The potential of reusing New Zealand's biowastes combined with native and exotic species for improved environmental and economic outcomes

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Obed Nedjo Lense

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Abstract of a thesis submitted in partial fulfilment of the

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Abstract

Biowastes are unwanted materials of biological origin include biosolids (sewage sludge), Treated Municipal Wastewater (TMW), wood-waste, Dairy Shed Effluent (DSE), and composts made from municipal wastes. Potentially, biowastes can improve soil fertility and reduce the requirement for mineral fertilizers for both degraded and productive lands. However, application to soil may result in the accumulation or leaching of the Nutrients and Contaminants Associated with Biowastes (NCAB) in the environment. Nutrients include nitrogen (N) and phosphorous (P) and other macronutrients, while common contaminants include cadmium (Cd), copper (Cu), and zinc (Zn). In New Zealand (NZ), most biowastes are discharged into waterways (e.g. treated municipal effluent) or disposed of in landfills (e.g. biosolids). This is expensive and represents a waste of a potentially valuable resource. While the application of biowastes to pristine agricultural land may be unacceptable, biowastes may be used to enhance the growth on degraded or marginal lands for the production of timber, fibre, energy, essential oils, or even NZ-native honey. Some of the negative environmental effects of adding biowastes to soil may be offset by the overlying vegetation if such plants take up nutrients that would otherwise leach, provided these plants do not accumulate unacceptable concentrations of contaminants.

I hypothesised NZ native and exotic plants that were selected for their potential economic or ecological value, may improve environmental outcomes of applying biowastes application to low-fertility soil through increased growth, while accumulating minimal concentrations of contaminants in their aerial parts. I also hypothesised that mixing distinct biowastes would reduce accumulation of contaminants and improve soil quality, thus stimulating growth of the plants. I aimed to determine the plant-soil

interactions on biowaste-amended soil using greenhouse experiments and field trials. Specifically, I tested *Leptospermum scoparium*, *Kunzea robusta*, *Kunzea serotina, Olearia paniculata, Coprosma robusta, Podocarpus cunninghamii, Grisilinea littoralis, Pseudopanax arboreus, Phormium tenax, Phormium cookianum, Cordyline australis, Pittosporum eugenioides, Pinus radiata, Brassica napus, Sorghum bicolor,* and *Lolium multiflorum.* Particular attention was paid to *L. scoparium* and *K. robusta* because these NZ-native species produce valuable honey and essential oils.

The biowastes included biosolids, TMW, sawdust, DSE, and compost made from municipal green-waste. Mineral fertilisers were used as comparison for some species. I measured the effects of the biowastes on plant growth and elemental uptake as well as the soil quality. Three glasshouse-based experiments and two field trials were conducted to support the objectives of this research.

Initially, the response of *L. scoparium* and *K. robusta* to individual nutrients was determined using mineral fertilisers on orthic brown soil with a clay-loam texture. Using agronomically-relevant application rates equivalent to 200 kg N ha^{-1}, 100 kg P ha^{-1}, 100 kg K ha^{-1}, 100 kg S ha^{-1}, my experiments showed that only N improved growth. However, the nutrient additions to soil resulted in increased foliar concentrations.

Amending the same soil with 2600 kg N ha^{-1} equivalent of biosolids and 200 kg N ha^{-1} equivalent of DSE improved the growth of both *L. scoparium* and *K. robusta* by 34% and 64%, respectively and increased foliar P, Ca, and S uptake by 33%, 37%, and 32%%. Concentrations of Cd, Cu and Zn increased, but remained within threshold values.

A second experiment, using 10 L lysimeters, showed that biosolids applied at 1200 kg N ha^{-1} equivalent improved the growth of *L. scoparium*, *K. robusta*, *P. radiata, S. bicolor, B. napus* and *L. multiflorum* by 60%, 27%, 61%, 29%, 61% and 77%, respectively. The beneficial effect of biosolids was slightly offset when it was mixed in equal volumes with sawdust. In general, the biowastes produced a larger growth response than urea applied at 200 kg N ha^{-1} equivalent, while the N leaching under biosolids was generally lower. There was a significant species effect on N-leaching, with *L. scoparium* and *K. robusta* leaching significantly less N than the other species. None of the species accumulated unacceptable concentrations of contaminants.

In a field trial on a Pawson Silt Loam, the irrigation of TMW at 500 mm yr^{-1} improved the growth of some, but not all species tested. A trial comprising 11 native species, namely *L. scoparium, K. robusta, O. paniculata, P. arboreus, C. robusta, P. cunninghamii, G. littoralis, P. eugenioides, C. australis, P. tenax,* and *P. cookianum* was established on ca. 1000 m^2 of land near the town of Duvauchelle. Trees irrigated with TMW grew better than or the same as unirrigated trees. There were no signs of toxicity.

The plants with the greatest positive response to TMW were *L. scoparium, O. paniculata, C. robusta, Podocarpus cunninghamii, Cordyline australis,* and *Phormium tenax.*

A second field trial at the former Eyrewell forest showed that only *K. serotina* responded positively to the application of municipal compost (1200 kg N ha^{-1} equiv.) and a DSE-sawdust mixture (2400 kg N ha^{-1} equiv.).

This thesis shows that a diverse range of NZ biowastes can be used to promote the growth of NZ-native and exotic species, without resulting in unacceptable concentrations of contaminants in the plants or soils. Whereas TMW and DSE could be continually applied to plants, the continual application of biosolids may result in the accumulation of contaminants in soil. Therefore, the biosolids application would be more suited to a single application to restore a low-fertility or degraded soil. Mixing the biosolids with sawdust may further reduce plant contaminant uptake or NO_3^- leaching. This beneficial reuse of biowastes will reduce disposal costs, while providing valuable economic or ecological benefits. There was some evidence in this thesis that some NZ-native plants, namely *L. scoparium* and *K. robusta*, may alter nutrient cycling in soil and therefore further reduce NO_3^- leaching. These rhizosphere studies should be the subject of future research.

Keywords: Biowastes, New Zealand native plants species, plant growth, nutrients uptake, soil quality, contaminants, NO_3^- leaching, rhizosphere, root exudate, nitrogen cycle

Publications

- Paramashivam, D., Timothy J. Clough, Nicholas M. Dickinson, Jacqui Horswell, **Obed Lense**, Lynne Clucas, Brett H. Robinson. (2016). The effect of pine waste and pine-biochar on nitrogen mobility in biosolids. *Journal of Environmental Quality.* 45:360–367. doi:10.2134/jeq2015.06.0298

- Esperschütz, J., **Lense, O**., Anderson, C., Bulman, S., Horswell, J., Dickinson, N., & Robinson, B. (2016). Biowaste Mixtures Affecting the Growth and Elemental Composition of Italian Ryegrass (Lolium multiflorum). *Journal of Environmental Quality,* 45:1054–1061. doi:10.2134/jeq2015.09.0459

- Esperschuetz, J., Anderson, C., Bulman, S., **Lense, O**., Horswell, J., Dickinson, N, Robinson, B. H. (2016). Production of Biomass Crops Using Biowastes on Low-Fertility Soil: 1. Influence of Biowastes on Plant and Soil Quality. *Journal of Environmental Quality, 45*(6), 1960-1969. doi:10.2134/jeq2015.12.0596

- Esperschuetz, J., Bulman, S., Anderson, C., **Lense, O**., Horswell, J., Dickinson, N., & Robinson, B. H. (2016). Production of Biomass Crops Using Biowastes on Low-Fertility Soil: 2. Effect of Biowastes on Nitrogen Transformation Processes. *Journal of Environmental Quality, 45*(6), 1970-1978. doi:10.2134/jeq2015.12.0597

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Table of Contents

List of Tables

List of Figures

List of Plates

Chapter 1

Introduction

1.1 General introduction

Biowastes are unwanted materials of biological origin. They include biosolids (sewage sludge), Treated Municipal Wastewater (TMW), municipal compost, Dairy Shed Effluent (DSE) and wood waste. They can contain high concentrations of plant nutrients, which potentially improve soil fertility and reduce the requirement for mineral fertilizers for both degraded and productive lands (Albihn and Vinnerås, 2007; Basta et al., 2015; Bruun et al., 2006; Hargreaves et al., 2008; Hawke and Summers, 2006; Lagae et al., 2009; Lopes et al., 2011; Minhas et al., 2015; Mohammad Rusan et al., 2007; Veeken and Hamelers, 2002). However, adding biowastes to soil may have negative environmental consequences including the accumulation in soil or leaching of Nutrients and Contaminants Associated with Biowastes (NCAB). Negative environmental consequences of NCAB addition to soil include excessive NO_3^- leaching, accumulation of heavy metals or xenobiotics, and pathogens that may endanger human health (Agopsowicz et al., 2008; Correa et al., 2006; Di et al., 1998; Hawke and Summers, 2006; Krogmann et al., 1997; Lavado et al., 2005; Mohammad Rusan et al., 2007; Qiang et al., 2004; Singh and Agrawal, 2008; Stoven and Schnug, 2009; Vogeler et al., 2006). Excessive NO_3^- leaching can contribute to the eutrophication of lakes and rivers as well as contaminate groundwater (Davis, 2014; De Vries et al., 2013; Fowler et al., 2013), detrimentally affecting human health (Agopsowicz et al., 2008; Galbally et al., 2013; McFarland et al., 2013). Biowastes contain a mixture of both organic and inorganic N (Angle et al., 1993). NO_3^- leaching is dependent upon the mineralisation of organic N to NH_4^+ and thence nitrification to NO_3^-, which is mobile in soil (Dick et al., 2000).

The role of plants to cope with the negative consequences of biowastes has attracted considerable scientific attention (Chague-Goff, 2005; Domínguez et al., 2008; Galbally et al., 2013; Lomonte et al., 2010; McCutcheon and Schnoor, 2004; Prosser, 2011; Robinson et al., 2007; Robinson et al., 2009; Tanner, 2001; Wang and Jia, 2010). For instance, the New Zealand native plants manuka (*Leptospermum scoparium*) and kanuka (*Kunzea robusta*) exude bioactive phytochemicals, either from the roots or from leaf fall, which significantly reducing the evolution of nitrous oxide (Fitzgerald, 2012; Hedley et al., 2013) and kill pathogens in biosolids-amended soil (Fitzgerald, 2012; Prosser, 2011). *L. scoparium* and *K. robusta* are pioneer species in the myrtaceae family that are widely distributed in New Zealand. They are commonly found in degraded environments and low fertility soils where the lands have received less agricultural inputs (Bertin et al., 2003; Stephens et al., 2005; Wardle, 1991).

L. scoparium, in particular, has been recognized as the most widely distributed, abundant, and environmentally tolerant native species among New Zealand woody plants (Ronghua et al., 1984; Stephens et al., 2005). Both *L. scoparium* and *K. robusta* have been used in land restoration of mine sites and degraded lands to improve soil quality, promote high invertebrate and species richness, increase soil ecosystem recovery, and promotes a self-sustaining plant community (Burrows et al., 1999; Craw et al., 2007; Thomas et al., 2014). These species rapidly colonise disturbed lands and erosion-prone pastoral hill country, resulting in erosion mitigation and soil conservation (Stephens et al., 2005). In addition to their fast growth, they recently become recognized in NZ as a potentially important C sink (Scott et al., 2000; Trotter et al., 2005). *L. scoparium* could provide commercial benefits through the production of high value honey (Beitlich et al., 2014; Steinhorn et al., 2011) and essential oils that have antimicrobial properties (Maddocks-Jennings et al., 2005; Song et al., 2013). *L. scoparium* honey provides ca. $315m per year to NZ's economy (MPI, 2016). Potentially, *L. scoparium* and *K. robusta* could be established on low-fertility or degraded soils that have been amended with biowastes such as biosolids and sawdust (Esperschuetz et al., 2017).

Other species that may be grown on soils amended with biowastes are sorghum (*Sorghum bicolor*) and oilseed rape (*Brassica napus*). These two species have been reported to remove contaminants from the soil and reduce NO_3^- leaching into waterways (Barceló and Poschenrieder, 2003; Licht and Schnoor, 1993; Pilipovic et al., 2006; Turan and Esringu, 2007; Wang et al., 2009).

Previous studies have shown that blending distinct biowastes may affect the fluxes of NCAB following their addition to soils. Blending biosolids with biochar significantly reduces NO_3^- leaching, while improving plant growth (Knowles et al., 2011). Lignite significantly reduces Cd accumulation by pasture on biosolids-amended soil (Simmler et al., 2013). Sawdust can reduce plant Cd uptake from biowaste-amended soil (Bugbee, 1999a; Daniels et al., 2001; Schmidt et al., 2001). In particular, mixing wood-waste (raw dried pine sawdust) with biosolids-amended soils showed a significant reduction in N mobility in biosolids and potentially reduced NO_3^- leaching (Paramashivam, 2015b). The study also demonstrated that mixing wood-waste (pine biochar) did not affect the NO_3^- leaching, but significantly decreased the mobility of NH_4^+ (Paramashivam, 2015b).

I hypothesised NZ native and exotic plants that were selected for their potential economic or ecological value, may improve environmental outcomes of applying biowastes application to low-fertility soil through increased growth, while accumulating minimal concentrations of contaminants in their aerial parts. I also hypothesised that mixing distinct biowastes would reduce accumulation of contaminants and improve soil quality, thus stimulating growth of the plants.

1.2 Aims, objectives, and benefit of the research

1.2.1 Aim

The aims of the research were to determine the effect of biowastes on the growth of the plants and to investigate how New Zealand native and exotic vegetation play a role in reducing the negative effect of (NCAB).

1.2.2 Objectives and thesis structure

The objectives of this research were to determine:

1. the effect of the application of individual macronutrients on the growth and elemental uptake of *L. scoparium* and *K. robusta* (Chapter 3).

2. the effect of adding biosolids and DSE to the soil on the growth and elemental uptake of *L. scoparium* and *K. robusta* (Chapter 4).

3. the growth, elemental uptake and NO_3^- leaching of *L. scoparium, K. robusta, L. multiflorum, S. bicolor, B. napus,* and *P. radiata* on soils amended with biosolids, biosolids+sawdust, and urea (Chapter 5).

4. how *L. Scoparium*, *K. robusta*, *O. paniculata, C. robusta, P. cunninghamii, G. littoralis, P. arboreus, P. tenax, P. cookianum, C. australis,* and *P. eugenioides* respond to the application of treated municipal wastewater (TMW) in a field trial (Chapter 6).

5. the response of *L. scoparium* and *K. serotina* to the application of compost and mixed of sawdust and dairy shed effluent on degraded/low fertility soil (Chapter 7)

This research seeks to improve our capacity to understand the relationship between plant species for alleviating negative environmental consequences associated with NCAB, which may lead to environmental or economic benefits.

Chapter 2

Literature Review

Based on the existing literature, I give an overview of how plants could play a significant role in mitigating environmental consequences following biowastes application to soil, with particular emphasis on several aspects related to the fluxes of Nutrients and Contaminants Associated with Biowastes (NCAB). I focus primarily on how plants play an important role in improving the negative environmental outcomes following the application of biowastes through evapotranspiration, root exudates, root-microbe interactions, and leaf litter contribution on the flux of NCAB.

2.1 The role of evapotranspiration in changing fluxes of NCAB

Evapotranspiration (ET) is the combination of two different processes whereby water is lost from the land and converted to water vapour, either by evaporation from a surface (such as lakes, rivers, pavements, and soils) or by plant transpiration (Allen et al., 1998). The movement of plant water through transpiration creates ideal soil-water conditions for the dissolution of contaminant molecules, and movement toward roots, thus increasing rhizosphere reactions (Brady, 2008). When a large amount of water is removed from soil by ET, the downward flow of water decreases through the soil, thereby reducing nutrient and contaminant leaching into surface and ground water (Pulford and Watson, 2003). Approximately 410 mm out of 710 mm of average annual rainfall that enters the soil is pumped back to the atmosphere through ET from vegetation (Clothier and Green, 1997; Harvey et al., 2002). Depending on the meteorological conditions, ET can reduce of the average water flux in the soil by 57%, and lead to a significant decrease in the volume of soil solution that exits the root-zone and therefore reduces the amount of water that is leached (Robinson et al., 2006). Plants therefore affect the mobilization and transport of certain nutrients (including NO_3^-) which are mobile in their soluble form, therefore their movement through the soil profile is strongly dependent on water transport through the soil (Harvey et al., 2002). In arid regions, this could be significant, particularly when ET may minimize NCAB mobility by reducing drainage (Robinson et al., 2006).

The effectiveness of plants in changing the flux of nutrients and contaminants associated with ET has been well studied (Grifoll and Cohen, 1996; Robinson et al., 2006; Robinson et al., 2007) and is strongly influenced by plant species and climate (Allen et al., 1998; McCulley et al., 2004; Robinson et al., 2007). Every plant species has its characteristic root system and ET characteristics, which directly affect the flux of nutrients and contaminants associated with biowastes**.** Under similar environmental conditions, differences in rooting characteristics resulted in different ET levels (Allen et al., 1998). In

drier conditions, for instance, plant species with deep-rooted systems usually had greater ET rates as they had better access to water during dry periods compared to those plants with shallow-rooted systems (Vogeler et al., 2001). As a result, plant species with deep-rooted systems would still be able to maintain their photosynthesis and increased plant water status and growth during drought (McCulley et al., 2004). Species such as poplar (*Populus* spp.) and willow (*Salix* spp.), which have high evapotranspiration rates, are fast-growing, and high-water use, were successfully employed in this role (Ferro et al., 1997; Robinson et al., 2007). Poplar (*Populus* spp.) grown on wood-waste sites decreased B leaching into surface and ground water (Robinson et al., 2007). Wood crop including white oak (*Quercus alba*), which had a greater rate of evapotranspiration relative to grass species, reduced the leaching of Strontium-90 (Sr-90) by approximately 16% (Garten Jr, 1999). High evapotranspiration rate willow trees (Pauliukonis and Schneider, 2001) grown on top soil and sand and treated with 2.2 L per week of the primary and secondary treated wastewater effluents took up a high proportion of the N and P applied as wastewater (Curneen and Gill, 2014).

In addition to plant species, environmental factors such as temperature and wind speed also affect evapotranspiration rates. Curneen and Gill (2014) found that there was a correlation (P=0.77) between air temperature and evapotranspiration, but there was little correlation (P=0.41) between the average wind speed and evapotranspiration. Additionally, an increase in average temperature during the growing season promoted higher evapotranspiration (Curneen and Gill, 2014). Another important factor that influences ET rate is soil water content, which is strongly dependant on the magnitude of the water deficit and the type of soil. In contrast, excessive amounts of water can lead to waterlogging, which may damage roots and reduce water and nutrient uptake by inhibiting the respiration process (Allen et al., 1998).

Several studies have reported that biowastes application could affect evapotranspiration rates of certain species. For example, as Curneen and Gill (2014) pointed out, the addition of wastewater effluent had a positive effect on the evapotranspiration rates of willow trees (*Salix* spp.). Willow trees grown on wastewater effluent treatment produced higher ET (average=3.9 mm day^{-1}) than trees receiving water treatment by 2.83 mm day^{-1}. Curneen and Gill (2014) demonstrated that willow trees under primary treated wastewater had higher ET values (4.56 mm day^{-1}) compared to the trees receiving secondary treated wastewater effluent (3.38 mm day^{-1}) (Curneen and Gill, 2014). **Figure 2.1** shows evapotranspiration rates (mm day^{-1}) of willow trees treated with wastewater effluent during the 2010, 2011 and 2012 growing seasons. Other studies reported significant ET rates (1790) following the application of wastewater between May and October (Guidi et al., 2008; Martin and Stephens, 2006). Pistocchi et al. (2009) pointed out that the evaporation rates of willow trees (*Salix* spp.) grown

on high concentrations of wastewater treatment were between 1.4 and 2 times greater than those grown on low strength wastewater effluent. A strong correlation between evapotranspiration and plant development was mainly due to the positive influence of greater nutrient availability on plant growth, rather than a specific plant characteristic of the willow trees (Guidi et al., 2008; Pistocchi et al., 2009).

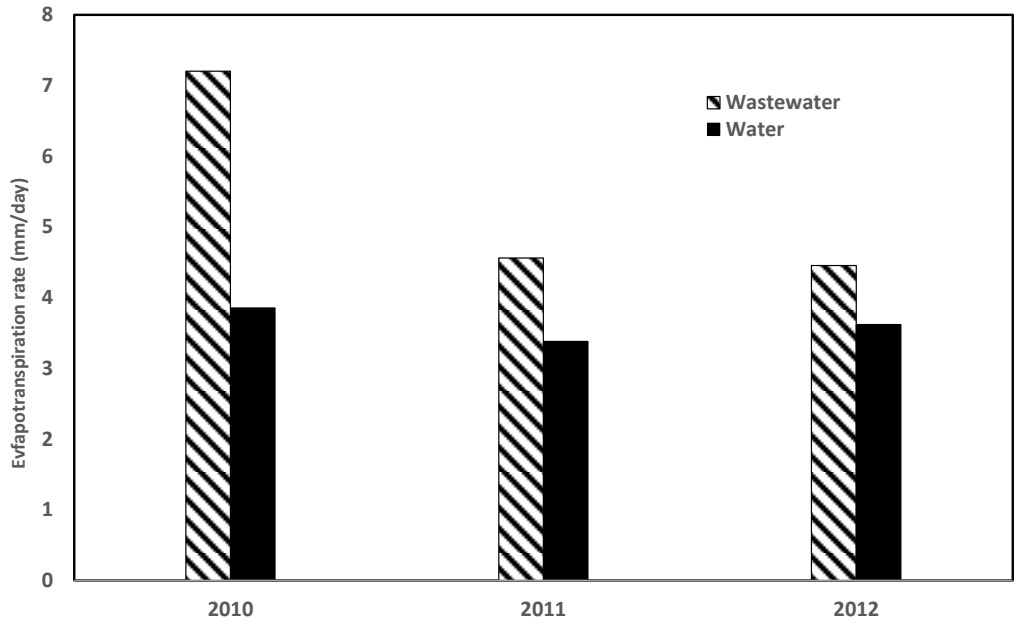


Figure 2. 1 Evapotranspiration rates (mm day^{-1}) of willow trees (*Salix* spp.) treated with different wastewater effluent during the 2010, 2011 and 2012 growing seasons (Curneen and Gill, 2014).

2.2 The physical effects of roots on NCAB transport in soil in terms of root architecture affecting erosion and preferential flow

Roots reduce soil erosion through their ability to modify soil properties including aggregate stability, hydraulic function, and shear strength (Li et al., 2014; Ola et al., 2015). Together with the chemical and biological activity in the rhizosphere, the physical action of the roots contributes to establishment of macropores (Ghestem et al., 2011). Many studies have investigated the role of plant roots in increasing soil preferential flow (Baets et al., 2007; Bogner et al., 2013; Germann et al., 2012; Ghestem et al., 2011; Jørgensen et al., 2002; Zhang et al., 2015). In particular, root architecture such as root diameter, length, orientation, and root density strongly affects soil preferential flow, especially through root channels, which enhance water and nutrient transport across soil profiles (Baets et al., 2007; Germann et al., 2012; Jørgensen et al., 2002). Ghestem et al. (2011) found that root decomposition resulted in greater root mass density and continuously created pores in the soil. This condition increased the transport of water, which may affect the movement of dissolved elements through the soil, via increased infiltration rates (Ghestem et al., 2011).

Root architecture, a key indicator of root development (Głąb, 2013; Mosaddeghi et al., 2009) and root biomass, have attracted attention because of their role in regulating water and nutrient cycling for plant growth (Zhang et al., 2015). As every species has its own root system architecture (diameter, length, orientation, and root density), the effect on soil erosion and preferential flow varies among species. For example, alfalfa (*Medicago sativa)* has a taproot system which increased infiltration rates (decreased surface run off) more than wheat *(Triticum turgidum*), which possesses fine, fibrous roots (Ghestem et al., 2011). Similarly, carrot (*Festuca rubra*), which also has a taproot, reduced erosion rates compared with rye grass (*Lolium perenne*), a fine-branched root species (Baets et al., 2007). Carrots (*Festuca rubra*) with very fine roots (less than 5 mm in diameter) showed a similar negative exponential relationship between root density and relative soil erosion rate to rye grass roots, and the reducing effect became less significant when the root diameter increased between 5 and 15 mm in diameter (Baets et al., 2007). Zhang et al. (2015) pointed out that the variety of root length densities (total root length per soil volume) and root biomasses of Chinese arborvitae (*Platycladus orientalis*)*,* Japanese emperor oak (*Quercus dentate*), and pagoda tree (*Sophora japonica*) had a strong effect on soil preferential flow, with root channels enhancing nutrition transport across soil profiles. This research found that fine root length density (less 5mm in diameter) decreased with increasing distance from soil surface**.**

2.3 Root uptake of NCAB

Plant roots serve several important key functions in the growth of the plant. One of the important roles is in moving and uptake of water and chemicals in soil (Bertin et al., 2003; Clothier and Green, 1997) which happens through interception, commonly known as root absorption, mass flow or through diffusion (Brady, 2008). Interception with plant root movement serves to shorten the distance between the roots and the presence of nutrients. The plant roots grow to a length and extend to get closer to where the elements are. In this specific mechanism, plant roots penetrate the soil pores (nutrients' location), so that between roots and soil, where the nutrients are located, so that the roots and soil nutrients are in close contact, and ion exchange can occur (Brady, 2008). Mass flow is the movement of nutrients from the soil to the roots simultaneously with the movement of the water mass. In this particular mechanism, water containing ionic nutrients flows toward the root or via the root itself. The plants in turn, absorb the nutrients. Absorption through mass flow can be affected by the concentration of nutrients in the soil solution, the amount of water lost through transpiration, and the volume of water that flows through the soil profile, which affects the amount of nutrients that can contact the roots. Lastly, nutrient uptake can occur through the mechanism of concentration

difference (diffusion) which occurs due to nutrient concentration in gradient (Brady, 2008). In addition, soil temperature affects the absorption of nutrients from the soil root (Clarke et al., 2015).

The movement of nutrients from the roots into the plant can be influenced by several factors such as the plant's uptake efficiency, transpiration rate, and the concentration of the nutrients in soil water (Brady, 2008; Erenoglu et al., 2011; Schnoor et al., 1995). As explained in the previous section, several plant species with high evapotranspiration rates such as poplars (*Populus* spp.), willows (*Salix* spp.), and white oak take up higher amounts of nutrients and contaminants associated with biowastes (Białowiec et al., 2007; Curneen and Gill, 2014; Martin and Stephens, 2006; Robinson et al., 2007; Wang and Oyaizu, 2009). The Zn uptake by roots of wheat (*Triticum durum*) was increased when the concentration of N supply increased from low to medium and from medium to high level (Erenoglu et al., 2011). Similarly, as Liu et al. (2015) pointed out, increasing NO_3^- levels from 2.0 to 20 m mol L^{-1} resulted in elevated root uptake rate of NO_3^- in two genotypes of spinach (*Spinacia oleracea*). This study also found that the high-oxalate-accumulating genotype of spinach (Heizhenzhu) showed a greater root uptake rate of NO_3^- compared with Weilv., which is a low-oxalate-accumulating genotype (Liu et al., 2015). Macduff and Wild (1989) found a close relationship between root temperature and the concentration of N which affected root uptake of NCAB. The study demonstrated that the uptake of NH_4^+ and NO_3^- was 50% higher at 13°C and N deficiency condition than that found under continuous N supply. On the contrary, under low N supply at 3°C, the uptake of NH_4^+ was 70% lower, whilst NO_3^- uptake was 50% more than that measured under continuous N application (Macduff and Wild, 1989). In addition to these factors, the role of soil microorganisms has played a key role in nutrient uptake by roots (Adesemoye and Kloepper, 2009; Courty et al., 2015; Hartmann et al., 2009; Lambers et al., 2009). This specific role is explained in more detail in the next sub headings.

