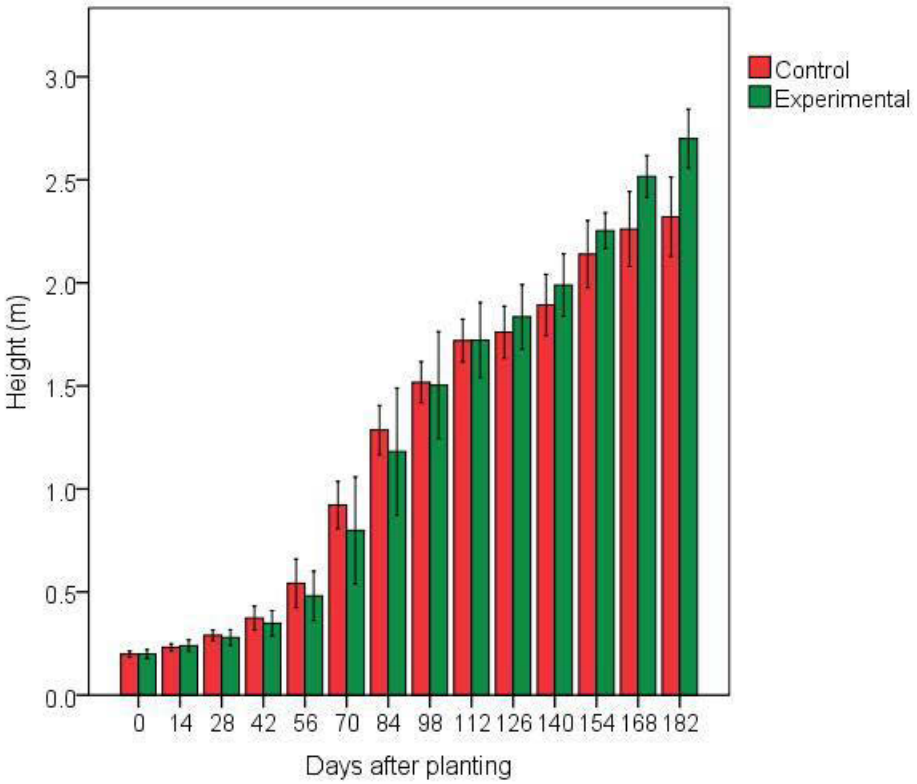

Figure 3.3: Mean height of *A. mearnsii* measured at two week intervals over the 26 week experimental period (*n*=4 and *n*=5 for experimental and control groups respectively).

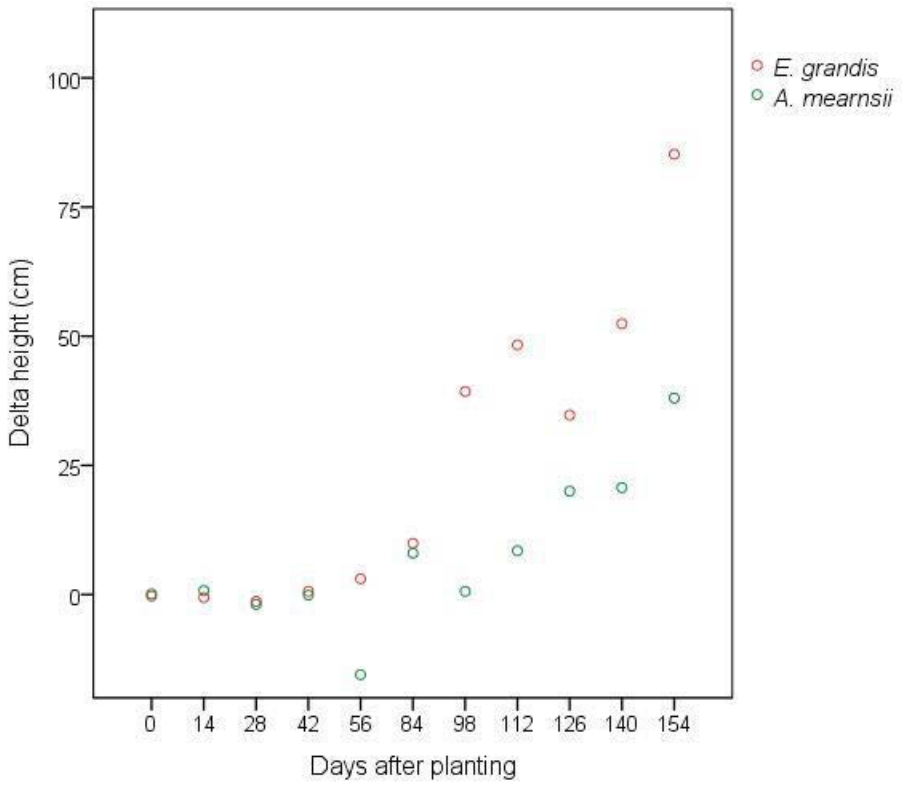

Figure 3.4: Mean delta height (experimental height – control height) of *E. grandis* and *A. mearnsii* over the 26 week experimental period.

Figure 3.5a and b: *E. grandis* of (a) control and (b) experimental groups prior to harvest.

Figure 3.6a and b: *A. mearnsii* of (a) control and (b) experimental groups prior to harvest.

3.1.3.2. Root collar diameter

Root collar diameter (RCD) in experimental saplings of *E. grandis* was significantly greater than that of control saplings of the same species at 12 weeks after planting (Fig. 3.7; p=0.05), occurring only shortly after significant differences in height emerged. At harvest RCD was 40 mm and 34 mm for the treatment and control groups, respectively (p=0.014), which was a relatively small difference in comparison with the considerable differences observed in tree height. RCD also continued to increase in the control by appreciable amounts throughout the growth period, unlike tree height which had more or less levelled soon after the midpoint of the experimental period. This indicates that biomass was preferentially partitioned in favour of greater stem diameter rather than stem length in control saplings of *E. grandis*.

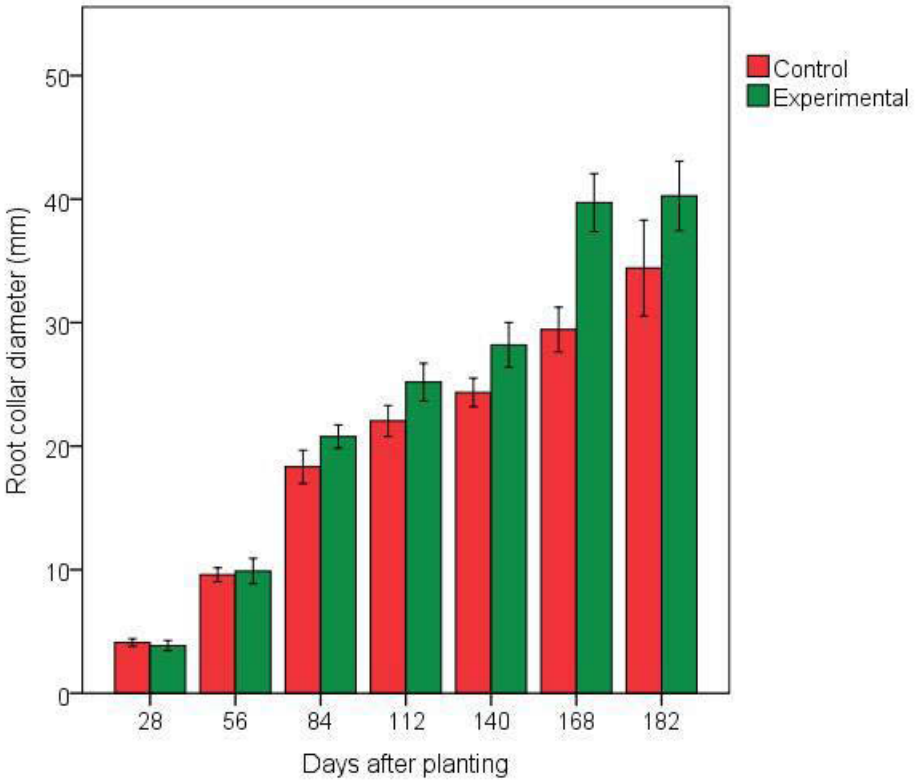

Figure 3.7: Mean root collar diameter of *E. grandis* measured at 4 week intervals over the 26 week experimental period (*n*=6).

Root collar diameter of *A. mearnsii* was similar in the control and experimental groups and at no point were differences in stem diameter significant (Fig. 3.8). Root collar diameter was 45 mm and 39 mm for the treatment and control groups, respectively, at harvesting. Tree height and RCD of this species showed similar trends and from week 4 to week 6 RCD was greater in the control, as was the case with tree height. During the last month RCD in the control group increased only marginally while RCD in the treatment group increased by a comparatively larger amount. The negligible increase in RCD and tree height in the final month of the growth period suggests that growth of *A. mearnsii* was restricted in the latter phase of the growth period as observed for *E. grandis* earlier in the growth period. Differences in RCD between species for control and experimental groups were not significant (p=0.213 and p=0.130, respectively).

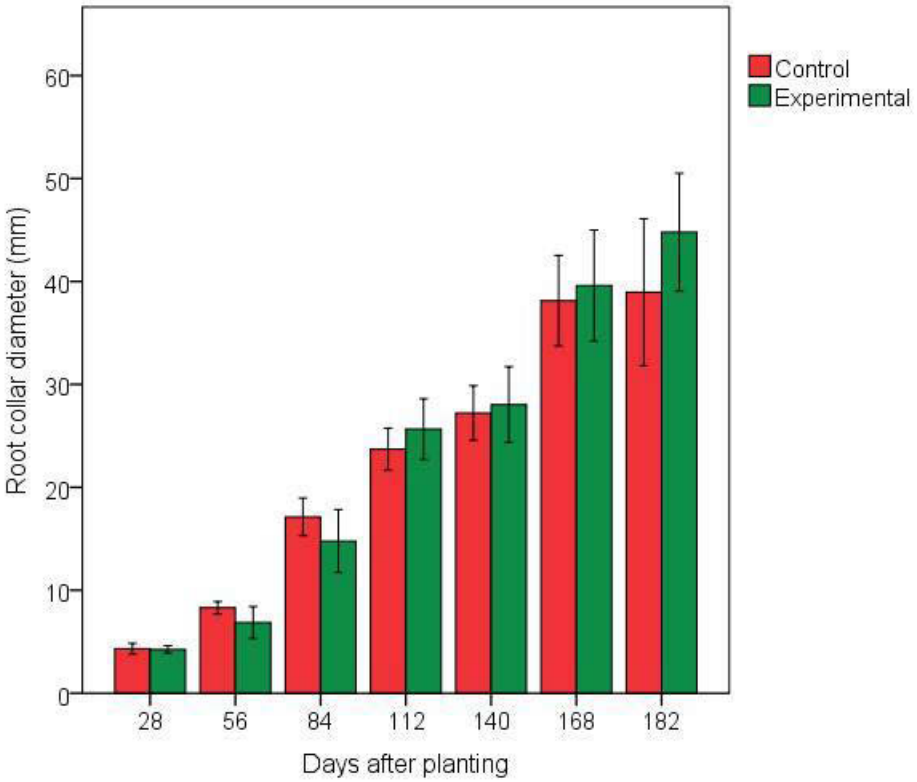

Figure 3.8: Mean root collar diameter of *A. mearnsii* measured at 4 week intervals over the 26 week experimental period (n=4 and n=5 for experimental and control groups, respectively).

3.1.3.3. Leaf area

Leaf area and specific leaf area of *E. grandis* and *A. mearnsii* for control and experimental groups are shown in Table 3.3. Leaf area was significantly lower in *E. grandis* in the control group than in the experimental group (p<0.005). Mean leaf area in the control was 1.83 m^2 compared to the experimental group which had a leaf area of 11.97 m^2, representing a 6.5-fold difference between treatments. The disparity in leaf area for *E. grandis* indicates that foliage production was severely restricted in the control group and leaves were visibly fewer in number and smaller than that of the experimental saplings. Furthermore, visible observations of the saplings suggest that leaf area (based on the observed amount of leaves) remained relatively unchanged for the last two months before harvest. This is consistent with height data which indicates a ceiling in growth.

The disparity in leaf area in *A. mearnsii* was less marked than that of the *E. grandis* but mean leaf area in the experimental group was still approximately twice that of the control group. Mean leaf area in control saplings was 1.95 m^2 compared to that of the experimental group of 3.73 m^2 (p=0.027). It is important to note that the CI-202 leaf area meter used was not portable and so leaf area was only able to be determined once leaves had been harvested and returned to the laboratory, by which time many of the leaves had closed. Leaf area figures reported here therefore do not represent absolute leaf area values but rather an approximation or ratio of leaf area; it is estimated that leaf area figures reported here are about 50% of the absolute leaf area based on the way in which the leaves close. Unlike *E. grandis*, *A. mearnsii* did not show premature leaf senescence although leaf production at the auxiliary buds appeared to be halted at approximately one month before harvest. Thus, this appears to be a different strategy to that adopted by *E. grandis*. As evidenced by leaf area, wattles grown in the experimental group showed extensive foliage production relative to the control, particularly at the base of the saplings where lateral growth was vigorous. Leaf area between the species in control groups was not significantly different (p=0.214) although the corresponding difference for experimental groups was significantly different (p<0.005) in favour of *E. grandis*.

Specific leaf area was considerably greater in experimental saplings of *E. grandis* compared with control saplings of the same species. In this respect, values of SLA for experimental and control groups were 18.91 and 10.64 $m^2.kg^{-1}$, respectively, and this difference was significant (p<0.005). Corresponding values of SLA for *A. mearnsii* were 5.94 and 4.67 $m^2.kg^{-1}$ but this difference was not significant (p=0.204). Interspecific differences in SLA were significant for both experimental and control groups, with *E. grandis* exhibiting the greatest SLA in both cases (p<0.005).

Table 3.3: Leaf area (LA) and specific leaf area (SLA) of *E. grandis* and *A. mearnsii* grown in control and experimental groups.

	E. grandis		*A. mearnsii*	
Parameter	Control	Experimental	Control	Experimental
LA (m^2)	1.83 ±0.23a	11.97 ±1.55b	1.95 ±1.14a	3.73 ±0.32b
SLA ($m^2.kg^{-1}$)	10.64 ±1.65a	18.91 ±2.71b	4.67 ±1.60a	5.94 ±0.89a

Values in the same row for the respective species followed by the same letter are not significantly different (p>0.05). Variations shown are ±SD around the mean (n=5).

3.1.3.4. Dry biomass partitioning

Dry biomass partitioning of *E. grandis* is shown in Fig. 3.9 and in Table 3.5. The experimental group showed a considerable increase in aboveground total dry biomass as well as greater dry biomass values for each of the measured components i.e. stem, twigs and leaves, relative to saplings grown in the control. Total aboveground biomass was 1.52 kg in the experimental group compared to only 0.36 kg for the control group. This represents an increase of over 4-fold in aboveground dry biomass in plants grown in faecal sludge and this difference was significant (p<0.005). Therefore, the application of faecal sludge considerably increased aboveground dry matter production relative to the control. All of the measured components showed significantly higher dry biomass in the experimental group (p<0.05).

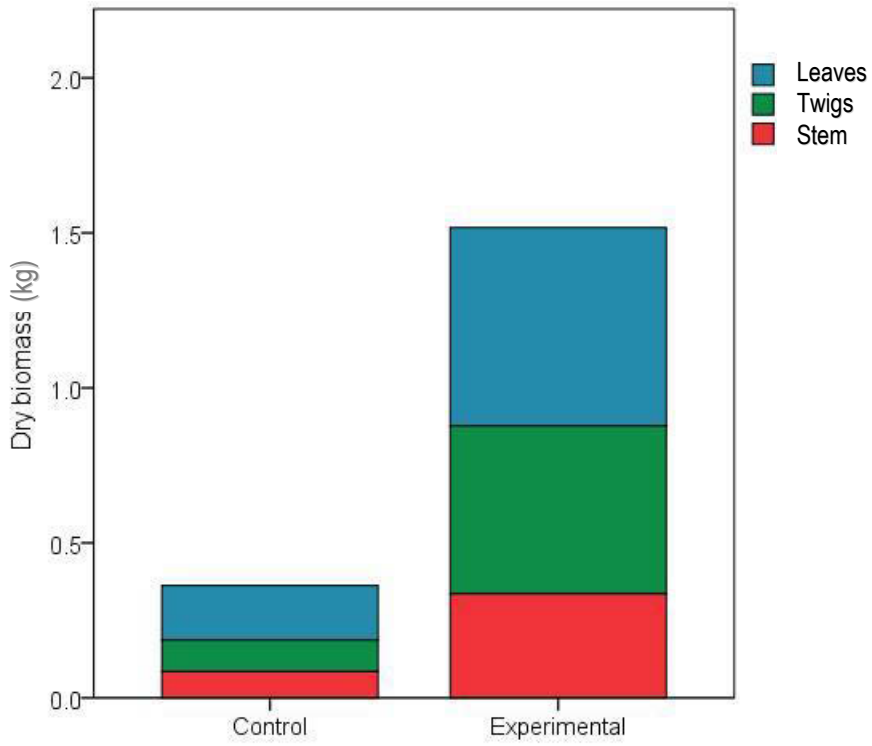

Figure 3.9: Mean dry biomass partitioning into leaves, twigs and stem for experimental and control groups of *E. grandis*.

The dry matter partitioning in *E. grandis* (based on percentage distribution of overall dry biomass) was significantly different between the treatment and control groups for the leaf and twig components but non-significant for the trunk (Fig. 3.10 and Table

3.3; p=0.236). Leaves represented the greatest sink in both experimental and control groups, constituting almost half of total dry biomass. In this respect mean dry leaf biomass constituted 42.1% of total dry biomass in the experimental group compared to that of the control which was 48.4% (p=0.007). Saplings in the experimental and control groups partitioned about one fifth of total dry biomass into the trunk (22.1% and 23.6%, respectively) approximately one third of total dry biomass into twigs (28.0% and 35.8%, respectively) but the latter difference was significant (p=0.001). Thus biomass partitioning was altered between the groups only with respect to the trunk, but not with leaves or twigs, such that for leaves and twigs: experimental>control; and for trunk: experimental=control. These results show that despite the disparity in overall biomass the partitioning of biomass was similar irrespective of treatment.

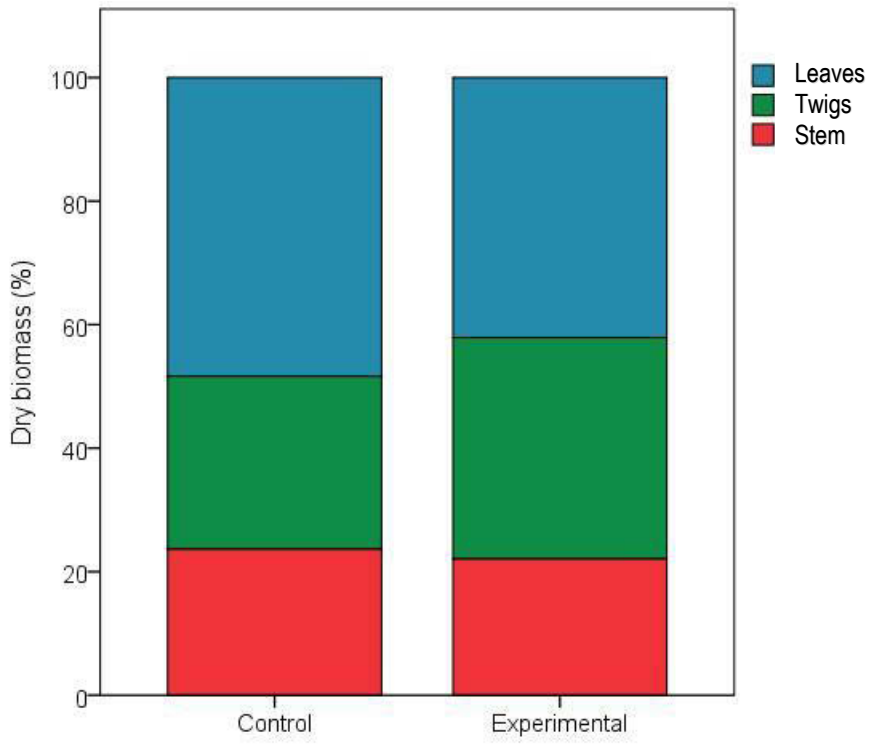

Figure 3.10: Mean percentage of total dry biomass partitioning into leaves, twigs and stem for experimental and control groups of *E. grandis*.

Dry biomass partitioning of *A. mearnsii* is shown in Fig. 3.11 and in Table 3.4. In contrast with *E. grandis* the difference in total dry biomass of *A. mearnsii* was comparatively less marked (1.0 and 1.6 kg for control and experimental groups,

respectively; p=0.016). All of the measured components showed a corresponding increase in dry biomass relative to the control, but the difference in trunk dry biomass was non-significant (although still significant at the p=0.1 level). The percentage of total dry biomass partitioned into leaves, twigs and trunks of *A. mearnsii* for both groups was almost identical for each component and all differences were within as little as 0.8% of total dry biomass (Fig. 3.12 and Table 3.5). In this respect, approximately 40% was partitioned into leaves and 30% was partitioned into the twigs and stem and all differences were non-significant.

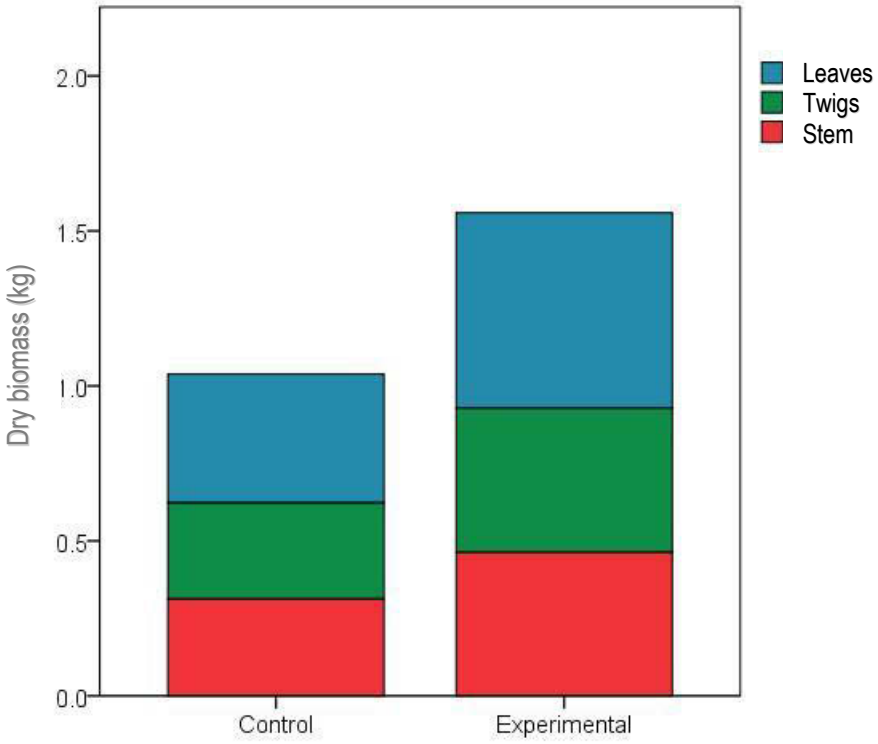

Figure 3.11: Mean dry biomass partitioning into leaves, twigs and stem for experimental and control groups of *A. mearnsii*.

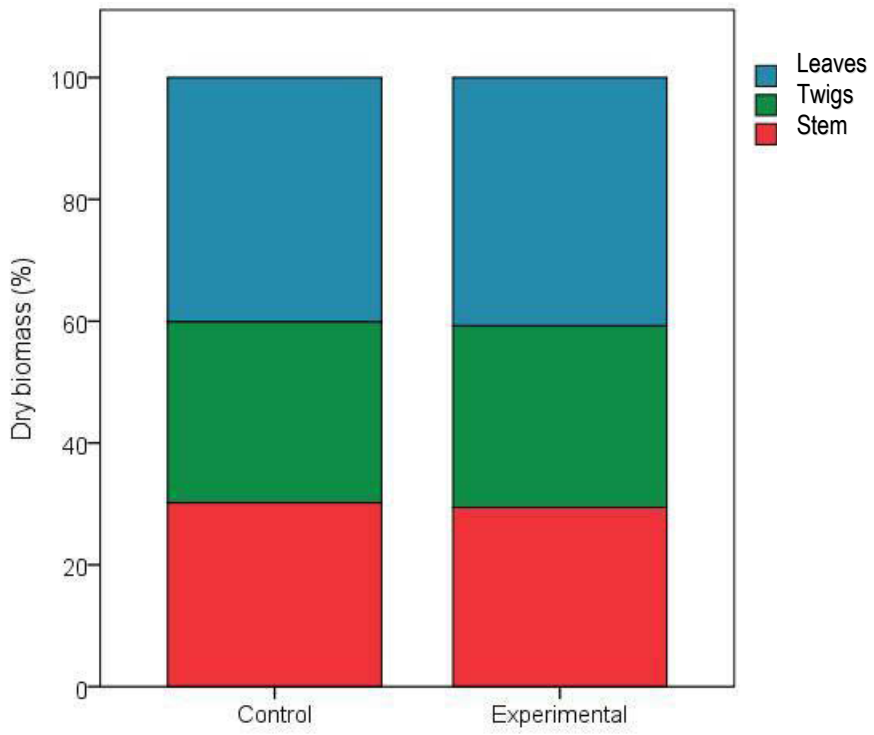

Figure 3.12: Mean percentage of total dry biomass partitioning into leaves, twigs and stem for experimental and control groups of *A. mearnsii*.

Table 3.4: Mean total dry biomass partitioned into leaves, twigs and stem of *E. grandis* and *A.mearnsii*. Variations shown are ±SD of the mean. (*n*=6, except for *A. mearnsii* where *n*=4 and *n*=5 for experimental and control groups, respectively).

	E. grandis		*A. mearnsii*	
Component	Control	Experimental	Control	Experimental
Leaves (g)	176 ± 41a	640 ± 95b	415 ± 116a	630 ± 49b
Twigs (g)	101 ± 20a	542 ± 48b	310 ± 96a	465 ± 69b
Stem (g)	85 ± 16a	336 ± 56b	313 ± 95a	463 ± 126a*
Total (g)	362 ± 73a	1517 ± 151b	1038 ± 295a	1558 ± 155b

*Significant at the 0.1 level

Values in the same row for the respective species followed by the same letter are not significantly different (p>0.05, Scheffe's multiple range test).