Table 2. 1 Selected properties of nutrients and contaminants associated with biowastes (biosolids, dairy shed effluent-DSE, municipal wastewater-MWW, and wood waste) reviewed in this chapter.

Properties	Biosolids		DSE		MWW		Wood waste		References
	Conc.	Reference	Conc.	Reference	Conc.	Reference	sawdust	biochar	Reference
pH	4.1-7.9	(Antolín et al., 2005; Fijalkowski et al., 2011; Knowles et al., 2011; Mok et al., 2013; Paramashivam, 2015b; Smith and Tibbett, 2004; Wang et al., 2005)	7.3-8.2	(Di et al., 1998; Zaman et al., 1999a; Zaman et al., 2002)	7.3	(Mohammad Rusan et al., 2007)	4.3-5.7	5.5	(Bugbee, 1999b; Paramashivam, 2015b)
CEC (cmol kg^{-1})	16.7-52.2	(Paramashivam, 2015b; Wang et al., 2005)	n.d		n.d		10.6	2.2	(Paramashivam, 2015b)
Total C (%)	0.1-38.2	(Antolín et al., 2005; Hue and Sobieszczyk, 1999; Knowles et al., 2011; Paramashivam, 2015b)	n.d		n.d		45-51	71	(Bugbee, 1999b; Paramashivam, 2015b)
Total N (%)	0.02-6.1	(Hue and Sobieszczyk, 1999; Knowles et al., 2011; Paramashivam, 2015b; Smith and Tibbett, 2004; Wang et al., 2005)	0.03-1.8	(Di et al., 1998; Zaman et al., 1999a; Zaman et al., 2002)	0.002-0.01	(Curneen and Gill, 2014; Gersberg et al., 1986; Monnet et al., 2002)	0.06-0.1	0.03	(Bugbee, 1999b; Paramashivam, 2015b)
Total P (mg kg^{-1})	3900- 6600	(Antolín et al., 2005; Fijalkowski et al., 2011; Knowles et al., 2011; Mok et al., 2013; Paramashivam, 2015b; Wang et al., 2005)	21-125	(Di et al., 1998)Longhurst et al., 2000,	10-15.5	(Curneen and Gill, 2014; Mohammad Rusan et al., 2007; Monnet et al., 2002)	n.d	n.d	

Table 2.1 continued

Properties	Biosolids		DSE		MWW		Wood waste		
	Conc.	Reference	Conc.	Reference	Conc.	Reference	sawdust	biochar	Reference
Total K (mg kg^{-1})	700-7300	(Antolín et al., 2005; Fijalkowski et al., 2011; Knowles et al., 2011; Mok et al., 2013; Paramashivam, 2015b; Wang et al., 2005)	n.d		22.6-33.3	(Curneen and Gill, 2014; Mohammad Rusan et al., 2007)	n.d	n.d	
S (mg kg^{-1})	800-16850	(Fijalkowski et al., 2011; Knowles et al., 2011; Mok et al., 2013)	n.d				n.d	n.d	
Cd (mg kg^{-1})	0.2-17	(Antolín et al., 2005; Knowles et al., 2011; Mok et al., 2013; Wang et al., 2005)	n.d		0.02	(Mohammad Rusan et al., 2007)	n.d	n.d	
Cu (mg kg^{-1})	205-5584	(Antolín et al., 2005; Fijalkowski et al., 2011; Knowles et al., 2011; Mok et al., 2013; Wang et al., 2005)	n.d		0.01	(Mohammad Rusan et al., 2007)	n.d	n.d	
Pb (mg kg^{-1})	8.7-385	(Antolín et al., 2005; Knowles et al., 2011; Wang et al., 2005)	n.d		0.77	(Mohammad Rusan et al., 2007)	n.d	n.d	
Hg (mg kg^{-1})	7.6	(Mok et al., 2013)	n.d				n.d	n.d	
Ni (mg kg^{-1})	25-126	(Antolín et al., 2005; Mok et al., 2013; Wang et al., 2005)	n.d				n.d	n.d	
Zn (mg kg^{-1})	54-1754.8	(Antolín et al., 2005; Fijalkowski et al., 2011; Knowles et al., 2011; Mok et al., 2013; Wang et al., 2005)	n.d		0.19	(Mohammad Rusan et al., 2007)	n.d	n.d	
Mg (mg kg^{-1})	300	(Fijalkowski et al., 2011)	n.d				n.d	n.d	
Mn (mg kg^{-1})	39.92	(Fijalkowski et al., 2011)	n.d		0.07-0.87	(Mohammad Rusan et al., 2007; Monnet et al., 2002)	n.d	n.d	

2.4 Root exudates and their role on biowaste degradation, speciation, and transport of NCAB in soil

The effect of root exudates on decomposition (degradation), speciation, and mobilization (transport) of organic and inorganic compounds in the soil matrix is well known (Bertin et al., 2003; Clothier and Green, 1997; Hodge and Millard, 1998; Kozdrój and van Elsas, 2000; Walker et al., 2003). The speciation and mobilization of organic and inorganic substances in the rhizosphere zone is driven by root exudates through: solubilisation by root exudate enzymes and cells; and mobilization by root exudate organic compounds (Bertin et al., 2003; Dakora and Phillips, 2002; Hodge and Millard, 1998; Schilling et al., 1998). Root exudates are one of the most imperative factors influencing microbial movement, biomass, and group structure. In the rhizosphere, root exudates produce certain compounds (**Table 2.2)** to stimulate microbial activities, which subsequently alters soil nutrient status through decomposition and mineralization of organic and inorganic substances (Hodge and Millard, 1998; Kozdrój and van Elsas, 2000).

Table 2. 2 Organic compounds and enzymes identified in root exudates of different plant species and their function in the rhizosphere (Dakora and Phillips, 2002; Faure et al., 2009).

Class of compound	Compounds	functions
Amino acids	α-alanine, β-alanine, asparagine, aspartate cysteine, cysteine, glutamate, glycine, isoleucine, leucine, lysine, methionine, serine, threonine, proline, valine, tryptophan, ornithine, histidine, arginine, homoserine, phenylalanine, γ-Aminobutyric acid, α-Aminoadipic acid	inhibit nematodes and root growth of different plant species, microbial growth stimulation, chemo-attractants, osmoprotectants, iron scavengers
Organic acids	Butyric, valeric, glycolic, piscidic, formic, aconitic, lactic, pyruvic, glutaric, malonic, aldonic, erythronic, tetronic, citric, oxalic malic, fumaric, succinic, acetic	plant growth regulation, chemoattractants, microbial growth stimulation
Sugar	Rhamnose, arabinose, raffinose, desoxyribose oligosaccharides, glucose, fructose, galactose, maltose, ribose	lubrication, protection of plants against toxin, microbial growth stimulation
Purine/ nucleosides	Adenine, guanine, cytidine, uridine	
Vitamins	Biotin, thiamine, niacin pantothenate, rhiboflavi	microbial growth stimulation
Enzymes	acid/alkaline-phosphatase, invertase H^+, amylase, protease	plant defence, Nod factor degradation
Inorganic ions and gaseous molecules	HCO_3^-, OH^-, CO_2, H_2	acquisition of mineral nutrients required for plant growth

Root exudates contain specific compounds which interact with organic and inorganic substances to regulate both the bioavailability and the transport of nutrients and contaminants in the soil matrix (Bertin et al., 2003; Dakora and Phillips, 2002; Degryse et al., 2008; Koo et al., 2010; Kozdrój and van Elsas, 2000; Walker et al., 2003). Organic acids are the main compounds of root exudates, including oxalic, tartaric, succinic, and the most important compounds for solubilisation and mobilization of plant nutrients and metals (Chang et al., 2002; Jones and Darrah, 1993; Koo et al., 2010). They assist in nutrient uptake by increasing the availability the of P and micronutrients including Fe and Zn (Gerke, 2000; Gerke et al., 2000; Hinsinger, 2001a; Hopkins et al., 1998; Jones, 1998; Keller and Romer, 2001; Römheld and Marschner, 1990; Ryan et al., 2001; Schilling et al., 1998). Fan et al. (2001) and Treeby et al. (1989) found that certain root exudate compounds including phytosiderophores, mugineic acid, and malate improved Fe availability. In addition, the enzyme activities of root exudates of ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) pasture, grown on Templeton sandy loam, significantly increased N mineralization due to the application of DSE (Zaman et al., 1999b). In contrast, root exudates may reduce the concentration of certain contaminants by forming a complex compound. For example, organic acids in root exudates reduced the concentration of Al, K, and metals around plant roots (Awad and Römheld, 2000; Chang and Roberts, 1991; Heim et al., 2001; Pellet et al., 1995) and formed a complex formation with metals including Fe, Mn, Cu, and Zn (Mench et al., 1988; Treeby et al., 1989; Zhang et al., 1991).

Different organic acids from root exudates have different implications for soil-root nutrient interactions. Nigam et al. (2001) reported that compared to carboxylic acid, amino acids were more effective in mobilizing Cd of maize (*Zea mays*) grown in sand and soil culture. The root exudates of two dicotyledonous plants, spinach (*Spinacia oleracea* L.) and tomato (*Lycopersicon esculentum* L.) grown on resin-buffered nutrient solutions at different free ion activities of Cu and Zn were able to mobilize Cu and Zn (Degryse et al., 2008). These mechanisms have implications for plant uptake of soil contaminants through the root system. Koo et al. (2010) found that root exudates played a key role in solubility and availability of Cd, Cu, Pb, Cr, Ni and Zn under application of biosolids. A complex compound of root exudates of Alpine Penny-cress (*Thlaspi caerulescens*) grown on weakly acidic sandy loam (pH 5.15; 68% sand, 20% silt, 12% clay) treated with MWW (septic tanks wastewater) increased the availability of Zn and Cd in the rhizosphere (Dessureault-Rompré et al., 2010).

In addition, through nitrification (**Figure 2.2)**, specific compounds of root exudates are crucial in mitigating the negative environmental consequences following the application of biowastes. When biowastes were applied to soil, a microbial process called nitrification converted most N into the highly mobile NO_3^- which caused low retention in the target system (Qiao et al., 2015). In most cases, NO_3^-

may be lost through leaching (Galloway et al., 2008; Ishikawa et al., 2003) or denitrification before plants can utilize it, thus reducing the Nitrogen Use Efficiency (NUE) in the system and increasing eutrophication of surface and groundwater contamination (Davis, 2014; De Vries et al., 2013; Fowler et al., 2013; Galloway et al., 2008). Several authors reported that a particular group of chemical compounds of root exudates, called Nitrification Inhibitors (NI), played an important role in reducing nitrification rates (Gopalakrishnan et al., 2007; Ishikawa et al., 2003; Qiao et al., 2015; Tanaka et al., 2010). They can suppress the first step of nitrification by inhibiting *Nitrosomonas* spp. bacteria that oxidize ammonium (NH_4^+) (a relatively immobile nitrogen form) to nitrite (NO_2^-), and therefore delay the nitrification process (Zerulla et al., 2001). For example, the root exudates of rice (*Oryza sativa*) and the tropical pasture of creeping signal grass (*Brachiaria humidicola*), significantly suppressed nitrification rates (Gopalakrishnan et al., 2007; Ishikawa et al., 2003; Tanaka et al., 2010), whereas this did not occur with two other tropical pastures, of signal grass (*B. decumbens*) and *stink grass* (*Melinis minutiflora*) (Ishikawa et al., 2003). Although the effects are strongly influenced by factors such as plant species, soil texture, and physicochemical characters of nitrification inhibitors, proper application rates of high N organic fertilizers such as biowastes often increased the efficiency of plant nitrogen utilization and alleviated negative environmental impacts including NO_3^- leaching (Qiao et al., 2015).

Surprisingly, several authors found that the amount and chemical composition of root exudates can be heavily affected by nutrient availability (Ahonen-Jonnarth et al., 2000; Hartmann et al., 2009; Jane et al., 1996; Lipton et al., 1987; Neumann et al., 1999). For example, alfalfa (*Medicago sativa*) and lupin (*Lupinus albus*) roots released 80% more of the root exudate citrate (Lipton et al., 1987) and released more carboxylate compound in later stage (Jane et al., 1996; Neumann et al., 1999) under P-stress conditions. The main compound of root exudates (organic acids, amino acids, and mugenic acid) of barley (*Hordeum vulgare*) increased 7-fold under medium Fe-stress conditions (Fan et al., 1997). Degryse et al. (2008) found that spinach (*Spinacia oleracea* L.) and tomato (*Lycopersicon esculentum* L.) responded to Zn deficiency by producing more root exudates. For particular plant species (including leguminous species), the deficiency of P increased the production of phenolic compounds of root exudate (Dinkelaker et al., 1995; Nair et al., 1991; Neumann et al., 1999). Certain organic acids (including oxalate, malate, and citrate) of root exudates from *Pinus sylvestris* increased significantly in soils containing Al (Ahonen-Jonnarth et al., 2000).

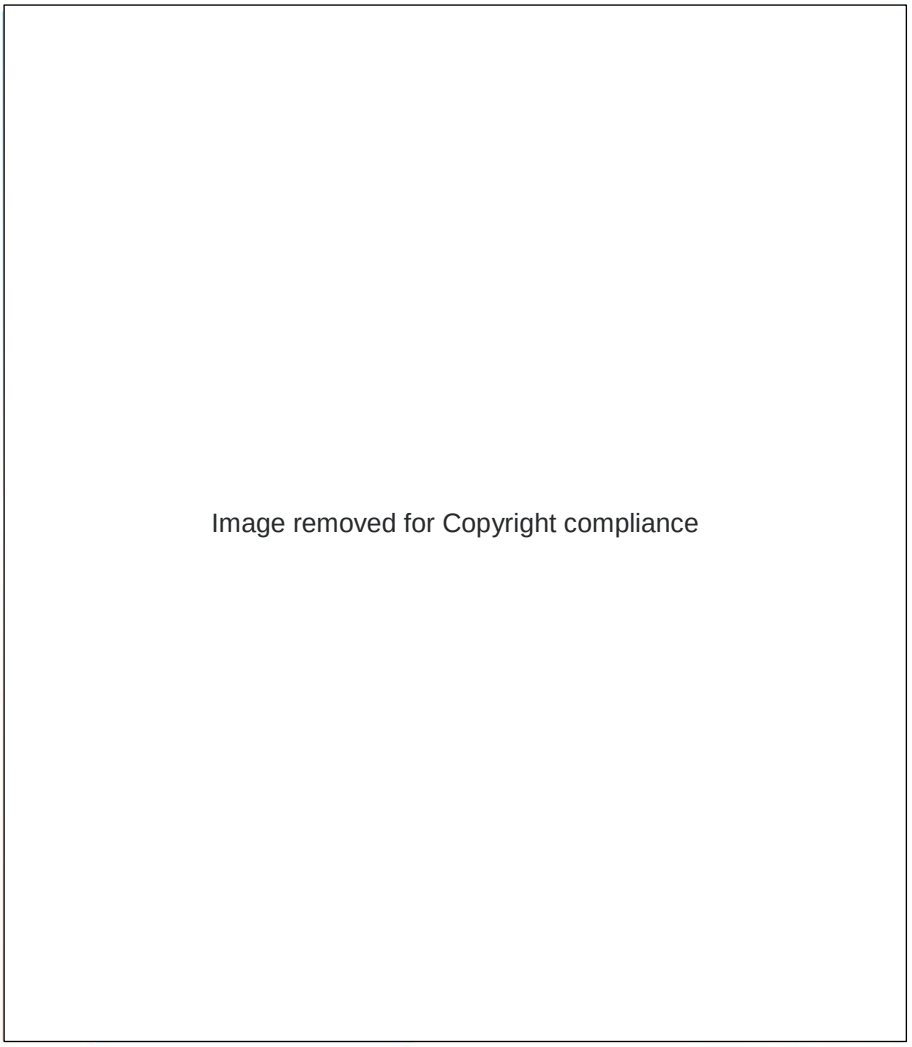


Figure 2. 2 The nitrogen cycle. Adapted from (Dixon, 2014).

2.5 The effect of root-microbe interactions on biowaste degradation and fluxes of NCAB

In the rhizosphere, some microbial activities play key roles in several biogeochemical processes (Mukerji et al., 2006; Stottmeister et al., 2003). These processes involve the root-associated microbial communities of plants, as certain microbes such as mycorrhizae are important for obtaining nutrients and water for plant growth (Hillel et al., 2005). One important mechanism that characterizes these underground zone activities of plants is the interaction between the root and the microbes (root-microbe interaction). These root-microbe interactions played significant roles in respect to stimulating degradation, availability, and immobilization of nutrients and contaminants associated with biowastes (Cohen et al., 2004; Harvey et al., 2002; Khan, 2006; Morikawa and Erkin, 2003; Stottmeister et al., 2003). In the rhizosphere, the root-microbe interactions are very important in establishing degraded

conditions. With the supporting oxygen supply from the plant (through fine roots), microbes degrade the contaminants in soil as part of their normal metabolic processes (Harvey et al., 2002). Plant roots excrete C compounds into the soil, which stimulate the growth of the rhizosphere bacteria, which in turn, degrade the organic contaminants (Brady, 2008). Hence, adding biowastes to soil could provide a source of food for the microbes. However, the contaminants applied as biowaste are usually still bound in the form of complex compounds that cannot be taken in directly by the plant (Brady, 2008). The complex compounds must be parsed again, to break them into ions that can be absorbed by plants. When organic material is eaten by bacteria for example, the structure of complex compounds is broken into elements more favourable for plant uptake (Khan, 2006). Plant roots interact extensively with soil microorganisms, which further affects the flux of nutrients, either directly, by influencing nutrient availability and uptake, or indirectly through plant (root) growth promotion (Richardson et al., 2009).

The effect of plants on the bioavailability and mobility of NCAB through root-microbes interaction is dependent on the species (Baldani and Döbereiner, 1980; Mazzola et al., 2002). Soil microbes are strongly influenced by certain NCAB (Chander and Brookes, 1991), which subsequently affects plant growth and quality. Depending on the quantity and type of biowastes, their application has an indirect influence in enhancing soil microbes, which are crucial in N cycling (Mukerji et al., 2006; Stottmeister et al., 2003). For example, the application of high Cu biosolids decreased the amount of soil microbial biomass by about 30% and 13% in sandy loam soil (15% clay) and silty loam soil (21% clay) respectively (Chander and Brookes, 1991). The application of biosolids combined with a eucalypt (*E. cladocalyx*) significantly increased mycorrhizal colonization (ectomycorrhizal and arbuscular mycorrhizal) in roots (Madejón et al., 2012). The application of DSE, for instance, resulted in a greater and more diverse microbial biomass in soil (Hawke and Summers, 2006). Similar findings using wood waste applied at a rate of 80 kg N ha^{-1} of coniferous sawdust on clay (36.5%), silt (41.0%), sandy (22.5%) and soil (Eutric Cambisol) found increased concentrations of bacteria and fungi, by 90% and 80%, respectively (Elfstrand et al., 2007). The application of a high rate (78 t ha^{-1}) of fresh paper mill residuals on Plainfield loamy sand (87% sand, 5% silt, and 8% clay) resulted in a 100% higher microbial biomass C compared to that of the low rate (22 t ha^{-1}) (Leon et al., 2006). In addition, over 3 years, application of 45 t ha^{-1} Dry Weight of biosolids on Gypsic Haploxerept (26.7% sand, 51.1% silt and 22.3% clay) under barley species (*Hordeum vulgare)* promoted the recycling of nutrients by improving soil microbiological properties, including basal respiration, microbial biomass and some soil enzyme activities (Antolín et al., 2005).

2.6 The effects of leaf litter on NCAB

Leaf litter significantly enhances the amount of organic matter in the surface layers of the soil, promoting nutrient cycling, soil aggregation and water holding capacity (Mukhopadhyay and Joy, 2010; Pulford and Watson, 2003; Schreeg et al., 2013). The decomposition of leaf litter plays a key role in flux of C and mineral nutrients, which is crucial for maintaining primary productivity in many systems (Schreeg et al., 2013). Several studies showed that in addition to improving nutrient status, leaf litter played a crucial role in driving soil-microbe interactions (Bowman et al., 2004; Cleveland et al., 2002; Wang et al., 2014; Wieder et al., 2008; Wurzburger and Hendrick, 2009) and affected the physicochemical interactions in the soil (Schreeg et al., 2013; Strobel et al., 2001). Leaf litter may affect both the mineralization process of Soil Organic Carbon (SOC) and the structure of the microbial community by changing the availability of soil nutrients and C (Brady, 2008; Villalobos-Vega et al., 2011; Wang et al., 2014). These particular interactions can then have further significant effects on the fluxes of nutrients and contaminants associated with biowastes (Cohen et al., 2004; Kozdrój and van Elsas, 2000). The effectiveness of leaf litter in related nutrient fluxes and soil-microbe interaction vary among plant species (Mukhopadhyay and Joy, 2010). Leaf litters of Cassia (*Cassia siamea*) increased the nutrient status and microbial activity in soil more than Shorea (*Shorea robusta*) and Acacia (*Acacia auriculiformis*) litters (Mukhopadhyay and Joy, 2010). Leaf litter of different plant species has different effects on nutrient fluxes, especially related to target elements. Leaf litters of two beans (*Sclerolobium macrocarpa* and *S. paniculatum*) and ouratea (*Ouratea hexasperma*) increased the availability of only one essential nutrient, Ca, in the upper soil layers (Villalobos-Vega et al., 2011). In contrast, the addition of leaf litter of Japanese cypress (*Chamaecyparis obtusa*) increased fluxes of Ca, Mg, K, and NH_4^+ in forest floor percolates (Chang et al., 2007). Fioretto et al. (2001) reported that leaf litter from the summer deciduous shrub, *Cistus incanus*, increased the availability of several macronutrients (N, P, K, S, and Ca) during the 18-month incubation period. In certain cases, leaf litter had positive effects in stimulating microbial activity while reducing nutrient availability effect. For instance, the leaf litter of rhizomatous forb, *Acomastylis rossii,* increased microbial activity, but affected the soil N cycling by decreasing the availability of N (Bowman et al., 2004). Leaf litter of certain plant species, such as alder (*Alnus glutinosa*) and poplar (*Populus tremula*), acted as a temporary storage for soil contaminants (Scheid et al., 2009). Over a 25 month incubation period of leaf litter of alder (*Alnus glutinosa*) and poplar (*Populus tremula*), the solubility of metals gradually decreased with time (Scheid et al., 2009). The effect of leaf litter on fluxes of nutrients is dependent on soil type. The availability of N and P decreased on less fertile soil (sandstone and heath forest soil) compared to the more fertile alluvial forest soil (Dent et al., 2006).

2.7 Nutrient cycling as related to NCAB

Biowastes that contain high concentrations of nutrients and organic matter are good low-cost fertilizers and conditioners for both plants and soils (Delibacak et al., 2009). The application of biowastes to soil influences nutrient cycling by increasing bioavailability and the uptake of nutrients to plants. In this specific process, biowastes may speed nutrient cycling by serving as both a short-term and long-term source of highly available nutrients (Murphy et al., 2007). As discussed above these nutrients can be a substrate for bacteria, fungi, and other decomposers contributing to nutrient cycling in the soil. The cycle begins with breaking the organic matter in to simpler compounds, thereby transforming them into plant nutrients available for uptake by roots. The application of biowastes may affect nutrient cycling by directly increasing the amount of available nutrients (Antolín et al., 2005; Morera et al., 2002; Singh and Agrawal, 2008). Biowastes modify physical soil properties, such as stability of aggregates and porosity, which can improve root environment and stimulate plant growth, and alter the chemical properties of soil (Singh and Agrawal, 2008). These changes and affect the growth of both plants and soil microbes (Cytryn et al., 2011; Rogers and Smith, 2007; Singh and Agrawal, 2008).

The influence of biowastes application on nutrient cycling, especially their direct contribution in supplying available nutrients has been well studied. Numerous studies reported that the application of organic materials, which are inherent in biowastes, increased the concentration of organic C and, therefore, increased the Capacity Exchange of Cations - CEC (Antolín et al., 2005; Brady, 2008; Weber et al., 2007). As organic C possess a high negative charge, it contributes to retaining nutrients and making them available to plants (Garcıa-Gil et al., 2004; Kaur et al., 2008). For instance, adding biosolids increased the availability of N, P, Zn, Cr for uptake by plants (Wong et al., 2001). Similarly, applying 90 t ha^{-1} biowaste (biosolids) to sandy loam (Typic Xerofluvethe) soil resulted in a significantly increased concentration of total N, Cu, Pb and Ni, and available P, K, Ca, Fe, Cu, Zn, Mn concentrations in soil but did not alter the concentration of available Mg and Na, total Fe, Zn, Mn, Cd, Co or Cr in (Delibacak et al., 2009). Minhas et al. (2015)reported that the application of MWW increased the concentration of Zn, Cu, Fe and Mn. Another study found that the application of DSE improved long-term soil fertility by increasing the concentration of total N, total P and plant available nutrients (Hawke and Summers, 2006).

Biowastes application affected the long-term availability of nutrients. Compared to mineral fertilizers, biowastes are generally slowly decomposed in the soil, and the continuous release of nutrients can sustain the microbial biomass population for longer periods of time (Murphy et al., 2007). For example, after four years, the application of biowaste (compost and manure) resulted in 20 to 40%

higher soil microbial biomass C compared with the N fertilizer treatment (Ginting et al., 2003). Zhang et al. (2006) found that adding 0 to 200 kg ha^{-1} biosolids to less fertile Gray Luvisolic soils increased the soil extractable P concentration from 7.2 to 86 mg kg^{-1} soil.

In addition to their direct contribution to increase the availability of nutrients, biowastes application has a variety of physical properties that affect soil nutrient transformations. Physical aspects such as aggregate stability, are key factors in maintaining proper soil structure, which can be increased by adding organic materials. This specific mechanism could improve soil porosity, which plays an important role for gas exchange, and water retention (Brady, 2008). Several authors found that biowaste application affected this particular aspect. For instance, Wong et al. (2001) showed that adding 8, 16, 44, and 88 kg ha^{-1} DW of biosolids to acidic loamy soil (using *Brassica chinensis*) improved soil texture by decreasing bulk density and elevating soil aeration, soil aggregation, and water holding capacity, which resulted in elevated total N, P, Zn, and Cr availability. Leon et al. (2006) found that the application of 38.1 and 78.4 t ha^{-1} of composted paper mill residuals over four years, resulted in an increase on average of 25% of water-stable aggregates compared with the non-treated soil.