Table 3.5: Mean percentage of total dry biomass partitioned into leaves, twigs and stem of experimental and control groups of *E. grandis* and *A. mearnsii*. Variations shown are ±SD of the mean. (*n*=6, except for *A. mearnsii* where *n*=4 and *n*=5 for experimental and control groups, respectively).

	E. grandis		*A. mearnsii*	
Component	Control	Experimental	Control	Experimental
Leaves (%)	48.4 ± 2.8a	42.1 ± 3.6b	40.1 ± 2.1a	40.7 ± 4.8a
Twigs (%)	28.0 ± 2.4a	35.8 ± 3.1b	29.7 ± 2.1a	29.8 ± 3.0a
Stem (%)	23.6 ± 1.9a	22.1 ± 2.3a	30.2 ± 3.3a	29.4 ± 6.0a

Values in the same row for the respective species followed by the same letter are not significantly different (p>0.05, Scheffe's multiple range test).

3.1.4. Gas exchange measurements

Light curves for both species showed two distinct trends depending on treatment (Figs. 3.13 and 3.14). At low PAR (>500µmol.m-2.s-1) photosynthetic rates were similar between control and experimental groups of both species indicating a fairly equal effect of light limitation irrespective of treatment. However, at higher PAR curves of both species diverged and at light saturation A was considerably suppressed in control groups of both species relative to that of experimental groups. Assimilation of control groups levelled at lower PAR compared with experimental groups, indicating that these saplings were unable to utilise increased PAR due to underlying biochemical limitations to photosynthesis.

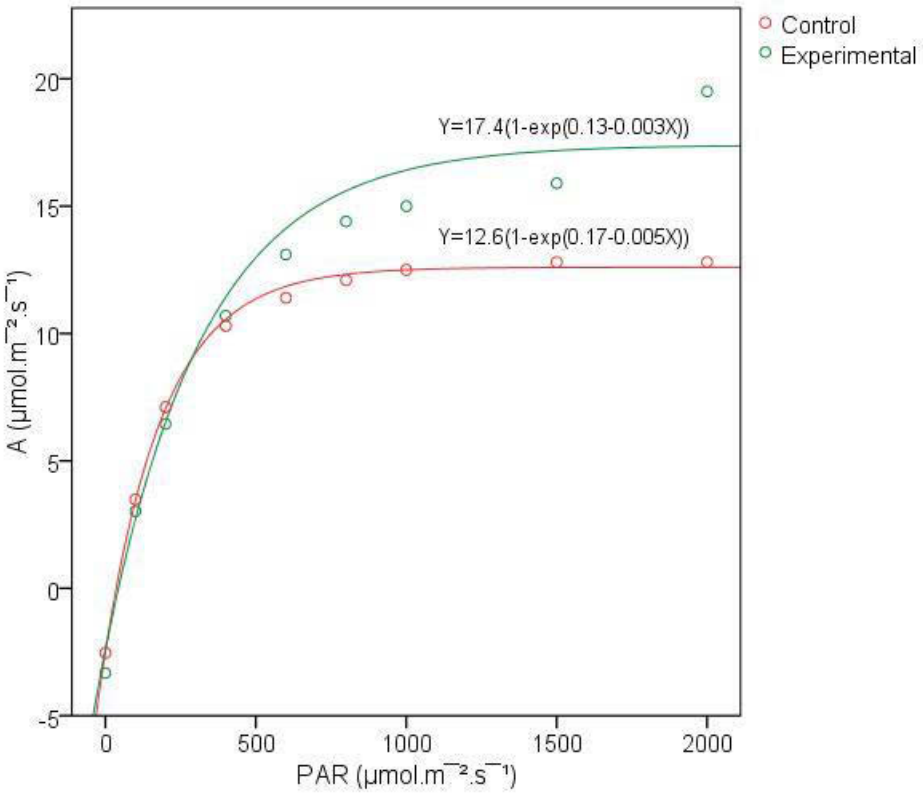

Figure 3.13: Representative light response curves of *E. grandis* for control and experimental groups (*n*=5).

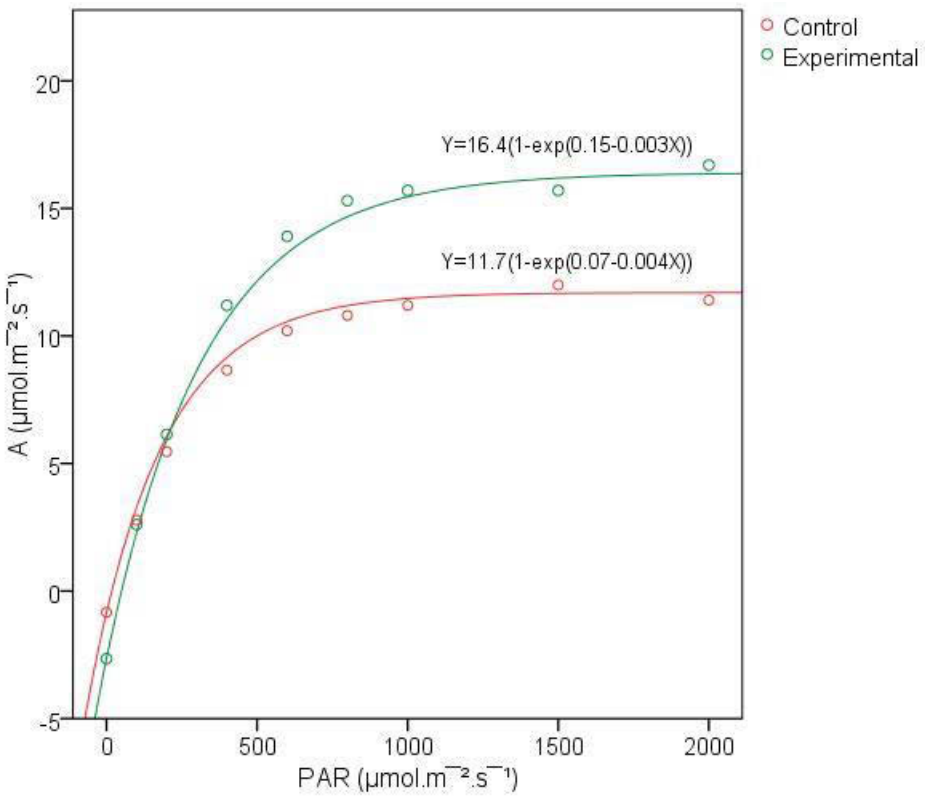

Figure 3.14: Representative light response curves of *A. mearnsii* for control and experimental groups (*n*=5).

A-C_i curves for both species indicate a typical bi-phase limitation to A whereby Rubisco is limiting at low Ci and RuBP regeneration is limiting at higher Ci (Figs. 3.15 and 3.16). Assimilation in control saplings was particularly suppressed at higher Ci relative to that in experimental saplings, suggesting that RuBP regeneration was a major limitation to photosynthesis in control saplings. Based on the initial slope of the CO_2 response curves, Rubisco limitation did not appear to be marked. Generally, no triose phosphate use (TPU) limitation could be discerned although in a few saplings of both species a slight decrease in A at high Ci could indicate a minor TPU limitation in some saplings.

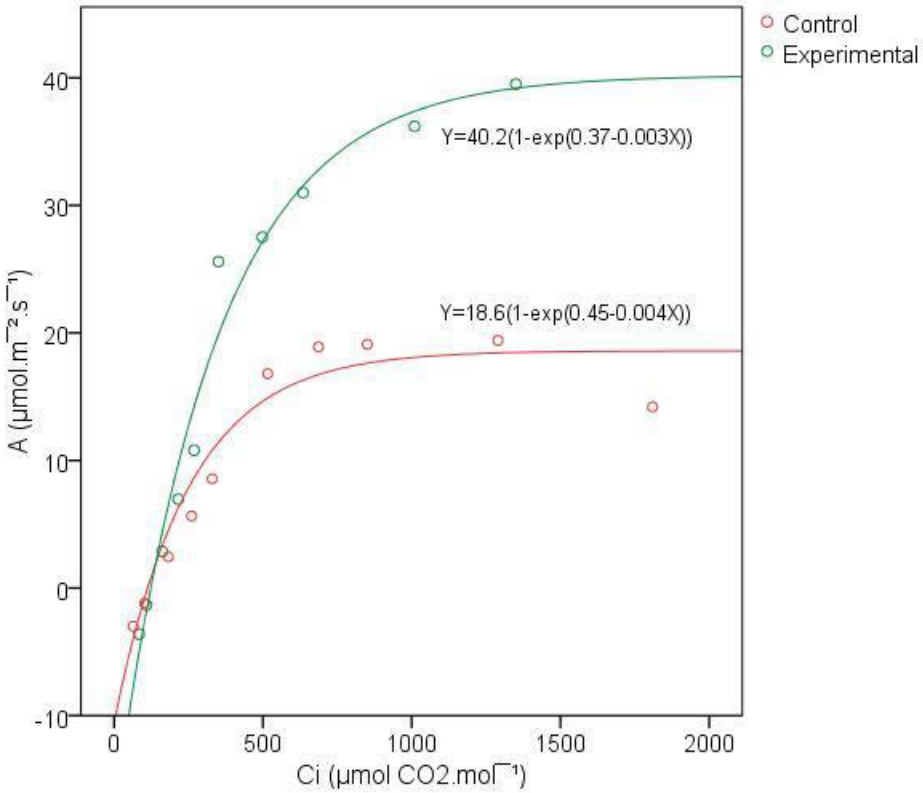

Figure 3.15: Representative CO_2 response curves of *E. grandis* for control and experimental groups (*n*=5).

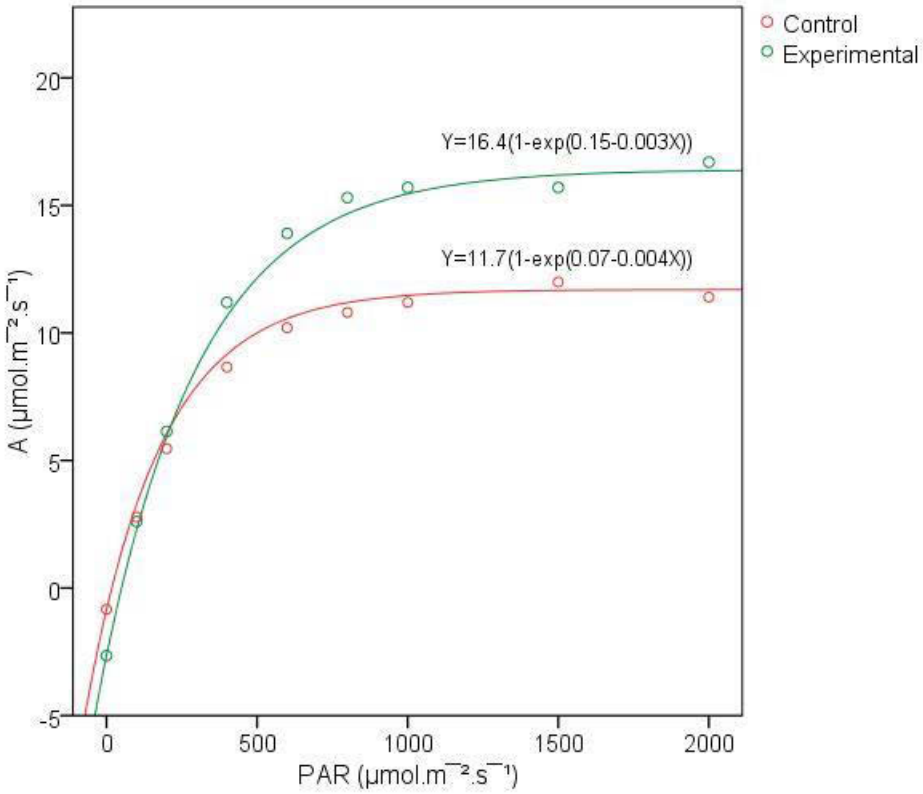

Figure 3.16: Representative CO_2 response curves of *A. mearnsii* for control and experimental groups (*n*=5).

Photosynthetic parameters calculated from light curves and A-c_i curves for *E. grandis* and *A. mearnsii* are presented in Table 3.6. The application of faecal sludge enhanced photosynthesis in general which reflects the positive response at the whole plant level. The response to faecal sludge was very similar between the species although the positive response to faecal sludge was slightly less marked in *A. mearnsii* which may be due to a lower nutrient demand by this species or the result of N_2 fixation. On an incident light basis, light use efficiency (LUE; $\mu mol.m^{-2}.s^{-1}.\mu mol\ photons^{-1}$) was not affected by treatment in either species. It is possible, however, that differences in leaf pigment compositions between treatments could have yielded differences in LUE on an absorbed light basis. CO_2 fixation at saturating light ($A_{max;}$ $\mu mol.m^{-2}.s^{-1}$) was considerably enhanced in both species grown above faecal sludge although in *A. mearnsii* this difference was smaller and significant only at the p=0.1 level. These differences in photosynthetic capacity were the result of differing nutrient availability between treatments (i.e. greater nutrient supply in the experimental group) which imposed major limitations to photosynthesis in saplings in the control group.

The underlying biochemical limitations to photosynthesis in control saplings were explored further using data calculated from A-c_i curves. Maximum electron transport in the regeneration of RuBP (J_{max}; $\mu mol.m^{-2}.s^{-1}$) was considerably increased in saplings grown above faecal sludge, particularly in *E. grandis* where J_{max} of saplings grown above faecal sludge was less than half that of control saplings. The maximum rate of carboxylation (V_{cmax}; $\mu mol.m^{-2}.s^{-1}$) was also increased in saplings grown above faecal sludge but, as with A_{max}, the difference in *A. mearnsii* was significant only at the p=0.1 level. Taken together, these results suggest that limitation to A in control saplings at high light was primarily the result of reduced electron transport capacity and, to a lesser extent, the amount or activity of Rubisco. Foliar nutrient concentrations, particularly that of N, support the latter (Table 3.7).

Photorespiration rates were considerably increased in both species grown above faecal sludge which was expected as it is not unusual for photorespiration rates to increase with photosynthetic capacity. However, these rates were exceptionally high relative to A for both species and may have been the result of the high temperatures at which measurements were conducted. The rate of dark respiration (R_{dark}) was higher in *E. grandis* grown above faecal sludge which was unsurprising given the considerably

lower specific leaf area in that treatment (i.e. greater tissue mass per unit area) which increased metabolic activity associated with maintenance or so-called 'maintenance respiration'. However, this difference was not significant in both species as were differences in light compensation point (I_C; µmol.m-2.s-1) and CO_2 compensation point (Γ; µmol).

The variability in E ($mmol.m^{-2}.s^{-1}$) was notably high in both species and these differences were not significant. Stomatal conductance (g_s) at saturating light in *E. grandis* in the experimental group was half that of control saplings of that species and g_s in *A. mearnsii* in the experimental group was greater than that of control saplings of that species by a factor of three (p=0.065). Calculated water use efficiencies (WUE; $\mu mol.m^{-2}.s^{-1}$ CO_2. $mmol.m^{-2}.s^{-1}$ H_20^{-1}) show that experimental saplings of *E. grandis* had a WUE of almost twice that of control saplings brought about by lower transpiration and greater CO_2 assimilation at saturating light. Unlike that observed in *E. grandis*, WUE in *A. mearnsii* was greater in the control group than the experimental group but this difference was relatively small and not of statistical significance.

Table 3.6: Photosynthetic parameters calculated from light curves and A-c_i curves taken on leaves of *E. grandis* and *A. mearnsii* in control and experimental groups.

	E. grandis		*A. mearnsii*	
	Control	Experimental	Control	Experimental
A_{max} ($\mu mol.m^{-2}.s^{-1}$)	12.7 ± 1.8a	17.3 ± 3.9b	11.7 ± 3.6a	16.4 ± 3.2a*
LUE ($\mu mol.m^{-2}.s^{-1}.\mu mol\ phot^{-1}$)	0.06 ± 0.02a	0.07 ± 0.02a	0.04 ± 0.01a	0.04 ± 0.01a
J_{max} ($\mu mol.m^{-2}.s^{-1}$)	18.2 ± 1.7a	41.2 ± 7.2b	29.2 ± 2a	43.1 ± 5.3b
V_{cmax} ($\mu mol.m^{-2}.s^{-1}$)	0.11 ± 0.01a	0.15 ± 0.03b	0.12 ± 0.04a	0.16 ± 0.02a*
Ic ($\mu mol.m^{-2}.s^{-1}$)	33.0 ± 5.4a	39.4 ± 13.7a	37.6 ± 33.1a	46.7 ± 37.5a
R_{dark} ($\mu mol.m^{-2}.s^{-1}$)	1.9 ± 0.6a	2.5 ± 0.6a	1.2 ± 0.7a	1.7 ± 1.3a
R_{day} ($\mu mol.m^{-2}.s^{-1}$)	9.1 ± 1.4a	15.7 ± 2.2b	10.7 ± 3.0a	14.8 ± 0.6b
Γ (µmol)	103.5 ± 17.4a	127.7 ± 18.4a*	106.0 ± 9.1a	108.0 ± 13.3a
g_s	0.6 ± 0.4a	0.3 ± 0.2a	0.1 ± 0.07a	0.4 ± 0.3a*
E ($mmol.m^{-2}.s^{-1}$)	4.1 ± 1.0a	3.2 ± 1.3a	2.7 ± 1.8a	4.2 ± 1.2a
WUE ($A_{max}.E^{-1}$)	3.2 ± 0.9a	6.2 ± 2.6b	5.4 ± 3.1a	4.0 ± 1.1a

Values for each species in the same row followed by the same letter are not significantly different (p>0.05, Scheffe's multiple range test). Variations shown are ±SD of the mean (n=5, except for T-1 where n=4).
*Significant at the 0.1 level

3.1.5. Foliar nutrient concentrations

Foliar nutrient concentrations of control and experimental saplings of *E. grandis* and *A. mearnsii* are shown in Table 3.7. With the exception of Ca all macronutrients occurred in greater concentration in foliage of *E. grandis* in the experimental group than in the control group, indicating the improved nutrition in saplings of that species when supplied with faecal sludge. In particular the foliar concentration of N in the experimental group was 32.9 $mg.g^{-1}$, representing a nearly three-fold increase compared with that of control saplings (p=0.001) and foliar P in experimental saplings was 3.1 $mg.g^{-1}$ which was approximately twice that of control saplings (p=0.012). Foliar K was 10.5 and 7.2 $mg.g^{-1}$ in experimental and control saplings of that species, respectively, and this difference was marginally non-significant at the p=0.05 level (p=0.058). Of the macronutrients, N was decreased by the greatest proportion in control saplings and probably had the greatest limiting effect on sapling growth in *E. grandis* together with P and possibly K. This is corroborated by the decrease in the N:P ratio from 11.1:1 in experimental saplings of *E. grandis* to 7.9:1 for control saplings (p=0.021). Control saplings of that species showed characteristic deficiency symptoms of both N and P as evidenced by correspondingly lighter green foliage and purple colouration on growing tips in the latter part of the growing phase (Fig. 3.17a and b). Foliar Mg was 3.1 and 4.8 $mg.g^{-1}$ in control and experimental saplings of *E. grandis*, respectively, and this difference was significant (p<0.005). Of the micronutrients in that species, concentrations of Fe and Al showed significant differences between control and experimental groups. In this respect, foliar Fe concentration was greater in experimental saplings (203 $mg.kg^{-1}$) compared with control saplings (163 $mg.kg^{-1}$; p<0.005). Corresponding concentrations of foliar Al were 127 and 171 $mg.kg^{-1}$ (p=0.005).

Concentrations of foliar macronutrients in control and experimental groups of *A. mearnsii* followed similar trends to that observed in *E. grandis*. In this respect, only Ca had a greater concentration in control saplings but this difference was not significant. Moreover, N, P, K occurred in greater concentrations in the foliage of experimental saplings. Experimental saplings had a foliar N concentration of 31.9 $mg.g^{-1}$ which was similar to that observed in *E. grandis* in the same group and greater than that of control saplings (23.3 $mg.g^{-1}$; p=0.006). The concentration of foliar P was

4.5 $mg.g^{-1}$ in experimental saplings which was about 4.5 times greater than that of control saplings (p=0.017) and foliar K in experimental saplings of 12.6 $mg.g^{-1}$ was approximately twice that of control saplings (p<0.005). The higher N:P ratio of foliage of control saplings (23.7:1) indicates that P was more deficient than N. No visible symptoms of K deficiency were evident in control saplings. Foliar Mg in experimental saplings was 27 $mg.kg^{-1}$ compared with that of control saplings of 18 $mg.kg^{-1}$ (p=0.021). Interspecific comparisons of foliar macronutrients showed that foliar concentrations of N, P, Ca and Mg in control saplings were statistically greater in *A. mearnsii* (p<0.05). Amongst experimental saplings, however, only Ca and Mg were statistically greater although these nutrients occurred in greater concentration in *E. grandis*.

Foliar Cu, Fe and Al were greater in control saplings of *A. mearnsii* and foliar Zn and Mn were greater in experimental saplings of that species. Foliar concentrations of Cu, Fe and Al in control saplings were 0.9, 25.8 and 16.9 $mg.g^{-1}$, respectively, and the corresponding concentrations of these nutrients in experimental saplings was 0.4, 17.9 and 12.0 $mg.g^{-1}$ (p<0.005, p=0.008 and p=0.020 for those respective differences). Of foliar Zn and Mn, only Zn concentration was statistically greater in experimental saplings with concentrations of 1.8 and 2.6 $mg.g^{-1}$ in control and experimental saplings, respectively (p=0.008).

Foliar Na was increased in experimental saplings of both species compared with control saplings, particularly in *E. grandis* where foliar Na of experimental saplings (7.1 $g.kg^{-1}$) was approximately twice that of control saplings (p=0.004). In *A. mearnsii* the difference in foliar Na between control and experimental saplings was much less marked than that observed in *E. grandis*, with concentrations of 3.0 and 3.8 $g.kg^{-1}$ for control and experimental saplings, respectively (p<0.004).

Table 3.7: Foliar nutrient concentrations of *E. grandis* and *A. mearnsii* grown in control and experimental groups.

	E. grandis		*A. mearnsii*	
Parameter	Control	Experimental	Control	Experimental
N ($mg.g^{-1}$)	11.2 ±0.7a	32.9 ±5.0b	23.3 ±4.2a	31.8 ±0.9b
P ($mg.g^{-1}$)	1.5 ±0.3a	3.1 ±1.1b	10.2 ±0.3a	4.5 ±2.5b
K ($mg.g^{-1}$)	7.2 ±0.9a	10.5 ±3.2a	6.2 ±1.3a	12.6 ±1.0b
Ca ($mg.g^{-1}$)	8.6 ±1.2a	7.4 ±1.2a	3.9 ±0.9a	3.7 ±1.0a
Mg ($mg.g^{-1}$)	3.1 ±0.4a	4.8 ±0.9b	1.8 ±0.3a	2.7 ±0.6b
Na ($mg.g^{-1}$)	3.6 ±0.6a	7.1 ±1.9b	3.0 ±0.2a	3.8 ±0.3b
Mn ($mg.g^{-1}$)	1.0 ±0.2a	0.8 ±0.2a	71.2 ±7.1a	91.5 ±26.7a
Zn ($mg.kg^{-1}$)	23.2 ±1.8a	24.4 ±11.2a	18.0 ±2.6a	25.8 ±3.8b
Cu ($mg.kg^{-1}$)	6.0 ±1.6a	8.5 ±2.6a	8.5 ±1.3a	4.2 ±0.3b
Fe ($mg.kg^{-1}$)	163.0 ±7.8a	203.0 ±22.0b	257.8 ±42.0a	178.8 ±6.0b
Al ($mg.kg^{-1}$)	171.4 ±13.4a	127.4 ±23.1b	168.6 ±30.9a	119.5 ±10.3b
N:P	7.9:1 ±1.5a	11.1:1 ±2.1b	23.7:1 ±5.0a	8.4:1 ±3.4b

Values in the same row for the respective species followed by the same letter are not significantly different (p>0.05, Scheffe's multiple range test). Variations shown are ±SD around the mean (n=5).

Figure 3.17a and b: Growing tips of *E. grandis* saplings from (a) control and (b) experimental groups at approximately 5.5 months after planting.

3.1.6. Root spatial distribution

Representations of root intersects at three horizontal planes above, below and midway through the sludge core for *E. grandis* and *A.mearnsii* are shown in Figs. 3.18 and 3.19, respectively. Only medium and coarse root intersections were enumerated at each level but fine root mass was extensive just beneath the surface where it formed dense matting in both species. Root mats consisting mostly of fine roots but also medium roots were also observed against the walls of all columns and, notably, fine

roots mats were observed on the immediate periphery of the sludge core (Fig. 3.20). Of the three factors evaluated by the general linear model i.e. treatment, depth, and intersection point (inner or outer ring), treatment and intersection point were significant in both species ($p<0.05$) but the number of root intersections was not significantly affected by depth. While depth was not a significant factor in the analysis for the medium and coarse roots measured here, observations suggest that fine roots decreased with depth (Fig. 3.21). That intersection point was a significant factor in the analysis for both species is evident from data presented in Tables 3.8 and 3.9. In this respect more root intersects occurred in the outer ring irrespective of treatment or species. The trend of more root intersections in the outer ring in treatment and control groups means that this lateral growth habit was normal and not the result of roots avoiding the sludge core in the experimental groups. It should be noted that a few roots of both species penetrated the sludge core centrally which suggests that sludge could support root growth (Fig. 3.22). To examine this further, the proportions of roots intersecting the inner ring at 500 mm were compared with that of the control groups. The percentage of roots intersects occurring in the sludge core or inner ring at 500 mm in *E. grandis* was 25.5 and 16.3 % in experimental and control groups, respectively ($p=0.503$). In *A. mearnsii* 31.3 % of all root intersects at 500 mm occurred in the sludge core compared with only 7.9 % in the inner ring in control saplings and this difference was marginally non-significant at the $p=0.1$ level ($p=0.103$). Therefore, in contrast with expectations, these data suggest that the sludge core itself was able to support root growth and, furthermore, may have been preferential for root growth.

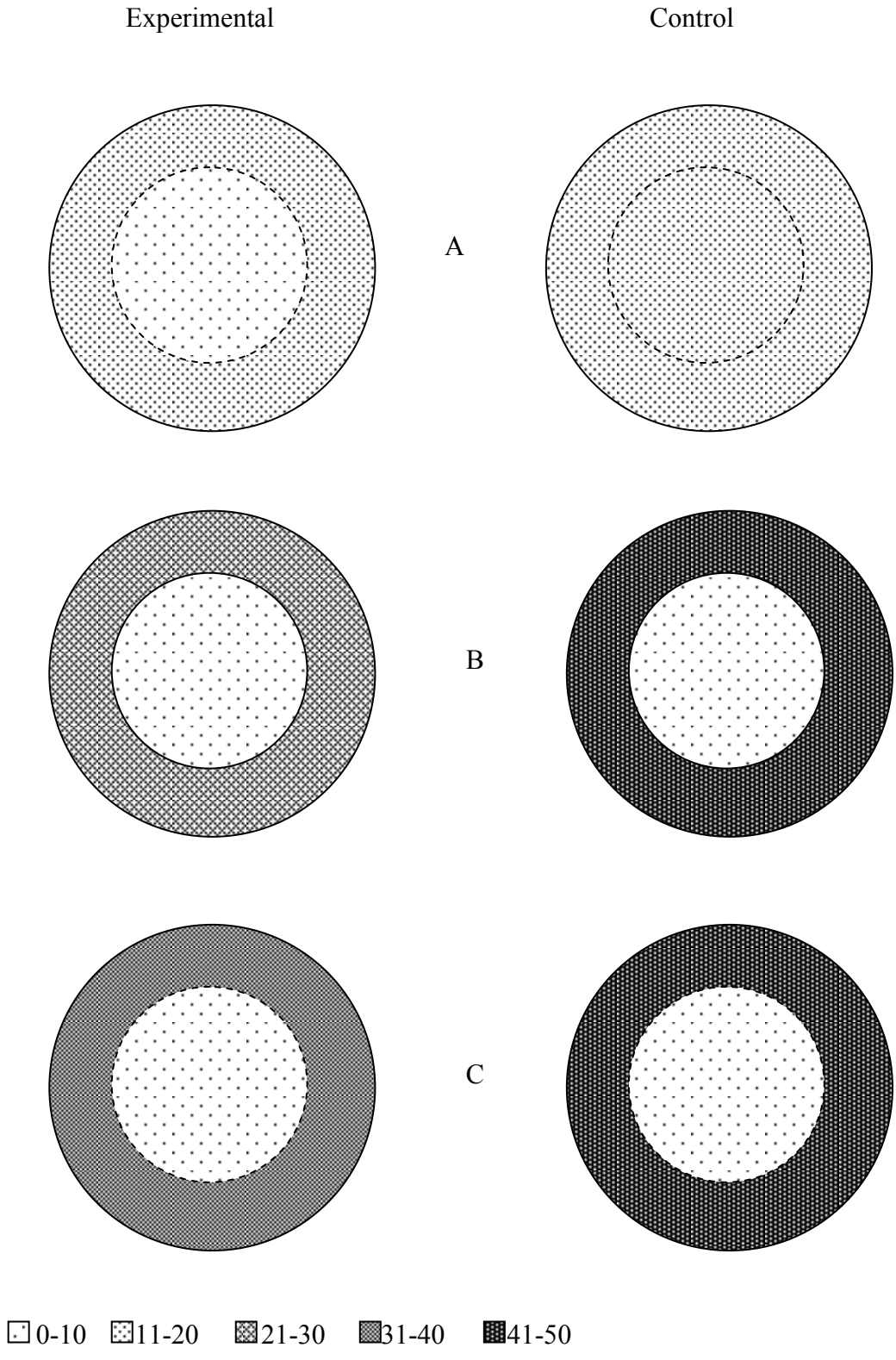

Figure 3.18, A-C: Representations of root intersections shown from vertical at approximately (A) 125 mm, (B) 500 mm and (C) 625 mm below the surface for control and experimental saplings of *E. grandis*. The inner rings correspond to sludge placement and outer rings represent river sand, to scale. Figures (A) and (C) show root intersection approximately 125 mm above and below the sludge core, respectively, and figure B shows intersection midway through the sludge core (n=4).

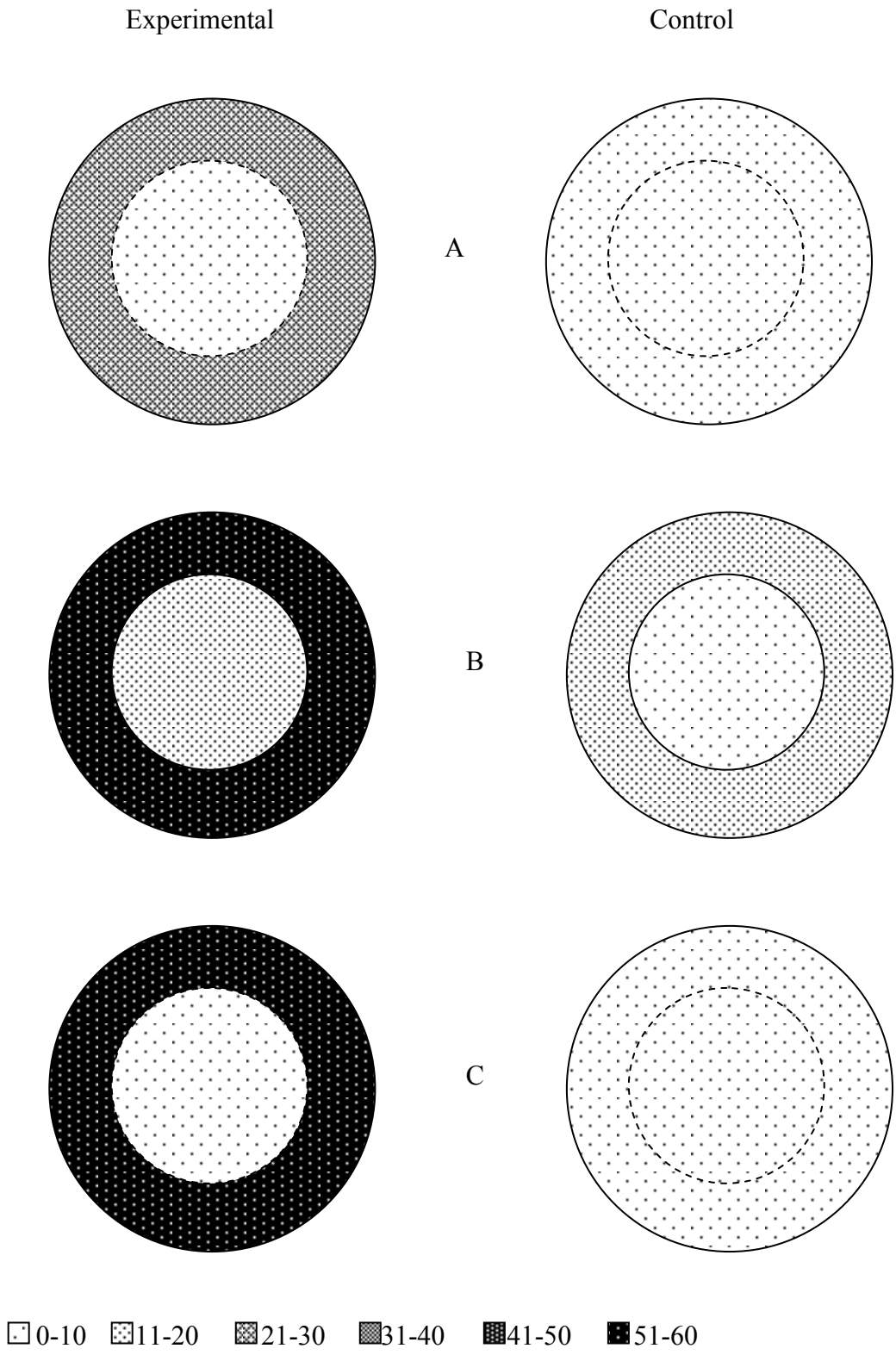

Figure 3.19, A-C: Representations of root intersections shown from vertical at approximately (A) 125 mm, (B) 500 mm and (C) 625 mm below the surface for control and experimental saplings of *A. mearnsii*. The inner rings correspond to sludge placement and outer rings represent river sand, to scale. Figures (A) and (C) show root intersection approximately 125 mm above and below the sludge core, respectively, and figure B shows intersection midway through the sludge core (n=4).

Table 3.8: Mean root intersections with inner and outer rings for control and experimental groups of *E. grandis*.

	Control		Experimental	
Depth (mm)	Inner ring	Outer ring	Inner ring	Outer ring
125	22.5 ± 4.5	28.8 ± 18.8	6.3 ± 6.5	18.8 ± 13.0
500	7.0 ± 0.8	49.8 ± 33.7	5.5 ± 2.9	22.5 ± 15.2
625	4.5 ± 3.4	42.0 ± 34.2	3.3 ± 3.3	33.0 ± 27.2

Variations shown are ±SD of the mean (*n*=4).

Table 3.9: Mean root intersections with inner and outer rings for control and experimental groups of *A. mearnsii*.

	Control		Experimental	
Depth (mm)	Inner ring	Outer ring	Inner ring	Outer ring
125	7.0 ± 5.0	9.3 ± 3.8	6.0 ± 2.2	24.8 ± 19.7
500	1.25 ± 1.3	18.0 ± 15.4	13.0 ± 3.2	56.3 ± 54.6
625	1.5 ± 1.3	8.5 ± 3.4	9.5 ± 4.0	54.8 ± 37.1

Variations shown are ±SD of the mean (*n*=4).

Figure 3.20: Fine root matting of *E. grandis* on periphery of sludge core.

Figure 3.21: Exposed sand profile of a control column in which *E. grandis* was grown. Markings across the profile indicate where concrete rings were joined.

Figure 3.22: Photographic representation of root distribution of *E. grandis* grown above faecal sludge, shown with sludge core exposed.

3.2. Growth of table vegetables in sand amended with faecal sludge

3.2.1. Growth conditions

Rainfall during the experimental period is shown in Fig. 3.23. A total of 22 mm of rain fell during the nine-week experimental period which was low for the region for that period of the year (late summer to late autumn). Temperature was not recorded but average temperature throughout the experiment was about 23ºC. Daytime temperatures were high and regularly exceeded 27ºC (based on recorded temperatures taken during photosynthetic measurements).

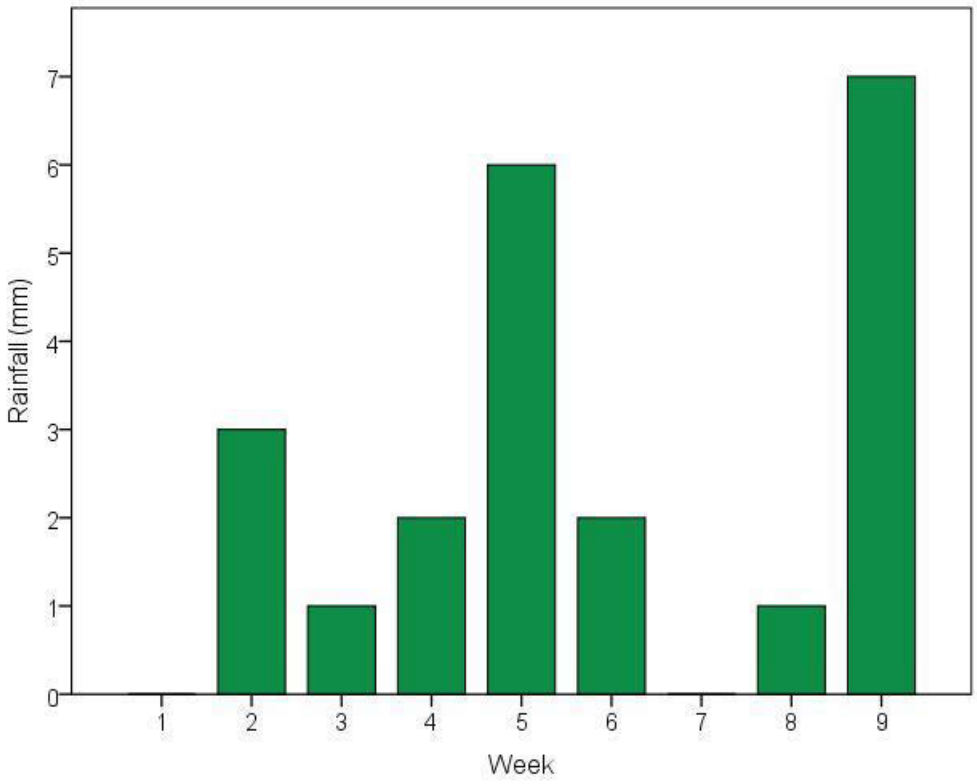

Figure 3.23: Rainfall during the 9 week experimental period.

3.2.2. Physical and chemical properties of sand and faecal sludge

Physical and chemical properties of sand and faecal sludge used in amendment preparations are shown in Table 3.10. Macronutrient concentrations of treatments T-0, T-1, T-2 and T-3 (expressed as the faecal sludge percentage: 0%, 10%, 20% and 30%, respectively; the remaining percentage was sand) at planting were generally quite variable and non-linear with respect to amendment rate (Table 3.11). In particular the relatively high N and K concentrations of T-1 were not in accordance with observed trends for those nutrients with increasing amendment rate and deviated significantly from linearity. The N concentration of T-1, for example, was significantly greater than T-2 (p=0.037). This observation is indicative of the heterogeneous nature of the faecal sludge. However, macronutrient concentrations did tend to show increases with increasing amendment rate, with the 0% treatment exhibiting the lowest macronutrient concentrations with the exception of Ca. Micronutrient concentrations at planting were similarly variable. In this regard, only Zn approximated a linear relationship of increasing concentration with increasing amendment rate. Pair-wise comparisons of amendments at planting and harvest yielded unexpected results, notably those of increases in concentrations of P, Bray P and Ca in treatments T-0 to T-3 at harvest compared with that at planting. However, with the exception of Ca concentration in T-3 (p=0.006) none of these observed differences were of statistical significance with Bonferroni correction applied.

Table 3.10: Physical and chemical properties of sand and faecal sludge

Parameter	Sand	Faecal sludge
Sand (0.05–2.0mm) (%)	95.33 ±0.58	
Silt (0.002mm–0.05) (%)	1.33 ±0.58	
Clay (<0.002mm) (%)	3.33 ±0.58	
N ($mg.kg^{-1}$)	0.045 ±0.01	2.97 ±0.52
Total P ($mg.kg^{-1}$)	2.8 ±0.7	4468.4 ±1018.5
Bray P ($mg.kg^{-1}$)	2.3 ±0.6	3691.8 ±820.6
K ($mg.kg^{-1}$)	2.98 ±0.39	586.16 ±48.74
Ca ($mg.kg^{-1}$)	13.79 ±0.26	266.69 ±45.16
Mg ($mg.kg^{-1}$)	4.59 ±0.56	88.74 ±8.49
Zn ($mg.kg^{-1}$)	0.37 ±0.17	186.16 ±32.16
Cu ($mg.kg^{-1}$)	0.36 ±0.21	9.45 ±4.36
Fe ($mg.kg^{-1}$)	48.69 ±7.95	149.04 ±110.81
Al ($mg.kg^{-1}$)	24.84 ±3.41	12.33 ±7.93
Mn ($mg.kg^{-1}$)	8.42 ±2.28	27.99 ±12.26
Na ($mg.kg^{-1}$)	1.23 ±0.43	550.53 ±28.01

n=3 and n=5 for sand and sludge analyses respectively. Variations shown are ±SD around the mean.

Table 3.11: Nutrient concentrations of river sand amended with sludge at rates of 10% (T-1), 20% (T-2) and 30% (T-3) (v/v) immediately prior to planting of *B. vulgaris*.

Parameter	T-1	T-2	T-3
N ($mg.kg^{-1}$)	0.09 ±0.00a	0.07 ±0.01b	0.08±0.01a
Total P ($mg.kg^{-1}$)	46.40 ±5.72a	39.80 ±8.60b	129.33 ±11.21c
Bray P ($mg.kg^{-1}$)	38.13 ±4.58a	33.03 ±7.40a	106.50 ±9.44b
K ($mg.kg^{-1}$)	22.34 ±2.47a	14.81 ±6.21a	19.93 ±6.75a
Ca ($mg.kg^{-1}$)	0.46 ±0.03a	0.60 ±0.32a	0.42 ±0.18a
Mg ($mg.kg^{-1}$)	64.62 ±2.04a	38.72 ±9.63b	57.28 ±8.48a
Zn ($mg.kg^{-1}$)	4.40 ±0.13a	7.69 ±0.84b	8.70 ±0.46ab
Cu ($mg.kg^{-1}$)	1.20 ±0.07a	0.67 ±0.19b	1.50 ±0.15ac
Fe ($mg.kg^{-1}$)	18.01 ±1.65a	14.13±1.01a	15.02 ±0.85a
Al ($mg.kg^{-1}$)	3.82 ±0.54a	2.92 ±0.49a	6.34 ±1.22a
Mn ($mg.kg^{-1}$)	6.22 ±0.55a	4.61 ±0.622a	8.36 ±0.32a
Na ($mg.kg^{-1}$)	6.37 ±0.43a	3.08 ±0.97a	5.68 ±1.92a

Values in the same row followed by the same letter are not significantly different (p>0.05, Scheffe's multiple range test). Variations shown are ±SD around the mean.

N concentrations of amended sand at the time of harvest where *B. vulgaris* was grown remained more or less similar compared with that at planting for all treatments with the exception of T-0 where N concentration was significantly decreased (Table 3.12; p=0.006). Interestingly, concentrations of P and Bray P in the fertiliser treatment by harvest were comparable to that of T-0 despite daily fertiliser additions and significantly less than that of T-3 (p<0.005). Concentrations of K had decreased considerably by harvest amongst treatments T-0 to T-3 although the observed

difference in only T-1 was significant (p=0.005). Only the treatment receiving fertiliser (T-4) showed a significant increase in K concentration since planting (p=0.008). Comparisons between K concentration of T-4 and other treatments at harvest revealed that with the exception of T-3, all differences were significant (p<0.005). Concentrations of Mg had decreased markedly by harvest amongst treatments T1, T-2 and T-3 (p<0.005) which suggests that uptake of this nutrient was high in these treatments. Interestingly, of the micronutrients measured, concentrations of Fe and Al had increased considerably by harvest compared with concentrations of those nutrients at planting (apart from Al in T-0). The reasons for this are not known although it is likely that sludge heterogeneity accounts for those unexpected results. This assumption is supported by the fact that those nutrients, including Zn, were the only micronutrients to show a consistent trend of increasing concentration with increasing amendment rate at harvest which would otherwise have been expected.

Table 3.12: Nutrient concentrations of river sand amended with sludge at rates of 0% (T-0), 10% (T-1), 20% (T-2) and 30% (T-3) (v/v) at the time of harvest of *B. vulgaris* and that of river sand which received commercial fertilizer for the duration of the experiment (T-4).

Parameter	T-0	T-1	T-2	T-3	T-4
N ($mg.kg^{-1}$)	0.05 ±0.01a	0.05 ±0.01a	0.07 ± 0.01ab	0.07 ±0.02ab	0.05 ±0.01a
Total P ($mg.kg^{-1}$)	6.63 ±0.40a	53.97 ±20.26a	138.97 ±71.30a	373.20 ±82.04b	7.62 ±2.25a
Bray P ($mg.kg^{-1}$)	5.63 ±0.23a	44.47 ±16.57a	113.97 ±58.57a	303.47 ±67.02b	6.40 ±1.9a
K ($mg.kg^{-1}$)	1.41 ±0.40a	1.50 ±0.13a	1.98 ±0.82a	5.23 ±2.13b	9.25 ±1.39b
Ca ($mg.kg^{-1}$)	15.03 ±1.45a	23.57 ±4.65ab	32.70 ±6.54b	38.36 ±5.34b	35.58 ±6.7b
Mg ($mg.kg^{-1}$)	4.44 ±0.06a	5.99 ±0.99a	9.38 ±3.83b	18.92 ±3.78b	6.18 ± 0.77a
Zn ($mg.kg^{-1}$)	0.24 ±0.03a	1.62 ±0.69ab	3.81 ±1.91b	9.45±0.46c	0.31 ±0.03ab
Cu ($mg.kg^{-1}$)	0.75 ±0.38a	0.62 ±0.18a	1.14 ±0.39a	1.28 ±0.21a	0.46 ±0.06a
Fe ($mg.kg^{-1}$)	32.23 ±4.83a	70.57 ±23.93	109.96 ±42.30	222.87 ±31.59	53.52 ±8.78
Al ($mg.kg^{-1}$)	12.10 ±1.31a	22.01 ±6.40a	32.41 ±9.83ab	47.90 ±10.62bc	24.06 ±2.99a
Mn ($mg.kg^{-1}$)	3.52 ±1.04a	5.10 ±2.30a	4.12 ±0.70a	8.52 ±2.75a	6.74 ±1.51a
Na ($mg.kg^{-1}$)	1.61 ±0.10a	1.42 ±0.37a	2.65 ±0.29a	1.85 ±1.42a	1.01 ±0.19a

Values in the same row followed by the same letter are not significantly different (p>0.05, Scheffe's multiple range test). Variations shown are ±SD around the mean (n=5).

By week 3 a number of plants of *S. melongena* in T-1 became diseased (suspected to be *Fusarium* spp., visual observations only), and the disease gradually spread to other treatments with the exception of T-3. Infected plants were removed and destroyed to help prevent further disease spread. Plants in T-4 which became infected were replaced with plants of similar height from the guard row which hitherto had been treated in exactly the same manner and no difference between replacement plants and

plants in T-4 could be discerned. Treatments T-0, T-1 and T-2 had less than three replicates at harvest and consequently nutrient concentrations of sludge amended sand and river sand for treatments T-3 and T-4 only are presented in Table 3.13. Interestingly, with the exception of Ca, the macronutrient concentrations of sand in T-4 at harvest were considerably less than that in T-3 ($p<0.005$). Concentrations of Zn and Cu at harvest were greater in T-3 while the converse was true for Fe and Al in the fertilizer treatment ($p<0.05$). Contrary to expectations concentrations of P, K, Ca, Mg, Cu, Fe and Al showed significant increases ($p<0.05$) at harvest compared to that at mixing. In the case of P and Bray P these increases were approximately 3.5 times the concentration of these nutrients at mixing and, correspondingly, 2.5 times for K. This may be the result of mineralisation or partly the result of sludge heterogeneity as already suspected for increases in nutrients by harvest where *B. vulgaris* had been grown. The addition of fertilizer had seemingly little effect on overall fertility of the river sand and only Ca showed a statistically significant increase ($p=0.022$) while concentrations of other measured nutrients remained more or less similar. This suggests that leaching of nutrients occurred in T-4 which is consistent with observations that the sand drained considerably well.

Table 3.13: Nutrient concentrations of river sand amended with sludge at a rate of 30% (v/v) immediately prior to planting, and at the time of harvest, of *S. melongena* and that of river sand which received commercial fertilizer for the duration of the experiment (T-4).

	T-3		
Parameter	At planting	At harvest	T-4
N ($mg.kg^{-1}$)	0.08 ±0.01a	0.087 ±0.01b	0.05 ±0.01a
Total P ($mg.kg^{-1}$)	129.33 ± 11.22a	474.13 ±75.27b	6.64 ±2.61a
Bray P ($mg.kg^{-1}$)	106.50 ±9.44a	387.63 ±61.12b	5.47 ±2.14a
K ($mg.kg^{-1}$)	19.93 ±6.75a	47.41 ±4.27b	5.42 ±3.84a
Ca ($mg.kg^{-1}$)	0.42 ±0.18a	1.34 ±0.17b	18.53 ±0.99a
Mg ($mg.kg^{-1}$)	57.28 ±8.48a	233.57 ±20.55b	8.49 ±2.76a
Zn ($mg.kg^{-1}$)	8.70 ±0.46a	7.49 ±0.74a	0.44 ±0.07b
Cu ($mg.kg^{-1}$)	1.50 ±0.15a	12.41 ±1.14b	0.40 ±0.08a
Fe ($mg.kg^{-1}$)	15.02 ±0.85a	36.62 ±1.71b	50.97 ±14.47b
Al ($mg.kg^{-1}$)	6.34 ±1.22a	5.46 ±3.07a	28.23 ±7.65a
Mn ($mg.kg^{-1}$)	8.36 ±0.32a	20.05 ±3.12b	9.28 ±0.50a
Na ($mg.kg^{-1}$)	5.68 ±1.92a	26.39 ±37.74a	3.91 ±2.23a

Values in the same row followed by the same letter are not significantly different ($p>0.05$) with the exception of the T-4 column where letters denote differences with T-3 at harvest. Variations shown are ±SD around the mean ($n=5$).

3.2.3. Plant growth measurements

3.2.3.1. Plant height

Shoot length of *B. vulgaris* increased weekly in all treatments throughout the experimental period with the exception of treatments T-0 and T-1 (Fig. 3.24). As early as week 2 a significant difference was observed between T-3 and T-0 (p=0.022). By the fourth week shoot length of all treatments was statistically different from T-0 with the exception of T-1 (p=0.60) and shoot length in T4 was statistically different from all treatments with the exception of plants receiving the highest of application of faecal sludge in T-3. The general decrease in shoot length in T-1 from week 4 onwards was the result of older leaves senescing leaving only smaller leaves which did not reach similar lengths probably due to an increasingly severe nutrient deficit. Shoot length in T-1 was statistically different from T-0 (p=0.03) at week 7, and from week 7 onwards the order of shoot length had established as T-0<T-1=T-2=T-3<T-4. However, at harvest the difference in shoot length between T-3 and treatments T-1 and T-4 was significant at the p=0.1 level of significance. Shoot length of T-1 and T-2 were more or less similar throughout the experimental period and increases in shoot length in those treatments had slowed quite considerably only 3 weeks after transplanting. Increases in shoot length of plants in T-3 appeared to have levelled off at week 8 and only plants in T-4 showed continue increases in shoot length until the completion of the experiment. Shoot length at harvest and amendment rate were strongly and positively related (R^2=0.739, p<0.005). This indicates that faecal sludge supported the growth of *B. vulgaris* and this was evident from visible observations (Figs. 3.25a-e). On the other hand, with the possible exception of the 30% amendment the amendment rates used here were not high enough to avoid nutrient limitations to plant growth.