Lastly, blending certain biowastes with another kind of biowaste may affect the flux of nutrients and contaminants associated with biowastes, particularly leaching of NCAB into surface and ground water. For instance, blending wood-waste (raw dried pine sawdust) with biosolids-amended soils showed a significant reduction in N mobility in biosolids and potentially reduced NO_3^- leaching (Paramashivam, 2015b). The study demonstrated that mixing wood-waste (pine biochar) did not affect the NO_3^- leaching, but significantly decreased the mobility of NH_4^+-N (Paramashivam, 2015b).

2.8 Conclusions

Plants have a significant role in mitigating the negative environmental consequences following the addition of biowastes to soil. There is plenty of evidence from the existing literature that ET, root architecture, root exudates, root-microbe interactions, and litter fall have significant roles. The following are key outcomes related to those aspects reviewed in this Chapter:

1. Depending on species and climate, ET could create the ideal soil-water environment to dissolve and make contaminants available for uptake by roots. ET is crucial in reducing the leaching of nutrients and contaminants into ground water.

2. Root architecture, including root diameter, length, orientation, and root density strongly affect water preferential flow of soil, especially through root channels, which enhance water

and nutrient transport across soil profiles. The increase of infiltration may affect the movement of dissolved elements through the soil matrix.

3. Root uptake of nutrients and contaminants associated with biowastes are strongly influenced by factors such as ET rates and the concentration of nutrients in soil water.

4. Root exudates regulate microbial activities which have further important roles in solubilisation, and mobilization of NCAB in the rhizosphere. Through nitrification, root exudates play an important role in reducing NO_3^- leaching following application of biowastes. In complementary fashion, the availability of NCAB affects the production and composition of root exudates in the rhizosphere.

5. Depending on plant species, the close interaction between roots and soil microbes could affect the flux of NCAB either through direct uptake of available nutrients or through root development. In contrast, adding biowastes, which are the source of NCAB to soil could affect soil microbes.

6. During their decomposition process, leaf litters play an important role in driving soil-microbe interactions which further affect the physicochemical activities in the soil. However, the effectiveness of this role varies among plant species.

7. The application of biowastes to soil may affect the nutrient cycle over both the short and long term.

Chapter 3

The response of manuka (*Leptospermum scoparium* J.R Forst) and kanuka (*Kunzea robusta* de Lange & Toelken) to individual macronutrients in a low-fertility soil

3.1 Introduction

3.1.1 Background

Manuka (*Leptospermum scoparium*) and kanuka (*Kunzea robusta*) are pioneering species that colonise disturbed areas or low-fertility agricultural land (Stephens et al., 2005; Wardle, 1991). *L. scoparium* is the most widely distributed, abundant, and environmentally tolerant native species among New Zealand's woody plants (Ronghua et al., 1984; Stephens et al., 2005). These species have been used in land restoration of mine sites and degraded lands to improve soil quality, promote invertebrate biodiversity, and increase ecosystem recovery (Burrows et al., 1999; Craw et al., 2007; Thomas et al., 2014). These species rapidly colonise disturbed land, especially steep, erosion-prone pastoral hill country, resulting in erosion mitigation and soil conservation (Stephens et al., 2005). These species are a potentially important C sink (Scott et al., 2000; Trotter et al., 2005). In addition, *L. scoparium* can tolerate soils with low fertility, high acidity, low or high moisture contents; and is able to withstand wind-exposed sites and salt sprays (Derraik, 2008).

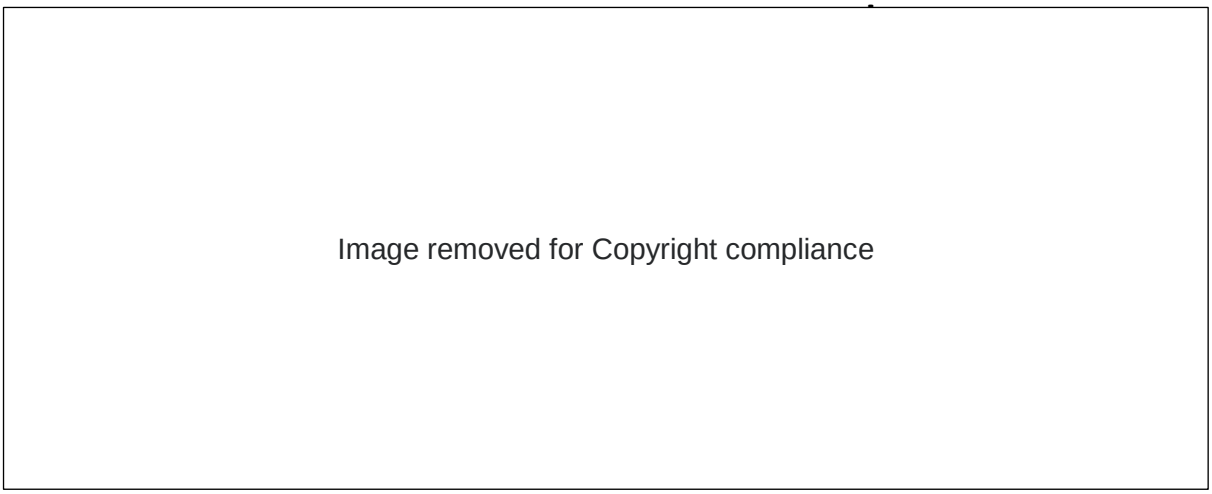


Plate 3. 1 (a) *L. scoparium* and (b) *K. robusta* with flowers (Photographs by Foster (2014))

Both *L. scoparium* and *K. robusta* can produce valuable essential oils, which have antimicrobial properties (Lis-Balchin et al., 2000; Lis-Balchin and Hart, 1998; Perry et al., 1997a; Perry et al., 1997b).

Maddocks-Jennings et al. (2009) reported that *L. scoparium* and *K. robusta* essential oils mouthwash used in a gargle can provide a positive effect on the development of radiation-induced mucositis of the oropharyngeal area during treatment for head and neck cancers. Lis-Balchin et al. (2000) reported that the essential oils of *L. scoparium* contain antibacterial agents, especially against gram-positive bacteria, that may end up in the soil via a number of pathways including rhizo-deposition from roots or through the decomposition of leaf-litter. Prosser (2011) demonstrated that *L. scoparium* and *K. robusta* promote the die off of human pathogens in soil. *L. scoparium* and *K. robusta* can exude bioactive phytochemicals, either from the roots or from leaf fall which affects the N cycle, significantly reducing the evolution of nitrous oxide (N_2O) (Fitzgerald, 2012; Hedley et al., 2013)and killing pathogens in biosolids-amended soil (Fitzgerald, 2012; Prosser, 2011). In addition, Craw et al. (2007) and Lee et al. (1983) reported that *L. scoparium,* in particular, tolerated up to 3.6, 3800, and 1000 mg/kg of As, Ni and Cr, respectively in soil, which may make these species useful for the phytostabilisation of contaminated sites. honey from *L. scoparium* is worth up to NZ$500 per kg (MPI, 2014) due to the perceived health benefits resulting from phenolic compounds such as trimethoxybenzoic acid, methylglyoxal, and 2-methoxybenzoic (Stephens et al., 2010; Weston et al., 1999). In the 2013/2014 period (up to June 2014), the New Zealand honey industry exported approximately 8.706 tonnes of honey (valued at $180 million), of which *L. scoparium* honey contributed 80 to 90% of the total export value (MPI, 2014). The concentration of non-peroxide antimicrobials in *L. scoparium* honey can be quantified analytically, and is known as the "Unique Manuka Factor" (UMF) (Stephens et al., 2005). In addition to honey product, wood of the *L. scoparium* tree has been used for fencing, tool handle manufacture, and firewood (Salmon, 1980). *L. scoparium* is usually a shrub or small tree.

3.1.2 Rationale of the study

My assumption was that *L. scoparium* and *K. robusta* occur on soils with low nutrient concentrations, especially the macronutrients N, P, K, and S. The information on the effects of macronutrients to the growth and quality of these two New Zealand native plants is unclear. Previous studies reported that a relative of *L. scoparium* and *K. robusta*, from the genera *Eucalyptus*, under the same family of myrtaceae, responded positively to the application of fertilizers (Albaugh et al., 2015; Bennett et al., 1996; Campion et al., 2006; Carlson et al., 2001; Cromer et al., 1993; Hunter, 2001; Judd et al., 1996; Messina, 1992; Mhando et al., 1993; Pankaj et al., 2008; Ringrose and Neilsen, 2005; Weggier et al., 2008; Xu et al., 2002). Like other members of the myrtaceae family, the leaves of *Leptospermum, Kunzea,* and *Eucalyptus* contain aromatic oils which can be smelled by crushing the leaves between the fingers (ANPSA, 2018). The majority of species in this group of plants are found in heath, woodland or open forest of mainly temperate areas. They are absent in rainforest and arid areas although many

species do occur in the tropics. The myrtaceae genera *Eucalyptus*, *Leptospermum*, and *Kunzea* are known to form ectomycorrhizal relationships (Wang et al., 2009). Several authors (Albaugh et al., 2015; Bennett et al., 1996; Campion et al., 2006; Carlson et al., 2001; Cromer et al., 1993; Hunter, 2001; Judd et al., 1996; Messina, 1992; Mhando et al., 1993; Pankaj et al., 2008; Ringrose and Neilsen, 2005; Weggier et al., 2008; Xu et al., 2002) reported that *Eucalyptus saligna, E. regnans, E. grandis, E. tereticornis, E. urophylla* responded positively to the application of fertilizers. The application of NPK treatments improved root-collar diameter, diameter at breast height and height growth compared with unfertilized treatments of *E. saligna* (Mhando et al., 1993). Ringrose and Neilsen (2005) found that Australian *E. regnans,* grown on nutrient-poor soils, responded significantly to the application of macronutrients (N, P, S, and Ca) by producing higher growth and higher foliar N and P concentrations. The application of N, P, and B at 1:1:0.005 ratio improved the volume growth of *E. grandis* by 91% during 3 year after treatment (Albaugh et al., 2015; Herbert, 1983). Crous et al. (2015) suggested that the addition of 50 kg P ha^{-1} yr^{-1} increased the P uptake significantly by 52% compared to non-fertilised treatment of *E. tereticornis* grown on P-limited soils. Campion et al. (2006) reported that the combination of irrigation and fertilizer treatment significantly increased total aboveground biomass and the available soil P of *E. grandis* by 58% and 9% respectively. The same species together with *E. urophylla* grown on high P sorption oxisol soils resulted in significantly higher tree growth, biomass production, and N, P, K uptake (Carlson et al., 2001; Xu et al., 2002). Seedlings of *E. camaldulensis and E. grandis* treated with various rates of NPK fertiliser had higher nutrient uptake and produced significantly higher above-ground biomass, by 23%, compared to the non-fertilised treatment (Hunter, 2001). Therefore, I hypothesized that adding macronutrients (N, P, K, and S) to low fertility soil would enhance the growth of *L. scoparium* and *K. robusta* as well as increase the uptake of these essential nutrients in plant parts.

3.1.3 Aims

This study aimed to determine whether the addition of N, P, K and S fertilizers significantly affected the growth, elemental uptake, and elemental composition in rhizosphere soil in combination with *L. scoparium* and *K. robusta*.

3.2 Materials and Methods

3.2.1 Experimental set up

The experiment was carried out in the Forester greenhouse, Lincoln University Nursery (43^0 38′ 42 ″ S 172^0 27′ 41″ E) from July 26th to November 26th, 2013. Low-fertility soil was collected from a

marginal farm area near Bideford, New Zealand (40^0 50′ 03″ S 175^0 59′ 36″ E). **Table 3.1** shows the properties of the soil used in the experiment. Fifty 5 L pots (22.5 cm in diameter with a height of 22 cm) were filled with 4 kg of homogenized soil. To improve drainage, 2 cm of gravel was put at the bottom of each pot (**Figure 3.2**). Pots were incubated at ambient conditions in the greenhouse for one week prior to treatment application. About 7-month old *K. robusta* and *L. scoparium* seedlings were then transplanted into each pot, 25 of each species. Each treatment consisted of 5 replicates, and received one of four macronutrients, either Nitrogen (N), Phosphorus (P), Potassium (K), or Sulphur (S). The 5 seedlings of the control received only water. The application rate of macronutrients N, P, K, and S treatments was based on 2:1:1:1 ratio (**Table 3.2**). The treatments were applied individually in solution form to each pot weekly. Prior to treatment application, the desired amount of salt (**Table 3.2**) of each nutrient was weighed and dissolved in a 1 L volumetric flask using Deionized (DI) water until the salt was completely dissolved. The nutrient solution was then transferred to a 100 mL volumetric cylinder and applied to each pot (**Plate 3.1b**).

Table 3. 1 Properties of soil used in the experiment. Values in brackets represent standard error of n=5 replicates.

Properties	concentration
pH	6.1
Moisture content [%]	26
dry matter [%]	80
C/N ratio	14
total available N [mg kg^{-1}]	43
CEC [me 100 g^{-1}]	21
total base saturation [%BS]	55
C [%]	6.5
N [%]	0.5
P [%]	0.1 (0.0)
K [%]	0.2 (0.0)
S [%]	0.1 (0.0)
Ca [%]	0.4 (0.0)
Mg [%]	0.2 (0.0)
B [mg kg^{-1}]	29 (0.3)
Cu [mg kg^{-1}]	4.2 (0.0)
Zn [mg kg^{-1}]	29 (0)
Mn [mg kg^{-1}]	134 (2.9)
Fe [mg kg^{-1}]	15461 (108)
Cd [mg kg^{-1}]	0.1 (0.0)

Table 3. 2 Macronutrients (kg ha^{-1}) applied to soil for the growth of *K. robusta* and *L. scoparium* seedlings.

Nutrient	Rate of application (kg/ha)	Chemical form added	Amount salt added (g)	
			Total salt added	Weekly application of salt
Nitrogen (N)	200	CH_4N_2O	13.5	1.7
Phosphorus (P)	100	KH_2PO_4	13.8	1.7
Potassium (K)	100	KCL	17.1	2.1

Sulphur (S)	100	K_2SO_4	6.0	0.8

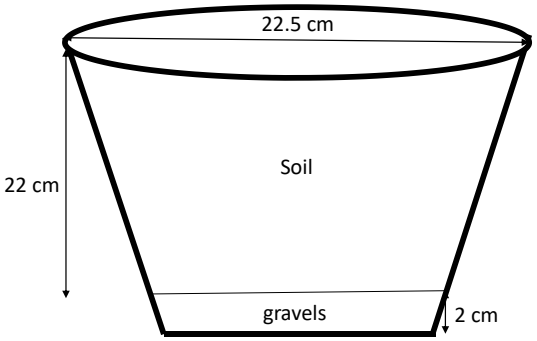


Figure 2 Pot design using in the experiment

Figure 3. 1 Pot design used in the experiment

The pots were watered to field capacity daily but not on the day of fertilizer application. They were weeded weekly. Monthly height measurements were taken. Pots were arranged in a randomized block design. The temperature inside the greenhouse varied between 18 - 23°C.

Figure 3 (**a**) Bidford low-fertility soil used in experiment; **(b)** Treatment application using

Plate 3. 2 (a) Bidford low-fertility soil used in experiment; (b) Treatment application using 100 mL volumetric cylinder

3.2.2 Analysis and statistical evaluation

After 16 weeks, the plants were harvested. Fresh plant biomass (root and above ground biomass) was carefully harvested and weighed. Both root and above ground fresh biomass samples were dried at 70^0 C until a constant weight was reached, and final dry weight was recorded. Rhizosphere soil which was attached to the plant roots (≤ 1 mm from the root surface) (Hinsinger, 2001a) was harvested

around plant roots, and sieved using a 2 mm plastic sieve. Around 500 g of fresh soil was stored in the fridge at ±4°C for mineral N (NO_3^- and NH_4^+) analysis. For metal elemental analysis, rhizosphere soil samples were dried at 70^0C for 24 hours. Mineral N (NO_3^- and NH_4^+) concentrations of soil were obtained using Flow Injection Analysis (FIA). Four g of air-dried ground soil sample of each treatment (3 replicates) were weighed, then transferred into 50 mL centrifuge tubes. The samples were then extracted by adding 40 mL of 2M KCL, shaken by end-over-over shaker for 1 hour and centrifuged at 2000 rpm for 10 minutes, and filtered using Whatman 52 filter paper. Extracts were stored in sealed containers in the freezer for further FIA analysis.

For plants, the dried above ground parts were ground using a Retch ZM200 grinder, while soil samples were crushed using ceramic pestle and sieved using a 2mm plastic sieve. Five g of each treatment (5 replicates) were weighed and transferred into 50 mL centrifuge tubes and extracted with 30 mL of 0.05 M 141 Ca $(NO_3)_2$, shaken by end-over-end shaker for 2 hours and centrifuged at 3200 rpm for 15 minutes, and then filtered using Whatman 52 filter paper. Extracts were stored in sealed containers in the fridge for further analyses. Concentrations of elements were determined using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES Varian 720 ES - USA). Reference soil and plant material came from Wageningen University, the Netherlands (International Soil analytical Exchange 921 and International Plant analytical Exchange 100), with recoverable concentrations of 81–112% of the certified values. Soil and plant total N and C concentrations were measured using an Elementar Vario MAX CN analyser.

The plant biomass, root to shoot ratio, and plant macronutrient concentrations were statistically analysed using analysis of variance (ANOVA). The model included plant species, macronutrients application and their interaction as fixed effects, and the experimental block as a random additive effect. Following the identification of a significant species x macronutrients interaction, one-way ANOVA was used to investigate the effect of macronutrients treatment on species biomass individually. The effect of applied macronutrients into foliar nutrients uptake was analysed by one-way ANOVA for each macronutrient application. Duncan post-hoc tests at P=0.05 was performed to evaluate the difference between treatments. The analyses were done in IBM SPSS v.**22** (International Business Machines Corp., New Orchard Road, Armonk, New York 10504 914-499-1900).

3.3 Results

3.3.1 Response in above ground dry biomass and root to shoot ratio

Figure 3.2 shows above ground biomass of *K. robusta* in combination with different macronutrient treatments.

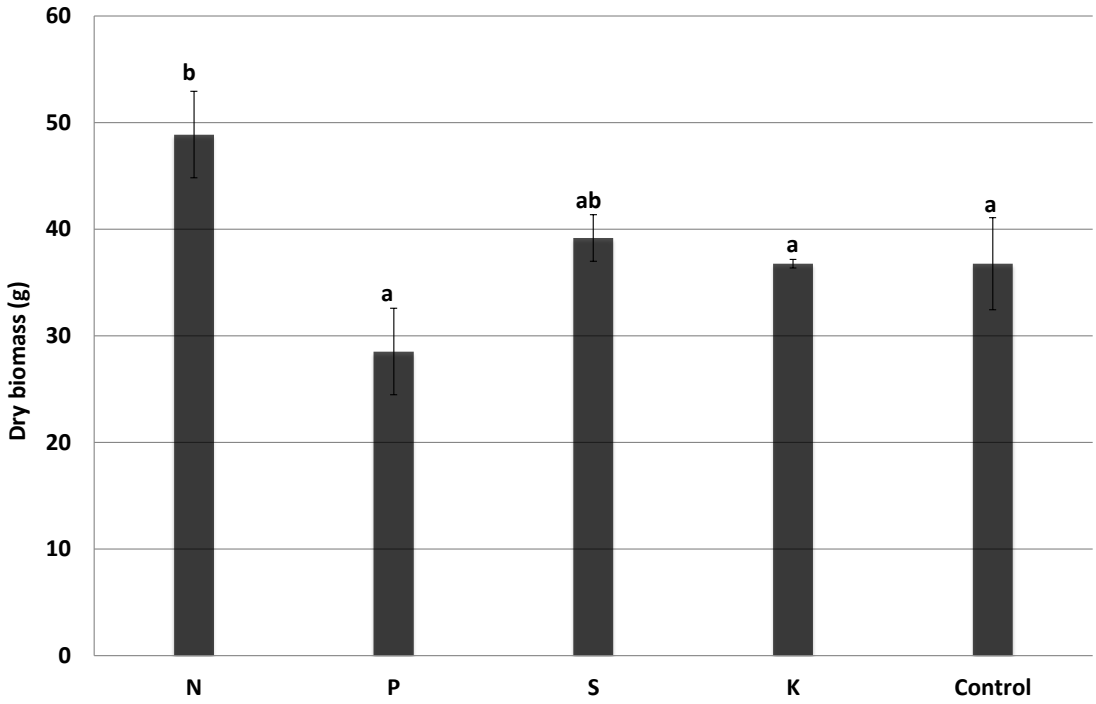


Figure 4. Above ground biomass of *K. robusta* in combination with different macronutrient treatment

Figure 3. 2 Above ground biomass of *K. robusta* in combination with different macronutrient treatments (n=5). Treatments that share letters have means that do not differ significantly.

After 16-month of the experimental period, in combination with *K. robusta*, the application of 200 kg ha^{-1} of N (in CH_4N_2O form) produced a significantly (*p<0.05*) higher above ground dried biomass compared to the control and other treatments of this species (**Figure 3.2)**. In contrast, there was no significant difference in above ground dry biomass between treatments in combination with *L. scoparium*. At the end of the experiment, *K. robusta* produced total above ground dry biomass up to 49 g pot^{-1} (equivalent to 12 t ha^{-1}), which is 33% higher than the control (**Figure 3.2)**.

Figure 3.2 shows that**,** with the exception of P treatment, in combination with *K. robusta*, amending the low fertility soil with macronutrients increased significantly above ground dry biomass compared to *L. scoparium*. The application of N, S, and K increased the above ground dry biomass of *K. robusta* by 40%, 25%, and 50% (respectively) higher than that of *L. scoparium* (**Figure 3.3**).

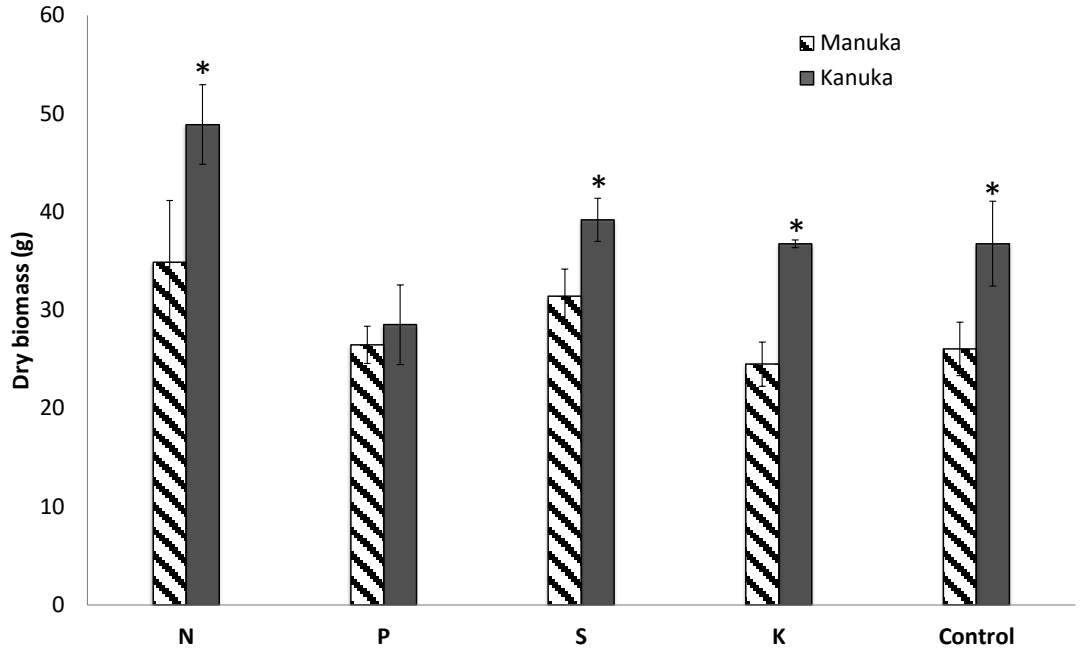


Figure 3. 3 Comparison of above ground biomass of *L. scoparium* and *K. robusta i*n combination with different macronutrient treatment (n=5). Asterisks (*) signify significant differences between species at *p≤0.05*.

This study shows that compared to control and other treatments, the addition of P had a significant (*p<0.05*) effect on the root to shoot ratio of *K. robusta* (**Figure 3.4**). *K. robusta* responded positively to the application of 100 kg ha^{-1} of P (in KH_2PO_4 form) by showing the highest root to shoot ratio value of 0.6 (**Figure 3.4**).

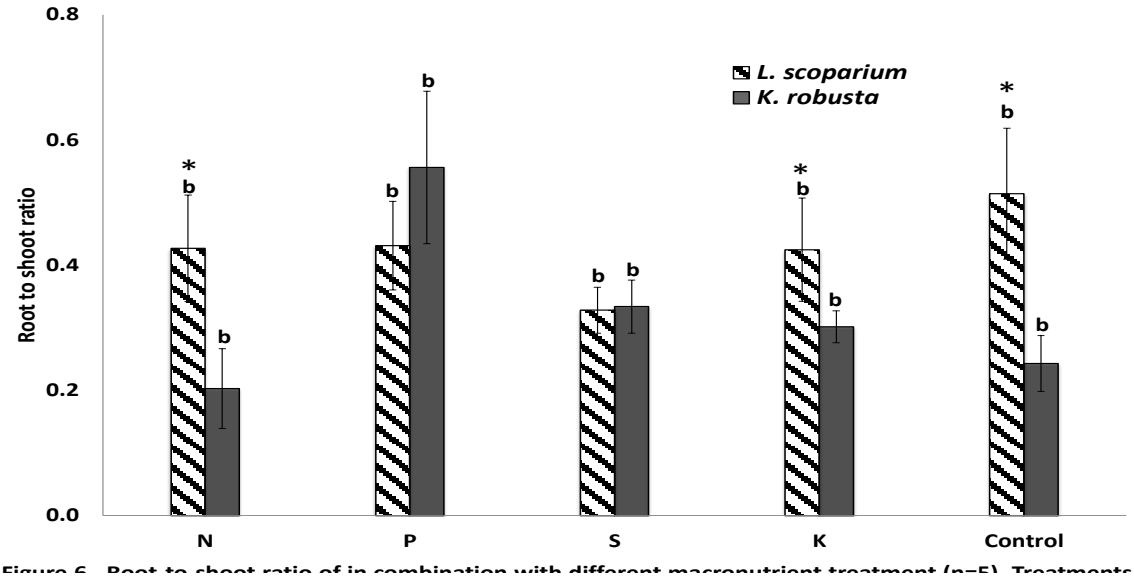


Figure 3. 4 Root-to-shoot ratio in combination with different macronutrient treatments (n=5). Treatments that share letters have means that do not differ significantly. Asterisks (*) signify significant differences between species at *p≤0.05*.

3.3.2 Foliar nutrients concentration

Nitrogen

Figure 3.5 shows N concentration of the leaves of *L. scoparium* and *K. robusta* in combination with macronutrient treatment. Foliar N concentrations of *L. scoparium* and *K. robusta* varied among treatments. In general, the concentration of N in the leaves of *L. scoparium* ranged from 1.5% to 1.9% between treatments, while the concentration of N in the leaves of *K. robusta* ranged from 0.9% to 1.6% (**Figure 3.5**). These two species tended to have similar N foliar concentrations. *L. scoparium* foliar N averaged 1.9%, while *K. robusta* averaged 1.6%. After 16 months of the experimental period, the concentration of N in *L. scoparium* and *K. robusta* increased by 19% and 78% respectively. These numbers indicate that that *K. robusta* accumulated more N than *L. scoparium*. The results indicate that *L. scoparium* and *K. robusta* had significantly ($p \leq 0.05$) higher N concentrations than the control (**Figure 3.5**).

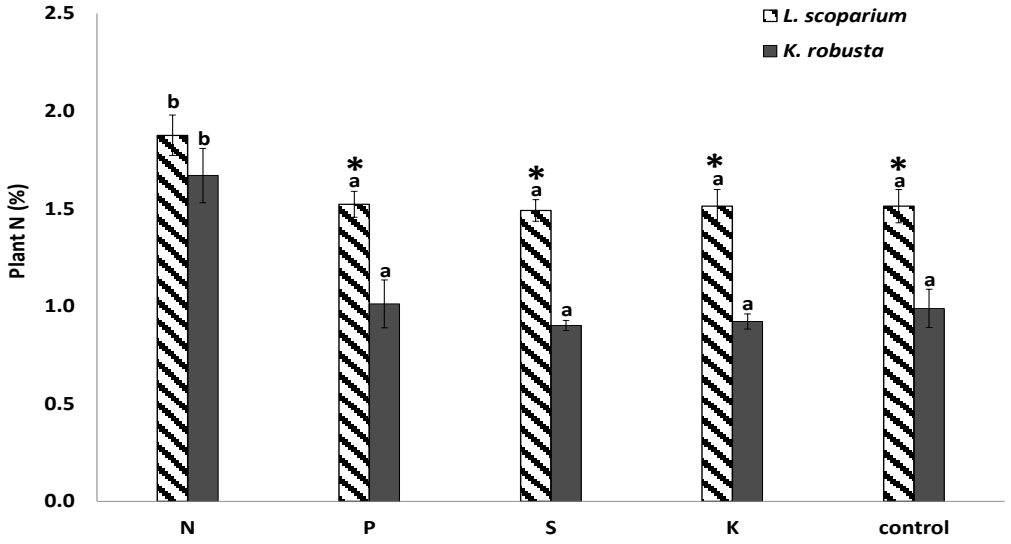


Figure 6. N concentration on foliar part in combination with basal macronutrient treatment (n=5).

Figure 3. 5 N concentration in the leaves in combination with macronutrient treatments (n=5). Treatments that share letters have means that do not differ significantly (*p≤0.05*). Asterisks (*) signify significant differences between species at *p≤0.05*.

With the exception of N treatment, the present study indicates that there was a significant difference in foliar N uptake between *L. scoparium* and *K. robusta* following the application of P, S, and K treatment (**Table 3.3** and **3.4**). In combination with P, S, and K, *L. scoparium* accumulated 50%, 64%, and 65% higher N concentrations, respectively, than that of *K. robusta.*

Table 3. 3 Foliar nutrient ratios of each element to N of *L. scoparium* measured at the end of the experiment. Values in brackets represent the standard error of the average concentration per pot throughout the experiment (n=5).

Treatment	Foliar nutrient ratios				
	N/P	N/K	**N/Ca**	**N/Mg**	**N/S**
N	17 (1)	3 (0)	0.4 (0)	3 (0)	4 (0.4)
P	12 (1)	2 (0)	0.5 (0)	3 (0)	5 (0.2)
S	13(0)	2 (0)	0.5 (0)	3 (0.)	5 (0.3)
K	14 (1)	2 (0)	0.6 (0)	4 (0)	6 (0.2)
Control	15 (1)	2 (0)	0.6 (0)	3 (0)	5 (0.3)

Table 3. 4 Foliar nutrient ratios of each element to N of *K. robusta* measured at the end of the experiment. Values in brackets represent the standard error of the average concentration per pot throughout the experiment (n=5).

Treatment	Foliar nutrient ratios				
	N/P	**N/K**	**N/Ca**	**N/Mg**	**N/S**
N	17 (1)	3 (0)	1 (0)	6 (0)	5 (0)
P	6 (0)	2 (0)	1 (0)	4(0)	6 (0)
S	10 (1)	1 (0)	1 (0)	5 (1)	7 (0)
K	9 (0)	2 (0)	1 (0)	3 (0)	4 (0)
Control	10 (0)	3 (0)	1 (0)	3 (0)	4 (0)

Phosphorus (P)

Figure 3.6 shows the foliar nutrient analysis concentration of macronutrients under various individual macronutrients fertilizer application.

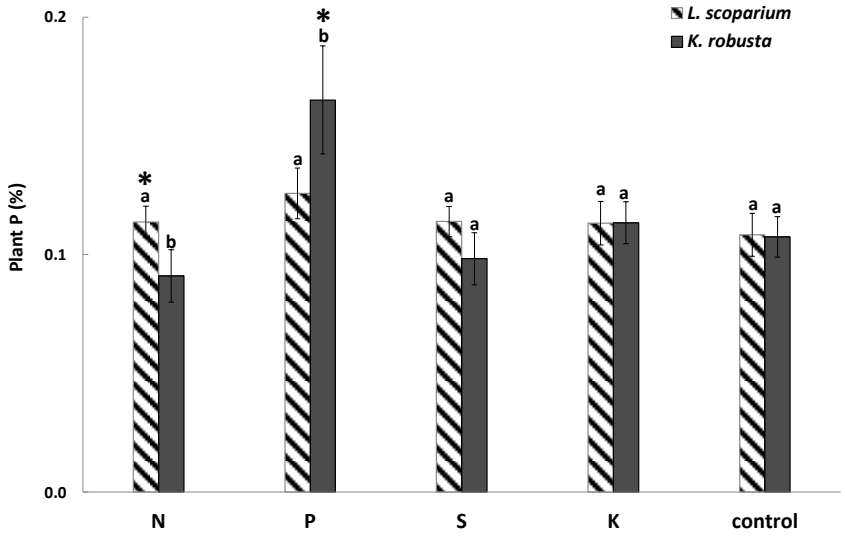


Figure 7. P concentration on foliar part in combination with basal macronutrient treatment (n=5).

Figure 3. 6 Leaf P concentration in combination with macronutrient treatment (n=5). Treatments that share letters have means that do not differ significantly (*p≤0.05*). Asterisks (*) signify significant differences between species at *p≤0.05*.

Figure 3.6 shows that the application of P to *K. robusta* in low fertility soil increased the concentration of foliar P by 0.2% compared to the rest of the treatments. The application of all macronutrients significantly altered the concentration of foliar P of *L. scoparium.* As shown by **Figure 3.6,** the application of individual 100 kg P ha^{-1} increased the concentration of P uptake in *K. robusta* by 100% compared to the control. This study found that *K. robusta* accumulated significantly higher foliar P than *L. scoparium* after amendment with 100 kg P ha^{-1} fertilizer.

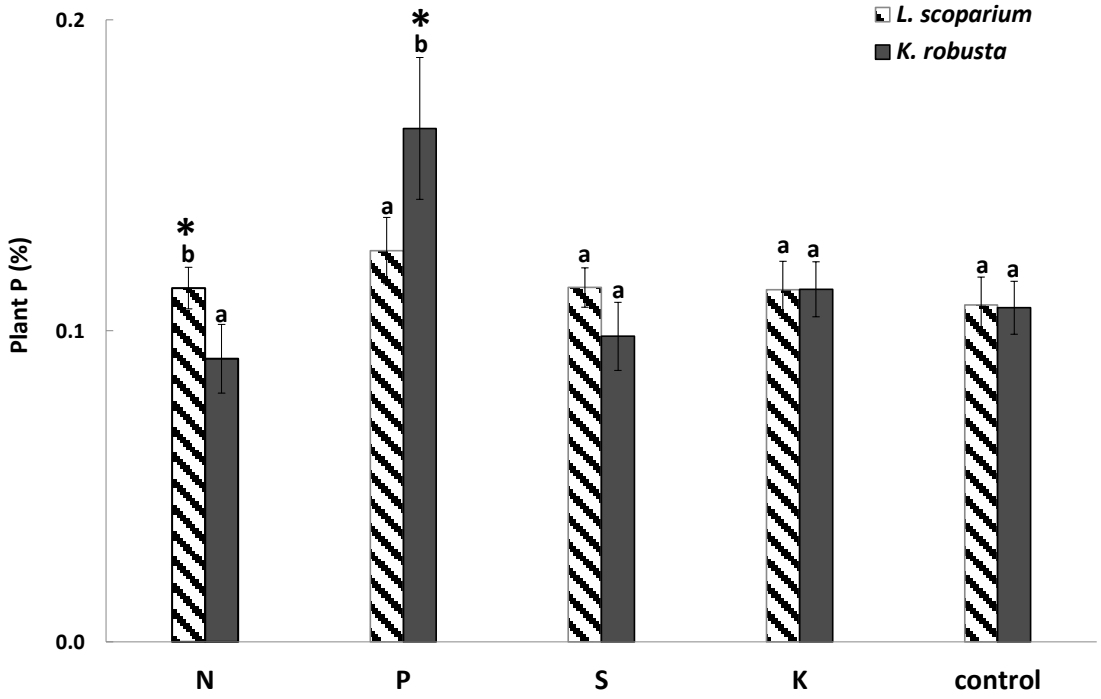


Figure 7. P concentration on foliar part in combination with basal macronutrient treatment (n=5).

Figure 3. 7 P concentration on foliar part in combination with basal macronutrient treatment (n=5). Treatments that share letters have means that do not differ significantly (*p≤0.05*). Asterisks (*) signify significant differences between species at *p≤0.05*.

Potassium (K)

In combination with *K. robusta,* the application of all individual fertilizers (N, P, K, and S) increased significantly (*p≤0.*05) foliar K compared to the control (**Figure 3.8**). The foliar K concentration in *K. robusta* was increased following the application of 100 kg K ha^{-1}, which ranged between 0.4% and 0.6% among treatments (**Figure 3.8**). On the other hand, amending low fertility soil with macronutrients did not significantly affect the accumulation of foliar K in *L. scoparium.*

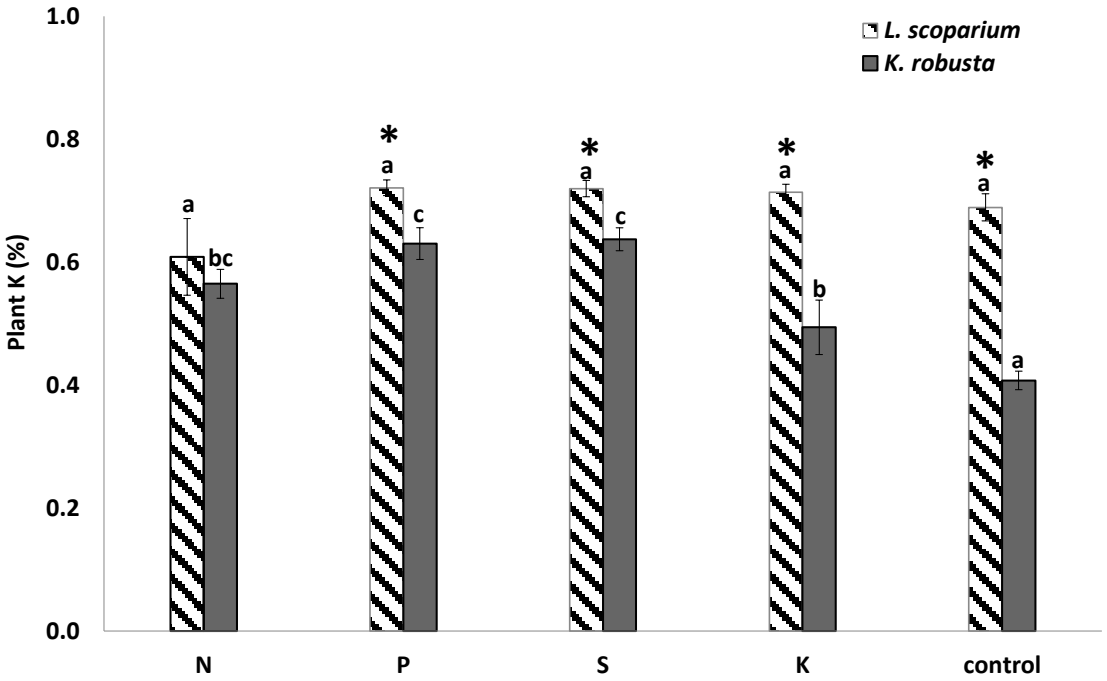


Figure 8. K concentration on foliar part in combination with basal macronutrient treatment (n=5). Figure 3. 8 Leaf K concentration in combination with macronutrient treatment (n=5). Treatments that share letters have means that do not differ significantly (*p≤0.05*). Asterisks (*) signify significant differences between species at *p≤0.05*.

The addition of all treatment (N, P, K, and S) significantly increased the K uptake by *K. robusta,* with treatment concentrations 25% - 50% higher than the control. **Figure 3.8** shows that the concentration of K not only increased in plants receiving 100 kg K ha^{-1} fertiliser but also increased in plants receiving 200 kg N ha^{-1}, 100 kg P ha^{-1}, and 100 kg S ha^{-1} fertilizers. This effect was not observed with *L. scoparium*, however, *L. scoparium* accumulated significantly higher foliar K than that *K. robusta* (**Figure 3.8**).

3.3.3 Rhizosphere soil nutrient concentration

Figures 3.9, 3.10, 3.11 and 3.12 show the concentrations distribution of nutrients in the N, P, K, and S treatments at the end of the experiment. In general, the P, S, K, Ca, and Mg concentrations in the soil of *L. scoparium* were relatively higher than of *K. robusta.* There was a significant difference (*p≤0.05*) of total soil P concentration between fertiliser treatments in combination with *L. scoparium* (**Figure 3.9**).

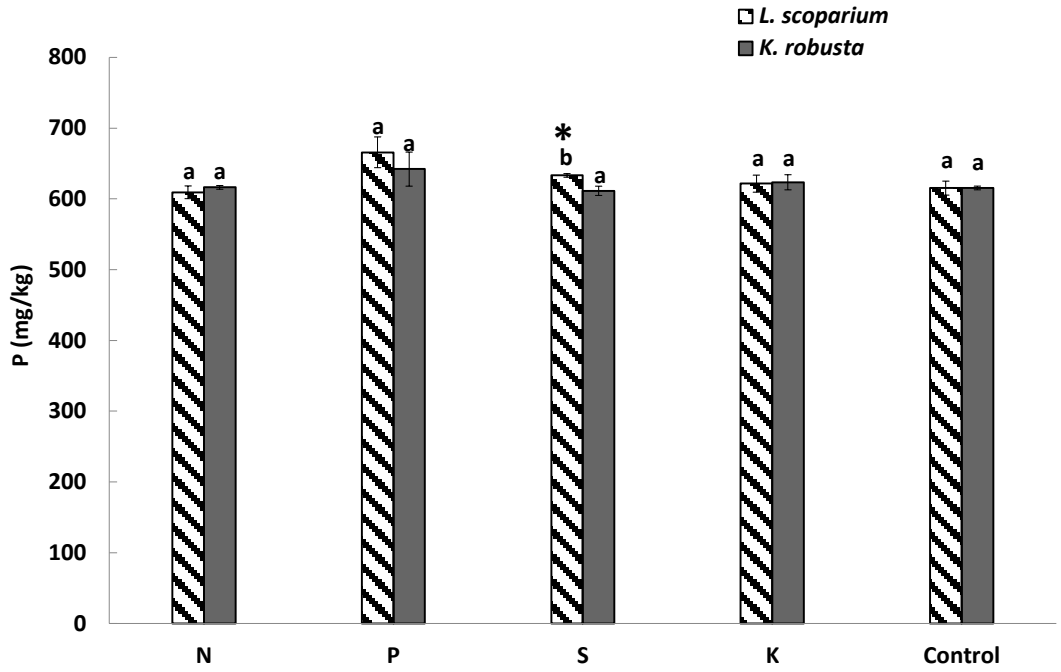


Figure 3. 9 Total soil P concentration in combination with macronutrient treatment (n=3). Treatments that share letters have means that do not differ significantly (*p≤0.05*). Asterisks (*) signify significant differences between species at *p≤0.05*.

The addition of 100 kg P ha^{-1} equiv. resulted in a significant increase in total P in the rhizosphere soil of *L. scoparium* by 15% compared to that of the unfertilised plant (**Figure 3.9**). On the other hand, *K. robusta* did not response positively to the application of nutrients with regard to total P concentration in rhizosphere soil. There was no difference in the total concentration of P following the application of individual P fertilizer in combination with both *L. scoparium* and *K. robusta*.

Following 100 kg K ha^{-1} equiv. application, there was no significant increase in K concentration compared to the control (**Figure 3.10**).

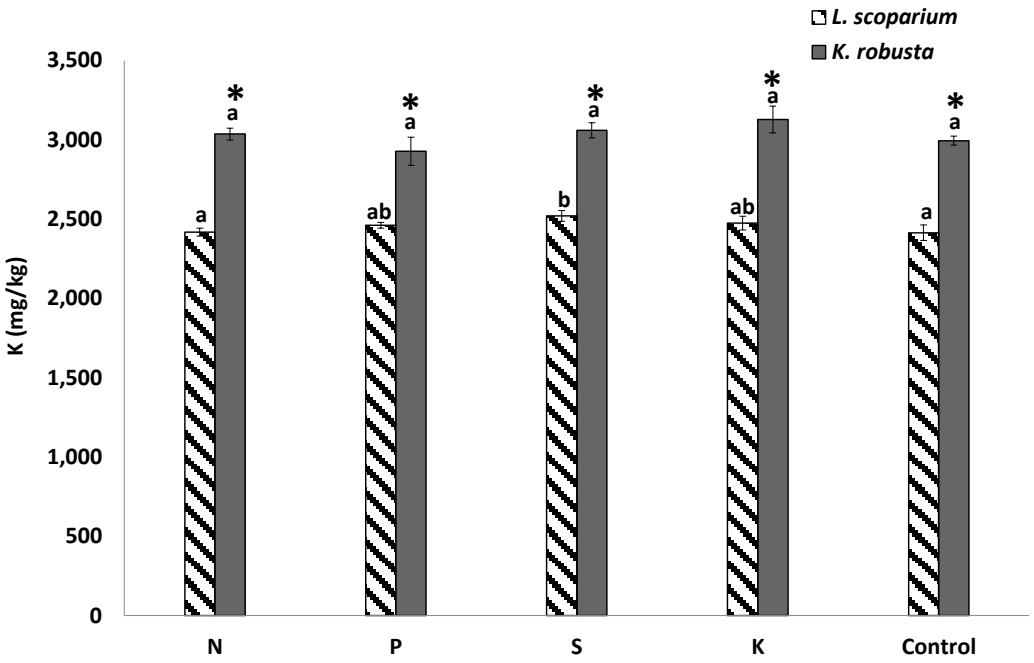


Figure 3. 10 Total soil K concentration in combination with macronutrient treatment (n=3). Treatments that share letters have means that do not differ significantly (*p≤0.05*). Asterisks (*) signify significant differences between species at *p≤0.05*.

Total S concentration

Fertilizer application increased the concentration of S in the rhizosphere of both *L. scoparium* and *K. robusta*. The addition of 100 kg S ha^{-1} fertilizer significantly increased the concentration of S within the rhizosphere soil of these species (**Figure 3.11**). The concentration of S in the rhizosphere soil of *L. scoparium* and *K. robusta* ranged between 0.04 and 0.05%. After the 16-week experimental period, the concentration of S in the rhizosphere soil of *L. scoparium* and *K. robusta* was increased by 23% and 21%, respectively (**Figure 3.11**).

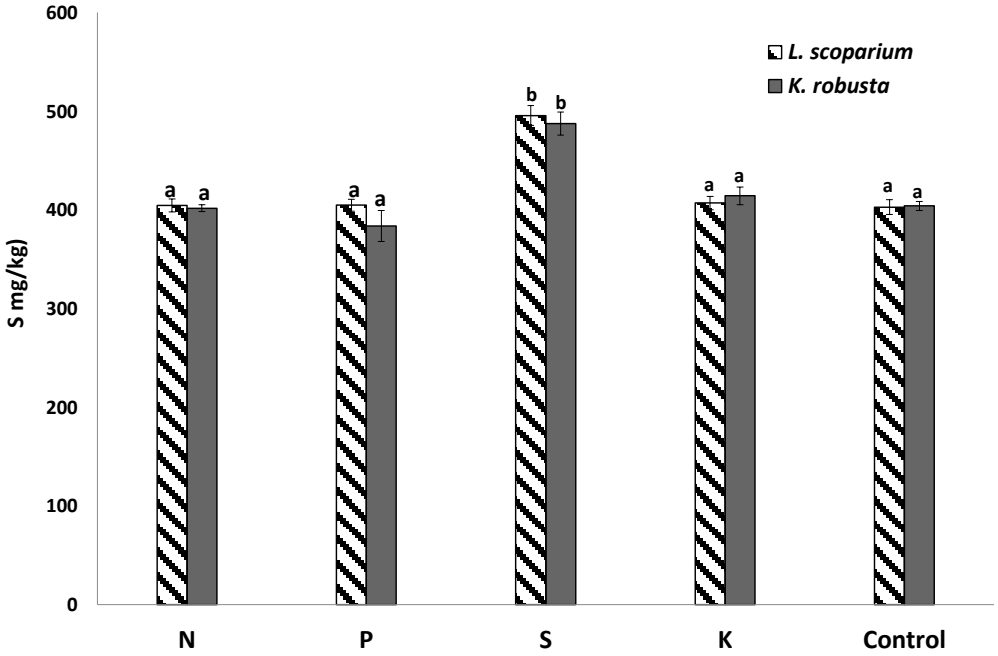


Figure 3. 11 Total soil S concentration in combination with macronutrient treatment (n=3). Treatments that share letters have means that do not differ significantly (*p≤0.05*).

Total Mg concentration

The study found that the application of all individual macronutrient fertilisers (200 kg N ha $^{-1}$, 100 kg P ha $^{-1}$, 100 kg K ha $^{-1}$, and 100 kg S ha $^{-1}$) significantly increased the concentration of Mg in the rhizosphere soil of *K. robusta*. At the end of the experiment, the total concentration of Mg in the soil of *K. robusta* treated with N, P, K, and S fertilisers was 0.2, 0.2, 0.19, and 0.2%, respectively, which were significantly (Duncan *p≤0.05*) higher than the unfertilised control (**Figure 3.1**).

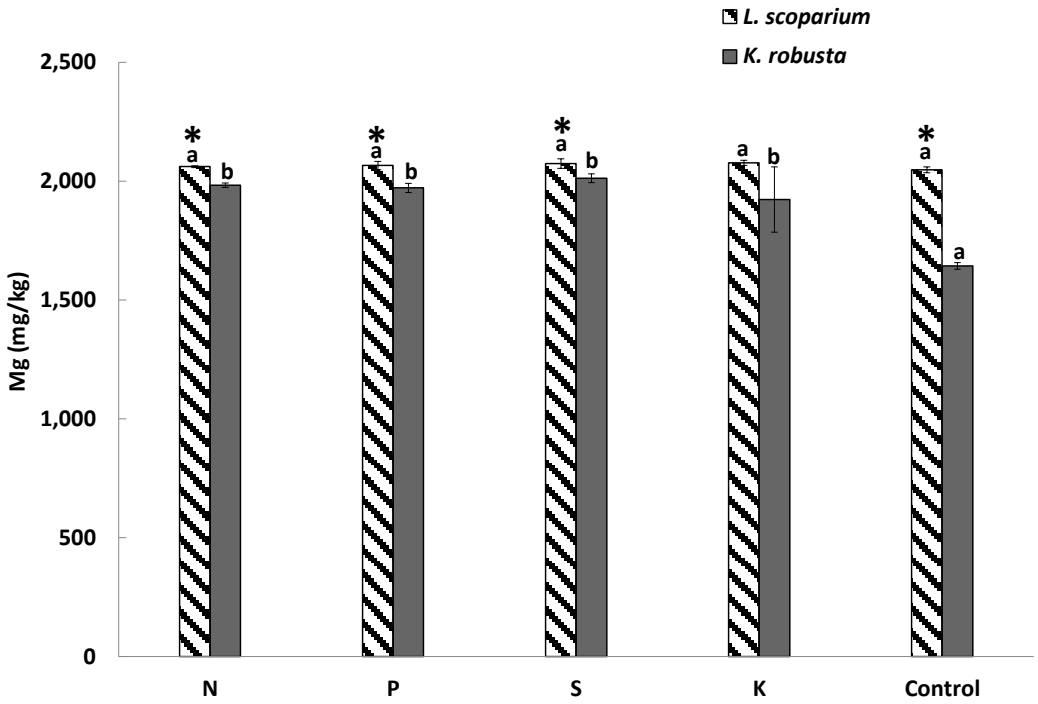


Figure 3. 12 Total soil Mg concentration in combination with macronutrient treatment (n=3). Treatments that share letters have means that do not differ significantly (*p≤0.05*). Asterisks (*) signify significant differences between species at *p≤0.05*.

Mineral N concentration

Analysis of variance showed that there was a highly significant (Duncan *p≤0.05*) difference in NO_3^--N concentration within the rhizosphere soil of *L. scoparium* and *K. robusta* (**Figure 3.13**). The application of 200 kg N ha^{-1} resulted in an increase of NO_3^--N from 0.2 to 3.5 mg kg^{-1} and from 0.2 to 3.1 mg kg^{-1} within the rhizosphere soil of *L. scoparium* and *K. robusta,* respectively**.**

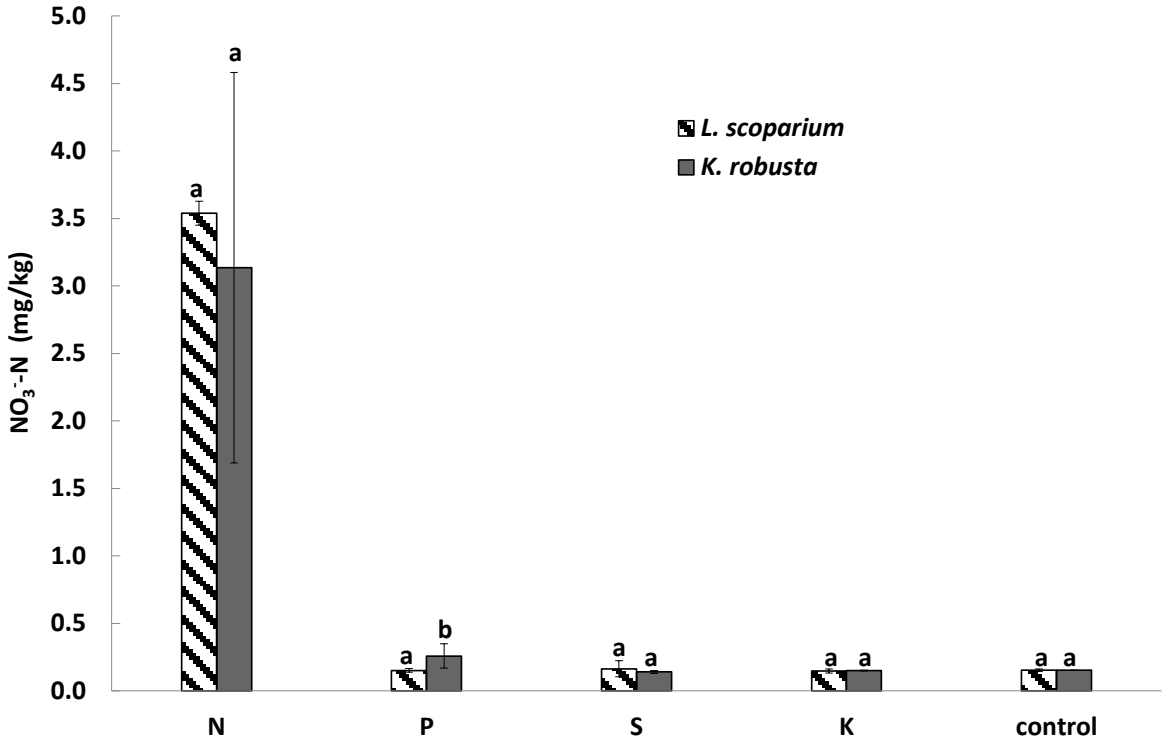


Figure 3. 13 Soil NO_3^--N in combination with macronutrient treatment (n=3). Treatments that share letters have means that do not differ significantly (*p≤0.05*).