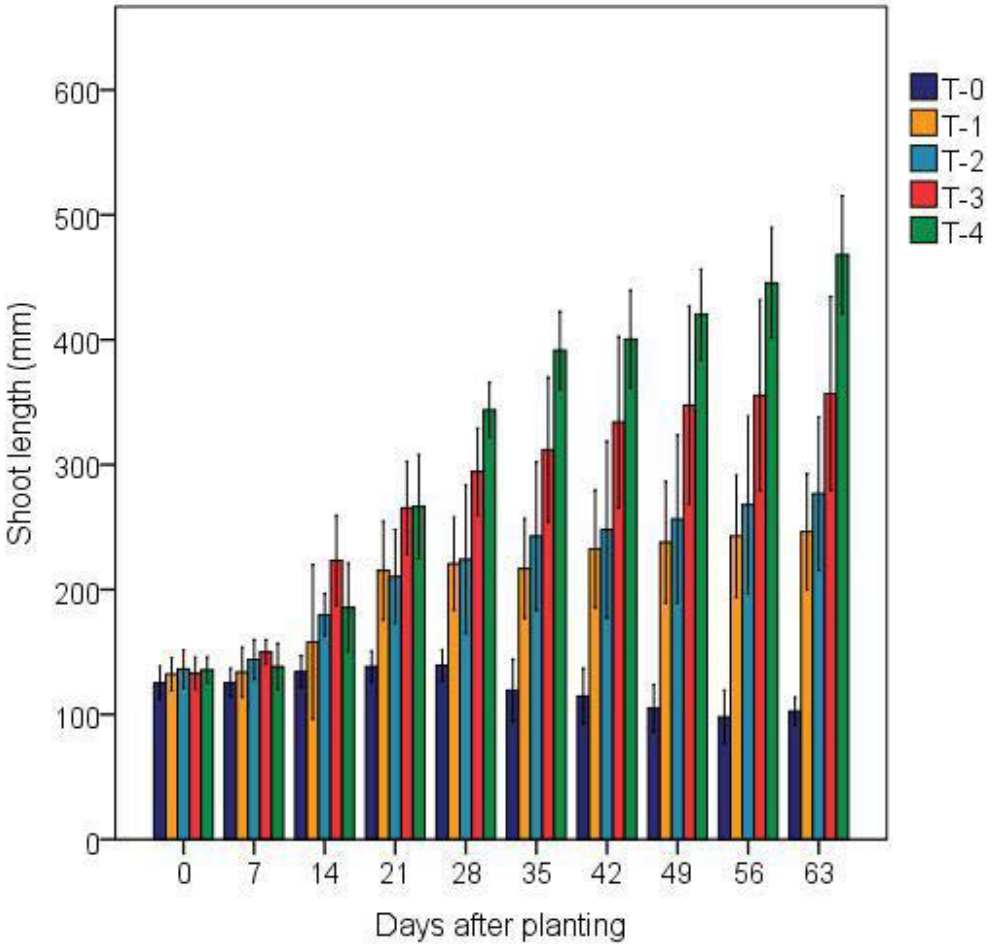

Figure 3.24: Shoot length of *B. vulgaris* measured at weekly intervals for treatments T-0 to T-4.

Figure 3.25a-d: *B. vulgaris* plants representative of mean leaf length for treatments (a) T-0, (b) T-1, (c) T-2, (d) T-3 and (e) T-4 at harvest.

Plant height in *S. melongena* increased weekly in all treatments for the duration of the experiment (Fig. 3.26). Where the number of replicates in a treatment became fewer than n=3 due to disease (as described earlier) the treatment was excluded from the experiment. Plants were harvested at 8 weeks after transplanting which was one week earlier than planned to avoid the possibility of further disease spread. From week 7 onwards only T-3 and T-4 had sufficient replicates (n>3). Consequently, comparisons between treatments T-0, T-1 and T-2 at harvest were not possible, however as early as week 2 plant height in T-3 was statistically greater than T-0 (p=0.022) and by week 3

plant height in T-1, T-3 and T-4 were statistically different to T-0 and T-3 was significantly greater than T-2 (p=0.008). Plant height in T-3 and T-4 closely approximated one another throughout the experimental period and at harvest mean plant height was 438mm and 465mm, respectively (p=0.399). This indicates that, at least in terms of plant height, sand amended with 30% faecal sludge was comparable to that of the synthetic fertilizer application. Photographic representations of *S. melongena* at harvest are shown in Fig. 3.27a,b.

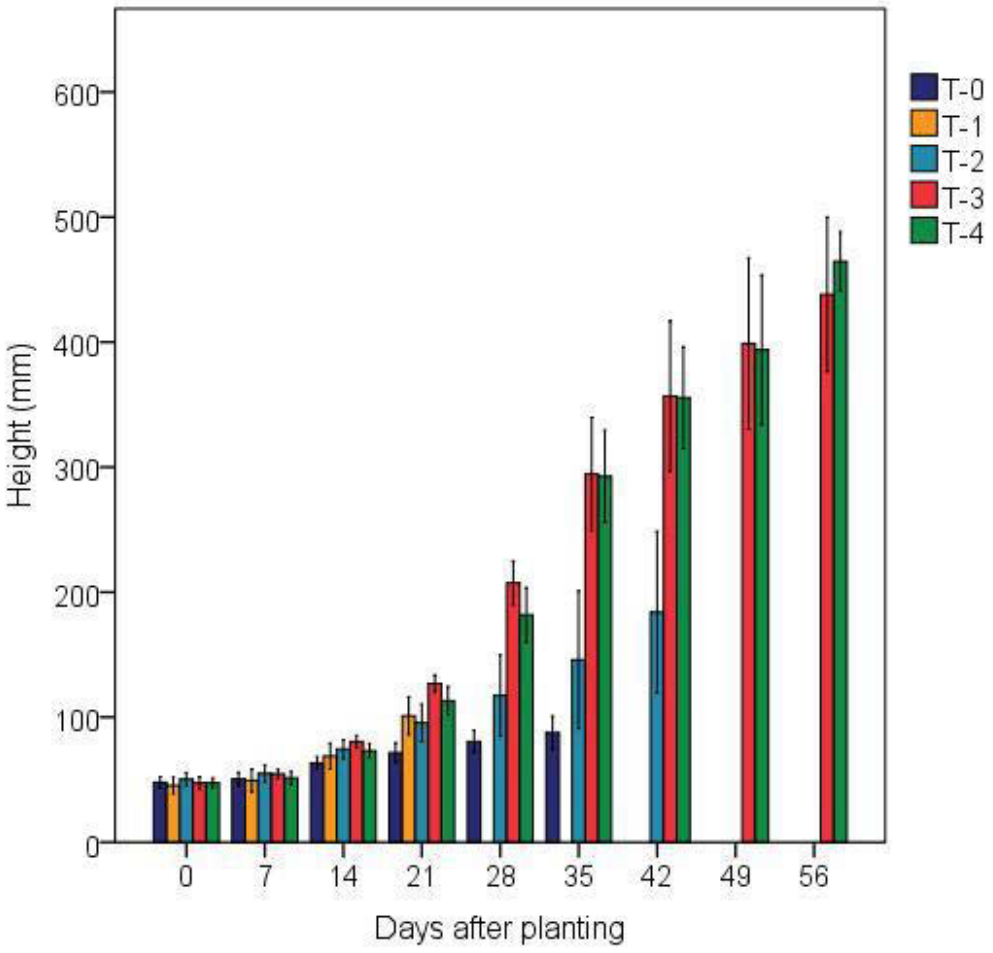

Figure 3.26: Mean height of *S. melongena* measured at weekly intervals for treatments T-0 to T-4. Missing bars are due to fewer than 3 replicates caused by disease.

Figure 3.27a and b: *S. melongena* in (a) T-3 and (b) T-4 at harvest.

3.2.3.2. Stem diameter

As expected, stem diameter showed similar trends to that observed for plant height (Fig. 3.28). Already by week 2 a number of statistical differences in stem diameter were observed amongst treatments. In this respect plants in T-3 had a greater stem diameter than all other treatments and T-4 had a greater stem diameter than T-0. At 3 weeks after transplanting stem diameter in T-0 was statistically less than all other treatments as a result of nutrient stress but the difference between T-3 and T-4 was no longer significant (p=0.19). At harvest there was no statistical difference in stem diameter between T-3 and T-4 (p=0.247). Although comparisons between plants at lower amendment rates was not possible, it can be inferred from both measured plant dimensions (height and stem diameter) when all treatments were still present that overall growth across different amendment rates is more or less consistent with that observed for *B. vulgaris*.

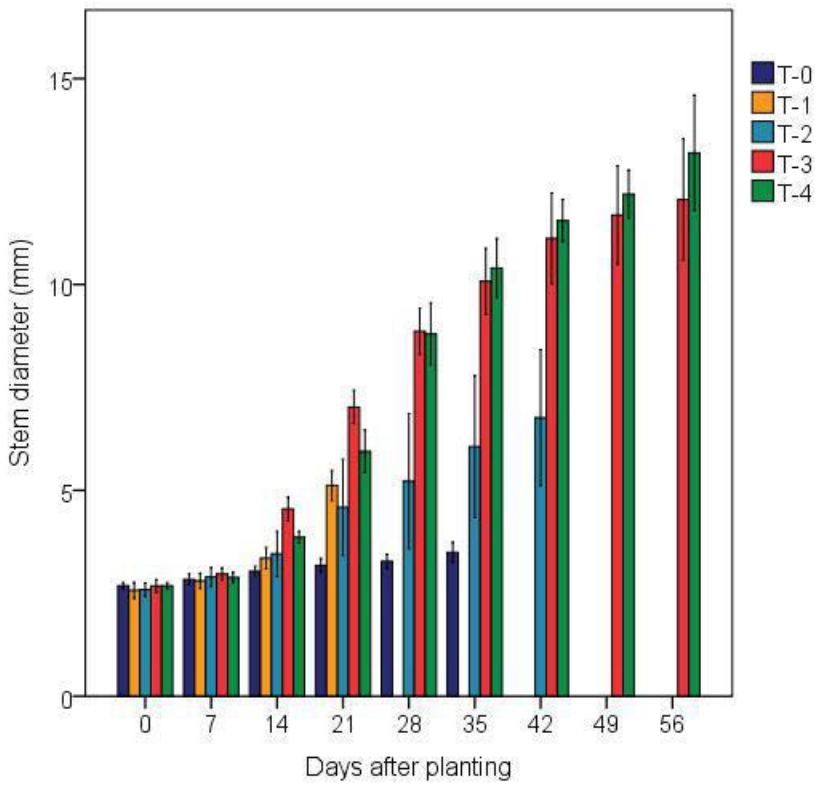

Figure 3.28: Stem diameter of *S. melongena* measured at weekly intervals for treatments T-0 to T-4. Missing bars are due to fewer than 3 replicates caused by disease. Error bars shown are ±SD.

3.2.3.3. Leaf count

Leaf counts (leaves.plant^{-1}) for *B. vulgaris* are shown in Fig. 3.29. Leaf counts decreased in *B. vulgaris* in a number of treatments during the first week after transplanting due in part to mild transplant shock which was exacerbated by several warm days after planting. At week 2 the mean number of leaves in T-3 was significantly greater than that of T-1 (p=0.40) and T-0 (p=0.001) which is likely the result of nutrient stress in those treatments. From week 2 onwards the mean number of leaves increased in all treatments with the exception of T-0 which began to show a small decline in the mean number of leaves per plant from week 6 onwards. The decrease in number of leaves in T-0 was due to the slow growth and limited production of new leaves which was compounded by the premature senescence of leaves. Premature senescence of leaves was particularly marked throughout the growing period in some plants of all treatments with the exception of T-4 and this may have been due to toxicity from phytotoxic compounds in the faecal sludge or as a

result of nutrient stress (or a combination of both). The mean number of leaves of T-3 and T-4 were similar throughout the experiment as was the case with T-2 and T-1, although to a slightly lesser extent. At harvest all treatments with the exception of T-1 had statistically more leaves than T-0 although the difference between T-0 and T-1 in this regard was marginally non-significant (p=0.072). No statistical differences were observed amongst other treatments at harvest, particularly since variability was notably high. Overall, however, the number of leaves at harvest for *B. vulgaris* was positively related to amendment rate (R^2=0.684, p<0.005).

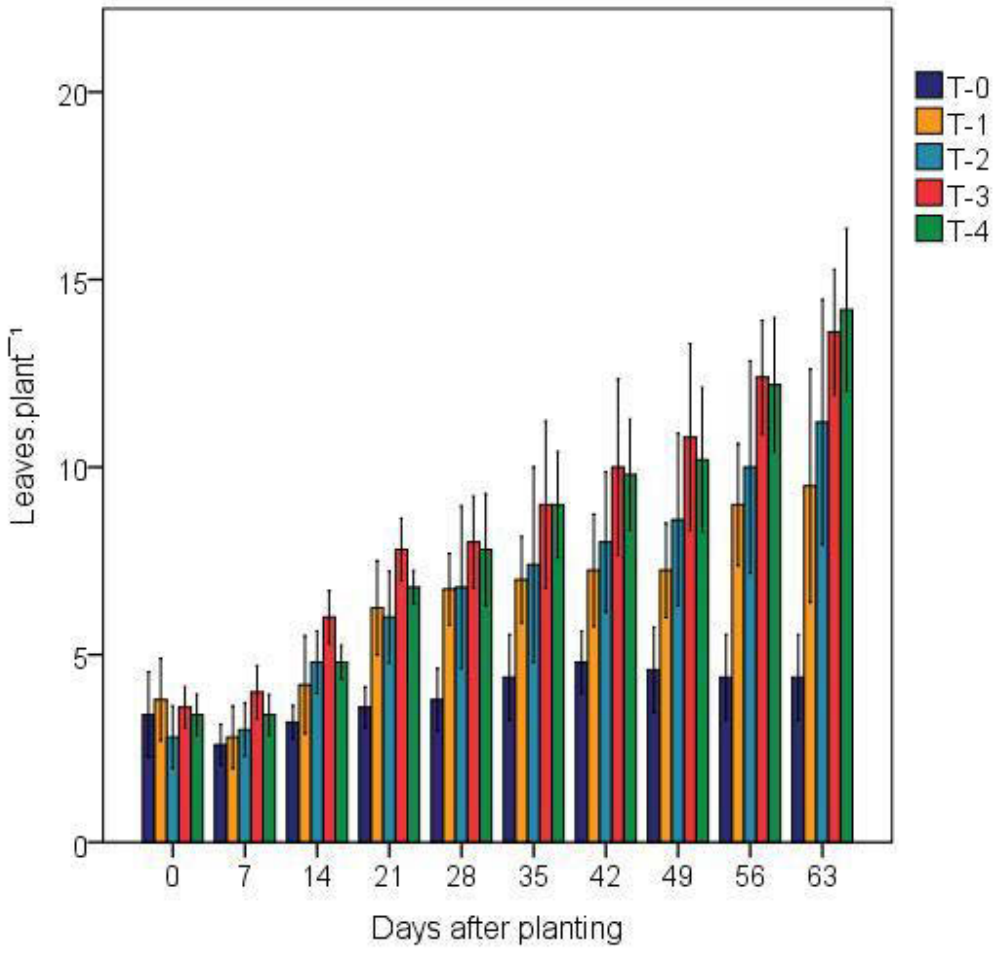

Figure 3.29: Number of leaves of *B. vulgaris* measured at weekly intervals for treatments T-0 to T-4 (n=5, except for T-1 where n=4).

Leaf counts for *S. melongena* are shown in Fig. 3.30. At two weeks after planting the number of leaves in T-3 and T-4 were already significantly greater than T-0 (p=0.001 and 0.015, respectively). The increase in foliar production in T-3 and T-4 relative to T-0 so early in the growth period indicates the immediate positive effect of the sand

amended with 30% faecal sludge and added fertilizer on foliar production in those treatments and, furthermore, the nutrient-stressed status of plants grown in river sand alone without fertilizer additions. By week 3, T-3 and T-4 had statistically greater numbers of leaves compared to all other treatments but no statistical differences were established amongst T-0, T-1 and T-2. At harvest T-4 had the highest number of leaves (105.8) compared with T-3 (82.0) but this difference was not statistically significant (p=0.203).

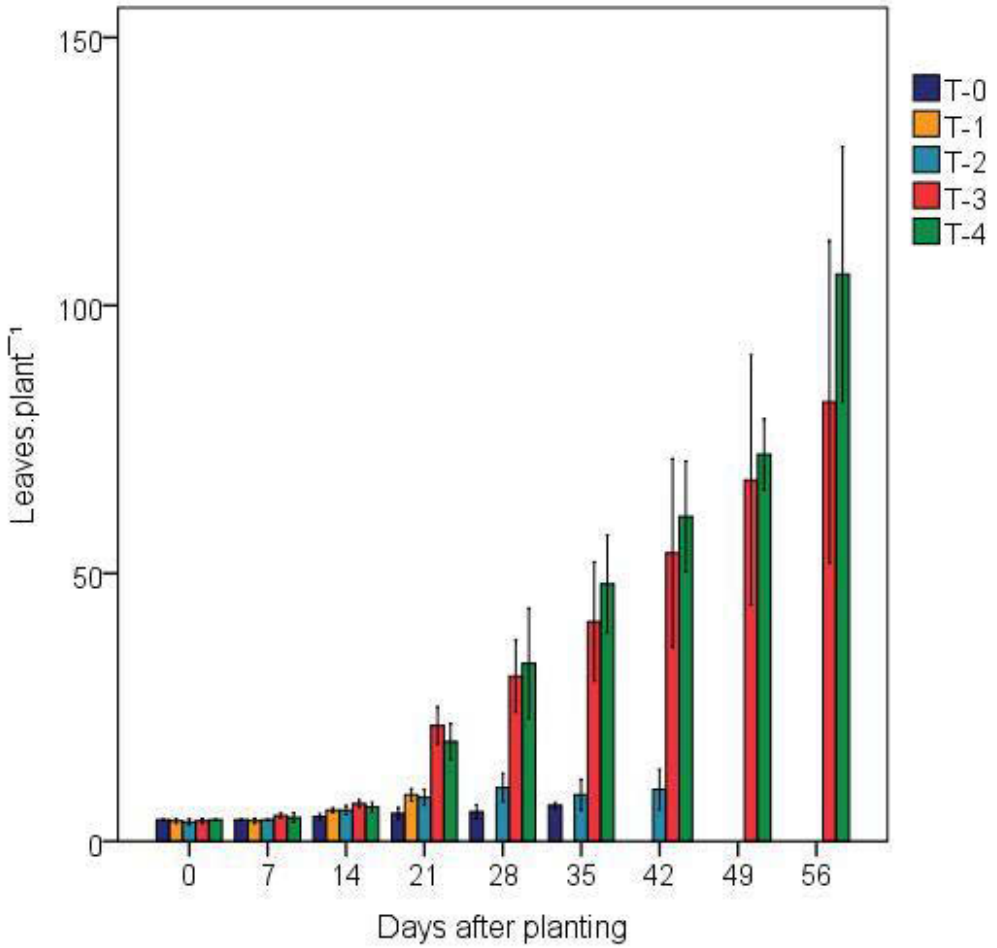

Figure 3.30: Number of leaves of *S. melongena* measured at weekly intervals for treatments T-0 to T-4. Missing bars are due to fewer than 3 replicates caused by disease.

3.2.3.4. Leaf area

Mean post-harvest total leaf area and specific leaf area of *B. vulgaris* was considerably greater in T-4 compared with all other treatments (Table 3.14). For

treatments T-0, T-1, T-2 and T-3 mean leaf area reduced with decreasing amendment rates. Mean leaf area for T-4 was 44.65 dm² which was more than double that of T-3 (20.26 dm²) and this difference was statistically significant (p<0.005), as were the differences between T-4 and all other treatments. Leaf area in T-3 was statistically greater than T-0 (p<0.005), T-1 (p=0.009) and T-2 (p=0.16). Leaf area in T-1 and T-2 was 5.24 dm² and 6.99 dm^2, respectively, which was considerably less than that of T-3. Leaf area in T-0 was very low (0.39 dm²) as a result of severely hindered growth in this group. However, differences amongst T-0, T-1 and T-2 were not significant. The greater leaf area in all treatments relative to T-0 is partly a result of an increased number of leaves per plant but also due to the production of larger leaves in these treatments.

Specific leaf area (SLA) varied quite considerably amongst treatments, increasing within the range of amendment rates of 10% to 30% but was greatest in T-0. Mean SLA of T-0 was 83.66 $cm^2.g^{-1}$ although this was associated with high variability about the mean. T-1 had the lowest SLA (46.35 $cm^2.g^{-1}$) of all treatments. None of the observed differences amongst the treatments was significant although the difference between T-0 and T-1 was significant at the p=0.1 level of significance (p=0.069).

Table 3.14: Leaf area (LA) and specific leaf area (SLA) of *B. vulgaris* for treatments T-0 to T-4.

Parameter	T-0	T-1	T-2	T-3	T-4
LA (dm^2)	0.39 ±0.16a	5.24 ±2.05a	6.99 ±3.15a	20.26 ±9.19b	44.65 ±5.66c
SLA ($cm^2.g^{-1}$)	83.66 ±31.32a	46.35 ±8.14a	60.54 ±11.83a	66.85 ±18.88a	50.97 ±10.09a

Values in the same row for the respective species followed by the same letter are not significantly different (p>0.05, Scheffe's multiple range test). Variations shown are ±SD around the mean. n=5 except for T-1 where n=4.

Post-harvest leaf area and specific leaf area of *S. melongena* is shown in Table 3.15. Mean leaf area was 31.92 and 40.31 dm^2 for T-3 and T-4, respectively, but this difference was not significant (p=0.246). The magnitude of difference observed between these treatments differs from what was observed for *B. vulgaris* for the equivalent treatments where leaf area was found to be considerably different. Leaf area of T-2 is not shown due to insufficient replicates (n=2) but mean leaf area for that

treatment was 5.1 dm^2 and in the only surviving plant in the T-0 treatment leaf area was 1.02 dm^2 which implies that leaf area was affected by amendment rate in much the same way as observed for *B. vulgaris* (i.e. a positive relationship). Specific leaf area was 74.46 and 54.63 $cm^2.g^{-1}$ in T-3 and T-4, respectively, and this difference was significant (p=0.036), indicating that leaves were thicker or denser in T-3.

Table 3.15: Leaf area (LA) and specific leaf area (SLA) of *S. melongena* for treatments T-3 and T-4.

Parameter	T-3	T-4
LA (dm^2)	31.92 ±12.40a	40.31 ±8.39a
SLA ($cm^2.g^{-1}$)	74.46 ±13.81a	54.63 ±10.85b

Values in the same row for the respective species followed by the same letter are not significantly different (p>0.05). Variations shown are ±SD around the mean (n=5).

3.2.3.5. Days to flower

Flower buds were generally visible in plants in T-3 before those in T-4 however the mean days to flower in plants in T-3 (43.8 days) and T-4 (46.4 days) was not statistically different (p=0.190). All plants in the T-3 and T-4 treatments had flowered by 48 days after planting but of the surviving plants in T-0 and T-2 (n=1 and n=2, respectively) no plants had flowered by this time and nor did they flower throughout the experiment.

3.2.3.6. Dry biomass partitioning

Dry biomass partitioning of *B. vulgaris* is shown in Fig. 3.31 and Table 3.16. Fertilized plants had the greatest total dry biomass (89 g) which was approximately 2.5 greater than that of plants in T-3 and 7.8 times greater than that of plants in T-2 and T-1 (p<0.05). The greater biomass in T-4 indicates that plants grew better than those where faecal sludge was applied, and this was evidenced by visibly larger plants in the middle to latter stages of the experiment. In treatments where faecal sludge was applied T-3 had the greatest mean total dry biomass (34 g) followed by T-1 and T-2 (12 and 12 g respectively) although the differences amongst these treatments were not significant. Treatment T-0 had the lowest dry biomass (0.46 g) and all treatments had

statistically greater dry matter production than T-0. All treatments had a statistically greater biomass for each of the measured plant parts than T-0. Treatment T-3 produced approximately three times the dry biomass of each measured plant part than T-2 despite a comparatively small difference in amendment rate. Only the dry biomass of petiole was statistically greater in T-4 than T-3. Treatments T-1 and T-2 had an almost identical dry biomass which signifies the non-linearity in the response of biomass to increasing amendment rate.

Dry biomass partitioning (expressed as percentages of total dry biomass for leaf, petiole and root components) in *B. vulgaris* is shown in Fig. 3.32 and Table 3.17. Dry biomass partitioning between leaves, petioles and roots was generally unaffected by treatment despite greatly differing amounts of dry biomass production and nutrient availability. Leaf biomass represented approximately half of total dry biomass irrespective of treatment with the percentage of dry biomass partitioned into leaves ranging from 47% to 56% for T-1 and T-3 respectively but there were no significant differences between any of the treatments in this regard (Fig. 3.32). Dry biomass was generally fairly equally distributed between petioles and roots with the exception of T-1, which partitioned almost twice as much biomass into the roots (35%) compared to the petioles (17%). However, no significant differences were observed between treatments with respect to percentage biomass partitioning into leaves or roots with the exception of the percentage petiole biomass in T-1 and T-4 (p=0.013) which was greater in T-4.