3.4 Discussion

3.4.1 Plant growth

The growth of *K. robusta* was greater than *L. scoparium* following the application of 200 kg N ha^{-1} urea fertilizer, producing significantly higher above-ground biomass. The application of nutrients may have resulted in the significantly higher accumulation of essential nutrients including N, P, and K, thus enabling the greater growth of *K. robusta,* whereas *L. scoparium* took up only N significantly compared to the control. The results indicate that the nutrient concentration, especially N, applied in this study, was sufficient for *K. robusta* to stimulate the uptake of this essential element, thus enhancing growth. This finding is in agreement with Hunter (2001), who reported that the application of 320 kg N ha^{-1} significantly increased the total dry above ground biomass of *Eucalyptus camaldulensis* and *Eucalyptus grandis,* relatives of *L. scoparium* and *K. robusta,* by 74% during a 37 month experiment. Xu et al. (2002) and Fernandez et al. (2000) reported that *E. grandis, E. urophylla,* and *E. camaldulensis* accumulated significantly higher P, thereby resulted in significantly higher biomass production compared to non-fertilized treatment. In addition, the increase of the total biomass production (33%) of *K. robusta* during the experiment under treatments was similar to increases reported in the literature (Hunter, 2001) for *E. camaldulensis* and *E. grandis* receiving of 320 kg N ha^{-1}.

Since *L. scoparium* responded positively to N fertilizer application, it is likely that applying higher amounts could stimulate growth further. Campion et al. (2006) found that *E. grandis* grown on low fertility soil did not produce significant difference in leaf biomass following the application of 106 kg N ha^{-1}, 113 kg P ha^{-1}, and 77 kg K ha^{-1} during 4-year trial period. This study indicates that applying higher rates of macronutrients as well as extending the length of experimental period of this present study may increase the growth of *L. scoparium*.

3.4.2 Element uptake

The increased uptake of N by both *L. scoparium* and *K. robusta*, and P and K by *K. robusta* in this present study is in agreement with previous studies on plants in the myrtaceae family. Judd et al. (1996) and Hunter (2001) reported that amending soil with 350 - 400 kg N ha^{-1} fertilizer during 3-4 year experimental period significantly increased the foliar N of *E. globulus, E. camaldulensis* and *E. grandis.* Judd et al. (1996) reported that these species increased P uptake in response to fertiliser application. Ringrose and Neilsen (2005) reported that the application of 700 kg P ha^{-1} increased the total foliar P of *E. Grandis*. Xu et al. (2002) reported that the application of 208 kg P ha^{-1} significantly increased the P uptake of *E. Grandis and E. urophylla* by. Campion et al. (2006) found that application of single superphosphate significantly elevated the P uptake of *E. Grandis* compared to control. In addition, Albaugh et al. (2015) reported that the same eucalyptus species responded to the application of 117 kg P ha^{-1} by increasing foliar P up to ± 25% during a one year growth period. The significant uptake of foliar K was reported by Hunter (2001) and Weggier et al. (2008), who found that *E. pilularis* and *E. camaldulensis* accumulated significantly higher foliar K concentrations in response to the application of 100 kg K ha^{-1}.

The significant uptake of foliar N, P, and K by *L. scoparium* and or *K. robusta* in this study is comparable to the results of previous studies using *E. camaldulensis* and *E. grandis,* which increased foliar N by 28 and 5%, respectively when receiving 350 kg N ha^{-1} fertilizer (Hunter, 2001). The significant uptake of foliar P by 100% in *K. robusta* is higher than that of found by Hunter (2001), Ringrose and Neilsen (2005), Campion et al. (2006), Judd et al. (1996), Albaugh et al. (2015) and Xu et al. (2002) who studied the effect of fertilisers application on several relatives species of *L. scoparium* and *K. robusta.* The application of 115, 208 and 700 kg P ha^{-1} increased the total foliar P of *E. Grandis* by 43% (Campion et al., 2006), 10% (Ringrose and Neilsen, 2005) and 56% (Xu et al., 2002), respectively. Albaugh et al. (2015) reported that the same eucalyptus species responded to the application of 117 kg P ha^{-1} by increasing foliar P up to ± 25% during a one year growth period. Xu et al. (2002) reported that the application of 208 kg P ha^{-1} significantly increased the P uptake of *E. urophylla* by 56%. The significant increase of foliar P uptake (100%) found in *K. robusta* in this study was higher than that reported by

Judd et al. (1996), who found that *E. globulus* responded to the application of 50-200 kg P ha^{-1} by increasing its foliar P uptake by 11% compared to unfertilized treatment. The significant increase of 50% of foliar K detected in *K. robusta* in this study is comparable to the previous studies done by Hunter (2001) and Judd et al. (1996). Application of 100 kg K ha^{-1} resulted in 0.5 and 36% of foliar K uptake in *E.camaldulensis* and *E. grandis,* respectively (Hunter, 2001), whereas amending the soil with 100 kg K ha^{-1} significantly increased the accumulation of foliar K by 13% (Judd et al., 1996).

In response to the treatments, both *L. scoparium* and *K. robusta* increased foliar N, whereas foliar P and K were only detected significantly higher in biomass of *K. robusta* compared to control. These findings are in agreement with (Baldani and Döbereiner, 1980), (Mason et al., 2000), and Mazzola et al. (2002) who found that the role of plants in the availability and mobility of nutrients through root-microbes interaction is dependent on the species. The treatments could have stimulated root exudation (Koo et al., 2013), including organic acids, which play an important role for solubilisation and mobilization of certain nutrients (Bertin et al., 2003). In addition, since the composition strongly varies with plant species (Walker et al., 2003), this can lead to different plant responses in terms of nutrient uptake.

3.4.3 Elemental composition in rhizosphere soil

The significant change of concentrations of total P, K, S, and Mg in rhizosphere soil following the application of fertilizers was in agreement with several previous studies using eucalyptus species. Ringrose and Neilsen (2005) reported that in combination with *E. regnans*, the application of individual fertilizers contained 322 kg P ha^{-1} and 364 kg S ha^{-1} significantly increased the concentrations of total P and S in top soil (0-30 cm). Dias et al. (2000) found that the in combination with *E. camaldulensis*, amending soil with 18 – 72 kg P ha^{-1}, which was in the form of superphosphate, increased significantly the available P in the top soil (0-15 cm depth) compared to control.

The response of both species on the concentration of total macronutrients in rhizosphere soil is comparable to the results found by previous authors. Although the increment of concentration of total P in soil in this study (15%) was lower than that of reported by Ringrose and Neilsen (2005), who found 100% increment of total P in soil, the total P concentration of 0.07% within the rhizosphere soil of *L. scoparium* found in this study was higher than that of 0.04% reported by (Ringrose and Neilsen, 2005) using *E. regnans* in combination with 322 kg P ha^{-1}, which is higher than the rate in this study.

3.5 Conclusions

Kunzea robusta responded to individual macronutrients by increasing the aboveground dry biomass as well as the foliar N, P, and K. Unlike *K. robusta* species, the application of macronutrients did not significantly affect the growth of *L. scoparium*, but significantly increased N uptake only. In response to applied N, P, K, and S, *K. robusta* accumulated higher foliar N, P, and K, whereas *L. scoparium* accumulated higher N only and neither *L. scoparium* nor *K. robusta* uptake significantly higher S compared to unfertilized plants. In addition, the treatments significantly increased the concentration of P and NO_3^- (in combination with *L. scoparium*), S (in combination with both species), and Mg (in combination with *K. robusta*) in rhizosphere soil. This study only shows the results of young seedlings. It is unclear whether older plants will respond similarly. Nevertheless, the results of these experiments indicate that it is likely that biowastes, which often contain elevated concentrations of N, P, K, and S, will increase the foliar concentrations of these elements in *L. scoparium* and *K. robusta* and may increase the growth, at least of *K. robusta.* This will be the focus of the following Chapters.

Chapter 4

The response of manuka (*Leptospermum scoparium* J.R Forst) and kanuka (*Kunzea robusta* de Lange & Toelken) to the application of biosolids and dairy shed effluent in a low fertility soil

4.1 Introduction

4.1.1 Background

Biosolids and Dairy Shed Effluent (DSE) can contain elevated concentrations of plant nutrients (Antoniadis et al., 2008b; Bai et al., 2014; Bai et al., 2013; Bright and Healey, 2003; Cogger et al., 2013; Di et al., 1998; Hawke and Summers, 2006; Haynes et al., 2009; Hedley et al., 2013; Moir et al., 2013; Paramashivam, 2015b; Zaman et al., 2002). The low C: N ratio of biosolids and DSE makes them a net N source, where the N and other nutrients are released slowly from these biowastes as they decompose in the soil (Gilmour et al., 2003; Murphy et al., 2007; Powlson et al., 2012). Therefore, the land application of these biodegradable materials can provide short and long-term benefits to soils (Ginting et al., 2003; Zhang et al., 2015) and crops, which can lead to a lower requirement for mineral fertilizers. Various studies have shown positive effects of DSE and biosolids application on forest tree species, which can subsequently provide economic returns through increased biomass and soil nutrients, while avoiding accumulation of biosolids derived contaminants above threshold values (Kimberley et al., 2004; Singh and Agrawal, 2008; Wang and Jia, 2010; Zaman et al., 2002). The application of biosolids provides nutrients, increases organic matter, improves soil structure, enhances nutrient absorption by plants (Antolín et al., 2005; Freeman and Cawthon, 1999; Morera et al., 2002; Singh and Agrawal, 2008; Weber et al., 2007), as well as increase the number and activities of soil microbes (Cytryn et al., 2011; Rogers and Smith, 2007; Singh and Agrawal, 2008). Biosolids have been used as fertilizers or composts in land applications to improve and maintain soil productivity, stimulate plant growth and establish sustainable vegetation at mine sites (Fresquez et al., 1990). They enhance the activities of soil enzymes as well as the number and biomass of soil organisms due to its high organic matter content and nutrient availability (Lteif et al., 2007; Singh and Agrawal, 2008). Frequent applications of biosolids has positive ecosystem effects with relatively low extractable metal levels in soil and support greater plant biomass and tissue quality (Sullivan et al., 2006). Moderate application rates of biosolids to low organic matter and clay content soils enhances soil organic carbon and increases nutrient retention (Antoniadis, 2008), enhances the adsorption capacity of soil to immobilize heavy metals such as Cu, and effectively reduced Pb availability in a high Pb urban soil

(Brown et al., 2003). The application of DSE, resulted in a greater and more diverse microbial biomass in soil (Hawke and Summers, 2006). In addition, the enzyme activities of root exudates of ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) pasture, grown on Templeton sandy loam, significantly increased N mineralization due to the application of DSE (Zaman et al., 1999b). Another study found that the application of DSE improved long-term soil fertility by increasing the concentration of total N, total P and plant available nutrients (Hawke and Summers, 2006). However, the application of biosolids and DSE to forest soil can result in decreased forest productivity because there is a strong dependence on the composition of biowastes, soil type and plant species (Cline et al., 2012).

In New Zealand, in 2010, there are approximately 2.5 million ha (**Figure 4.1)** of land in forest in which *Pinus radiata* are the most fastest growing commercial plantations (Paramashivam, 2015a). Several thousands of hectares are classified as degraded or low-fertility soils as during the logging, most of the top soil, which contain a significant higher organic matter, are being removed. As a result, the soil has become acidic and depleted in nutrients (Paramashivam, 2015a). Hence, these kinds of lands can be an appropriate alternative for biowastes addition as the contaminants associated with biowastes are less to enter the food chain.

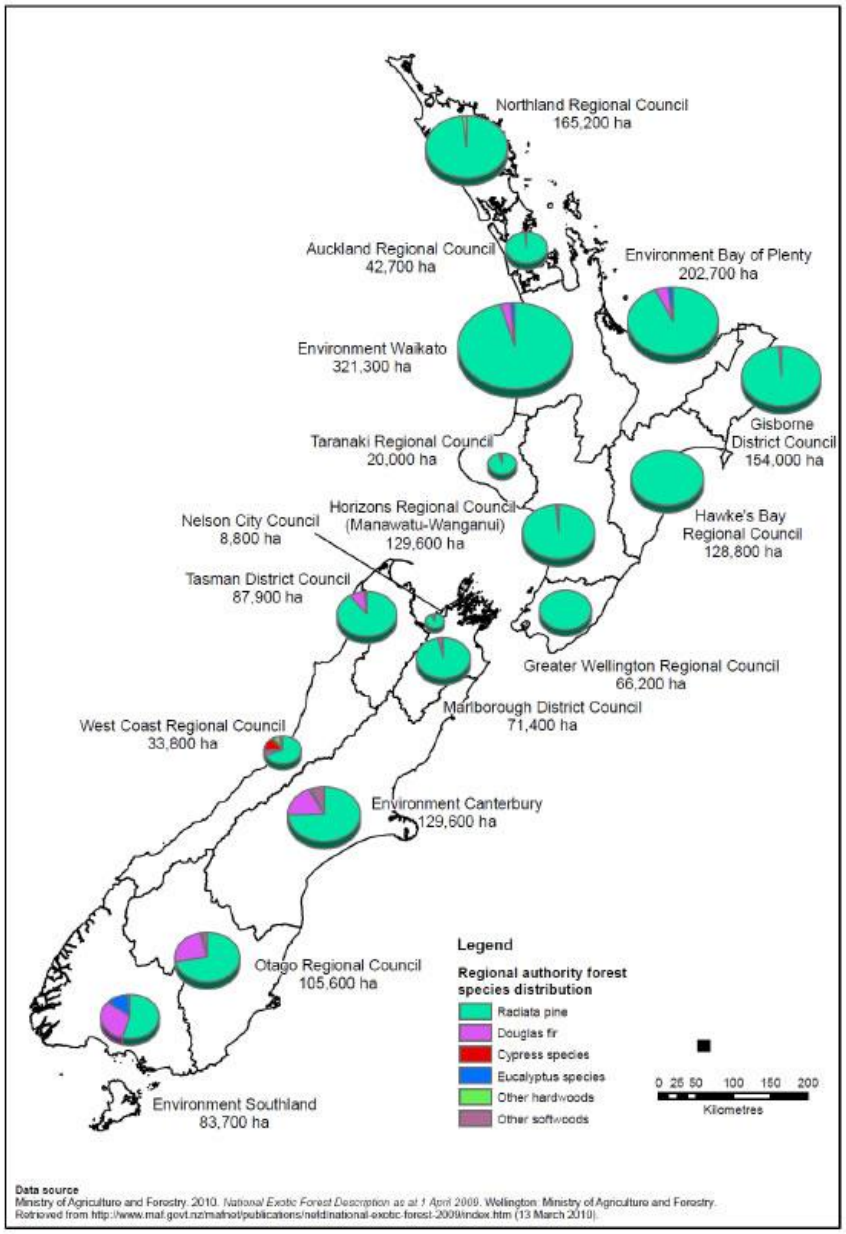


Figure 1. Distribution nof commercial

Figure 4. 1 Distribution of commercial forest species by region in New Zealand (MAF, 2010)

Chapter 3 of this thesis showed that *L. scoparium* and *K. robusta* responded positively to the addition of macronutrients. These two New Zealand's native plants produced significantly higher above ground dried biomass as well as elevated N, P, and K uptake under individual application of macronutrients. The application of 200 kg N ha^{-1} increased the above ground dry biomass of kanuka by 33% and increased the N uptake of both manuka and kanuka by 19% and 78% respectively. The addition of 100 kg P ha^{-1} and 100 kg K ha^{-1} significantly increased the foliar P and K of *K. robusta* by 100% and 50% respectively. The study found that the application of macronutrients significantly increased the concentration of P, S, Mg and NO_3^- in the rhizosphere soil. Although in combination with *L. scoparium* it did not significantly affect its growth, the application of macronutrients significantly increased N

uptake. I hypothesized that fresh biosolids and DSE will enhance the growth of *L. scoparium* and *K. robusta* in low fertility soil because DSE and biosolids which high concentrations of these macronutrients (Antolín et al., 2005; Bradley, 2011; Hawke and Summers, 2006; Kimberley et al., 2004; Singh and Agrawal, 2008; Wang et al., 2009; Zaman et al., 2002). Further, I hypothesized that biosolids, but not DSE, will lead to elevated concentrations of Cd, Cu and Zn in the plants, as these elements occur at elevated concentrations in biosolids (Simmler et al., 2013).

4.1.2 Aims

I aimed to measure the growth and the elemental composition of the leaves of *L. scoparium* and *K. robusta* following the application of fresh biosolids and fresh DSE.

4.2 Materials and Methods

4.2.1 Experimental setup

The experiment was conducted at Lincoln University greenhouse facility (43°38’42.3”S 172°27’41.0”E). Low fertility soil with yellow-grey earths, mostly classified as Lismore stony silt-loam derived from Greywacke gravels and thin loess deposits from a former pine plantation of Eyrewell (**Plate 4.1A** - 43^0 25’19” S, 172^0 15’52”E), New Zealand, was used as the planting medium. Fresh Dairy Shed Effluent (DSE) was collected from Lincoln University Dairy Farm, New Zealand (**Plate 4.1B** - 43°38’40"S, 172°26’ 32” E; 17 m a.s.l) in January 2015. Biosolids were obtained from the Kaikoura Wastewater Treatment Plant, New Zealand (**Plate 4.1C** - 42°21'37.40"S, 173°41'27.35"E) in July 2014. The initial treatment consisted of sedimentation and anaerobic digestion in settlement ponds for 6-8 months.

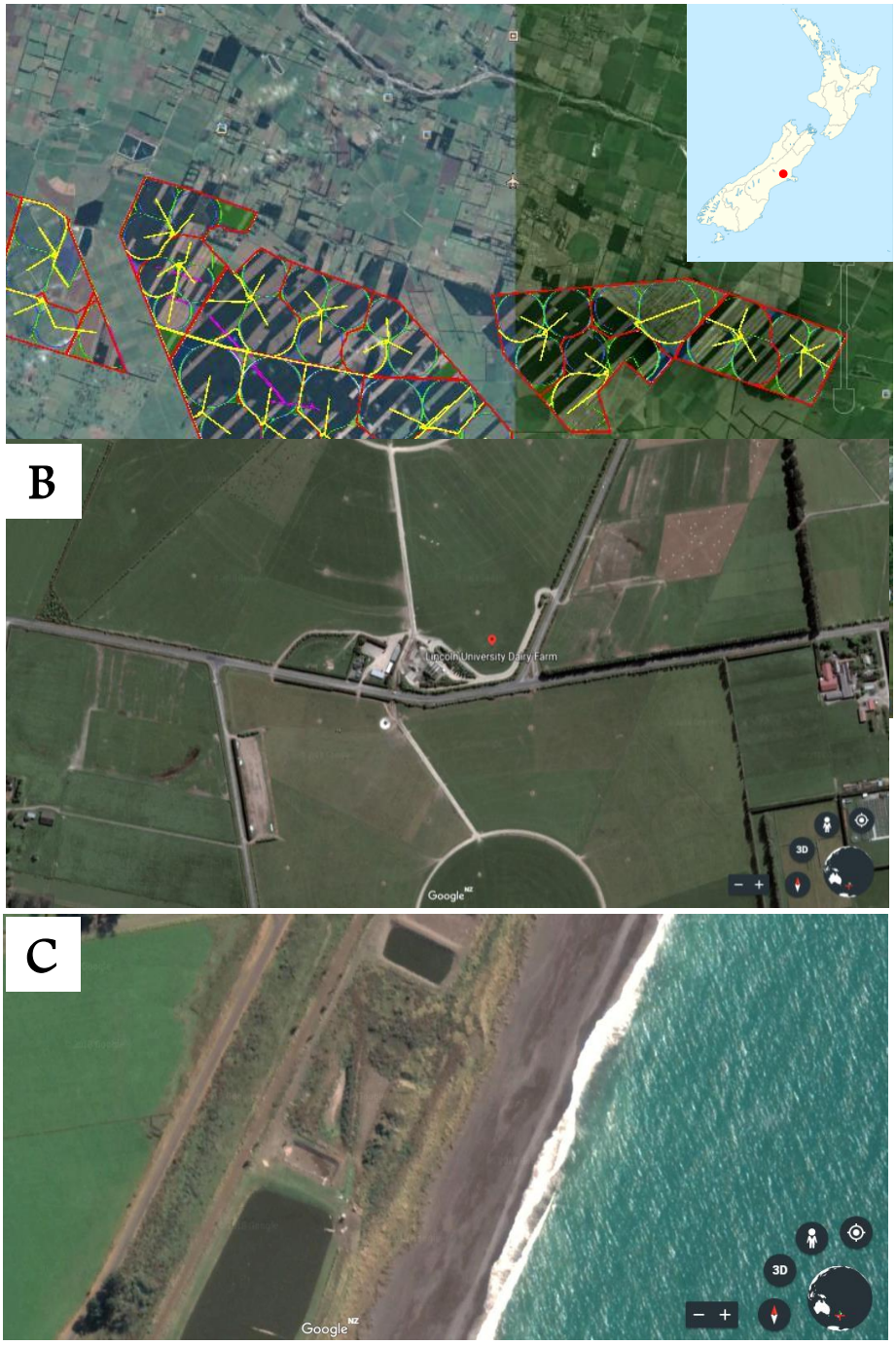


Figure 2 (A) a former pine plantation of Eyrewell for obtaining soil

Plate 4. 1 (A) A former pine plantation at Eyrewell where low-fertility soil was obtained; (B) Lincoln University Dairy Farm, New Zealand for sourcing DSE; (C) Kaikoura Wastewater Treatment Plant for collecting biosolids (Google Earth).

The key properties of soil, DSE, and biosolids used in this experiment are presented in **Table 4.1**.

Table 4. 1 Concentration of nutrients, trace elements and contaminants in soils, DSE, and biosolids used in the present study. Values in brackets represent standard error (n=15[1]; n=6[2]; [3]n=5[3])

Properties	Soil[1]		DSE[2]		Biosolids[3]	
pH	4.5	(0.3)	7.5	(0.01)	4.5	(0.0)
C [%]	4.3	(0.4)	0.11	(0.0)	27	0.7)
N [%]	0.17	(0.02)	0.02	(0.0)	2.5	(0.6)
P [%]	0.05	(0.00)	0.001	(0.0)	0.50	(0.0)
K [%]	0.2	(0.01)	0.002	(0.0)	0.14	(0.01)
S [%]	0.03	(0.00)	0.001	(0.0)	0.87	(0.01)
Ca [%]	0.2	(0.01)	0.003	(0.0)	0.63	(0.01)
Mg [%]	0.3	(0.00)	0.001	(0.0)	0.30	(0.00)
B [mg kg^{-1}]	5.0	(0.3)	0.04	(0.0)	27	(0.1)
Cu [mg kg^{-1}]	4.1	(0.2)	0.0	(0.0)	891.0	(18.9)
Zn [mg kg^{-1}]	72	(1.5)	0.08	(0.0)	1073	(27)
Mn [mg kg^{-1}]	265	(15)	0.04	(0.0)	185	(4.5)
Fe [mg kg^{-1}]	21121	(291)	0.05	(0.0)	14534	(92)
Cd [mg kg^{-1}]	0.2	(0.01)	0.04	(0.0)	4.0	(0.1)

Thirty-six 10 L pots (25 cm in diameter with a height of 29 cm) were used (**Figure 4.2**). The treatments contained a total of 6 L Dairy Shed Effluent (DSE) which is 220 kg N ha^{-1} equiv. and 1 kg fresh biosolids per pot, which was 2600 kg N ha^{-1} equiv. The justification of applying different N loadings between DSE and biosolids was the speciation of N in the material. Biosolids mostly contain organic-N, which is unavailable for plant uptake and not subject to leaching (Gilmour et al., 2003; Pu et al., 2012). Only small amounts of N are present in forms of nitrate (NO_3^-) and ammonium (NH_4^+) (Eldridge et al., 2008); therefore, high rates of biosolids are necessary to establish plant growth and ecosystem function in low-fertility soils and degraded environments. While the organic N in biosolids will eventually mineralise and release ammonium and then nitrate, this process was not significant on the timescale of these experiments.

The DSE and biosolids were first homogenised thoroughly using a 100 L plastic tank and black tarpaulin respectively (**Plate 4.2).** DSE then further stored in the fridge for further application in the greenhouse. The biosolids were mixed with soils at the beginning of the experiment. For each individual pot, 1 kg fresh biosolids was mixed completely with 9 kg fresh soil using a 20 L bucket. The soil was then filled into the pot in layers to give a soil bulk density of approximately 1.3 g cm^{-3}. *L. scoparium* and *K. robusta* seedlings were obtained from Waiora Nursery Ltd., Christchurch, New Zealand. All plants were transplanted directly after all pots were filled with medium (soil and plus biosolids). The pots were arranged in the glasshouse in a randomized block design.

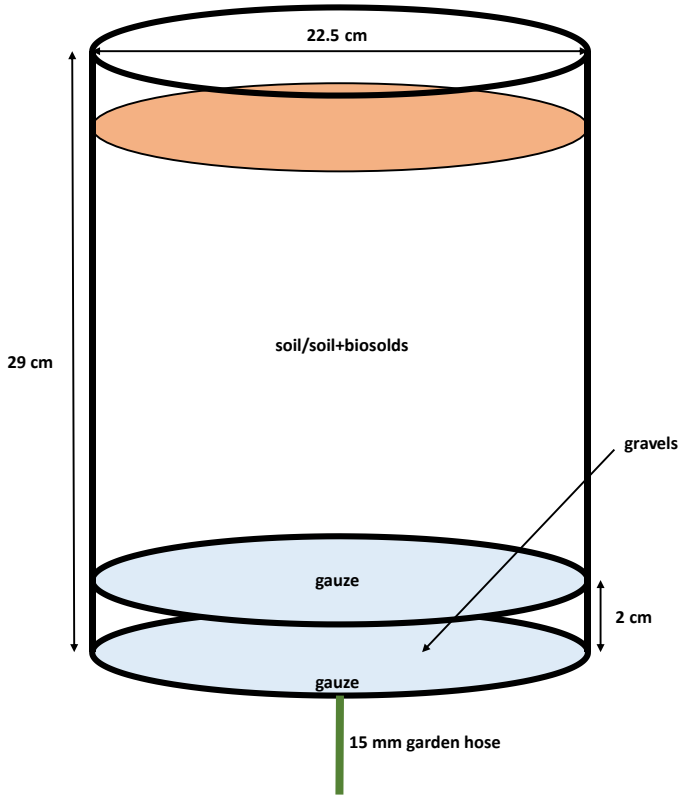


Figure 2. Pot design used in the present
Figure 4. 2 Pot design used in the present study

To avoid preferential flow, DSE was applied gently on to the soil surface of the pots which contained 9 kg of fresh soil with soil bulk density of approximately 1.3 g cm^{-3}. DSE was applied weekly (500 mL $week^{-1}$). In the first two weeks (January 12th, 2015 and January 19th, 2015), the DSE was applied daily (from Monday to Friday) of 100 mL of each application, 3 hours after irrigating the pots. During the next three weeks (Jan 26th, 2015; Feb 2nd and 9th, 2015) the DSE was applied on Monday, Wednesday, and Friday at rates of 150 mL, and 200 mL respectively. From February 2nd, 2015 to March 3rd, 2015, it was applied twice per week (Monday and Friday) of 250 mL of each application. In the last two weeks before harvesting the experiment, 500 mL of fresh DSE was applied weekly only (Mondays). Each treatment had 4 replicates. The controls received neither biosolids nor Dairy Shed Effluent. During the experiment, the pots were irrigated with measured amount of water using an automated irrigation system. Each pot received 200 mL of water twice a day over the experimental period to ensure optimal plant growth at conditions near field capacity. The temperature in the greenhouse ranged from 9 to 20°C during the night (10 pm until 6 am) and from 14°C to 28°C during the day.

After 12 weeks, the above ground biomass was carefully harvested and weighed. Plant samples was dried at 70^0C until constant weight was obtained and ground using a Retch ZM200 grinder.

Plate 4. 2 (a) Homogenising of DSE and: (b) biosolids used in the experiment

Soil pH was determined using pH meter (MTSE). A 10 g portion of soil of soil was mixed with 25 mL deionised water and then shaken for two hours using an end-over-end shaker (at 20 rpm). The plant-available elements were determined using a 0.05 M $Ca(NO_3)_2$ extraction (Esperschuetz et al., 2017). Concentrations of Ca, K, S, Cd, Cu, Mn, and Zn were determined using inductively coupled plasma optical emission spectrometry (ICP-OES Varian 720 ES - USA). Reference soil and plant material from Wageningen University, the Netherlands (International Soil analytical Exchange 921 and International Plant analytical Exchange 100) was analysed with the samples. Recoverable concentrations were 81–112% of the certified values.

4.2.2 Data and statistical analysis

The aboveground plant biomass and foliar nutrients concentrations were statistically analysed using analysis of variance (ANOVA) at α=0.05. The fixed effect were plant species and macronutrients application and their interaction, and experimental block as a random additive effect. One-way ANOVA was also used to investigate the effect of macronutrients treatment on species biomass and nutrient uptake individually followed by Duncan post-hoc tests at *P=0.05*. The analyses were done in IBM SPSS v.**22** (International Business Machines Corp., New Orchard Road, Armonk, New York 10504 914-499-1900).

4.3 Results

4.3.1 Aerial biomass production

Figure 4.4 shows the cumulative biomass (g per pot) of *L. scoparium*, and *K. robusta* in combination with DSE, biosolids, and control.

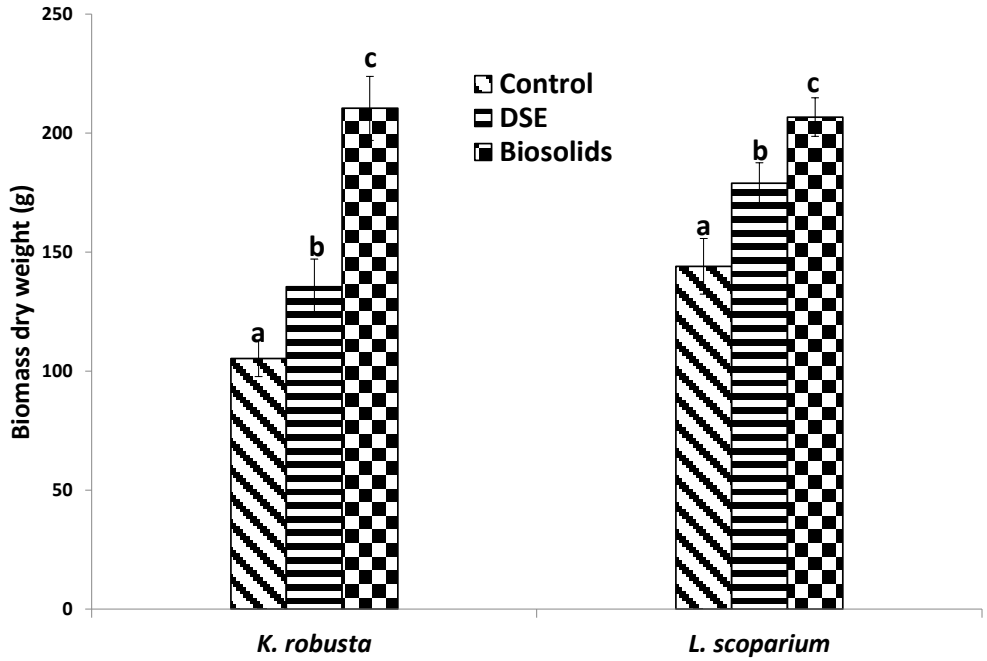


Figure 4. Cumulative above ground biomass of *L. scoparium, K. robusta, and L.*

Figure 4. 3 Cumulative above ground biomass of *L. scoparium, K. robusta, and L. perenne* in combination with DSE, biosolids, and the control (n=4). Treatments that share letters have means that do not differ significantly (*p≤0.05*).

Figure 4.3 shows that compared to the control, the addition of 2600 kg N ha^{-1} equiv. of biosolids and 200 kg N ha^{-1} equiv. of DSE significantly (*p≤ 0.05*) increased the cumulative biomass production of *L. scoparium* and *K. robusta.* Twelve weeks after applying treatments, significant differences were detected in the growth response of *L. scoparium* and *K. robusta* as a result of different treatments, ranking in order of biosolids > DSE > control (**Figure 4.3** and **4.4**).

In combination with *K. robusta*, biosolids application resulted in the highest increment (100%) of biomass, from 105 g per pot, equivalent to 21 t ha^{-1} to 210 g per pot, equivalent to 43 t ha^{-1}. In combination with *L. scoparium* by comparison, biosolids application significantly increased its biomass by 44% higher than the control, from 144 g per pot to 207 g per pot, equivalent to 41 t ha^{-1}.

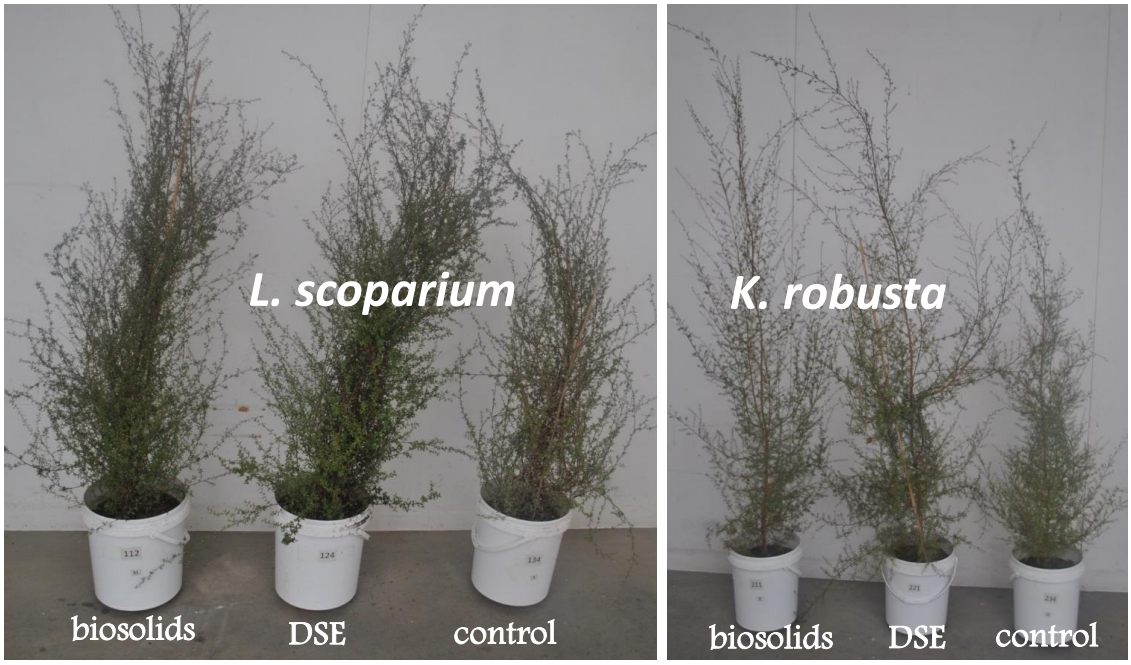


Figure 4. 4 Plant growth responses under different treatments of 12 weeks experiment period under Eyrewell soil medium.

DSE increased the above ground biomass of *K. robusta* by 24%, up to 135g per pot, equivalent to 28 t ha^{-1}. Whereas in combination with *L scoparium*, amending soil with DSE resulted in a significant increase of the above ground dried biomass by 29%, up to 179 g per pot, equivalent to 36 t ha^{-1}. There was a significant difference in above ground biomass between *L. scoparium* and *K. robusta* in combination with DSE (**Figure 4.5**). In combination with DSE, *L. scoparium* produced 25% higher above ground dried biomass than that of in *K. robusta.*

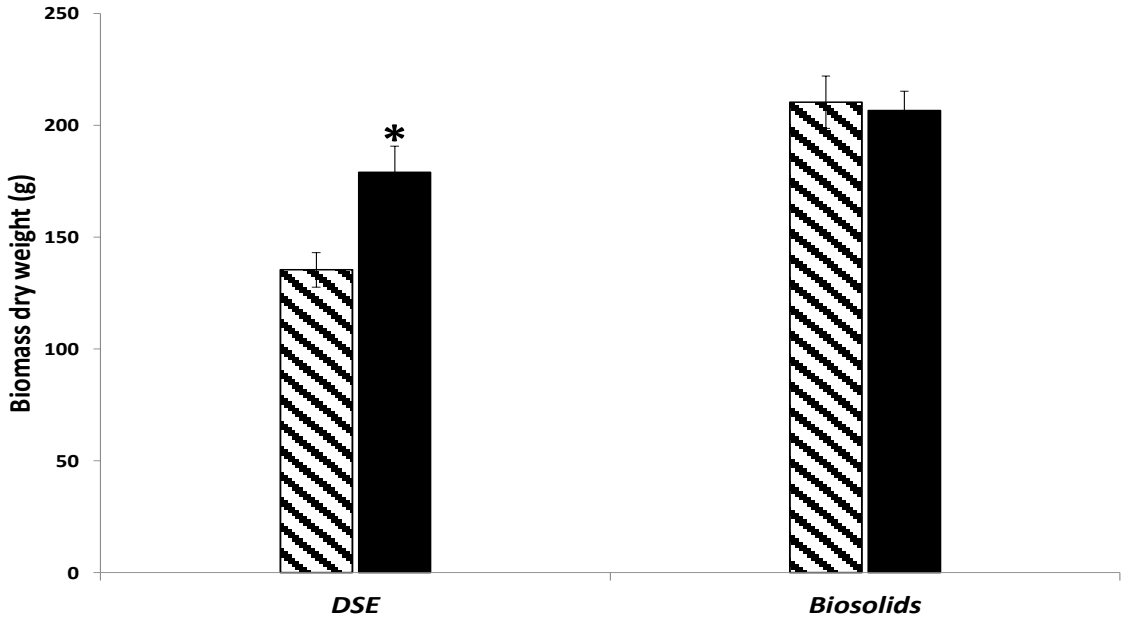


Figure 6. Comparison cumulative above ground biomass of *L. scoparium* and *K.*

Figure 4. 5 Comparison cumulative above ground biomass of *L. scoparium* and *K. robusta* combination with DSE and biosolids (n=4). Asterisks (*) signify significant differences between *K. robusta* (striped bars) and *L. scoparium* (solid bars) at *p≤0.05*.

4.3.2 Element uptake

Macronutrients

The foliar macronutrient concentrations and ratios of *L. scoparium* and *K. robusta* measured at the end of the experiment are presented in **Figures 4.6** and **4.7.** Compared to the control, in combination with *L. scoparium*, the application of both DSE and biosolids significantly (*p≤0.05*) increased the uptake of the concentration of foliar Ca by 21% and 29% higher than the control, respectively (**Figure 4.6**). Whereas in combination with *K. robusta,* DSE and biosolids addition resulted in 22% and 51% higher concentration of foliar Ca than control. There was no significant different of Ca uptake between DSE and biosolids treatment in combination with *L. scoparium* (**Figure 4.6**).

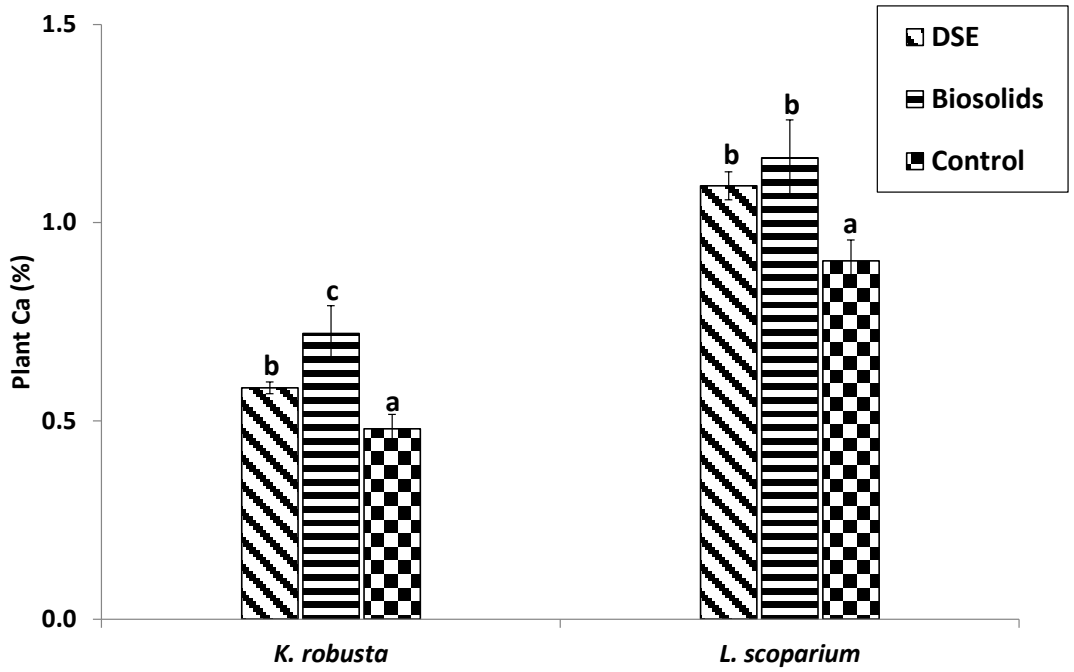


Figure 8 Total concentrations of foliar Ca (%) of *L. scoparium* and *K. robusta* measured

Figure 4.6 Total concentrations of foliar Ca (%) of *L. scoparium* and *K. robusta* measured at the end of experiment. Error bars represent the standard error of the mean. Treatment that share letters have means that do not differ significantly (p<0.05).

In combination with biosolids, *K. robusta* accumulated 32% higher foliar S concentration than the control **(Figure 4.7B**). In contrast, **Figure 4.7B** shows DSE did not significantly affect foliar S uptake. In combination with *K. robusta*, DSE and biosolids application significantly reduced the concentration of foliar K (**Figure 4.7A**).

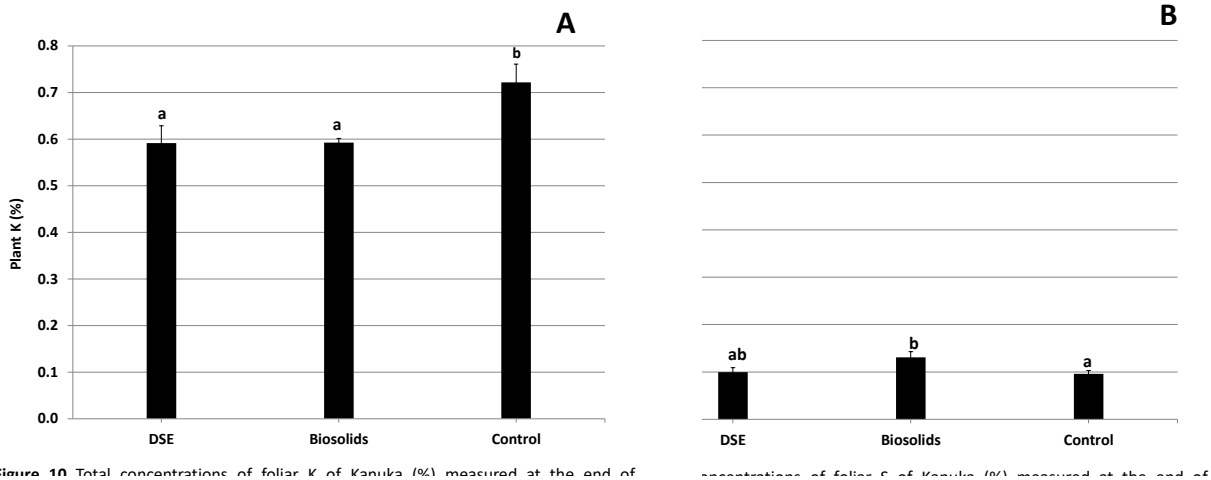


Figure 10 Total concentrations of foliar K of Kanuka (%) measured at the end of concentrations of foliar S of Kanuka (%) measured at the end of

Figure 4. 7 Total concentrations of foliar (A) K and (B) S (%) of *K. robusta* measured at the end of experiment. Error bars represent the standard error of the mean. Treatment that share letters have means that do not differ significantly (p<0.05).

Although in combination with *L. scoparium* and *K. robusta* there was no significant difference in N uptake between treatments, these New Zealand native species responded differently in accumulating foliar N (**Figure 4.8)**. In combination with *L. scoparium*, biowastes application increased significantly increased the foliar N uptake compared to that of when combined with *K. robusta*. Amending DSE and biosolids increased the foliar N uptake of *L. scoparium* by 23% and 29%, respectively compared to *K. robusta*.

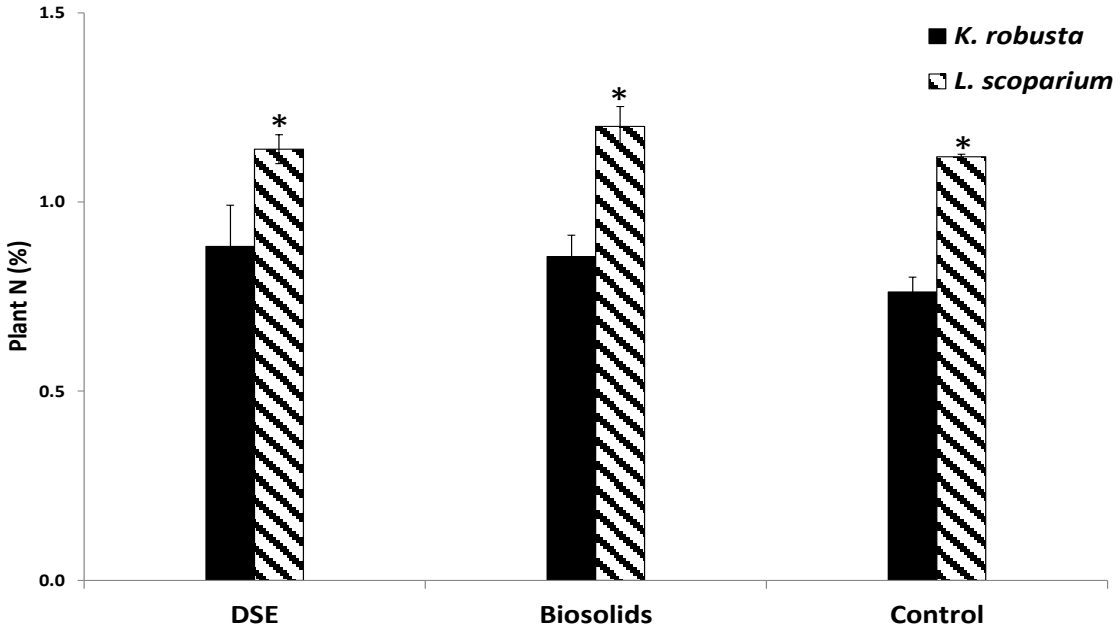


Figure 10. Total concentrations of foliar N (%) of *L. scoparium* and *K. robusta* measured at the
Figure 4. 8 Total concentrations of foliar N (%) of *L. scoparium* and *K. robusta* measured at the end of experiment. Error bars represent the standard error of the mean. Asterisks (*) signify significant differences between the effluents (striped bars) and controls (solid bars) at *p≤0.05*.

Micronutrients

Figure 4.9 shows total concentrations of foliar trace elements (mg/kg) of *L. scoparium* and *K. robusta* measured at the end of experiment.

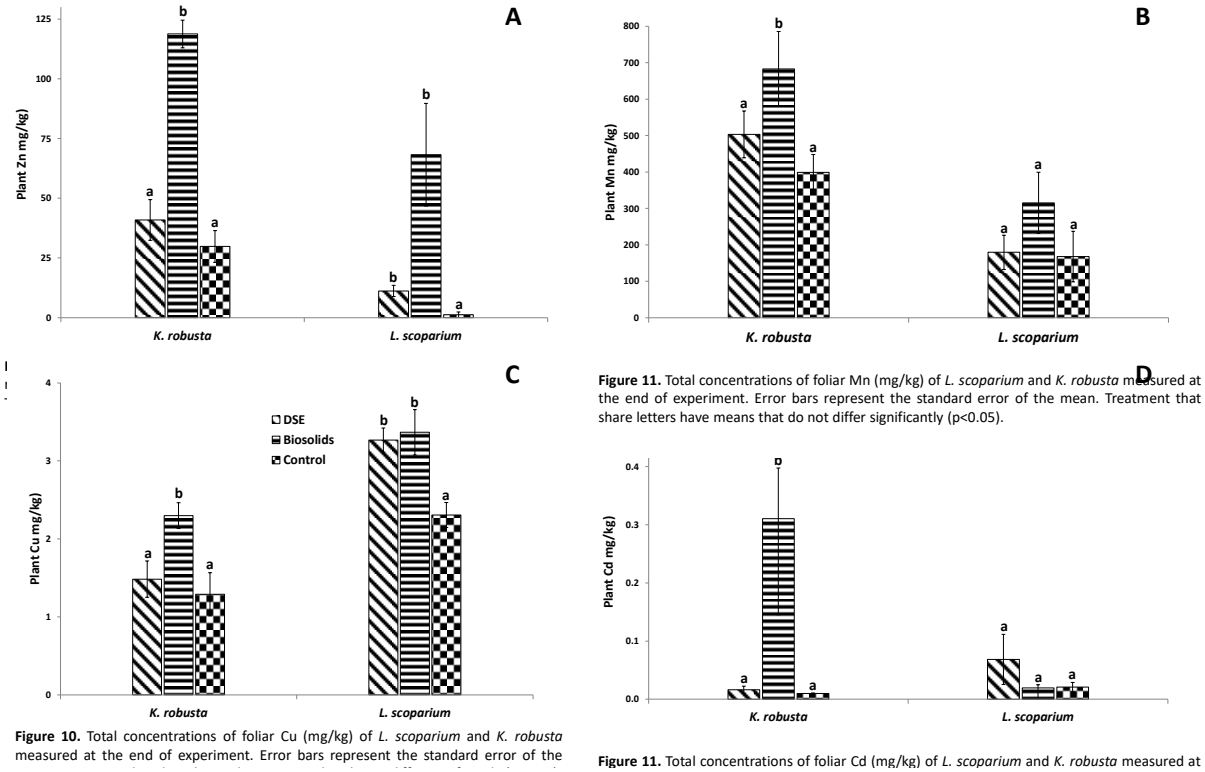


Figure 10. Total concentrations of foliar Cu (mg/kg) of *L. scoparium* and *K. robusta* measured at the end of experiment. Error bars represent the standard error of the

Figure 11. Total concentrations of foliar Mn (mg/kg) of *L. scoparium* and *K. robusta* measured at the end of experiment. Error bars represent the standard error of the mean. Treatment that share letters have means that do not differ significantly (p<0.05).

Figure 11. Total concentrations of foliar Cd (mg/kg) of *L. scoparium* and *K. robusta* measured at

Figure 4. 9 Total concentrations of foliar trace elements (mg/kg) of *L. scoparium* and *K. robusta* measured at the end of experiment. Error bars represent the standard error of the mean. Treatment that share letters have means that do not differ significantly (p<0.05)

The application of biosolids and DSE to *K. robusta* increased the concentration of foliar Cu by 78% and 15%, whereas these treatments increased Cu in *L. scoparium* by Cu by 42 and 46%, respectively (**Figure 4.9B**). Biosolids significantly increased the uptake of Zn by both *L. scoparium* and *K. robusta* by 569% and 298% respectively (**Figure 4.9A**). In comparison, the DSE did not significantly change the Zn concentration in the leaves of *K. robusta* and only produced a 37% increase in *L. scoparium* (**Figure 4.9A**).

K. robusta accumulated significantly (*p≤0.05*) higher Cd in the biosolids treatment (**Figure 4.9D).** In contrast, **Figure 4.9D** shows that in the DSE treatment*, K. robusta* was not different to the control. *K. robusta* responded to the application of biowastes in related to Mn uptake. Biosolids application significantly increased (*p≤0.05*) the uptake of Mn (**Figure 4.9B**). The application of biosolids increased the concentration of foliar Mn in *K. robusta* by 71% compared to the control. In contrast, **Figure 4.9B** shows that in combination with *K. robusta*, there was no significant difference in total concentration of foliar Mn between DSE and the control. In addition, there were no significant differences of foliar Cd and Mn in both *L. scoparium* and *K. robusta* compared to the control (**Figure 4.9B** and **4.9D**).