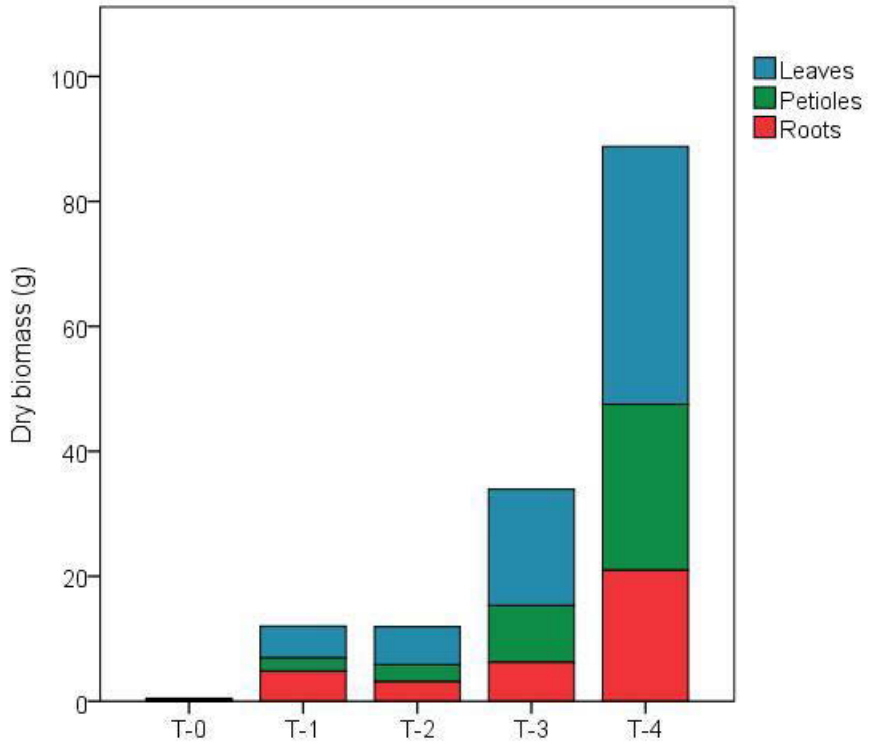

Figure 3.31: Mean dry biomass partitioning into leaves, petioles and roots of *B. vulgaris* for treatments T-0 to T-4 (*n*=5, except for T-1 where *n*=4).

Table 3.16: Mean total dry biomass partitioned into leaves, petioles and roots of *B. vulgaris* for treatments T-0 to T-4.

	Leaves (g)	Petioles (g)	Roots (g)	Total (g)
T-0	0.24 ± 0.05a	0.10 ± 0.00a	0.12 ± 0.07a	0.46 ± 0.11a
T-1	5.08 ± 1.57a	2.12 ± 1.33a	4.82 ± 4.03a	12.02 ± 6.65a
T-2	6.10 ± 2.97a	2.72 ± 1.27a	3.11 ± 1.66a	11.94 ± 5.09a
T-3	18.62 ± 10.46b	9.06 ± 4.75b	6.24 ± 4.81a	33.92 ± 18.26b
T-4	41.24 ± 2.78c	26.56 ± 4.72c	20.98 ± 7.88b	88.78 ± 9.14c

Values in the same column followed by the same letter are not significantly different (p>0.05). Variations shown are ±SD of the mean (*n*=5, except for T-1 where *n*=4).

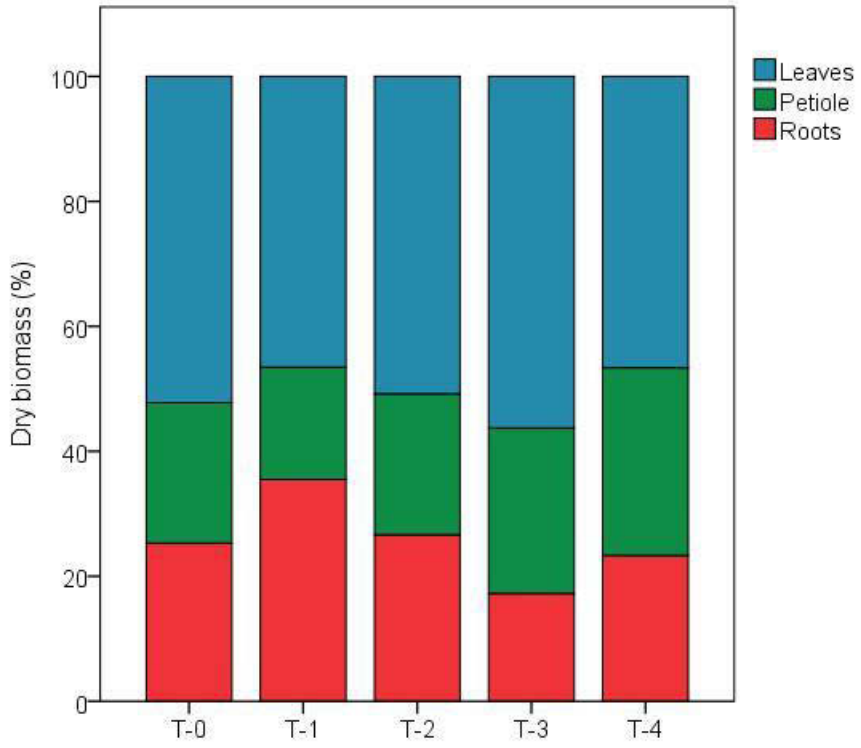

Figure 3.32: Mean percentage of total dry biomass partitioned into leaves, petioles and roots of *B. vulgaris* for treatments T-0 to T-4 (*n*=5, except for T-1 where *n*=4).

Table 3.17: Mean percentage of total dry biomass partitioned into leaves, petioles and roots of *B. vulgaris* for treatments T-0 to T-4.

	% Leaves	% Petioles	% Roots
T-0	52.2 ± 5.9a	22.5 ± 4.4a	25.3 ± 7.6a
T-1	46.6 ± 11.5a	17.9 ± 4.1ab	35.5 ± 12.9a
T-2	50.8 ± 7.1a	22.6 ± 5.4a	26.6 ± 8.3a
T-3	56.3 ± 9.4a	26.5 ± 2.2a	17.2 ± 8.4a
T-4	46.7 ± 3.5a	30.1 ± 5.1ac	23.3 ± 6.9a

Values in the same column followed by the same letter are not significantly different (p>0.05). Variations shown are ±SD of the mean (n=5, except for T-1 where n=4).

Dry biomass of each measured plant part of *S. melongena* was greater in fertilized plants compared to T-3 (Fig. 3.33 and table 3.10). Dry root biomass in T-4 was 30 g which was notably higher than that of T-3 which was 8 g although variation around the mean for T-4 was considerable and this difference was not significant. Nor were the observed differences significant for any of the other measured plant parts (leaves, stem and flowers and fruit). Total (whole plant) dry biomass of plants in T-4 was 79 g which was almost the double of that of T-3 of 42 g and this difference was significant (p=0.049). Dry biomass of flowers and fruit was relatively low for both treatments

since plants had only recently reached maturity and fruits were still small when plants were harvested. Percentage dry biomass partitioning for *S. melongena* is shown in Fig. 3.34 and Table 3.18. Percentage total dry biomass partitioned into leaves was 42% and 33% for T-3 and T-4, respectively, and this difference was significant (p=0.04). The corresponding values for roots were 20% and 33% although this difference was not statistically significant. Percentage biomass partitioned into flowers and fruit and stem were similar for both treatments.

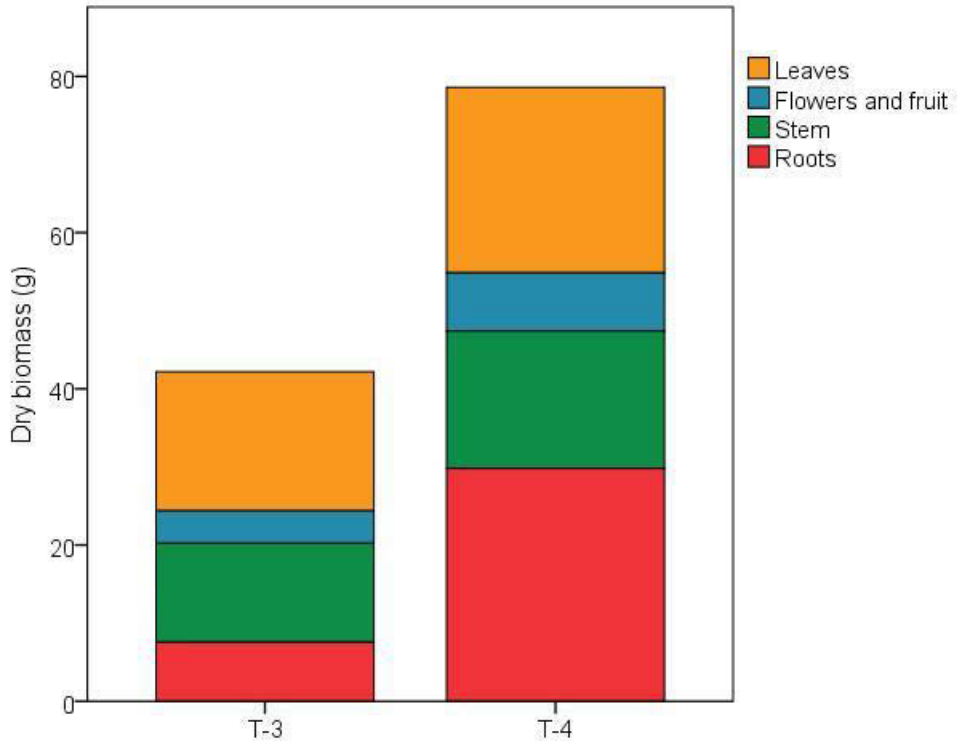

Figure 3.33: Mean dry biomass partitioning into leaves, petioles and roots of *S. melongena* for treatments T-3 and T-4 (n=5).

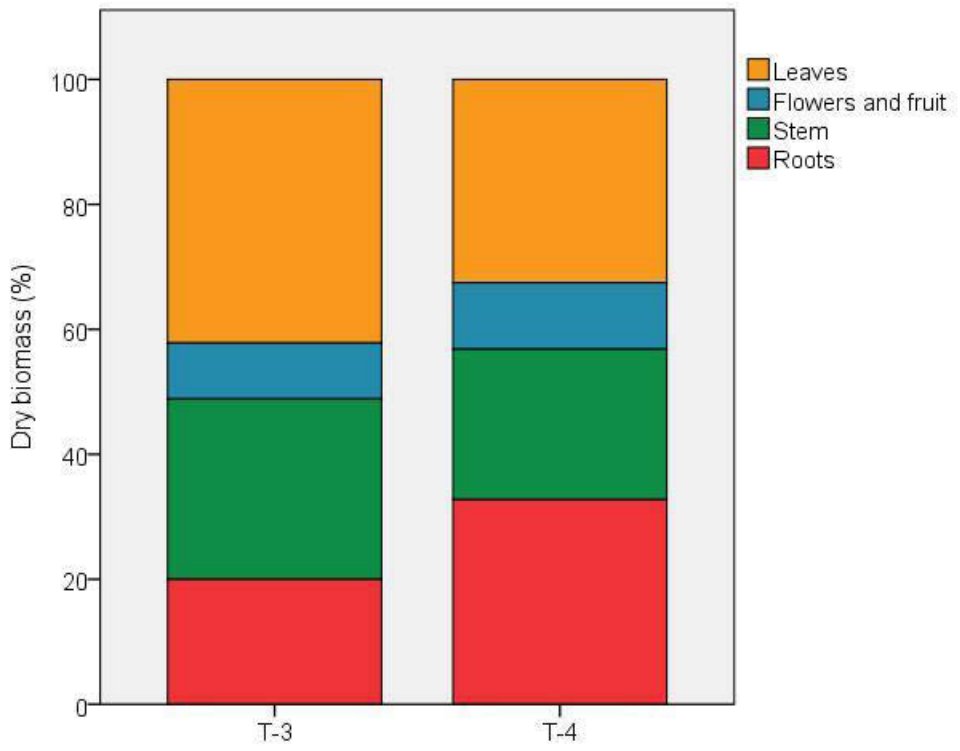

Figure 3.34: Mean percentage of total dry biomass partitioned into leaves, petioles and roots of *S. melongena* for treatments T-3 and T-4.

Table 3.18: Mean total dry biomass partitioned into leaves, petioles and roots of *S. melongena* for treatments T-3 and T-4.

	Flowers + fruit	Leaves	Stem	Roots	Total
T-3 (g)	4.16 ± 2.75a	17.81 ± 5.90a	12.66 ± 5.63a	7.56 ± 1.90a	42.19 ± 13.65a
T-4 (g)	7.48 ± 3.81a	23.75 ± 4.38a	17.56 ± 3.40a	29.82 ± 26.70a	78.60 ± 32.25a
T-3 (%)	9.0 ± 5.4a	42.2 ± 3.5a	28.9 ± 5.9a	20.0 ± 9.3a	-
T-4 (%)	10.6 ± 6.1a	32.6 ± 8.0b	24.1 ± 6.2a	32.7 ± 17.7a	-

Values for the same measurement in the same column followed by the same letter are not significantly different (p>0.05). Variations shown are ±SD of the mean (n=5).

3.2.4. Gas exchange measurements

Representative light response curves for *B. vulgaris* are shown in Fig. 3.35. Light response curves were characterised by a pattern of increasing assimilation at light saturation with increasing amendment rate. At low PAR (<200µmol.m-2.s-1) assimilation was similar across treatments with the exception of T-0 indicating that light limitation had a fairly equal effect on rates of carbon assimilation in those treatments. Representative light response curves for *S. melongena* (Fig. 3.36) indicate that assimilation was greater at light saturation in T-4 compared with T-3.

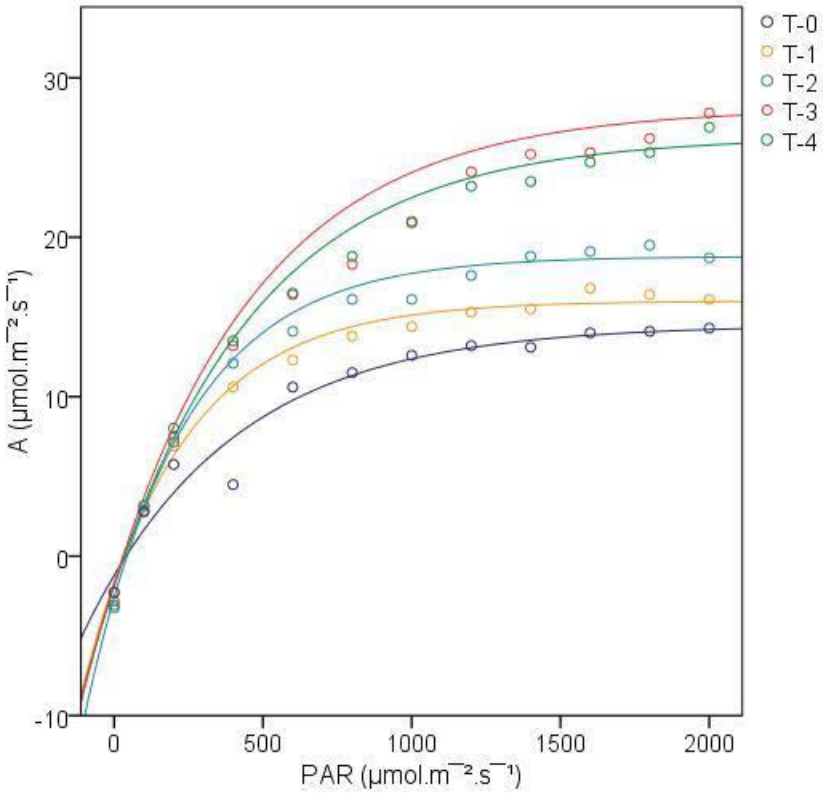

Figure 3.35: Representative light response curves of *B. vulgaris* for treatments T-0 to T-4 (*n*=3).

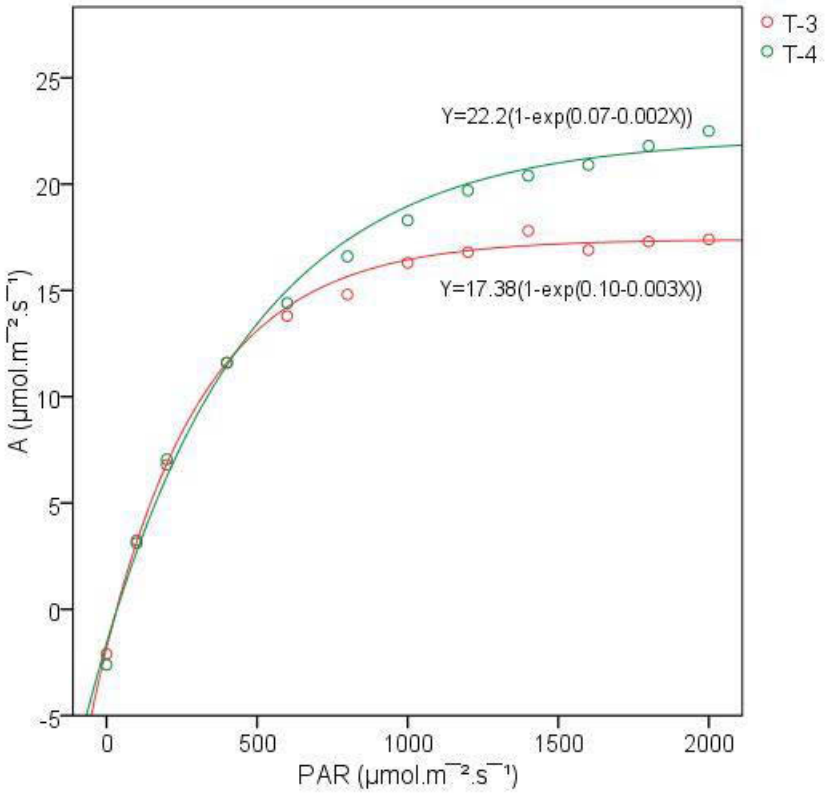

Figure 3.36: Representative light response curves of *S. melongena* for treatments T-3 and T-4 (n=4).

Representative A-C_i curves for *B. vulgaris* and *S. melongena* are shown in Figs. 3.37 and 3.38, respectively. For *B. vulgaris* it is immediately apparent that increasing amendment rates resulted in markedly different relationships between A and C_i. In this regard the gradients of the linear part of the curves (i.e. at low C_i) were progressively greater with increasing amendment rate and the maximum assimilation at high C_i followed the same trend. Based on the model of Farquhar *et al.* (1980), where the activity or amount of Rubisco limits A at low Ci and where electron transport in the regeneration of RuBP limits A at high C_i, both of these factors were enhanced, and positively related to, amendment rate. For both species A responded most positively to increasing C_i in fertilized plants, suggesting that the activity or amount of Rubisco and electron transport were comparatively less limiting at high and low C_i, respectively.

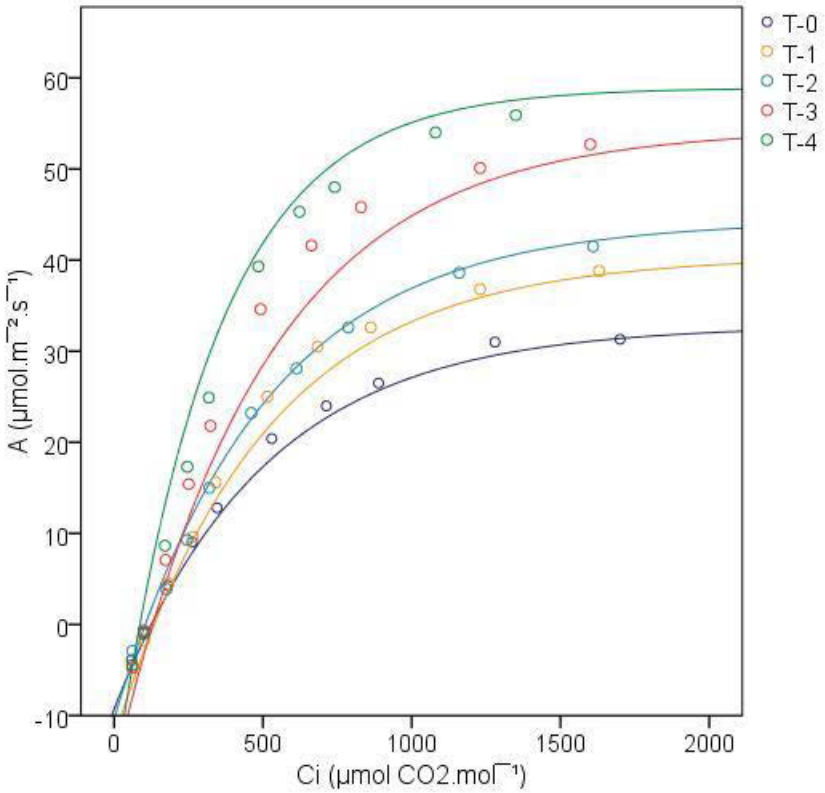

Figure 3.37: Representative CO_2 response curves of *B. vulgaris* for treatments T-0 to T-4 (*n*=3).

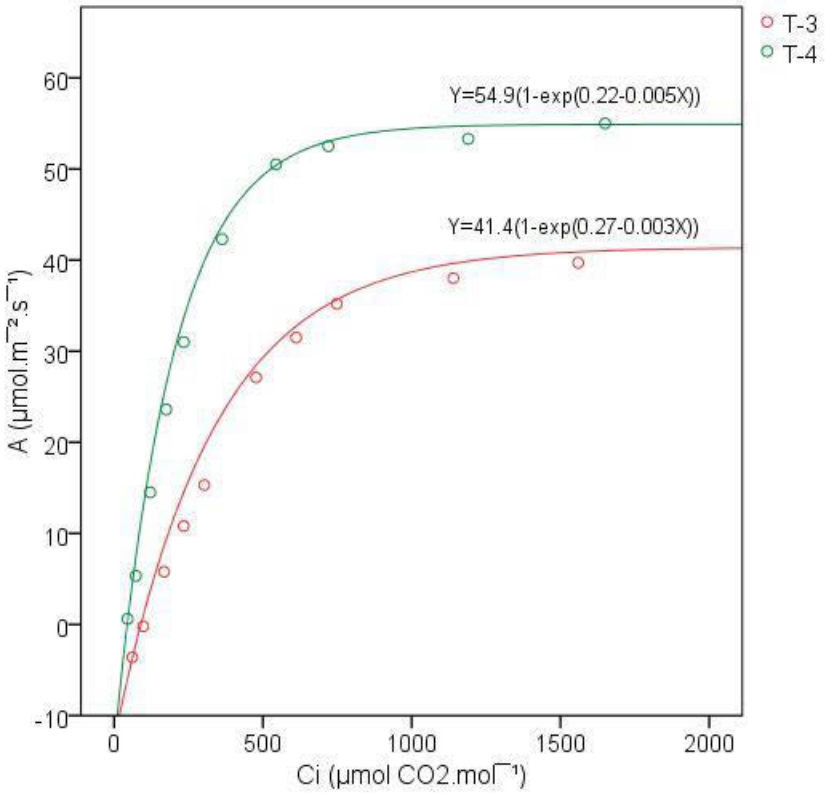

Figure 3.38: Representative CO_2 response curves of *S. melongena* for treatments T-3 and T-4 (*n*=4).

Photosynthetic parameters for *B. vulgaris* and *S. melongena* which were derived from light curves and A-c_i curves are shown in Tables 3.19 and 3.20, respectively. Since only two treatments of *S. melongena* had sufficient replicates for analysis, the relationship between sludge amendment rate and various photosynthetic parameters could be determined only for *B. vulgaris* (Table 3.21). Nonetheless, A_{max}, J_{max} and amendment rate were strongly and positively related which clearly demonstrates that photosynthetic capacity was enhanced by the addition of faecal sludge. This was the result of increasing nutrient availability as amendment rate increased and possibly other factors related to soil properties. Since well-fertilized plants in T-4 had similar A_{max} and J_{max} values to that of T-3, plants in T-3 could not have experienced an acute nutrient shortage unlike plants in the remaining treatments. It is nevertheless surprising that such a high amendment rate was necessary to ensure a comparable photosynthetic capacity to that of well-fertilized plants. R_{day} and R_{dark} were positively but weakly related which was expected since both parameters normally increase with photosynthetic capacity. LUE was very weakly related to amendment rate and differences were negligibly small. A generally positive effect was also observed on V_{cmax} although only fertilized plants had a statistically greater V_{cmax} than other treatments and the results of the linear regression model were not significant.

Photosynthetic parameters were generally similar in *S. melongena* with the exception of A_{max} and J_{max} where significant differences were observed. This differs from that observed in *B. vulgaris* where values for these parameters were comparable and no significant differences could be established. In particular, A_{max} and J_{max} were less in plants in T-3 than fertilized plants which suggests that these plants may have been slightly nutrient limited and this is supported by foliar nutrient concentrations shown on page 117. It is possible that nutrient demand by *S. melongena* was considerably greater than that of *B. vulgaris* leading to these differences even despite the high amendment rate. The only other parameter to show a sizeable, although non-significant, difference was V_{cmax} which was lower in plants in T-3.

Minimum fluorescence (F_o) in *B. vulgaris* decreased with amendment rate and was lowest in the fertilized plants. Surprisingly, however, F_v/F_m in that species was remarkably similar amongst treatments and only very weakly and positively related to amendment rate. In this respect, values ranged from 0.8 to 0.83 which would normally

be indicative of healthy plants and a high photosynthetic capacity. This is however not consistent with photosynthetic and growth data which suggest the contrary. Nonetheless, these data indicate that the functioning of PSII was not seriously compromised despite nutrient stress in some plants although the reasons for this are not immediately apparent. Fluorescence parameters were not statistically different in *S. melongena*.

Table 3.19: Photosynthetic parameters calculated from light curves and A-c_i curves taken on leaves of *B. vulgaris* grown in sand amended with different proportions of sludge or which received fertilizer.