4.4 Discussion

4.4.1 Plant growth

The positive growth effects of biosolids and DSE may be due to their contribution of available nutrients, especially, N, P, K and S. As organic materials, amending these biowastes increased the concentration of organic C and, therefore, increased the Cation Exchange Capacity - CEC (Antolín et al., 2005; Brady, 2008; Weber et al., 2007), contributed in retaining nutrients and making them available to plants (Delibacak et al., 2009; Garcıa-Gil et al., 2004; Kaur et al., 2008; Wong et al., 2001). As a source of valuable nutrients, the application of DSE improved long-term soil fertility by increasing the plant available nutrients (Hawke and Summers, 2006). Esperschuetz et al. (2016c) reported that adding 1250 kg N ha^{-1} equiv. of biosolids improved the growth of *Brassica napus* and *Sorghum bicolor* compared to the control. The effect of applying biosolids and DSE on plant growth could be related to role in stimulating root-microbe interactions processes (Khan, 2006), in which adding biowastes such as DSE to soil could provide a source of food for the microbes (Hawke and Summers, 2006). Mok et al. (2013) pointed out that other myrtaceae family members, *Eucalyptus polybractea* and *Eucalyptus cladocalyx* grown on biosolids produced high biomass. (Moyersoen and Fitter, 1999) and (Weijtmans et al., 2007) reported that Ectomycorrhizal has been identified with *L. scoparium* and *K. robusta*.

4.4.2 Nutrients and trace elements in plant biomass

The application of biosolids and DSE to soil influenced nutrient cycling by increasing bioavailability and the uptake of Ca, K, S, Cu, Zn, and Mn to plants. The biowastes may have increased nutrient cycling, making more nutrients available (Antolín et al., 2005; Morera et al., 2002; Murphy et al., 2007; Singh and Agrawal, 2008). Nutrients incorporated into organic matter can be consumed by bacteria, fungi, and other decomposers and transformed into plant-available forms. The present study found that the uptake of nutrients and contaminants associated with biowastes (NCAB) is species dependent. In combination with biosolids and DSE, both *L. scoparium* and *K. robusta* accumulated Ca, Cu, and Zn, whereas plant K, S, Mn, and Cd were only detected in biomass of *K. robusta.* These findings are in agreement with (Baldani and Döbereiner, 1980) and Mazzola et al. (2002) who found that the role of plants in the availability and mobility of nutrients and contaminants associated with biowastes through root-microbes interaction is dependent on the species. Biosolids and DSE application could have stimulated root exudation (Koo et al., 2013), including organic acids, which have played an important role for solubilisation and mobilization of NCAB (Bertin et al., 2003), particularly elevating the availability of Zn (Hinsinger, 2001a; Keller and Römer, 2001b). Since exudate composition strongly varies with plant species (Walker et al., 2003), this can lead to different plant responses in terms of NCAB uptake.

Copper and Zn uptake by *L. scoparium* and *K. robusta* were higher than that reported by Beshah et al. (2015) for other species. They found that the application of 65 t ha^{-1} dried biosolids significantly increased the accumulation of foliar Zn of oats (*Avena sativa*) by 280% (from 16 to 61 mg kg^{-1}) which are lower than our results for *L. scoparium* by 569% (increased from 1.2 to 68.2 mg kg^{-1}) and *K. robusta* by 298.3% (increased from 29.8 to 118.7 mg kg^{-1}). Mok et al. (2013) reported that two myrtaceae members, *Eucalyptus cladocalyx,* and *E. polybractea*, which were grown in a pot trial in heavy metal-contaminated biosolids reported that these species accumulated Cu (5.3 – 16.3 mg kg^{-1}) and Zn (215.4 – 2074 mg kg^{-1}), which were higher than in this study. Another similar study showed that adding 65 t ha^{-1} dried biosolids significantly increased foliar Cu (Beshah et al., 2015). As reported by Beshah et al. (2015), both *Brassica napus* and *Avena sativa* increased herbage Cu by 100% (from 10 to 20 mg kg^{-1} and from 3.5 to 7.0 mg kg^{-1}), which was higher than the increases in this study. Prosser (2011) reported that the application of biosolids contained 0, 300, and 600 mg kg^{-1} Zn and 0, 100, and 200 mg kg^{-1} Cu within 6-month experimental period resulted in the accumulation of total foliar Cu and Zn in *L. scoparium* by 30-58 mg kg^{-1} and 79 – 140 mg kg^{-1} respectively, which were higher than in the present study. Here, the DSE and biosolids contained somewhat lower concentrations of Cu and Zn. Increasing the application rate and extending the experimental period could promote higher foliar Cu and Zn of this species. Although these elements were increased, the levels in all treatments were in the reported range of toxic thresholds (Alloway, 2013; Broadley et al., 2007). The lower concentration of foliar K found in *K. robusta* was probably influenced by either structural roles in cell walls and membranes or inter- and intracellular functions (Marschner, 1995). It is suspected that adding biosolids may have changed either chemical properties or growth environment of root. This condition is in agreement with White and Broadley (2003) who reported that the uptake of K mainly occurs via root tips.

4.4.3 Contaminants accumulation in the leaves

Concentrations of Cd in *K. robusta* were between 0.02 and 0.3 mg kg^{-1}, which has been reported as a normal range in plants (Alloway, 2013). The significant increase of Cd found in *K. robusta* biomass due to biosolids application compared to control, was not in the range that would pose a risk to human or animal health (Alloway, 2013; Esperschuetz et al., 2016c). While the concentration of Cd in honey or essential oils were not measured, the low foliar concentrations indicates that transfer of excessive Cd into saleable plant products is unlikely. This indicates that biosolids can enhance uptake of essential trace elements in plant parts while not increasing toxic elements like Cd to levels dangerous for animal and human health. *L. scoparium* which did not accumulate increased contaminants from the biosolids treatment, may be safely amended with higher rates of biosolids.

4.5 Conclusions

Amending the low fertility soil with 2600 kg N ha^{-1} equivalent of biosolids and 200 kg N ha^{-1} equivalent of DSE improved the growth of both *L. scoparium* and *K. robusta* through higher production of biomass and increased of Ca, K, and S uptake. *L. scoparium* were growing better than *K. robusta* in combination with DSE, whereas they both gave same positive response on growth parameter in combination with biosolids. Biowastes application increased the uptake of certain essential trace-elements and contaminants but did not result in unacceptable levels. Differences in the biomass increase between *L. scoparium* and *K. robusta* in combination with DSE compared to biosolids treatment might result from a stimulation of different mycorrhiza types, associated with the respective species, which will be an interesting area for future research. Since biosolids may have influenced plant rhizodeposition, it is recommended for future studies to investigate plant root-microbe interactions with regard to plant element uptake.

Chapter 5

A lysimeter study to reveal the response of *Leptospermum scoparium* J.R Forst, *Kunzea robusta* de Lange & Toelken*, Pinus radiata* D. Don*, Lolium multiflorum* Lam, *Brassica napus* L. 'MAKRO', and *Sorghum bicolor* L. on nutrient fluxes in biowaste-amended soil

My role in this study was helping Dr Juergen Esperschuetz with the experimental maintenance, data collection, final harvesting, soil and plant samples preparation for analysis, and some data analysis. I am a co-author on the following three papers that have been published from this study as follow:

Esperschuetz J, Lense O, Anderson C, Bulman S, Horswell J, Dickinson N, Robinson BH (2016). Biowaste mixtures affecting the growth and elemental composition of Italian Ryegrass (Lolium multiflorum). Journal of Environmental Quality 45(3), 1054-1061.

Esperschuetz J, Bulman S, Anderson C, Lense O, Horswell J, Dickinson N, Robinson BH (2016). Production of biomass crops using biowastes on low fertility soil – Part I: Influence of biowastes on plant and soil quality. Journal of Environmental Quality 45(6) 1960-1968.

Esperschuetz J, Bulman S, Anderson C, Lense O, Horswell J, Dickinson N, Robinson BH (2016). Production of biomass crops using biowastes on low fertility soil – Part II: Effect of biowastes on nitrogen transformation processes. Journal of Environmental Quality 45(6), 1970-1978.

5.1 Introduction

Previous studies have shown that biosolids application increases the growth and the uptake of Cd, Cu and Zn of *Lolium multiflorum* (Ahumada et al., 2009; Bai et al., 2013; Santibanez et al., 2008). Therefore, contaminants such as Cd may enter grazing animals and result in concentrations in excess of food safety standards in animal products (Reiser et al., 2014). On the other hand, Anderson et al. (2012) reported that the increase in Cu and Zn in the plant biomass can be beneficial to the health of grazing animals in areas where these elements are deficient, or where high Zn concentrations are needed such as a prophylaxis to facial eczema. Given their multi-benefits such as edible oil, fodder crops as well as bioenergy production, Sweet sorghum (*S. bicolor*) and Oilseed rape (*B. napus*) are species of economic interest (Gomes, 2012; Wang et al., 2009). In addition, these species have been effective in removing contaminants from the soil and preventing nutrient leaching into waterways

(Barceló and Poschenrieder, 2003; Licht and Schnoor, 1993; Pilipovic et al., 2006; Turan and Esringu, 2007; Wang et al., 2009). Recent studies have shown that some of the negative effects of biosolids addition to soil can be mitigated by blending the biosolids with other biowastes including biochar (Knowles et al., 2011), lignite (Simmler et al., 2013), organic acid (Zaleckas et al., 2009), and sawdust (Bugbee, 1999a; Daniels et al., 2001; Schmidt et al., 2001). Hence, we hypothesized that (1) applying biosolids will improve the growth and nutrients uptake of *L. multiflorum, B. napus,* and *S. bicolor;* (2) blending biosolids with sawdust can improve soil fertility while reducing plant nutrients loss through leaching.

5.2 Aim

The aim of the study was to determine the effect of biosolids and biosolids and sawdust mixture addition on the growth, plant nutrients uptake, and nutrients loss in combination with *L. scoparium, K. robusta, P. radiata, L. multiflorum*, *B. napus*, and *S. bicolor*

5.3 Materials and methods

5.3.1 Experiment set up

In April 2013, 10-L lysimeters were constructed and installed at the Lincoln University plant growth facility (43°38’42’’ S, 172°27’41’’E). Low-fertility soil, as defined according to its low Olsen P of 11 mg L^{-1}, was collected from the North Island, near Bideford, New Zealand (40°45’56’’ S, 175°54’42’’ E). It has no history of fertilizer addition and mainly classified as orthic brown soil with a clay-loam texture (Esperschuetz et al., 2016a; Esperschuetz et al., 2016b; Esperschuetz et al., 2016c). Soil analyses showed a medium pH (pH 6.1), with medium carbon (6.5%) and nitrogen (0.46%) levels and a C/N ratio of 14.3. The Cation Exchange Capacity (CEC) was 21 meq 100 g^{-1}. Potassium, Mg, and Na occurred at concentrations of 0.30, 0.63, and 0.14 meq 100 g^{-1}, respectively. The soil was homogenized before it was placed into lysimeters (25 cm in diameter; 29 cm in height). To measure NO_3^- leaching, a leachate-sampling device was installed in the bottom of each lysimeter. The device was covered by fleece sheets and a gravel drainage layer to avoid stagnant moisture. Each lysimeter was filled with 10 L of soil at an average soil bulk density of 1.3 g cm^3. Soil was packed in three layers to avoid gradients. Lysimeters were incubated at near field capacity conditions and ambient conditions in the greenhouse for 14 w before treatment application. The experiment was set up in four soil treatments (control, biosolids, biosolids-sawdust, and urea) and arranged in a randomized block design. Biosolids (untreated pond sludge, characterized as Grade “Bb” according to) (NZWWA, 2003) were collected from settlement ponds of the Kaikoura Sewage Treatment Plant; sawdust (*Pinus radiata* D. Don, untreated) was obtained from an adjacent wood-waste disposal area (Kaikoura, New Zealand, 42°21'37.40"S,

173°41'27.35"E). Biosolids were homogenized thoroughly after sieving (diameter 10 mm). The treatments comprised urea (2.11 g dry weight [DW]), biosolids (245 g DW), and the same amount of biosolids mixed with sawdust (123 g DW). The application rates for urea and biosolids were equivalent to 200 and 1250 kg N ha^{-1}, respectively; the biosolids application rate was equivalent to 50 t ha^{-1} dry weight. For a mixture of biosolids and sawdust treatments, the sawdust was mixed with the biosolids before application at a ratio of 1:0.5 (biosolids/sawdust). The biosolids and biosolids-sawdust mixtures were applied to the surface of the pots before sowing. Urea (50 kg N ha^{-1} equivalent) was applied four times over the experimental period.

Seeds of *L. multiflorum* LAM. Feast II Tetraploid Italian ryegrass (2 g), *S. bicolor* (L.) Moench 'Sudanese', and *B. napus* L. 'MAKRO' were sown directly into the lysimeters after treatment application. After germination, *S. bicolor* and *B. napus* were thinned to three and five plants per lysimeter, respectively. A leachate-sampling device was installed in the bottom of each lysimeter to measure NO_3^- leaching.

The lysimeters were arranged in the glasshouse based in a randomized block design. An irrigation system allowed the independent watering of each plant species by pressure-compensated drippers. Manual irrigation was used to apply additional water to treatments within species. The lysimeters were maintained for 18 weeks in the greenhouse with temperatures ranging between 9 and 20°C during the night time (10 PM until 6 AM) and between 14 and 28°C during the daytime. Lysimeters were watered to produce 1-3 L of drainage per week. Aliquots were stored at -20°C until further analyses (Esperschuetz et al., 2016c). The lysimeters were weeded fortnightly.

5.3.2 Analyses and measurements

A final destructive harvest of all lysimeters was performed after 18 weeks. The total plant biomass was weighed to investigate the growth responses of each plant species to soil amendments after oven-drying at 70°C until constant weight. Dried plant parts were further separated into roots, stems, and leaves. Further details of samples analyses, measurements and statistical analyses were clearly described by Esperschuetz et al. (2016b); Esperschuetz et al. (2016c); Esperschuetz et al. (2016a); and Esperschuetz et al. (2017).

5.4 Results and discussion

5.4.1 Biomass production

Figure 5.1 shows that compared to untreated soil, biosolids+sawdust treatment significantly increased the growth of *L. multiflorum* during 18 weeks experimental period of spring and summer weeks. Blending biosolids with sawdust increased the cumulative biomass of *L. multiflorum* to 3 t ha^{-1} which

is almost 1 t ha^{-1} higher than the control (2.14 t ha^{-1}). However, **Figure 5.1** shows that the biosolids + sawdust treatment had significantly lower aerial biomass compared to urea (4.93 t ha^{-1}) and biosolids alone (4.14 t ha^{-1}). The results indicate that *L. multiflorum* started to give significant response at six weeks after sowing and resulted different treatments ranking in order of urea > biosolids > a mixture of biosolids and sawdust > control in which remain the same until the end of the experiment (**Figure 5.1**).

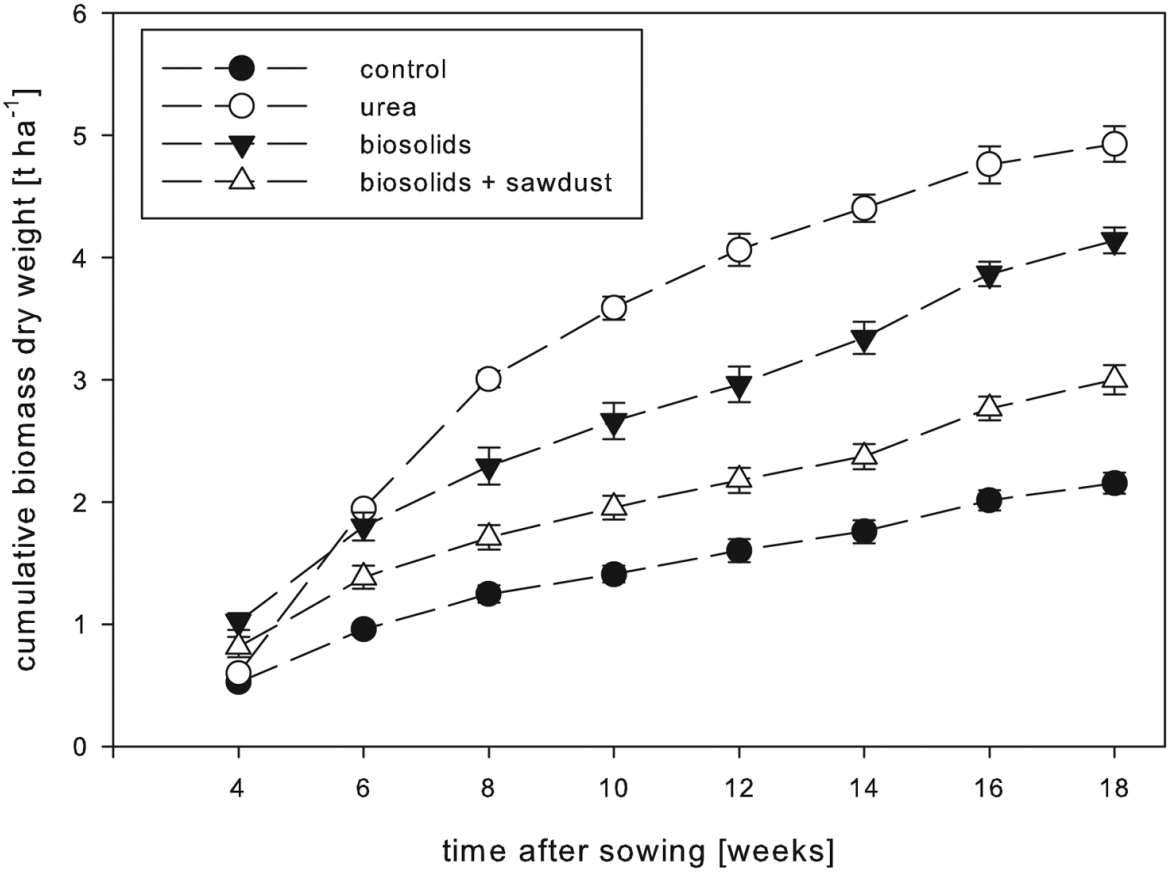


Figure 5. 1 Cumulative biomass (dry weight) in t ha^{-1} equivalent during the 18-wk experimental period. Each point is the average of six replicates with bars representing the standard error of the mean. Non-overlapping bars indicate significant differences ($p \leq 0.05$). (Esperschuetz et al., 2016b)

The growth of *L. multiflorum,* which is indicated by the production of aerial biomass is comparable to other studies using biosolids. Smith and Tibbett (2004) found that the application of 4, 8, and 16 t ha^{-1} of dried biosolids resulted the production of annual biomass production of 1.7, 2.0, and 2.4 t ha^{-1} (the present study using which is approximately 50 t ha^{-1} of dried biosolids). Other studies conducted by Moir et al. (2013) and Hanson et al. (2006) reported the average biomass production of 2.2 and 8.7 t ha^{-1}, depending on the growth period, reported for 'Feast II'. The lower biomass production of the

biosolids + sawdust treatment compared to the biosolids-alone treatment is probably due to sawdust immobilizing N (Bugbee, 1999b).

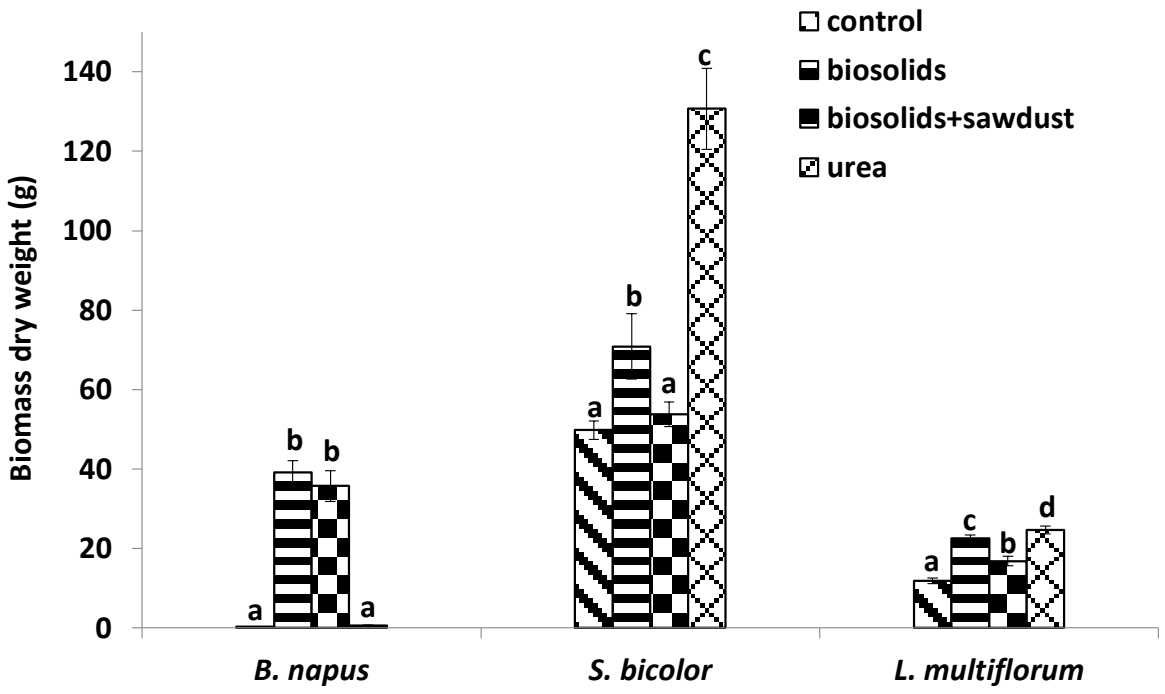


Figure 5. 2 Total aboveground plant biomass of *B. napus, S. bicolor* and *L. multiflorum* at the end of the experiment. Significant differences ($p \leq 0.05$) are represented by lowercase letters. (Esperschuetz, et al., 2016b)

Figure 5.2 shows that during the 18-week experimental period, applying 50 t ha^{-1} of biosolids, equivalent to 1250 kg N ha^{-1}, resulted in a positive growth response in *L. multiflorum, B. napus,* and *S. bicolor* compared to the control. **Figure 5.2** shows that blending biosolids with sawdust significantly increased the above ground biomass of *L. multiflorum* and *B. napus* but not *S. bicolor*. Compared to urea treatment, *B. napus* produced significantly higher biomass in both the biosolids biosolids+sawdust treatments (**Figure 5.2**). Applying 200 kg N ha^{-1} fertilizer has boosted the growth of *S. bicolor* and *L. multiflorum* compared to biosolids and biosolids+sawdust. This is because urea contains higher plant-available N (200 kg N ha^{-1}), which rapidly hydrolyses to NH_4^+ (Paul, 2014) compared to biosolids, which contain >95% organic N (Gilmour et al., 2003). The poor growth performance of *B. napus* in the control and urea treatment was probably due the limitation of another element other than N. Previous studies reported that compared to other species including wheat or maize, *B. napus* requires higher S and P (Abdallah et al., 2010; Ahmad et al., 2007; Chen et al., 2015; Jackson, 2000). Amending of the biosolids which were equivalent of 375 kg ha^{-1} of total S and 250 kg

ha^{-1} of total P resulted plant available S and P of rapeseed by 41.5 and 2.5 kg ha^{-1} in the biosolids and biosolids+sawdust treatments, respectively.

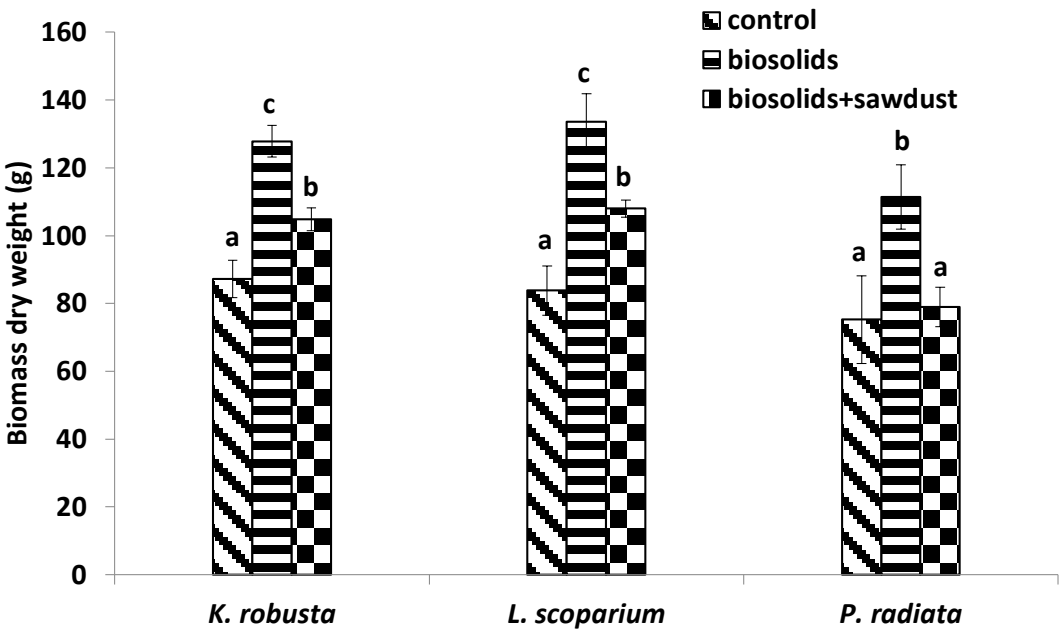


Figure 5. 3 Aboveground plant biomass [g DW] after a growing period of 18 weeks in control, biosolids and biosolids+sawdust treatments (n=4±se). Significant differences between treatments at p ≤ 0.05 are indicated by letters (a, b, c) within plant species. Esperschuetz, et al., (2017)

Pinus radiata responded positively to the application of biosolids by producing significantly higher aboveground biomass (**Figure 5.3**). Compared to control, the species produced 61% higher above ground biomass following biosolids application (**Figure 5.3**). **Figure 5.3** shows that biosolids treatment stimulated the growth of *L. scoparium* and *K. robusta* with an increase in aboveground biomass of 60% and 27%, respectively compared to the control. The biosolids+sawdust treatment increased the aboveground biomass of *L. scoparium* and *K. robusta* by 57% and 52% respectively. In contrast, the biosolids+sawdust treatment had no effect on the growth of *P. radiata* (**Figure 5.3**).

The positive response of *P. radiata* to the application of biosolids has been reported by Kimberley et al. (2004), who found that adding biosolids increased the growth of the species. It is comparable with the application mineral fertilizer (Prescott and Brown, 1998; Weetman et al., 1993). Although *L. scoparium* and *K. robusta* species are naturally adapted to low fertility soil, their growth can be increased by adding high N biosolids. Altering the soil's physical properties and stimulating soil microbial activity, particularly mycorrhiza, in soil by adding high source C fresh sawdust gave positive results and stimulated the growth of *K. robusta,* presumably due to higher porosity of the soil

compared to biosolids treatment alone. This is in agreement with Haynes and Goh (1987) and Watson and Mardern (2004) who found that mixing sawdust with biosolids resulted in higher porosity of the growth media, hence increased root biomass of *K. robusta*. Smith et al. (2011) reported that adding biosolids into soil may have stimulated ectomycorrhizal fungi, which in turn, increased plant nutrient uptake. Moyersoen and Fitter (1999), Weijtmans et al. (2007), and Walbert et al. (2010) found that ectomycorrhizal has been associated with the growth of *L. scoparium, K. robusta,* and *P. radiata.* Arbuscular mycorrhiza has played an important role in promoting growth following the application of biosolids and sawdust mixture (Smith et al., 2011; Whiteside et al., 2012). Hence, adding both biosolids (high organic N) and a mixture of biosolids and sawdust (high source of organic C) may have promoted the growth of both ectomycorrhizal and arbuscular mycorrhiza. This is supported by Moyersoen and Fitter (1999) and Weijtmans et al. (2007) who found that both ectomycorrhizal and arbuscular mycorrhiza colonisation were observed in *K. robusta* and *L. scoparium,* whereas only ectomycorrhizal was found in *P. radiata* after the application biosolids and a biosolids and sawdust mixture.