	T-0	T-1	T-2	T-3	T-4
A_{max}	13.4 ± 3.4a	15.5 ± 1.4a	18.1 ± 4.2ac	27.2 ± 2.4b	26.8 ± 2.7bc
LUE	0.04 ± 0.01a	0.05 ± 0.00a	0.06 ± 0.01a	0.06 ± 0.00a	0.06 ± 0.01a
J_{max}	31.6 ± 3.9a	39.7 ± 3.5a	43.1 ± 3.2a	56.9 ± 5.1b	57.8 ± 5.0b
V_{cmax}	0.11 ± 0.02a	0.10 ± 0.01a	0.13 ± 0.04ab	0.20 ± 0.05ab	0.23 ± 0.01b
Ic	36.8 ± 3.3a	35.8 ± 2.5a	39.9 ± 5.2a	40.3 ± 10.6a	38.5 ± 2.6a
R_{dark}	1.5 ± 0.3a	1.8 ± 0.0a	2.2 ± 0.3a	2.3 ± 0.4a	2.1 ± 0.3a
R_{day}	8.6 ± 2.6a	11.7 ± 1.0ac	11.9 ± 2.3ac	17.4 ± 2.4bc	18.2 ± 0.7b
Γ	95.6 ± 38.7a	128.8 ± 7.8a	106.4 ± 14.5a	102.8 ± 21.5a	91.7 ± 10.3a
F_o	61.8 ± 4.3a	60.0 ± 3.6ab	56.5 ± 4.5ab	52.0 ± 3.8b	43.2 ± 2.3c
F_v/F_m	0.81 ± 0.01a	0.81 ± 0.01a	0.81 ± 0.01a	0.82 ± 0.01a	0.83 ± 0.01a

Values in the same row followed by the same letter are not significantly different (p>0.05, Scheffe's multiple range test). Variations shown are ±SD of the mean (n=5, except for T-1 where n=4).

Table 3.20: Photosynthetic parameters calculated from light curves and A-c_i curves taken on leaves of *S. melongena* grown in sand amended with 30% sludge or which received fertilizer.

	T-3	T-4
A_{max}	17.5 ± 2.5a	22.7 ± 2.7b
LUE	0.05 ± 0.01a	0.06 ± 0.01a
J_{max}	41.7 ± 4.1a	55.1 ± 5.5b
V_{cmax}	0.18 ± 0.04a	0.25 ± 0.07a
Ic	37.8 ± 4.4a	42.3 ± 11.0a
R_{dark}	1.9 ± 0.1a	2.2 ± 0.5a
R_{day}	12.4 ± 1.4a	15.4 ± 5.3a
Γ	83.6 ± 23.2a	73.6 ± 27.3a
F_o	61.8 ± 4.3a	60.0 ± 3.6a
F_v/F_m	0.81 ± 0.01a	0.81 ± 0.01a

Values in the same row followed by the same letter are not significantly different (p>0.05). Variations shown are ±SD of the mean (n=4).

Table 3.21: Linear regression models relating photosynthetic parameters of *B. vulgaris* to amendment rate[1,2]

Parameter	Intercept	Slope	R^2	*p*
A_{max}	12.00	0.44	0.71	<0.005
LUE	0.045	0.001	0.44	0.019
J_{max}	30.95	0.79	0.84	<0.005
R_{dark}	1.51	0.03	0.58	<0.005
R_{day}	8.42	0.27	0.67	<0.005
F_o	62.51	-0.33	0.50	0.001
F_v/F_m	0.80	0.0005	0.25	0.024
WUE	1.30	0.023	0.36	0.007

[1]Only significant linear relationships shown.
[2]*n*=5

3.2.4.1. Spot measurements

Spot measurements were taken as a means to provide an accurate measure of photosynthesis *in situ* by using a transparent chamber (i.e. measuring spectrum was the same as for growth) and by containing leaves only briefly in a chamber where environmental parameters were not controlled and similar to ambient. These measurements are shown in Tables 3.22 and 3.23 for *B. vulgaris* and *S. melongena*, respectively. It is particularly surprising that A in *B. vulgaris* was not significantly different amongst all treatments which was unexpected given the differences in photosynthetic capacity as determined from light curves. Spot measurements of A were slightly lower in all treatments than corresponding values of A_{max} which could be explained by the measuring light intensity (mean 1481.1 μmol.mol^{-1} with little variance as skies were clear) which was not completely saturating for all treatments. Nonetheless, A was considerably less than expected in treatments T-2, T-3 and T-4 considering data of A for these treatments taken at comparable light intensities during the light curves. E and g_s were similar amongst treatments and no significant differences were observed. WUE was weakly and positively related to amendment rate although only plants in T-4 had a significantly different WUE than other treatments.

A was greater than A_{max} in both treatments of *S. melongena* and considerably so in T-4 even though light intensity (mean 1506.9 μmol.mol^{-1}) was not saturating based on data obtained from the light curves. Other parameters were comparable between treatments apart from WUE which was greater in T-4 but not significantly different from T-3.

Table 3.22: Spot measurements taken on leaves of *B. vulgaris* grown in sand amended with different proportions of sludge or which received fertilizer.

	T-0	T-1	T-2	T-3	T-4
A	10.8 ± 3.0a	12.6 ± 1.8a	12.3 ± 1.6a	14.7 ± 4.7a	17.1 ± 2.9a
g_s	0.7 ± 0.2a	0.8 ± 0.3a	0.5 ± 0.1a	0.7 ± 0.6a	0.6 ± 0.3a
E	8.4 ± 1.2a	8.0 ± 2.0a	7.3 ± 1.3a	7.4 ± 1.6a	8.1 ± 1.3a
WUE	1.3 ± 0.2a	1.6 ± 0.5ab	1.7 ± 0.2ab	2.0 ± 0.6ab	2.1 ± 0.1b

Values in the same row followed by the same letter are not significantly different (p>0.05, Scheffe's multiple range test). Variations shown are ±SD of the mean (n=5, except for T-1 where n=4).

Table 3.23: Spot measurements taken on leaves of *S. melongena* grown in sand amended with different proportions of sludge or which received fertilizer.

	T-3	T-4
A	19.7 ± 3.3a	27.3 ± 1.8b
g_s	1.3 ± 0.6a	1.5 ± 0.6a
E	8.2 ± 1.0a	8.9 ± 1.5a
WUE	2.4 ± 0.4a	3.2 ± 0.8a

Values in the same row followed by the same letter are not significantly different (p>0.05). Variations shown are ±SD of the mean (n=5, except for T-1 where n=4).

3.2.5. Foliar nutrient concentrations

Foliar nutrient concentrations of *B. vulgaris* are shown in Table 3.24. Replicates of T-0 were combined for all nutrient analyses due to small sample sizes. Data for T-0 was therefore excluded from statistical analyses since sample variability could not be established. Foliar N ranged from 22.6 to 41.6 $mg.g^{-1}$ for treatments T-1 and T-4 respectively and was positively related to amendment rate where sludge was applied i.e. excluding T-0 which had a greater foliar N than T-1 (Table 3.25; R^2=0.59, p=0.001). Foliar N in T-4 was significantly greater than T-1 and T-2 (p<0.005) and marginally non-significant from T-3 at the p=0.05 level. In treatments where sludge was applied, only plants in T-3 had a statistically higher foliar N than T-1. Foliar P was similar between treatments T-1, T-2 and T-3 (32.7-35.8 $mg.g^{-1}$) which was about three times greater than T-0 and, interestingly, T-4 (10.0 and 11.0 $mg.g^{-1}$ respectively). In this respect, T-1, T-2 and T-3 all had statistically greater foliar P than T-4 (Mann-Whitney U with Bonferroni correction applied, p<0.017). Foliar K was highly variable amongst treatments, ranging from 32.0 to 78.4 $mg.g^{-1}$ for treatments T-3 and T-4 respectively. Foliar K in T-4 was statistically greater than all other treatments (p<0.05) and foliar K was statistically lower in T-3 than in T-2 (p=0.011). The greatest N:P ratio of 3.9:1 was observed in the T-4 treatment. By comparison, the

N:P ratio for plants in treatments T-1, T-2 and T-3 were significantly depressed, occurring in a similar range of 1:0.7-0.9, and these ratios were significantly different from that of T-4 (p<0.005). Plants in the T-0 treatment exhibited an intermediate N:P ratio of 2.7:1. Treatments T-0, T-1, T-2 and T-4 had identical N:K ratios of ~1:0.5 indicating that uptake of N and K occurred in the same proportion despite greatly varying uptake of these nutrients. Foliar concentrations of N and K in T-3 were similar hence the N:K ratio of 1:1.1, and this was significantly greater than treatments T-1, T2 and T3 (p<0.005). Ratios of K:P were consistent with trends observed for ratios of N:P. Accordingly, the greatest K:P ratio of 1:7.5 was observed in T-4 followed by T-0 (1:5.1) and treatments T-1, T-2 and T-3 (1:0.9-1.7) and only differences between T-4 and treatments T1, T-2 and T-3 were significant (p<0.005).

Foliar Ca was similar for treatments T-0 and T-1 and declined with increasing amendment rate where faecal sludge was applied (R^2=0.79, p<0.005) but only the difference between T-4 and T-2 was significantly different (Mann-Whitney U with Bonferroni correction applied, p<0.008). The same trend observed for foliar Ca was observed for foliar Mg (R^2=0.47, p=0.007) and the differences between T-4 and T-2 was significant (p=0.006). Differences in foliar Mg between T-4 and T-2 and T-1 and T-3 were significant at the p=0.1 level (p=0.08 and p=0.062 for those respective differences). Plants in the unamended sand had the greatest foliar concentration of Cu (14 $mg.g^{-1}$), Mn (800 $mg.kg^{-1}$), Fe (505 $mg.kg^{-1}$) and Al (232 $mg.kg^{-1}$) of all treatments. Foliar Zn was lowest in T-4 (140 $mg.kg^{-1}$) and decreased with increasing amendment rate amongst treatments where sludge was applied (R^2=0.37, p=0.022), ranging from 77 to 214 $mg.kg^{-1}$ for T-3 and T-1, respectively. Foliar Zn in the unamended treatment was 140 $mg.kg^{-1}$. Only the differences in foliar Zn between both T-3 and T-4 and T-1 was significant (p<0.05). Foliar Cu was quite variable amongst treatments and no trends could be established with increasing amendment rate. The lowest foliar Cu concentration occurred in T-4 (6 $mg.kg^{-1}$) and this difference was significantly different to T-1 and T-2 (p=0.049 and p=0.025 for those respective differences). Foliar Mn decreased with increasing amendment rate for treatments T-1 to T-3 (R^2=0.39, p=0.016) and the differences between T-3 and treatments T-1 and T-2 were significant at the p=0.1 level. Foliar Mn of T-4 was 557 $mg.kg^{-1}$ which was within a similar range to that of T-1 and T-2 and no significant differences were established between T-4 and other treatments. Foliar Fe was lowest in T-4 (154

mg.kg^{-1}) and greatest in T-2 (340 mg.kg^{-1}) amongst treatments where sludge was applied and this difference was significant (p=0.037). Foliar Al occurred in a similar range in treatments T-1 to T-4, ranging from 88 to 97 mg.kg^{-1} and none of these differences were significant.

Table 3.24: Foliar nutrient concentrations of *B. vulgaris* for treatments T-0 to T-4.

Parameter	T-0	T-1	T-2	T-3	T-4
N (mg.kg^{-1})	26.60	22.55 ±0.91a	27.60 ±4.97ab	32.76 ±3.96bc	41.64 ±5.92cd
P (mg.kg^{-1})	10.00	33.53 ±3.33a	32.66 ±6.33a	35.78 ±1.57a	11.02 ±2.64b
K (mg.kg^{-1})	51.10	44.30 ±2.24a	53.54 ±12.00ab	32.04 ±9.40ac	78.38 ±6.32d
Ca (mg.kg^{-1})	13.60	14.18 ±0.65a	10.06 ±2.34	7.44 ±0.56	6.50 ±0.82
Mg (mg.kg^{-1})	16.80	16.78 ±2.62a	14.74 ±1.40ab	12.96 ±1.38ab	11.32 ±2.08b
Zn (mg.kg^{-1})	140.0	213.50 ±33.91a	200.20 ±121.32a	77.40 ±32.85b	56.40 ±7.83b
Cu (mg.kg^{-1})	14.00	11.18 ±1.99a	12.38 ±5.30a	7.10 ±1.76ab	5.64 ±2.03b
Fe (mg.kg^{-1})	800.00	626.50 ±43.31a	605.80 ±137.92ab	431.40 ±98.82a	557.00 ±74.74ac
Al (mg.kg^{-1})	505.00	280.00 ±51.51a	340.20 ±152.13a	197.80 ±45.74a	154.20 ±47.21a
Mn (mg.kg^{-1})	232.00	87.50 ±20.73a	96.80 ±37.92a	74.40 ±30.68a	88.80 ±26.62a
N:P	2.7:1	0.7:1 ±0.1a	0.9:1 ±0.3a	0.9:1 ±0.1a	3.9:1 ±0.6b
N:K	1:0.5	1:0.5 ±0.0a	1:0.5 ±0.1a	1:1.1 ±0.3b	1:0.5 ±0.1a
K:P	1:5.1	1:1.3 ±0.2a	1:1.7 ±0.6a	1:0.9 ±0.3b	1:7.5 ±2.3c

Values in the same row followed by the same letter are not significantly different (p>0.05, Scheffe's multiple range test). Variations shown are ±SD around the mean. n=5 except for T-1 where n=4. Individual foliar samples of T-0 were too small to allow for analysis and the results shown for T-0 represent the analysis of the combination of all five replicates.

Table 3.25: Linear regression models relating foliar nutrient concentrations to amendment rate[1,2]

Nutrient (mg.g^{-1})	Intercept	Slope	R^2	p
N	17.42	0.51	0.59	0.001
Ca	17.21	-0.33	0.79	>0.005
Mg	18.63	-0.19	0.47	0.007
Zn	305.64	-7.02	0.37	0.022
Mn	757.86	-10.06	0.39	0.016

[1] Only significant linear relationships shown
[2] n=5

Foliar nutrients of *S. melongena* are shown in Table 3.26. Foliar N in T-4 was 45 mg.kg^{-1} which was about 50% greater than that of T-3, and foliar K in T-4 of 47 mg.kg^{-1} was more than twice that of T-3. Both foliar N and K were significantly different between T-3 and T-4 (p<0.005), suggesting that these nutrients may have been limiting in T-3. As for foliar P, this was 8 and 5 mg.kg^{-1} in T-3 and T-4,

respectively, and this difference was significant (p<0.005). These results for P suggest that unlike N and K, P was not limiting in T-3. Foliar Ca was similar between treatments and this difference was not significantly different (p=0.365). Foliar Mg in T-3 was 17 mg.kg^{-1} which was about 2.5 times greater than that in T-4 of 7 mg.kg^{-1} and this difference was significant (p<0.005). The ratio of N:P in T-4 of 8.8:1 was significantly greater than that of T-3 (p<0.005). An even greater disparity was observed in the ratio of K:P between treatments. In this regard, T-4 had the highest K:P ratio of 1:9.3, which was more than three times greater than that of T-3 (p<0.005). Plants in T-3 exhibited an elevated N:K ratio of 1:1.4 compared with that of T-4 where this ratio was 1:1.0, indicating an equal uptake of these nutrients (p<0.005).

Foliar Zn, Cu and Mn were 50%, 14% and 102% higher, respectively, in T-3 than in T-4 and these differences were significant (p<0.05). The higher uptake of these micronutrients, particularly Zn and Mn, may have been in excess due to other nutrient deficiencies which resulted in metabolic imbalances. Foliar Fe and Al were comparable between treatments and no significant difference could be established for either of these elements (p=0.67 and p=0.51, respectively).

Table 3.26: Foliar nutrient concentrations of *S. melongena* for treatments T-3 and T-4.

Parameter	T-3	T-4
N (mg.kg^{-1})	30.76 ±3.28a	44.62 ±3.80b
P (mg.kg^{-1})	8.1 ±0.63a	5.16 ±0.82b
K (mg.kg^{-1})	21.98 ±1.42a	47.06 ±4.38b
Ca (mg.kg^{-1})	15.84 ±1.90a	17.44 ±3.2a
Mg (mg.kg^{-1})	17.44 ±1.67a	6.60 ±0.63b
Zn (mg.kg^{-1})	36.20 ±7.29a	23.60 ±3.51b
Cu (mg.kg^{-1})	8.24 ±0.50a	6.90 ±0.48b
Fe (mg.kg^{-1})	341.80 ±17.25a	354.00 ±57.16a
Al (mg.kg^{-1})	220.80 ±28.75a	241.80 ±60.40a
Mn (mg.kg^{-1})	355.40 ±66.32a	176.20 ±59.47b
N:P	1:3.8 ±0.2a	1:8.8 ±1.4b
N:K	1:1.4 ±0.2a	1:1.0 ±0.1b
K:P	1:2.7 ±0.3a	1:9.3 ±1.1b

Values in the same row followed by the same letter are not significantly different (p>0.05). Variations shown are ±SD around the mean (n=5).

4. Discussion

This study was motivated by the need to find an effective and sustainable management option for evacuated faecal sludge. The conventional option of treating faecal sludge at WWTWs is not feasible and other options such as marine outfalls are increasingly falling out of favour in accordance with international best practise. The limited number of current options and their lack of practicality in dealing with evacuated faecal sludge demand a new holistic approach to the problem. Working within the framework provided by ecological sanitation this study recognises the potential of faecal sludge in plant production. While traditionally viewed as a waste that requires disposal, faecal sludge contains micro- and macro-nutrients (Schouw *et al.*, 2002) which are required for plant growth and can improve a number of beneficial soil properties. This study examined the potential of faecal sludge in agroforestry by employing two pot experiments. The first experiment investigated tree growth and photosynthesis of *E. grandis* and *A. mearnsii* above buried faecal sludge. These species were chosen since they are fast-growing and of economic importance, particularly as a source of timber. Furthermore timber has the added benefit of not being consumed which reduces health risks which may arise from contamination. The second experiment examined the potential of faecal sludge as a fertilizer to produce the food crops *B. vulgaris* and *S. melongena*.

4.1. Tree growth in experimental columns

4.1.1. Chemical composition of faecal sludge

Faecal sludge used in the tree growth experiment had a total N concentration of 2.18 $mg.kg^{-1}$ on a dry mass basis which was considerably less than that of 18.3 $mg.kg^{-1}$ for faecal sludge sourced from pit latrines in KwaZulu-Natal and irradiated with infrared radiation to aid drying (Still, unpublished data). Concentrations of N as high as 35 100 $mg.kg^{-1}$ on a dry mass basis have been reported for faecal sludge sourced from settling lagoons (Warman and Termeer, 2005). However, the total N concentration of faecal sludge used in the present study was comparable to that of a mixture of faecal sludge sourced from pit latrines, septic tanks and public toilets of 1.1 $mg.kg^{-1}$ (Kegne *et al.*, 2008). Nonetheless, total N content in the present study was far lower than expected,

particularly considering the high content of nitrogenous waste in raw human excreta. For example, Cross and Strauss (1985) reported total N concentrations of human excreta ranging from 50-70 $g.kg^{-1}$ and in a study by Schouw *et al.* (2002) N ranged from 63.5-159.2 $g.kg^{-1}$.

Therefore much of the N from human excreta had been lost during storage in pit latrines and total N concentration was low at application. It is well known that loss of N commonly occurs during storage due to denitrification and more stable forms of faecal sludge typically exhibit lower N concentrations than raw faecal sludge (Aalbers, 1999). Concerns regarding the loss of N during composting of human excreta have also been raised (Schouw *et al.*, 2002). The storage time of faecal sludge in the present study could not be established but was probably in the range of five to nine years based on estimates for South Africa (Still *et al*., 2009). Consequently, the sludge could be considered as fairly highly stabilised which is reflected by the low total N concentration. It is unknown from which part of pit latrines the samples may have originated from. One obvious consequence of the design of pit latrines is that freshest faecal sludge (and hence less stable) will be found in the uppermost layer of sludge in the pit (Heinonen-Tanski and van Wijk-Sijbesma, 2005). However, since sampling was conducted randomly it is assumed that the N concentrations presented here represent that of faecal sludge from multiple depths and are thus representative of the average. The low variability about the mean (SD=0.11) is suggestive that N concentration was more or less uniform irrespective of sourcing depth from the pit latrine, perhaps with the exception of the uppermost layer.

Concentrations of total P and K (4501 and 462 $mg.kg^{-1}$, respectively) were considerably less than that of faecal sludge sourced from settling lagoons (9750 and 2600 $mg.kg^{-1}$, respectively; Warman and Termeer, 2005) and infrared-irradiated faecal sludge (9300 and 1700 $mg.kg^{-1}$, Still, unpublished data). Higher concentrations of P, and particularly K, have been reported for dewatered faecal sludge (1050 and 3900 $mg.kg^{-1}$, respectively; Cofie *et al.*, 2009). Therefore, as with N, total P and K concentrations were on the low end of the range typically reported for various types of faecal sludge. Total P and K could have been expected to be quite high since raw human excreta is usually high in these nutrients. Using a mass balance approach, Cordell *et al*. (2009) found that, based on the normal bodily requirement of 1.2g P per

person per day, the total population consumes roughly 3 million tonnes (MT) P annually of which almost all is excreted (Jönsson *et al*., 2004). The daily excretion rate of K is in a similar range to that of P (Schouw *et al.*, 2002).

The ratios of N:P and N:K of the faecal sludge were low which is further evidence of denitrification during storage. Therefore, relative to N, P and K were quite high. The latter has been observed by Mnkeni and Austin (2009) who reported that UD waste was a better source of P and K than N, and for better agronomic efficacy recommended the co-application of inorganic N. Of the remaining macronutrients, Ca concentration was approximately half that of infra-red irradiated faecal sludge, while Mg concentration was similar (Still, unpublished data). Compared with that faecal sludge all micronutrients tested occurred in lower concentrations, particularly those of Fe and Al which were orders of magnitude lower. This is probably reflective of the high variability of sludge chemistry rather than a result of the drying process employed by Still *et al.* (unpublished data).

The river sand used in the study was characterised by a paucity of nutrients and despite weekly fertilizer applications the river sand in the control did not show appreciable increases in fertility. Of the macronutrients only N and K concentrations of the sand in the control group had showed increases at harvest although these increases were marginal. Nevertheless, most micronutrients had increased in concentration by the end of the experiment. From a mass balance perspective nutrient flux in the control group would have been largely determined by fertilizer addition, plant uptake and losses through leaching. Fertilizer addition may have been insufficient given the nature of the river sand which was especially prone to leaching. Additionally, the frequent rainfall midway through the experiment amounting to as much as 100 mm in a single week could have purged the control columns of nutrients.

Changes in the concentrations of macronutrients in the faecal sludge between planting and harvest (as determined from samples taken in the centre of the sludge cores) were varied. In this respect, N concentration remained more or less similar while K decreased markedly and P and Bray P increased irrespective of species. Interestingly, N concentration in the experimental and control groups where *A. mearnsii* had been grown had increased by harvest and were higher than, but not significantly different,

from the corresponding values for *E. grandis*. The latter may indicate the presence of N_2-fixing activity in those saplings which elevated soil N. The increase in Bray P of the faecal sludge at harvest indicates the occurrence of mineralization over the experimental period. Many nutrients in human excreta are organically bound and therefore require mineralization before they can be utilized by plants (Kirchman and Petterson, 1995). Accordingly, it is speculated that the faecal sludge acted as a slow release fertilizer as nutrients were mineralised and slowly became available to plants.

4.1.2. Sapling growth

A comparison of control saplings with those grown above a core of buried faecal sludge indicates that growth of both species was enhanced by the application of faecal sludge, particularly in *E. grandis*. Growth of control saplings was poorer than expected despite fertilizer additions for three main reasons. First, it is believed that excessive leaching of nutrients occurred in control treatments due to the physical properties of the sand. Second, the nutrient demand by the saplings, particularly that of *E. grandis*, was even greater than expected which, combined with the fact that fertilizer levels remained unchanged throughout the growth period, meant that saplings became progressively more nutrient stressed as they grew. Lastly, though the positive growth response to faecal sludge application in both species can be attributed mainly to increased nutrient supply compared to the control, the contribution of improved soil properties such as increased carbon content, cation exchange capacity, microbial activity and water holding capacity cannot be underestimated. The resultant growth of *E. grandis* in the control group was notably hindered and those saplings developed symptoms of nutrient deficiency as early as 12 weeks after planting. In contrast, no deficiency symptoms could be observed in experimental saplings of *E. grandis* and growth was vigorous which is typical of that species (Campinhos, 1980). At the end of the experimental period mean height of those saplings was 2.44 m which was more than double that of control saplings and compares favourably with reported heights of plantation-grown *E. grandis* and other *Eucalyptus* clones over similar growth durations. For example, du Toit (2008) reported heights of *E. grandis* of 0.71 and 1.59 m at 0.4 and 0.8 years, respectively, in an intensively managed stand on a site of moderate productivity in KwaZulu-Natal, South Africa and Bouillet *et al.* (2002) reported a height of 1.8 m for 6 month old hybrid *Eucalyptus* clones in an

intensively managed stand in the Pointe-Noire region of the Democratic Republic of Congo. Leuning *et al.* (1991) grew *E. grandis* in a plantation with and without added fertilizer and reported that at six months after planting fertilized saplings had attained a height of 3.8 m while saplings grown without added fertilizer attained a height of 0.7 m. The disparity in height observed here reflects the marked positive response of *E. grandis* towards fertilization regimes. Such a positive response of *E. grandis* to fertilization has been widely reported (e.g. Judd *et al.*, 1996, Misra *et al.*, 1998; Hunter, 2001). Root collar diameter (RCD) was less positively affected by the application of faecal sludge and the RCD of both experimental and control saplings at harvest of 40 and 34 mm, respectively, is comparable to that reported by Mulizane *et al.* (2005) who observed mean RCD of 34.1, 26.3 and 27.7 mm for three successively planted one-year old stands of *E. grandis*.

The growth of *A. mearnsii* above buried faecal sludge was not dissimilar to that observed for *E. grandis*. Mean height of *A. mearnsii* in the experimental group was 2.7 m at the end of the experimental period and, in contrast to that observed for *E. grandis*, the mean height of control saplings was not considerably less than that of those plants in the experimental group. Growth rates of *A. mearnsii* of both control and experimental saplings observed here is comparable to that reported for the same species by Clulow (2010) who observed mean increases in height of 0.37m month^{-1} or 4.5 m year^{-1} over 13 months after establishment, but greater than that observed by Forrester *et al.* (2004) who observed that the maximum mean annual height increase of an 11-year old stand was 2.3 m year^{-1}. Khanna (1997) reported similar annual increases in height of a stand of *A. mearnsii* to that of Forrester *et al.* (2004) of about 2 m year^{-1} over a 33 month period. Indications are that growth of control saplings of *A. mearnsii* had levelled shortly before harvest based on height and stem data. In this respect, the difference in height between control and experimental saplings was significant at harvest and while RCD was not significantly different at harvest, RCD did not show appreciable increase during the month before harvest. Therefore, while saplings were harvested for practical considerations due to their size, a longer experimental period may have revealed significant restrictions to growth like that observed for *E. grandis*, but on a longer timescale.

Both species showed considerable increases in leaf area where faecal sludge was applied but the response in leaf area was far greater in *E. grandis* which showed a 6.5-fold increase in leaf area while in *A. mearnsii* leaf area of experimental saplings was almost twice that of control saplings. Leaf area in *E. grandis* has been shown to be greatly increased by improved plant nutrition. Leuning *et al.* (1991) found that leaf biomass (and by inference, leaf area) increased from 1.4 to 5.9 $t.ha^{-1}$ in unfertilized and fertilized trees, respectively. Thomas *et al.* (2006) raised seedlings of *E. grandis* in pots for 19 weeks at varying P supply and reported leaf area ranging from 0.01 m^2 to 0.6 m^2, representing a 60-fold increase. Those authors found that the increase in leaf area occurred through the combined increases of area of individual leaves and number of leaves per plant. These data were not recorded in the present study although leaves were visually smaller and fewer in control saplings of *E. grandis*. In *A. mearnsii* leaf size was not observed to be affected by faecal sludge application although the number of leaves per plant was visually less. Contributing to the reduced number of leaves in control saplings of *E. grandis* was notably high amounts of leaf senescence indicating low leaf lifespan. Stape *et al.* (2008) recorded a mean leaf lifespan of 1 year in a clonal *Eucalyptus* stand but leaf lifespans of up to 3 years have been recorded in stands of *E. maculata* and *E. globulus* (Pook, 1984; Cannell, 1989, reviewed by Whitehead and Beadle, 2004). Therefore, leaf senescence was not normal age-related developmental senescence (i.e. senescence was premature). This gave the saplings a distinct bare appearance with branches holding leaves only further away from the trunk where leaves were younger. The premature senescence observed in control saplings of *E. grandis* was probably due to nutrient stress and plants responded by senescing older leaves to allow for nutrient remobilization to new sinks (Munné-Bosch and Alegre, 2004; Guiboileau *et al*., 2010). Using this 'altruistic' mechanism plants are able to ensure, or enhance, overall survival by sacrificing plant parts and translocating nutrients accumulated in senescing tissue to more productive plant parts (Munné-Bosch and Alegre, 2004; Guiboileau *et al*., 2010). Premature leaf senescence was not observed in *A. mearnsii* but instead plants appeared to respond by considerably reducing the production of new foliage.

Specific leaf area was significantly increased from 10.6 to 18.9 $m^2.kg^{-1}$ in control and experimental saplings of *E. grandis*, respectively, but was not significantly increased by the application of faecal sludge in *A. mearnsii*. The lower SLA in control saplings

of *E. grandis* was the result of denser or thicker leaves, indicating changes in leaf structure (Milla *et al.*, 2008; Reich *et al.*, 1998). SLA is widely regarded as an important leaf trait which reflects a compromise between light capture and investment into leaf tissue, affecting leaf economy (Milla *et al.*, 2008, Shipley *et al.*, 2006; Wright *et al.*, 2004). Ideally, SLA would be infinitely high to maximise light capture for photosynthesis but this is met by functional constraints including supporting, protective and transport tissues which require investment of carbon (Milla *et al.*, 2008). The *Eucalyptus* genus is generally characterised by thick leaves with low SLA in their native habitats but SLA is typically greatly increased under experimental conditions where water and nutrient availability are supplied in abundance (Whitehead and Beadle, 2004; Kirschbaum and Tompkins, 1990; Kirschbaum *et al.*, 1990). In this respect, SLA has been shown to be plastic with increased N fertilization (Knops and Reinhart, 2000; Grassi *et al.*, 2002) but not that of P (Thomas *et al.*, 2006). Grassi *et al.* (2002) observed denser or thicker leaves in seedlings of *E. grandis* grown at a low nitrogen supply compared with higher nitrogen supply. Accordingly, the marked difference in SLA in *E. grandis* in the present study was probably the result of greatly differing nutrient availability, or specific nutrient deficiencies, which decreased SLA in control saplings and consequently the SLA of only experimental saplings of *E. grandis* is within the reported range of 15-37 $m^2.kg^{-1}$ for that species grown in controlled conditions (reviewed by Whitehead and Beadle, 2004). The greater SLA of experimental saplings allowed experimental saplings of *E. grandis* to maximise productivity due to abundant resource availability and accounts for the rapid growth of those saplings (Grotkopp and Rejmánek, 2007; Poorter & Van der Werf, 1988; Van der Werf *et al*., 1988).

The improvement in growth in both species, particularly in *E. grandis*, where faecal sludge was applied is consistent with the positive response in growth noted by Morgan (2007) using the 'arborloo' system described in the introduction. Similar nutrient-rich wastes such as the one used in the present study have yielded positive effects on growth. Da Silva *et al.* (2011) used four treatments, *viz.* mineral fertilizer, wet sewage, dry sewage and no fertilizer, to determine the effects of the sewage sludge application on biomass production in *E. grandis*. At 12 months after planting those authors observed increases in the biomass of leaf, bark, wood, and total biomass in saplings grown in the experimental treatments. In particular, leaf biomass was

increased by about 2-fold in the experimental treatments and total biomass in the dry sewage sludge was 35% and 145% greater than saplings with and without fertilizer, respectively. Myers *et al.* (1996) irrigated a young plantation of *E. grandis* and *Pinus radiata* with municipal effluent corresponding to the estimated water use of those saplings, with addition rates of half or twice that rate. Trees of *E. grandis* which received the normal addition rate over a three-year period developed leaf area more rapidly and showed greater volume and biomass than trees irrigated with bore-hole water. However, those authors noted the absence of such a response in *P. radiata*, postulating that available N of the site was in excess of requirements, commensurate with the slow early growth of that species.

In contrast with *E. grandis*, the growth of *A. mearnsii* in response to faecal sludge application was relatively poor which may be explained by differences in the N_2 fixing activity between control and experimental plants of that species. Nitrogen-fixing bacteria can be found alone in soil or in a symbiotic relationship with members of the Leguminosae where they fix N_2 into a usable form (ammonium) by plants in root nodules (Gage, 2004). As part of the symbiotic relationship, host plants infected with N_2-fixing bacteria provide photosynthate at the root system as a source of energy to sustain bacterial metabolism and thus maintain the activity of N_2 fixation (Antolin *et al.*, 2010*a*; Antolin *et al.*, 2010*b*, Khan *et al*., 1995). It is well known that nitrate has an inhibitory effect on the growth and nitrogenase activity of root nodules (Lucinski *et al.*, 2002; reviewed by Streeter, 1988). Streeter (1985) reported an 80% reduction in nitrogenase activity in nodulated soybean plants (*Glycine max*) temporarily supplied with a high nitrate solution. (Thomas *et al.*, 2000) observed a reduction in nitrogenase activity, nodule mass and number, nodule activity and the proportion of plant N derived from N_2 fixation in *Gliricidium sepium*, a tropical tree species, fertilized with a N fertilizer (NH_4NO_3). Therefore, in the presence of high nitrate leguminous plants acquire most N from the soil solution rather than through the rhizobia-legume symbiosis. The high inorganic N content of sewage sludge led Antolin *et al.* (2010*a*) to hypothesise that impairment of nodule metabolism could occur in alfalfa (*Medicago sativa*) treated with sewage sludge. While those authors found that sewage sludge had no effect on nodulation, evidence was suggestive of reduced N_2-fixation efficiency in those plants. In the present study, root nodulation within the sludge core could not be determined as the sludge was too dense to allow

the separation of roots from the sludge with the required delicacy. Root nodulation was however visible in both treatments (i.e. in the annular ring and in the control) to an equal but small extent. Assuming that nodulation was equal throughout experimental columns, the nitrogenase activity may have been different between the treatments. Thus the relatively poor response in growth of *A. mearnsii* to faecal sludge application could be explained by the high nitrate content of the faecal sludge which inhibited N_2-fixation in experimental saplings. In contrast, it is likely that N_2-fixing activity in control saplings was high, especially since the sand was low in N. The benefit of high N_2-fixation activity in control saplings must have partially offset the limiting effect on growth due to the poor N status of the soil, including the investment of photosynthate to sustain bacterial metabolism.

The effect of possibly increased N_2 fixing activity in control saplings of *A. mearnsii* was further evidenced by interspecific comparisons. In this respect, control saplings of *A. mearnsii* had statistically greater leaf, twig, trunk and total dry biomass than corresponding saplings of *E. grandis*. However, interspecific comparisons amongst experimental saplings showed no significant differences in total dry biomass or for measured components. Thus *A. mearnsii* was able to grow better than *E. grandis* in the control group due presumably to enhanced N_2-fixing activity which *E. grandis* lacks, but under the high N conditions posed by the faecal sludge the suppression or reduction thereof resulted in similar growth between the species. The relatively small difference in foliar N between control and experimental saplings of *A. mearnsii* compared with that of *E. grandis* is supportive of N_2 fixation in control saplings of that species which enabled these saplings to maintain reasonable concentrations of foliar N despite low soil N.

4.1.3. Foliar nutrients

Foliar macronutrients occurred in considerably greater concentration in both species where faecal sludge was applied. In this respect, foliar N, P and K were increased by 200%, 107% and 46% percent in experimental saplings of *E. grandis*, respectively, and the corresponding increases for *A. mearnsii* were 39%, 350% and 103%. N and P deficiencies were clearly evident in *E. grandis* and symptoms included a distinct purple discolouration of leaves and twigs, particularly at the growing tips, which is

characteristic of a P deficiency and a generally lighter green appearance relative to the experimental group which is indicative of N deficiency. However, no obvious deficiencies could be discerned in control saplings of *A. mearnsii* despite greatly decreased foliar macronutrient concentrations. N and P are most often limiting nutrients to plant growth and the use of N and P stoichiometry, or N:P ratios, as a diagnostic tool has been shown to be informative in determining whether N or P are limiting, or co-limiting, provided K is not primarily limiting (Fisher and Binkley, 2000; Koerselman and Meuleman, 1996; Han *et al.*, 2006). Skewed N:P ratios can be the result of luxury consumption of the non-limiting, or less limiting nutrient, and as such higher N:P ratios are indicative of a P deficiency while a lower N:P ratio suggests a N deficiency (Koerselman and Meuleman, 1996). Despite greatly decreased foliar K in control saplings of both species, particularly in *A. mearnsii*, no visible signs of K deficiency were present in any saplings and so K was assumed not to be the primary limiting nutrient for purposes of analysis. In this respect, foliar K concentrations of control and experimental saplings of *E. grandis* (10.5 and 7.2 $mg.g^{-1}$, respectively) were greater than that of the same species and age fertilized with KCl (Laclau *et al.*, 2009) and greater than, or comparable to, the adequate foliar K concentration of 7.5 $mg.g^{-1}$ for that species (Herbert and Schonau, 1989; reviewed by Drechsel and Zech, 1991). Furthermore, foliar K concentrations of control and experimental saplings of *A. mearnsii* (12.6 and 6.2 $mg.g^{-1}$, respectively) were notably greater than the adequate foliar K concentration of 3.7 $mg.g^{-1}$ in 16-year old trees of that species (Schmitt, 1987; reviewed by Drechsel and Zech, 1991).

The N:P ratio of 11.1:1 in experimental saplings of *E. grandis* was within the proposed optimum range of 11:1-18:1 for that species which indicates balanced N and P nutrition in those saplings. However, the N:P ratio of control saplings of *E. grandis* was 7.9:1 which points to reduced N uptake (Graciano *et al.*, 2006). This would suggest that N was the primary limiting nutrient in control saplings although since both N and P deficiencies were clearly evident it is more likely that the ratio indicates that P was co-limiting to some extent (Koerselman and Meuleman, 1996). The foliar N and P concentrations of experimental saplings of *E. grandis* (32.9 and 3.1 $mg.g^{-1}$, respectively) were greater or comparable with that of well-fertilised saplings of the same species cultivated in pots or the field (Judd *et al.*, 1996, Graciano *et al.*, 2006, Leuning *et al.*, 1991, Thomas *et al.*, 2006) and greater than the adequate foliar N and

P concentrations of 2.9 and 1.4 $mg.g^{-1}$, respectively, for that species (Herbert and Schonau, 1989; reviewed by Drechsel and Zech, 1991). By comparison, the foliar N concentration in control saplings of *E. grandis* (1.1 $mg.g^{-1}$) was appreciably low compared with those studies although foliar P concentration (1.5 $mg.g^{-1}$) was generally less or comparable. In this respect, the foliar P concentration of control saplings was similar to that of 1.6 $mg.g^{-1}$ reported by Thomas *et al.* (2006) for 19-week old saplings of the same species supplemented with the highest P supply of 1000 $mg.kg^{-1}$ soil. Those authors noted the absence of any nutrient deficiency at lower foliar P concentration of 1.2 $mg.g^{-1}$. Therefore, it is probable that P deficiency was not as acute as N deficiency in control saplings of *E. grandis* and this is supported by the N:P stoichiometry which suggests that N was the primary limiting nutrient in control saplings with a degree of co-limitation occurring from P. In contrast, N and P concentrations in experimental saplings was high and uptake of these nutrients was balanced, indicating that sludge was able to supply an abundance of N and P for tree growth.

In the absence of a proposed normal N:P ratio for *A. mearnsii* in literature, a N:P ratio of 22.6:1 was derived from normal foliar nutrient concentrations reported by Schmitt (1987) for the same species. This ratio for control saplings of *A. mearnsii* was 23.7:1 which indicates that uptake of N and P in those saplings was balanced despite appreciably lower foliar N and P concentrations (23.3 and 1.0 $mg.g^{-1}$, respectively) than that reported by Schmitt (1987) of 31.7 and 1.4 $mg.g^{-1}$, respectively. Therefore, N and P may have been co-limiting nutrients in control saplings of *A. mearnsii* but since no visible signs of deficiency were evident in that species it is assumed that N and P were not seriously deficient. This is reflected by the reasonably similar growth between control and experimental saplings of *A. mearnsii*. It is important to note that the difference in foliar N concentration between experimental and control saplings of *A. mearnsii* was considerably less marked than that of *E. grandis* which may have been the result of increased nitrogen-fixing activity in control saplings of *A. mearnsii* in the nutrient poor conditions, as discussed. The N:P ratio of experimental saplings (8.4:1) was significantly less than that of control saplings due to high P uptake. In this respect, foliar P concentration in experimental saplings of *A. mearnsii* (4.5 $mg.g^{-1}$) was greater than that reported by Schmitt (1987) by a factor of 3.2 but foliar N concentration was almost identical (31.8 $mg.g^{-1}$) to that reported by the same author.

Such high foliar P concentrations do not necessarily imply excessively high P uptake since plants can increase storage of P mostly as inorganic P or polyphosphate in vacuoles when an adequate P supply is available (Chapin *et al.*, 1990; Thomas *et al.*, 2006).

An extension of the N:P ratio is the ratio of foliar macronutrients and secondary macronutrients relative to N (N:P:K:Ca:Mg) and has also been used as a diagnostic tool to optimise fertilization practices in silviculture (Campion and Scholes, 2007). Campion and Scholes (2007) determined that the foliar N:P:K:Ca:Mg ratio of 100 N:8 P:35 K: 2.5 Ca: 4 Mg was optimal for growth in *E. grandis* and confirmed this ratio in trees of the same species grown in the province of KwaZulu-Natal, South Africa. In the present study the N:P:K:Ca:Mg ratio of experimental saplings of *E. grandis* was 100 N:9.3 P:31.6 K: 22.7 Ca: 14.6 Mg which closely conforms to the proposed optimal ratio with respect to macronutrient ratios (N:P:K) but not secondary macronutrient ratios relative to N (N:Ca:Mg). This would suggest excessive uptake of Ca and Mg in experimental saplings of *E. grandis*, although the foliar Ca concentration (7.4 $mg.g^{-1}$) was less than the adequate foliar Ca concentration of 16 $mg.g^{-1}$ for that species while foliar Mg concentration (4.8 $mg.g^{-1}$) was slightly greater than the adequate foliar concentration of 3.5 $mg.g^{-1}$ for the same species (Herbert and Schonau, 1989; reviewed by Drechsel and Zech, 1991). In control saplings the N:P:K:Ca:Mg ratio was 100 N:13.1 P:64.6 K:77.2 Ca:27.3 Mg which indicates sub-optimally high uptake of P and particularly K relative to N which is further supportive of N being the primary limiting nutrient in control saplings. As observed in experimental saplings, Ca and Mg uptake in control saplings was high relative to N, particularly with respect to Ca. The greater overall deviation of control saplings from the optimal N:P:K:Ca:Mg ratio is indicative of nutrient imbalances in those saplings possibly induced by the reduced uptake of N and other co-limiting nutrients such as P (Koerselman and Meuleman, 1996).

The N:P:K:Ca:Mg ratio in control and experimental saplings of *A. mearnsii* was 100 N:14.1 P:39.4 K:11.4 Ca:8.5 Mg and 100 N:4.4 P:26.8 K:17 Ca:7.8 Mg, respectively. The N:P:K:Ca:Mg ratio derived from adequate foliar concentrations reported by Schmitt (1987) for the same species was 100 N:4.4 P:11.7 K:9.1 Ca:2.8 Mg although foliar Mg concentration in that study was low but not deficient

(reviewed by Drechsel and Zech, 1991). By comparison with the latter ratio, K uptake relative to N in both experimental and control saplings was particularly high and Ca uptake relative to N was slightly elevated in control saplings compared with that of Schmitt (1987) but significantly elevated in experimental saplings. Actual foliar Ca concentrations in control and experimental saplings (3.9 and 3.7 $mg.g^{-1}$, respectively) were appreciably greater than that of 2.9 mg.g-1 reported by Schmitt (1987) for the same species. Therefore, consumption of K and Ca (and possibly Mg) was likely in excess of requirements in experimental and control saplings, constituting luxury consumption of these nutrients as they were apparently non-limiting (Koerselman and Meuleman, 1996).

Foliar micronutrient concentrations in both species were generally similar to that reported for the respective species with the exception of foliar Mn and Fe concentrations in *E. grandis* (Herbert and Schonau, 1989) and foliar Mn and Al in *A. mearnsii* (Schmitt, 1987) which deviated quite substantially from that reported by those respective authors. In this respect, foliar Mn and Fe were greater in both groups (control and experimental) of *E. grandis* while Mn and Al were lower in both groups of *A. mearnsii*. Foliar Mn and Al concentrations were greater in control saplings of *E. grandis* and Fe, Al and Cu concentrations were greater in control saplings of *A. mearnsii* but there was no evidence to suggest that these nutrients were limiting in experimental saplings of those species and therefore luxury consumption of these nutrients may have occurred predominantly in control saplings, possibly arising from nutrient imbalances (Koerselman and Meuleman, 1996).

4.1.4. Gas exchange

Measured photosynthetic parameters generally showed a positive response to faecal sludge application in both species. A-ci curves of control saplings of both species indicate that photosynthesis in control saplings was relatively more RuBP regeneration-limited in both species compared with experimental saplings (Long and Bernacchi, 2003). Thus, photosynthetic rate in control saplings was limited at the electron transport level as reflected by notably decreased J_{max} values. The Rubisco limitation in control saplings of *A. mearnsii* was evidenced by the significantly greater V_{cmax} (p=0.1 level) in experimental saplings of that species which reflects changes in

Rubisco concentration, or the activation state thereof (Warren and Adams, 2002). Together with J_{max}, A_{max} measured in light response curves also showed marked increases in experimental saplings of both species. The enhanced photosynthetic capacity in both species was a consequence of generally increased foliar macronutrient concentrations due to the higher nutrient availability in pots containing faecal sludge (Leuning *et al.*, 1991). The positive correlations between foliar N and P concentrations and A_{max}, J_{max} and V_{cmax} (significant at the p=0.1 level) in *E. grandis* suggest that these nutrients were major determinants of photosynthetic capacity in that species, although the relative effects of N or P could not be distinguished. In *A. mearnsii* the correlations between foliar N and P concentrations and A_{max} and V_{cmax} were not significant which could suggest that N and P were not acutely limiting to those parameters. However, foliar N concentration in that species was strongly positively correlated with J_{max} which indicates that foliar N concentration had an effect at the electron transport level. Furthermore, a reasonably strong correlation between V_{cmax} and foliar N concentration, but not with foliar P concentration, was observed in that species, suggesting that N was a more important determinant of photosynthetic capacity than P in that species.

The relationship between foliar N concentration and photosynthetic capacity is well recognised (Field and Mooney, 1986; Evans, 1989, 1996). Leuning *et al.* (1991) reported a positive relationship between foliar N concentration and J_{max} and V_{cmax} in six month-old saplings of *E. grandis*. Grassi *et al.* (2002) observed a positive relationship between foliar N concentration and A_{max}, J_{max} and V_{cmax} in seedlings of the same species which led those authors to conclude that foliar N concentration was a significant determinant of photosynthetic capacity in that species. Within the eucalyptus genus, the enhancement of photosynthetic capacity in response to higher foliar N concentration has also been observed in *E. nitens* (Medhurst and Beadle, 2005) and *E. globulus* (Sheriff and Nambiar, 1991; Periera *et al.*, 1994). Mitchell and Hinckley (1993) observed significantly increased photosynthetic rates in shoots of Douglas-fir with a high N concentration compared with shoots with a relatively low foliar N concentration. The positive relationship between foliar N concentration and photosynthetic capacity is unsurprising since a considerable proportion of leaf N is present in thylakoids and Calvin cycle proteins (Field and Mooney, 1986; Evans,

1989). In this respect, a strong positive correlation exists between foliar N concentration and contents of RuBP carboxylase and chlorophyll (Evans, 1989).

Foliar P concentration has been shown to be positively correlated with A_{max}. Kirschbaum and Tompkins (1990) observed that A was sensitive to P nutrition in aeroponically-grown seedlings of *E. grandis*, and A_{max} declined with decreasing foliar P concentration. A_{max} was strongly correlated with phyllode P concentration in a 5-year old stand of *Acacia melanoxylon* subjected to varying amounts of pruning Medhurst *et al.* (2006). However, foliar P concentration has been shown to be generally less important than foliar N concentration in determining photosynthetic capacity. For example, Leuning *et al.* (1991) observed a relatively poor correlation between foliar P concentration and A_{max} in contrast with the relationship observed with foliar N concentration. Tuohy *et al.* (1991) examined the relationship between A (taken as spot measurements at light intensities of >750 $\mu mol.m^{-2}.s^{-1}$) and foliar N and P concentrations in 14 ecologically important species in Zimbabwe, including eight leguminous species. Those authors reported no correlation between A and foliar P concentration although A and foliar N concentration showed a very strong positive correlation. In seedlings of *E. gumnifera*, *E. pilularis* and *E. grandis*, Mulligan and Sands (1988) found that very low foliar P concentrations (greater than 0.02 $mg.g^{-1}$) did not limit A_{max}. Therefore, the enhanced photosynthetic capacity in experimental saplings of *E. grandis*, and possibly in *A. mearnsii*, was likely influenced more by improved N nutrition from the faecal sludge which increased foliar N concentration.

Water use efficiency under saturating light was almost doubled in experimental saplings of *E. grandis* relative to control saplings of that species while the corresponding values in *A. mearnsii* showed a slight decrease in WUE in experimental saplings although this difference was not significant. WUE in experimental saplings of *E. grandis* (6.2 $\mu mol.mmol^{-1}$) was higher than the range reported by Shem *et al.* (2009) of 3-5 $\mu mol.mmol^{-1}$ for field and pot-grown saplings of three species, including *E. grandis*, grown at various soil moisture contents. Those authors found that A and E decreased in pot-grown *E. grandis* in response to decreased soil moisture, with a resultant decrease in WUE. The apparent influence of soil water content on WUE in *E. grandis* means that the observed differences in WUE between experimental and control groups may have been the result of different soil water

contents. In this respect it is conceivable that faecal sludge application improved water retention in experimental pots which may have improved WUE in those saplings. Another possible explanation for the difference in WUE between experimental and control saplings of *E. grandis* was the difference in photosynthetic capacity brought about by differences in nutrient availability. Graciano *et al.* (2005) reported that P fertilization increased WUE in well-watered plants in a sandy soil. Sheriff *et al.* (1986) observed a positive relationship between foliar N and P concentrations and WUE in a seven-year-old stand of *Pinus radiata*. In the present study, foliar N and P concentrations in *E. grandis* were poorly correlated with WUE and E, although these nutrients had a strong positive correlation with A_{max}, as discussed. Thus, it appears that foliar N and P concentrations altered WUE mostly through their positive effect on A_{max} rather than E in *E. grandis*.

4.1.5. Root spatial distribution

Medium and coarse roots of both species had explored the entire soil volume irrespective of treatment and could be found at the bottom of pots (approx. 0.9 m below surface). Moreover, depth had no significant effect on the number of medium and coarse root intersections in either species and therefore roots were more or less equally distributed throughout the column profile. In a 6-month old stand of a *Eucalyptus* clone 11 out of 15 intersects were found in the top 25 cm of soil and the remaining four intersects occurred at 0.9 m to 1.5 m in depth, highlighting the predominantly superficial root distribution at that age (Bouillet *et al.*, 2002). It is however important to note that in the present study roots found at the bottom of pots were mostly lateral roots which diverted downwards by the pot walls rather than of tap root origin. Paulinho *et al.* (2003) reported that the percentage of all roots in the top 25 cm of soil in a 3-year old stand of *A. mearnsii* ranged from 56.27% to 62.95%, depending on the type of container used for seedling growth, which demonstrates the superficial rooting habit of that species. This makes *A. mearnsii* particularly prone to lodging in high winds (Paulinho *et al.*, 2000) and this was observed in several trees of the same species in the present study. Furthermore, in that same study roots were found to a depth as shallow as 0.75 m to 1 m.

Medium and coarse root intersects for both species were generally greater in the annulus of sand irrespective of depth and treatment which suggests that this was the normal rooting habit of these species as raised rather than the result of faecal sludge application. In this respect, medium and coarse roots of both species generally grew laterally before diverting vertically at the column walls while only a few roots grew below the stump. This is in contrast with Bouillet *et al.* (2002) who found that the medium root density in a clonal *Eucalyptus* stand was generally greatest below the stump. Furthermore, those authors found that the lateral rooting of the same species was not pronounced with 12 out of 15 intersects in a 6-month old stand occurring within 30 cm of the planting row although lateral root extension was rapid with roots in a 3-month old stand found from 1.25 to 2.50 m from the planting row. It is likely that the lateral rooting habit of *A. mearnsii* and *E. grandis* in the present study was an artefact of early containerised growth which tends to promote lateral root growth and a poor tap root system in these species (Barroso *et al.*, 2000; Smith *et al.*, 2001; Sherry, 1971). Barroso *et al.* (2000) reported that *Eucalyptus* spp. seedlings raised in plastic tubes exhibited a forked and undefined tap root as observed in both species in the present study. Such poorly developed root systems can be a consequence of root restriction and resulting deformations in containerised seedlings which affects rooting pattern in later growth (Reis *et al.*, 1989). In this regard, Leles *et al.* (2000) indicate that the use of more substrate (larger containers) is beneficial. The dense root matting observed in the growing substrate at planting could indicate that at that age the containers were insufficiently large with resultant root restriction. Normally developed tap roots in both species would have forced a stronger interaction of primary, secondary and tertiary roots with the faecal sludge. Nonetheless, a few coarse and medium roots of both species penetrated the sludge core centrally from below the stump.

The comparison of root distribution between experimental and control saplings was based on the assumption that if faecal sludge could support root growth the distribution of medium and coarse roots in experimental pots would be the same as control pots. It is acknowledged that the differing nutrient heterogeneity in control and experimental pots could have led to inherently different medium and coarse root distributions since root distribution is known to be affected by the distribution of nutrients (Hodge, 2004). However, this was not shown to be the case in either species

with the apparent exception of fine root distribution. Therefore, the comparison of medium and coarse root distribution in control and experimental saplings was informative in determining the suitability of faecal sludge as a medium to support growth of those particular roots.

A greater proportion of root intersections in both species were found to intersect the faecal sludge core at a depth of 500 mm compared with the number of intersections in the same area in control saplings. This was particularly evident in *A. mearnsii* where 31.3 % of roots at that depth had intersected the faecal sludge core while only 7.9 % of roots in control pots were found in the equivalent area. While this difference was marginally non-significant at the p=0.1 level, these data indicate that at the least, root distribution in that area did not deviate from that of control saplings and therefore faecal sludge did not inhibit root growth. Moreover, it is probable, based on the increased root intersections in that area in *A. mearnsii* in particular, that roots actively penetrated the faecal sludge. Therefore, the hypothesis that roots would not proliferate in the faecal sludge core is rejected. It is however noteworthy that the faecal sludge was very wet on application and in that state was unlikely to have been conducive to root growth itself due to the apparently anoxic condition of the sludge. Eventual root penetration may only have been possible due to the progressive drying of the sludge following burial (Foxon, unpublished data).

Fine root (<1 mm) intersections were not enumerated in this study although in both species a superficial fine root mat was visible, but fine roots decreased sharply with depth. This is the usual pattern of fine root distribution in forest species (da Silva *et al.*; 2009). Bouillet *et al.* (2002) found that in a 1 year old stand most fine roots (<1 mm) were found in the top 20 cm of soil below which fine roots were scarce. In 6-month old monospecific stands of *E. grandis* and *A. mangium* the greatest fine root (<3mm) density occurred in the top 30 cm of soil (da Silva *et al.*; 2009). A fine root mat was visible along the periphery of the faecal sludge core which is indicative of the high nutrient status and possibly the relatively high water content of the sludge. Fine roots have been shown to proliferate in nutrient patches (Gross *et al.*, 1993; Hackett, 1972; Drew and Saker, 1975, 1978; Jackson and Caldwell, 1989) including patches of increased water supply (Pregitzer *et al.*, 1993).

Root biomass could not practically be determined in experimental pots since roots could not be separated from the faecal sludge which was dense and unworkable. Therefore, comparisons of root biomass between experimental and control saplings could not be made. However, root biomass can be inferred from the total number of root intersections enumerated at the three depths used. In this respect, a mean of 155 and 89 root intersects occurred in control and experimental saplings of *E. grandis*, respectively, which suggests that control saplings had a greater root biomass than experimental saplings of that species. It is well-known that plants allocate more biomass to roots in response to decreasing nutrient availability (Poorter and Nagel, 2000) and the relatively nutrient poor environment in control pots may have resulted in increased root biomass in those particular saplings. This allows plants to forage for nutrients over a greater volume of soil to meet nutrient requirements for growth (Evans and Edwards, 2001). Cromer and Jarvis (1990) observed an increase in the ratio of biomass allocation to roots in seedlings of *E. grandis* in response to reduced nitrogen availability. Working with the same species, Kirschbaum *et al.* (1992) reported that the ratio of root to dry leaf mass was negatively related to phosphorous availability. Accordingly, the apparently reduced root biomass in experimental saplings of *E. grandis* may have been the result of the relative abundance of nutrients in the faecal sludge compared with the control. However, in saplings of *A. mearnsii* a mean of 46 and 164 root intersects occurred in control and experimental saplings, respectively, which is opposite to the trend observed in *E. grandis*. This represents a 357% increase in root intersects in experimental saplings of *A. mearnsii*. Despite this, more fine roots could be observed in control saplings of that species and these roots are involved in nutrient and water acquisition.

4.2. Growth of table vegetables in sand amended with faecal sludge

4.2.1. Plant growth

Faecal sludge application enhanced growth in *B. vulgaris* and *S. melongena* relative to unamended river sand. For the sake of brevity the chemical composition of the faecal sludge and river sand is not discussed here as it was broadly similar to that used in the

tree growth experiment. Two key growth parameters, shoot length and number of leaves per plant, were positively and linearly related to amendment rate in *B. vulgaris* (and, tentatively, *S. melongena*). The immediate positive growth response of plants to faecal sludge application relative to the unamended treatment indicates that sufficient nutrients were immediately available to plants from the applied faecal sludge. The river sand used was not suitable for crop production in its unamended form or without added nutrients and T-0 showed the least growth. The beneficial effect of faecal sludge on plant growth observed here is in accordance with the findings of Morgan (2007) using the arborloo system, and consistent with findings using not dissimilar kinds of waste such as UD waste (Guzha *et al*., 2005; Rodda, pers. comm) and sewage sludge (Li *et al*., 2009; Wang *et al*., 2008). Plants grown in river sand with applied fertilizer generally showed the greatest growth which could suggest a slight nutrient deficiency in plants grown in the amendments, even at the highest amendment rate. It is however probable that greater growth could have been achieved at higher application rates, perhaps even matching or exceeding that observed for fertilized plants. Linear regression models for shoot length and leaf count indicate that at about 40% amendment rate both these parameters would approximate that observed in fertilized plants, assuming that linearity is maintained to allow for extrapolation. High application rates of sewage sludge have been shown to produce the largest increase in growth and yield. Li *et al*. (2009) reported that maximum growth in Canna (*C. indica*) occurred in a clay-sludge amendment of 75% sewage sludge (dry weight basis) or 495 $t.ha^{-1}$ but plants did not survive in 100% sewage sludge. Wang (2008) found that the highest biomass of the grass *Zoysia japonica* was at the highest sewage sludge application rate of 150 $t.ha^{-1}$ at the first harvest after application but was highest in 15 and 60 $t.ha^{-1}$ at second harvest the following year. However, Singh and Agrawal (2007) reported decreases in growth in *B. vulgaris* with increasing sewage sludge application of 20% and 40% relative to unamended soil. Those authors attributed the decrease in growth to metal stress arising from the high metal content in the sewage sludge used in the amendments.

Leaf senescence was premature in *B. vulgaris* grown in the amendments, particularly in plants in the 0%, 10% and 20% amendments but also in the 30% amendment. The premature senescence observed in those treatments was probably due to varying degrees of nutrient stress and plants responded by senescing older leaves to allow for

nutrient remobilization to new sinks (Munné-Bosch and Alegre, 2004; Guiboileau *et al*., 2010). Using this 'altruistic' mechanism plants are able to ensure, or enhance, overall survival by sacrificing plant parts and translocating nutrients accumulated in senescing tissue to more productive plant parts (Munné-Bosch and Alegre, 2004; Guiboileau *et al*., 2010). In the unamended treatment leaf count remained relatively unchanged from the middle to latter stages of growth but leaf turnover was visibly high (data not recorded) suggesting that, in such an extreme case of nutrient stress, plants were unable to produce new leaves without recycling nutrients by initiating senescence of older leaves.

Comparisons of growth of *S. melongena* at the different amendments were only possible in the early part of the experiment since a number of plants quickly became diseased. Visual observations suggest that this was *Fusarium* spp. which thrived in the warm and humid growth conditions. Interestingly, no plants in T-3 became infected at any point during growth, which may have been the result of unsuitable conditions for the establishment of *Fusarium* spp. in the 30% amendment. Only growth of fertilized plants and plants in the 30% amendment could be evaluated at the end of the experiment. Nevertheless, the indications from early growth of *S. melongena* (while all treatments were present) are that growth was enhanced by sludge application, especially at the highest application rate. The biomass of fertilized *S. melongena* was almost twice that observed for plants in the 30% amendment, which is a similar magnitude of difference observed for biomass of *B. vulgaris* in equivalent treatments. As noted for *B. vulgaris* it is possible that higher application rates may have further improved growth in *S. melongena* relative to fertilized plants. In a similar study to the one presented, Bletsos and Gantidis (2004) grew *S. melongena* in peat or river sand amended with 25%, 50%, 75% and 100% sewage sludge and observed the greatest height in the 50% amendment while stem diameter was greatest in peat followed by the 50% amendment.

Plants grown in the 30% faecal sludge were able to reach maturity (bloom) and the time to maturity was not significantly different from those plants which received chemical fertilizer. Despite lack of replicates, it could be stated tentatively that plants in T-2 (and possibly T-0 and T-1) were not able to reach maturity, at least within a normal timeframe. Plants grown in the 30% amendment began to bloom 40 days after

planting which compares favourably to that of 52 days for *S. melongena* irrigated with treated wastewater in Jordan (Al-Nakshbandi *et al*., 1997). However, blossom drop was observed to be particularly high in plants in the 30% amendment (data not recorded) and so fruit set was quite low and this was reflected in the lower biomass of flowers and fruit at harvest compared to fertilized plants. Based on this, while plants were harvested before fruit could reach appreciable size, it could be surmised that the longer-term yield would likely have been reduced in plants in the 30% amendment (relative to fertilized plants), unless those plants had adopted a different fruiting strategy (fewer but larger fruit). Nevertheless, the application of nutrient-rich waste has been shown to increase yield in *S. melongena*. Irrigation of *S. melongena* with effluent has been demonstrated to increase yield (Chimonidou *et al*, 1988) and Al-Nakshbandi *et al*. (1997) report a yield of 56.3 $t.ha^{-1}$ for effluent-irrigated *S. melongena* which was about twice the average yield of 28.5 $t.ha^{-1}$ using the normal fertilizer application in Jordan. Al-Nakshbandi *et al*. (1997) partly attributed that increase in yield to the abundance of available N, P and K in the irrigation effluent.

Leaf area of *B. vulgaris* was greatly affected by amendment rate and this response was non-linear. In this respect, plants in the 20% amendment had a leaf area almost 3 times less than plants in the 30% amendment but was similar to that of plants in the 10% amendment. Leaf area was several-fold greater in all treatments but less in the unamended treatment than that reported by Singh and Agrawal (2007) for *B. vulgaris* grown in sewage sludge. A similar non-linear response to amendment rate was observed for biomass. Based on the response of biomass in *B. vulgaris*, overall plant growth was non-linearly related to amendment rate and shoot length and leaf count were therefore relatively poor predictors of overall plant growth.

4.2.2. Foliar nutrients

Of the macronutrients N, P and K, only N showed a generally consistent trend of increasing concentration with increasing amendment rate in *B. vulgaris* while P and K were notably variable across treatments. The higher foliar N concentration in fertilized plants compared with other treatments was evidenced by visibly darker green leaves. Foliar N in *B. vulgaris* was found to be positively related to biomass (R^2=0.52) which indicates that foliar N was most likely a limiting nutrient, or

determinant of growth, in *B. vulgaris* due to its important role in leaf proteins and chlorophyll production. Foliar P occurred in a similar range for *B. vulgaris* in 10%, 20% and 30% amendments but was about three times lower in fertilized plants and plants in the unamended treatment. Foliar K was quite considerably greater in fertilized plants compared to other treatments and this may indicate that K was deficient in remaining treatments. In this respect, a few plants in the unamended treatment displayed a purple-red colouration characteristic of potassium deficiency although plants in other treatments did not present similar symptoms despite similar foliar K concentrations. Supportive of a N and K co-limitation was the low N:P and K:P ratios of treatments T-0, T-1, T-2 and T-3 relative to T-4 (Koerselman and Meulemann, 2004; Gotelli *et al.*, 2008). This is despite the absence of apriori determinations of normal macronutrient ratios for that species since foliar macronutrient concentrations and their relative concentrations in T-4 compare favourably with that reported by Dzida and Pitura (2008) for well fertilized plants of the same species. The similar ratios of N:K in T-0, T-1 and T-2 together with reduced foliar concentrations of N and K compared with T-4 suggest that neither N or K limitations predominated in these treatments. However, the high N:K ratio in T-3 implies that K was more limiting than N in that particular treatment.

That N was suspected to be limiting or co-limiting to growth in all treatments but the fertilized treatment is however contradicted by concentrations of these nutrients in the amendments or sand which were generally higher by harvest in treatments T-0 to T-3 than that of fertilized plants although this was not the case regarding K. This would suggest that K was limiting and not N. It is however suggested that sludge heterogeneity could have led to substantial over estimations of nutrients in the amendments and that foliar measurements were a more reliable predictor of specific nutrient limitations to growth.

Foliar N and K in *B. vulgaris* in the unamended treatment was relatively high compared to other treatments despite far poorer nutrient availability. It is suggested that plants in the unamended treatment were able to maintain relatively high nutrient levels by adjusting sink size based on available resources through leaf senescence. Accordingly, and to different extents, plants in the 10%, 20% and 30% treatments must have employed premature leaf senescence as part of a similar strategy. Plants in

the unamended treatment also had the highest foliar Cu, Mn, Fe and Al but micronutrients are generally regarded as more or less immobile (Grusak *et al*., 1999) and consequently these nutrients would mostly not have been recycled through leaf senescence. In spite of this, foliar micronutrients were not limiting (based on their concentrations in fertilized plants) and so relatively high concentrations of these nutrients could be maintained.

Similar trends in foliar nutrients to that observed in *B. vulgaris* were observed in *S. melongena*. In this respect, foliar N and K were considerably greater in fertilized plants, especially foliar K which was about two times greater in fertilized plants. Fawzy *et al*. (2007) examined the effects of varying K application rates on *S. melongena* and found that foliar K concentration was 24.8 $mg.g^{-1}$ when vegetative growth and total yield was highest. Al-Nakshabandi *et al*. (1997) reported a foliar K concentration of 16.0 and 32.8 $mg.g^{-1}$ for field-grown *S. melongena* irrigated with water or treated effluent, respectively, with the greatest yield occurring in the latter. In the present study, the foliar K concentration of plants in the 30% amendment was 22 $mg.g^{-1}$ which was only slightly lower than that reported by Fawzy *et al.* (2007) and within the range reported by Al-Nakshabandi *et al*. (1997). Additionally, Al-Nakshabandi *et al*. (1997) reported foliar N and P concentrations of 23.66 and 3.41 $mg.g^{-1}$, respectively, for *S. melongena* irrigated with effluent which was less than that found in plants in the 30% amendment of 31 and 8 $mg.g^{-1}$ for those respective nutrients. However, despite the indications in literature that N and K were not limiting in *S. melongena* in the 30% amendment, these were the only foliar nutrients of those measured to show considerably lower concentrations than fertilized plants. In this regard, foliar Zn, Cu, Mn and P concentrations were greater ($p<0.05$) in plants in the 30% amendment compared to fertilized plants suggesting that these nutrients could not have been deficient in that treatment. As with *B. vulgaris*, the low N:P and K:P ratios compared with fertilized plants are suggestive of co-limitation by N and K although the relatively high N:K ratio in T-3 implies that K was more limiting than N. However, while leaves in the 30% amendment showed no visible symptoms of K deficiency, leaves were lighter green than T-4 (as observed in *B. vulgaris*), which implies that foliar N was low. It can therefore be inferred that N, and possibly K, were limiting in the 30% amendment. However, concentrations of N and K were higher in the amendment than in the fertilized sand which contradicts this conclusion. This is

similar to what was observed for *B. vulgaris* and the reason for the incongruence between N and K concentrations of leaves and the amendment/sand is likewise the possible result of sludge heterogeneity.

4.2.3. Gas exchange

Photosynthetic parameters generally improved with increasing sludge application rate in *B. vulgaris*. In this respect, A_{max} and J_{max} were greatly increased by sludge application relative to the unamended treatment with the greatest values for these parameters occurring at the highest application rate of 30%. Singh and Agrawal (2010) reported increasing photosynthetic rates in rice with increasing sewage sludge amendment rates. Those authors attributed those increases in photosynthetic rate to increased nutrients arising from applied sludge which increased total chlorophyll content. While chlorophyll content was not measured in the present study, it has been shown, together with CO_2 assimilation, to be positively related to foliar N levels in sunflower (*Helianthus annuus* L.; Ciompi *et al*., 1996) sorghum (*Sorghum bicolour* L.; Zhao *et al*., 2005) and spring wheat (*Triticum aestivum* L.; Evans, 1983). Sinclair and Horie (1989) reported that leaf N and CO_2 assimilation were strongly correlated in three crops viz. soybean (*Glycine max* [L.] Men.), rice (*Oryza sativa* L.), and maize (*Zea mays* L.). Therefore higher foliar N in the present study probably increased chlorophyll content which in turn enhanced photosynthetic rate. In this respect, foliar N accounted for 42% of variability observed in A_{max}. While A_{max} and J_{max} were similar in *B. vulgaris* between fertilized plants and plants in the 30% amendment, for the equivalent treatments in *S. melongena* both these parameters were greater in fertilized plants ($p<0.05$). Foliar N was 45% higher in fertilized plants compared to plants in the 30% amendment which could account for the difference in A_{max} for the reasons discussed above. The A-C_i curves for *B. vulgaris* indicate a typical bi-phase limitation to A (Long and Bernacchi, 2003). In this respect, Rubisco became increasingly limiting at low C_i as amendment rate decreased, followed in suit by a RuBP regeneration limitation at higher C_i. The greater J_{max} associated with the regeneration of RuBP in sludge amendments (compared to the unamended treatment) was probably an effect of nutrient availability. This is supported by the fact that fertilized plants of both species had a greater J_{max} than the other treatments, although

this difference was significant only in *S. melongena*, probably since *S. melongena* has high nutrient requirements (Hedge, 1997).

Spot measurements of A for both species yielded unexpected results. In this respect, A was lower (and considerably so in treatments T-2, T-3 and T-4) in all treatments of *B. vulgaris* than A measured at similar light intensities during the light curves. In *S. melongena* spot measurements of A were greater than A_{max} in both treatments despite the fact that light intensity was not saturating. It is likely that spectrum accounts for these somewhat unexpected results as environmental conditions were similar to that used during the light curves. Furthermore, the shape of light curves could have been quite different if daylight had been used as actinic light. Therefore these findings demonstrate that care should be taken when interpreting gas exchange data taken using an actinic spectrum which differs from the growth spectrum. In this respect, the use of red and blue light emitting diodes has become the norm for actinic light in photosynthetic apparatus (such as in the one used here) and results are frequently assumed to be comparable to that occurring *in situ*.

Photorespiration rate increased in *B. vulgaris* with increasing sludge amendment rate but occurred in rough proportion to net CO_2 assimilation for both species. Lawlor *et al*. (1987) reported that photorespiration was proportional to CO_2 assimilation in wheat (*Triticum aestivum*). Photorespiration rate was however exceedingly high amongst treatments of *B. vulgaris*, ranging from 68 to 76% of net photosynthesis and was equally high in *S. melongena*. The high photorespiration rate may be partly attributed to the fairly high temperature (28ºC) at which measurements were taken (Lawlor *et al*., 1987) resulting in higher oxygenase activity of Rubisco (Sage and Sharkey, 1987). However for both species observed photorespiration rates far exceed high estimates of 50% of net photosynthesis (Zelitch, 1975) and this would have had a detrimental impact on biomass accumulation in all treatments (Zelitch, 1992) since about 90% of the dry weight of plants is derived from CO_2 assimilated through photosynthesis (Zelitch, 1982).

V_{cmax} in *B. vulgaris* was positively related to amendment rate which may be a consequence of improved foliar nutrient levels as a result of higher nutrient availability. In this respect, foliar N was found to be positively related to V_{cmax}

(R^2=0.48). Moon *et al*. (1990) found that carboxylation efficiency was enhanced at higher foliar N levels in wild strawberry (*Fragaria chiloensis*). A considerable proportion of leaf N is present in thylakoids and Calvin cycle proteins (Evans, 1989) of which about 13 to 37% is present in Rubisco (Makino *et al*., 1992). Rubisco content has also been shown to increase with greater foliar N in pea (Makino *et al*., 1991) and spinach (Evans and Terashima, 1988). Therefore, it is proposed that the mechanism of increase in V_{cmax} was through higher foliar N which increased Rubisco content.

4.2.4. Chlorophyll fluorescence

Chlorophyll fluorescence parameters F_o and F_v/F_m were measured on plants shortly before harvest to determine the possible effects of sludge application on the performance of photosystem II (PSII). F_o was negatively related to sludge application in *B. vulgaris* (R^2=0.50). A lower F_o can indicate decreased chlorophyll content due to lower reabsorption of fluorescence by remaining chlorophyll (Lichtenthaler, 1988). Therefore, it is possible that chlorophyll content decreased in plants with decreasing amendment rate primarily due to nutrient stress and this caused the corresponding reductions in F_o. Singh and Agrawal (2006) reported increasing F_o in *B. vulgaris* with increasing sewage sludge amendment rate although that was the result of damage to PSII due to heavy metal toxicity while Li *et al*. (2009) found that F_o decreased with increasing sewage sludge amendment rate in Canna (*Canna indica*). The F_v/F_m ratio in *B. vulgaris* generally increased with increasing amendment rate but increments were small and only the difference between fertilized plants and plants in the 30% amendment was significant at the p=0.1 level. Li *et al*. (2009) observed similarly insignificant changes of F_v/F_m in *C. indica* despite large increases in amendment rate. The F_v/F_m ratio for both species and all treatments combined ranged from 0.80 to 0.83 which is within the range of 0.75 to 0.85 for healthy plants (Demming and Bjarkman, 1987). This was surprising since all other indications were that plants in the 10% and 20% amendments (and possibly even 30%) and especially those in the unamended sand were not healthy, to varying extent, and the maximum quantum yield of PSII was expected to decline accordingly. Nitrogen stress has been shown to negatively affect maximum quantum yield of PSII in Maize (Sepehri and Modarres Sanavy, 2003) and this could account for differences observed in both species although these

differences were negligibly small. A possible explanation for the similar values of F_v/F_m is that the number of PSII reaction centres was reduced in treatments which were nutrient limited but PSII efficiency was not affected. In this way, nutrient stressed plants do not compromise PSII function but instead decrease the number of PSII reaction centres as this represents more prudent use of limited resources.

5. Conclusion

The present study showed that faecal sludge was able to support plant growth by supplying plants with nutrients and may have had additional soil enhancing properties. Growth and photosynthesis of *E. grandis* and *A. mearnsii* where faecal sludge was applied was enhanced compared to a control treatment where commercial fertilizer was applied. In a separate experiment the relationships between the rate of sludge application and measured growth and photosynthetic parameters in *B. vulgaris* and *S. melongena* were positive, consistent with the noted benefits of sludge application. Unlike sewage sludge which can be high in metals and other potentially phytotoxic chemicals, the faecal sludge used here had the benefit of being low in metals and no phytotoxicity could be observed in plants. Future work needs to carefully investigate suitable application rates of faecal sludge to avoid the possibility of excessively high application rates which could lead to leaching of nutrients and consequent groundwater pollution. In this respect, the amount of faecal sludge applied will have to take into account soil type, environmental conditions and species. Only river sand was used in the present study which, due to its infertility, would have been biased towards higher applications of faecal sludge. In the food crop trial, plants which received commercial fertilizer accumulated more biomass than plants grown in the amendments; it is however likely that far smaller amendment rates would be required to attain similar growth to fertilized plants if even a moderately infertile soil was used.

Often overlooked is that faecal sludge has a potential economic value as a nutrient source; fertilizer shortages predicted in the next few decades, especially of phosphorous from mined phosphate rock, mean that this may need to be reconsidered. Perhaps the only notable drawback of faecal sludge in plant production is the risk it poses to human health. Research is required to quantify the health risk and to determine how such risk can be effectively managed. It can therefore presently be concluded that, notwithstanding the risks posed by faecal sludge to human health, faecal sludge showed great potential for plant production and represents a chiefly unexploited source of valuable plant nutrients.

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Appendix

	Mondi orange	Nitrosol	Trelmix	Chemicult
N ($g.kg^{-1}$)	129	80	-	65
P ($g.kg^{-1}$)	176	20	-	27
K ($g.kg^{-1}$)	111	58	-	130
Ca ($g.kg^{-1}$)	-	6	-	70
Mg ($g.kg^{-1}$)	-	6	0.3	22
S ($g.kg^{-1}$)	-	4	-	75
Fe ($g.kg^{-1}$)	-	0.006	22.6	1.5
Mn ($g.kg^{-1}$)	-	0.040	2.9	0.240
B ($g.kg^{-1}$)	-	0.023	1.1	0.240
Zn ($g.kg^{-1}$)	-	0.001	2.4	0.050
Cu ($g.kg^{-1}$)	-	0.001	3.2	0.020
Mo ($g.kg^{-1}$)	-	0.015	0.3	0.010
Gibberellic acid ($g.kg^{-1}$)	-	0.003	-	-