5.4.2 Elemental uptake

Adding biowastes on to soil significantly increased the concentration of several macro - and micro-nutrients in the leaves of *L. multiflorum* as shown in **Table 5.1** and **5.2**.

Table 5. 1 Average concentration of macronutrients in *L. multiflorum* over the experimental period. Values in parentheses represent the standard error of the average concentration per pot (*n* = 6) throughout the experiment (*n* = 8). Esperschuetz et al. (2016b).

	Control	Urea	biosolids	Biosolids+sawdust
		% w/w		
N	2.39 (0.04)^{a}	3.35(0.09)^{c}	2.56(0.05) ^{ab}	2.63(0.12)^{b}
P	0.30 (0.01)^{b}	0.17 (0.00)^{a}	0.43 (0.02)^{d}	0.35 (0.02)^{c}
K	3.21 (0.03)^{c}	1.93 (0.02) ^{a}	2.73 (0.06)^{b}	3.00 (0.12) ^{c}
S	0.38 (0.01)^{bc}	0.26 (0.00)^{a}	0.40 (0.01)^{c}	0.35 (0.02)^{b}
Ca	0.80 (0.01)^{c}	0.77 (0.02)^{bc}	0.73 (0.01)^{b}	0.66 (0.02)^{a}
Mg	0.23 (0.00)^{a}	0.24 (0.01)^{bc}	0.23 (0.00)^{b}	0.21 (0.01)^{a}

Notes: Different lowercase letters indicate significant differences between treatments at $p \leq 0.05$

Table 5. 2 Average concentration of trace elements in *L. multiflorum* over the experimental period. Values in parentheses represent the standard error of the average concentration per pot (*n* = 6) throughout the experiment (*n* = 8). Esperschuetz et al. (2016b).

	Control	Urea	biosolids	Biosolids+sawdust
		mg kg^{-1} dry wt		
B	11.4 (1.0)^{b}	8.9 (0.3)^{a}	10.5 (0.3)^{ab}	9.9 (0.8)^{ab}
Cu	5.9 (0.1)^{a}	6.0 (0.2)^{a}	10.3 (0.6)^{c}	8.7 (0.4)^{b}
Zn	21.6 (2.3)^{a}	19.8 (0.7)^{a}	150.4 (8.3)^{c}	91.7 (3.7)^{b}
Mn	37.4 (1.0)^{a}	35.2 (0.8)^{a}	60.2 (1.7)^{c}	51.0 (2.4)^{b}
Fe	96.0 (3.9)^{a}	105.8 (13.6)^{a}	118.7 (14.4)^{a}	105.5 (7.1)^{a}
Cd	0.03 (0.01)^{ab}	0.02 (0.00)^{a}	0.26 (0.06)^{c}	0.13 (0.00)^{b}

Notes: Different lowercase letters indicate significant differences between treatments at $p \leq 0.05$

In combination with *L. multiflorum,* application of both biosolids and biosolids + sawdust significantly increased the concentrations of foliar P and S compared to the control (**Table 5.1**). **Table 5.1** shows that adding biosolids alone, did not significantly increase foliar N concentration of *L. multiflorum.* In contrast, biosolids + sawdust treatment significantly increased both N and P. A lower concentration of foliar N of *L. multiflorum* indicates that other components in the biosolids, such as heavy metals, may have reduced the effectiveness of the added N. In the biosolids and biosolids + sawdust treatments, only a limited amount of the total N applied with biosolids (1250 kg ha^{-1}) was immediately plant available. It is probably because most of the N in biosolids is locked up in organic compounds which need to undergo (microbial) transformation processes to become available (Sommers, 1977). The biosolids treatment decreased the concentration of foliar K. The results indicated that K concentration in the plant biomass showed a decreasing trend in all treatments (**Figure 5.4b**). P and S reached their peak concentration in 10, 12 w, and at the end of the experiment (**Figure 5.4c** and **5.4d**). The concentrations macronutrients including K, P, and S (35, 30, and 35 g kg^{-1}, respectively) in the present study are comparable to similar study conducted by (Harrington et al., 2006) and were higher than deficiency threshold concentrations (28, 2.1, and 1.8 g kg^{-1}, respectively) in *L. perenne* as reported by (McNaught, 1970; Smith et al., 1985).

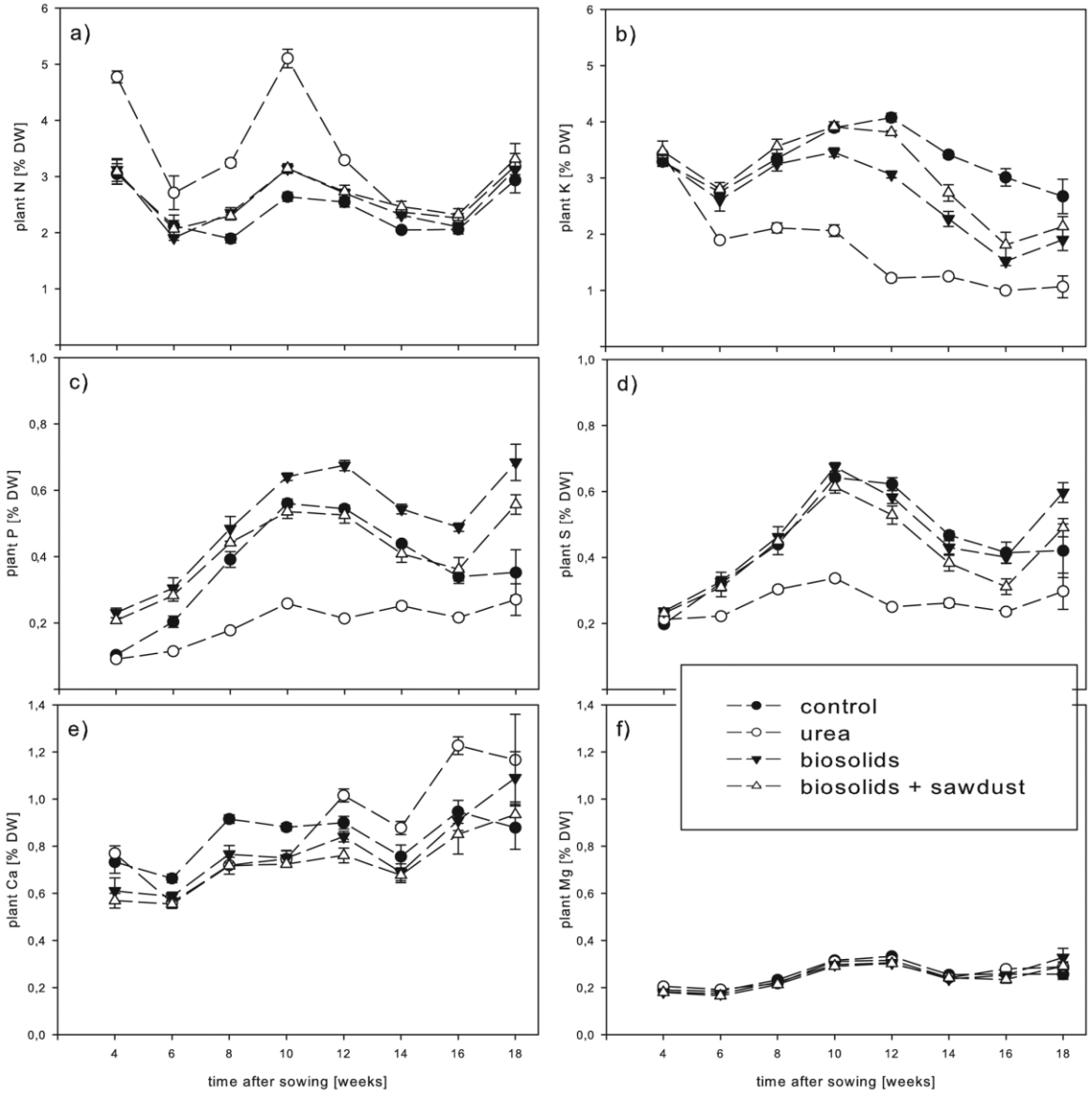


Fig. 2 Average concentrations of macroelements elements over the experimental period. Error bars

Figure 5. 4 Average concentrations of macro elements over the experimental period. Error bars represent the standard error of the mean. Non-overlapping error bars indicate significant difference between means ($p \leq 0.05$). Adopted from Esperschuetz et al. (2016b)

Lolium multiflorum accumulated significantly higher concentrations of Cd, Cu and Zn in both the biosolids and biosolids+ sawdust treatments compared to control and urea treatments (**Table 5.2**). **Table 5.2** shows that blending sawdust with biosolids significantly reduced the accumulation of Cd in the leaves of *L. multiflorum* compared to biosolids alone treatment. This could be beneficial for *L. multiflorum* or other edible plants as sawdust addition can reduce the entry of this toxic element in their tissues. The present study shows that Cd concentrations of the leaves of *L. multiflorum* were within the range of acceptable daily intake of Cd concentration based on both food standards of New Zealand (≤1.25 mg kg^{-1} for kidney and ≤2.5 mg kg^{-1} for liver) and the European Union (≤1.0 mg kg^{-1} for kidney and ≤0.5 mg kg^{-1} for liver) (Reiser et al., 2014). The average Cd concentrations in this present

study were lower compared to other studies where biosolids had been used as a soil conditioner at similar rates as reported by Antoniadis et al. (2008b) and Black et al. (2012). The lower concentration of Cd found in this study is probably related to the higher concentration of Zn (Khoshgoftar et al., 2004; Oliver et al., 2005). Khoshgoftar et al. (2004) reported that Cd absorption by plants occurs through a process that through root Zn transporter in which a low supply of plant available Zn could promotes the absorption of Cd by the plant. It is supported by Oliver et al. (2005) who found that Applying Zn fertilizer inhibits Cd uptake and translocation, especially in soils with low plant available Zn. For instance, applying Zn fertilizer to wheat elevated the foliar Zn concentration from 26 to 56 mg kg^{-1} and reduced foliar Cd concentration from 0.90 to 0.09 mg kg^{-1}. In terms of concentrations, the concentrations of Zn in the biosolids treatment in this study were similar to those of *L. perenne* (129 to 390 mg kg^{-1}) reported by (Santibanez et al., 2008) and (Torri and Lavado, 2009), who used higher rates of biosolids (150–400 t ha_{-1}) and even higher than similar studies using lower rates of biosolids treatment (Ahumada et al., 2009; Antoniadis et al., 2008a; Black et al., 2012). The concentrations of Cu were increased in the biosolids+sawdust treatment (**Table 5.2**). Although the Cu concentrations in this study were generally lower than those reported for *L. perenne* (Ahumada et al., 2009; Antoniadis et al., 2008a; Black et al., 2012), this can provide benefits to mitigate the global issues on Cu deficiency in all agricultural systems (White and Broadley, 2009).

In both the biosolids and biosolids+sawdust treatments, there were no significant differences in the foliar concentration of N, P, K, S, Ca, and Mg of *B. napus* compared to the control (**Table 5.3**). In contrast, *S. bicolor* accumulated significantly higher S and Mg in the biosolids and biosolids+sawdust treatment compared to the control.

Table 5. 3 Total macronutrients in *B. napus* and *S. bicolor* biomass in response to different soil amendments. Different lowercase letters indicate significant differences between treatments at $p \leq 0.05$. Esperschuetz et al. (2016b)

	Control	Urea	Biosolids + sawdust	Biosolids
		% w/w		
B. napus				
N	4.50 ± 0.09b	4.55 ± 0.36b	0.58 ± 0.11a	0.47 ± 0.04a
P	0.07 ± 0.02a	0.09 ± 0.01a	0.08 ± 0.01a	0.07 ± 0.01a
K	1.29 ± 0.56ab	1.48 ± 0.49b	0.53 ± 0.13ab	0.39 ± 0.04a
S	1.49 ± 0.09c	0.99 ± 0.04b	0.24 ± 0.03a	0.21 ± 0.02a
Ca	4.58 ± 0.29b	5.00 ± 0.51b	1.29 ± 0.18a	1.19 ± 0.05a
Mg	0.30 ± 0.02b	0.33 ± 0.00b	0.13 ± 0.02a	0.11 ± 0.01a
S. bicolor				
N	4.50 ± 0.09b	4.55 ± 0.36b	0.58 ± 0.11a	0.47 ± 0.04a
P	0.07 ± 0.02a	0.09 ± 0.01a	0.08 ± 0.01a	0.07 ± 0.01a
K	1.29 ± 0.56ab	1.48 ± 0.49b	0.53 ± 0.13ab	0.39 ± 0.04a
S	1.49 ± 0.09c	0.99 ± 0.04b	0.24 ± 0.03a	0.21 ± 0.02a
Ca	4.58 ± 0.29b	5.00 ± 0.51b	1.29 ± 0.18a	1.19 ± 0.05a
Mg	0.30 ± 0.02b	0.33 ± 0.00b	0.13 ± 0.02a	0.11 ± 0.01a

Note: The average macronutrient concentrations are based on a weighted average across individual harvests. Lowercase letters indicate significant differences between treatments at $p \geq 0.05$.

With regard to trace elements uptake (**Table 5.4**), compared to the control, *B. napus* had significantly a higher Zn concentration, but had significantly lower B and Fe concentrations in both the biosolids and biosolids+sawdust treatments. Compared to the control, the biosolids and biosolids+sawdust treatments increased Zn concentrations fivefold and eightfold, respectively. Blending biosolids with sawdust significantly increased the concentrations of Cu and Mn by 30% and 40%, respectively, compared to biosolids alone.

Table 5. 4 Total trace elements in *B. napus* and *S. bicolor* biomass in response to different soil amendments. Different lowercase letters indicate significant differences between treatments at $p \leq 0.05$. Esperschuetz et al. (2016b)

	Control	Urea	Biosolids + sawdust	Biosolids
		mg kg^{-1} dry wt		
B. napus				
B	41.6 ± 3.6b‡	64.7 ± 13.2a	19.2 ± 2.2c	16.5 ± 0.6c
Cu	2.1 ± 0.4a	2.5 ± 0.1a	2.7 ± 0.3a	2.3 ± 0.2a
Fe	46.5 ± 6.3b	53.0 ± 3.0b	20.5 ± 3.4a	15.8 ± 1.7a
Mn	21.4 ± 0.2a	41.2 ± 6.8b	25.3 ± 3.6a	15.7 ± 1.7a
Zn	21.7 ± 6.8a	31.4 ± 6.9a	249.7 ± 16.9b	232.0 ± 18.4b
Mo	3.11 ± 1.66a	0.76 ± 0.08a	2.03 ± 0.58a	3.05 ± 0.54a
S. bicolor				
B	2.7 ± 0.3a	2.9 ± 0.3a	2.7 ± 0.3a	2.8 ± 0.3a
Cu	2.0 ± 0.1a	2.2 ± 0.3ab	3.7 ± 0.1c	2.8 ± 0.2b
Fe	22.8 ± 3.1a	35.2 ± 6.7a	22.9 ± 0.6a	26.2 ± 4.5a
Mn	11.9 ± 0.4a	10.9 ± 1.6a	19.6 ± 2.1b	13.9 ± 0.9a
Zn	9.4 ± 0.3a	6.5 ± 1.0a	81.6 ± 6.6c	54.8 ± 2.1b
Mo	0.46 ± 0.16b	0.33 ± 0.02a	0.93 ± 0.08c	1.18 ± 0.12c

Note: The average macronutrient concentrations are based on a weighted average across individual harvests. Lowercase letters indicate significant differences between treatments at $p \geq 0.05$.

The results of the present study are in agreement with those of Riedell (2010) who reported lower shoot P and K concentrations in maize after high-N application. The increased of Ca (1000 to 50,000 mg kg^{-1}), Mg (1500 to 3500 mg kg^{-1}), S (1000 to 5000 mg kg^{-1}), and Cu (1 to 10 mg kg^{-1}) concentration of *B. napus* and *S. bicolor* after biosolids application in the present study fall within the range of food crops (Alloway, 2013). Amending the soil with biosolids and biosolids+sawdust elevated the concentration of Zn to above the typical range found in crop species (Alloway, 2013) of both *B. napus* and *S. bicolor*. In contrast, *S. bicolor* accumulated lower Zn concentration by 15 and 20 mg kg^{-1} in the control and urea treatment, respectively in which still within the range for adequate growth in most crop species. Plum et al. (2010) reported that although Zn is an important element for various biological functions, high concentrations of Zn^{2+}, as with other trace elements is toxic. Broadley et al. (2007) found that the tolerable Zn toxicity in plants is above 300 mg kg^{-1}. In this study, the application of biosolids and biosolids+sawdust boosted the concentrations of Ni and Cd (0.1 to 0.3 mg kg^{-1}) in *B. napus* and *S. bicolor,* however, they were still within the range for food crops for both human and animal health (Alloway, 2013; Gerstl, 1993). This indicates that amending high rates of biosolids and biosolids+sawdust onto soil can enhance accumulation of essential trace elements without causing Ni and Cd to exceed threshold levels for food products.

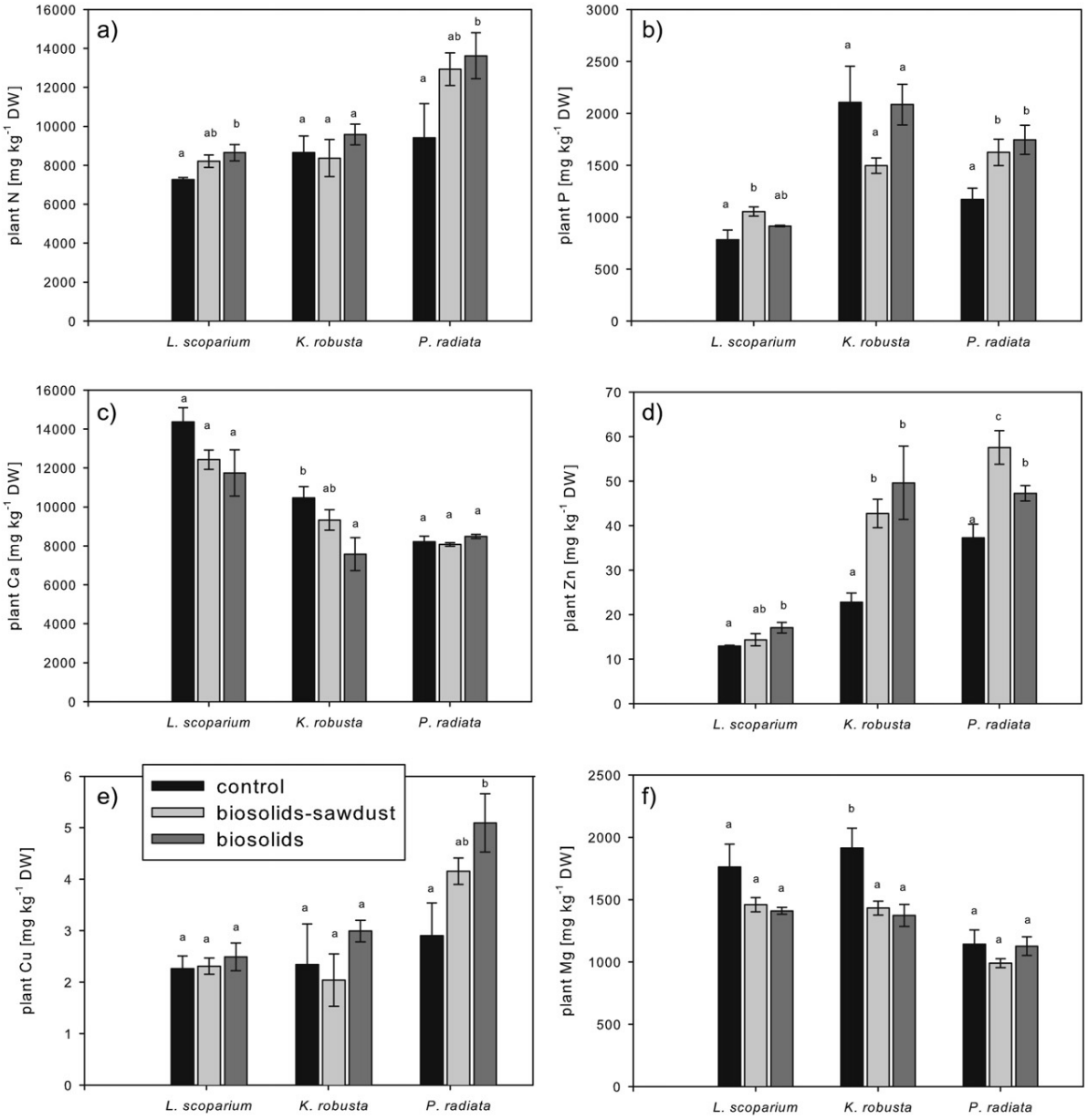


Fig. 6. Concentration of selected macro- and micronutrients in plant leaves [mg kg–1

Figure 5. 5 Concentration of selected macro- and micronutrients in plant leaves [mg kg–1 DW] after a growing period of 18 weeks in control, biosolids-sawdust and biosolids amended treatments (n=4±se). Significant differences between treatments at p ≤ 0.05 are indicated by letters (a, b, c) within plant species. Adopted from Esperschuetz, et al., (2017)

Figure 5.5 shows that the biosolids and biosolids+sawdust treatments significantly increased plant Zn, but lowered Ca, Mg, and Mn. Several authors have reported that amending biosolids into soil may have boosted root exudation such as organic acids which played an important role to transform nutrients into mobile and soluble form, thus increase the available P and Zn (Bertin et al., 2003; Koo et al., 2010). The present study found that *K. robusta* accumulated higher (118%) Zn concentration than that of in *L. scoparium* (27%) and *P. radiata* (32%) (**Figure 5.5d**) after biosolids application. This

presumably due to exudate composition variance between species that influenced the plant-availability of nutrients (Walker et al., 2003). Lower concentrations of foliar Ca and Mg found in *K. robusta* was probably due to lower metabolic requirement in this species (Marschner, 1995). Adding biosolids would have changed the physical environment of the roots affecting both the morphology and physiology of the root tips where Ca is taken up (White and Broadley, 2003)..

5.4.3 Rhizosphere chemistry

Table 5.5 shows the extractable (Ca $(NO_3)_2$) nutrient and trace element concentrations in soil detected in combination with different plant species and soil amendments.

Table 5. 5 Extractable (Ca $(NO_3)_2$) nutrient and trace element concentrations in soil detected in combination with different plant species and soil amendments. Different lowercase letters indicate significant differences between treatments at $p \leq 0.05$. Esperschuetz et al. (2016b)

	Control	Urea	Biosolids + sawdust	Biosolids
		mg kg^{-1} dry wt		
S. bicolor				
P	0.65 ± 0.06a↑	0.60 ± 0.00a	0.79 ± 0.13b	0.60 ± 0.01a
K	17.1 ± 0.66ab↓	14.4 ± 0.55a↓	18.9 ± 2.61b	16.0 ± 1.03ab↓
S	4.39 ± 0.24a	3.53 ± 0.35a	10.19 ± 2.26b	7.88 ± 1.33b↓
Mg	90.0 ± 3.43b	70.5 ± 1.40a↓	90.6 ± 4.01b	84.9 ± 2.57b↓
Cu	0.01 ± 0.00a	0.01 ± 0.00a	0.02 ± 0.01a	0.01 ± 0.00a↓
Fe	1.10 ± 0.09a	2.15 ± 0.32 b	1.01 ± 0.05a	1.29 ± 0.06a↑
Mn	7.29 ± 1.00b ↑	3.10 ± 0.25a↓	3.05 ± 0.76a	4.85 ± 1.67ab
Zn	0.05 ± 0.03a	0.02 ± 0.02a↓	0.33 ± 0.14a	0.36 ± 0.18a↓
L. multiflorum				
P	0.48 ± 0.02a	↓ 0.57 ± 0.05ab	0.62 ± 0.03b	0.62 ± 0.01b
K	23.1 ± 1.86ab↑	26.4 ± 1.92b↑	22.7 ± 1.44ab	19.8 ± 0.69a↑S
S	5.19 ± 0.35a	3.34 ± 0.15a	11.85 ± 1.59b	14.54 ± 0.93c↑
Mg	5.19 ± 0.35a	3.34 ± 0.15a	11.85 ± 1.59b	14.54 ± 0.93c↑
Cu	0.02 ± 0.01a	0.02 ± 0.01a	0.05 ± 0.02a	0.03 ± 0.00a↑
Fe	0.88 ± 0.07a	4.64 ± 0.27b	0.97 ± 0.04a	0.96 ± 0.04a↓
Mn	2.07 ± 0.05a↓	6.57 ± 0.46c↑	2.88 ± 0.11b	2.98 ± 0.16b
Zn	0.13 ± 0.06a	0.11 ± 0.01a↑	0.84 ± 0.20a	1.74 ± 0.46b↑

Note: The average macronutrient concentrations are based on a weighted average across individual harvests. Lowercase letters indicate significant differences between treatments at $p \geq 0.05$.

The plant available P of *S. bicolor* rhizosphere soil increased after the application of biosolids alone, whereas amending the soil with both biosolids and biosolids+sawdust increased plant available S (**Table 5.5**). Lower concentrations of K, Mg, Mn, and Zn were found in the rhizosphere soil of *S. bicolor* following the application of urea. Mg and Zn concentration of rhizosphere soil under *L. multiflorum* after the application of biosolids alone, while concentration of P, S, and Mn were higher in both biosolids and biosolids+sawdust treatment (**Table 5.5**). **Table 5.5** shows that with regard to rhizosphere soil's extractable elements, each plant species has different response to the applied

treatments. Following biosolids addition, the concentration of available K, S, Mg, Cu, and Zn of *S. bicolor* were lower than that of in *L. multiflorum*. Certain trace elements including Cd, Cr, Ni, and Pb were below detection limits (<0.1 mg/kg).

Previous studies have reported that mixing sawdust with other biowastes has altered the availability of certain soil nutrients such as P and S by exerting effect of microbial activity due to leaching of organic compound including phenols, tannins, lignin, and terpenes (Hall, 2007; Hedmark and Scholz, 2008; Keeling and Bohlmann, 2006; Sanati, 2005). The higher concentrations of Mg, Mn, and Zn in *L. multiflorum* rhizosphere soil after biosolids application were presumably because the species did not require high concentration of these elements in producing biomass compared with *S. bicolor*. The lower concentrations of certain trace elements such as Cd, Ni, and Cr indicate that the application of 50 t ha^{-1}(equivalent to 1250 kg N ha^{-1}) is still an ideal rate for *S. bicolor* and *L. multiflorum*. The present study shows that *S. bicolor* and ryegrass utilised different way in exerting the macro- and micronutrients in soil probably due the root exudation and growth (Do Nascimento and Xing, 2006). For instance, the concentrations of $Ca(NO_3)_2$-extractable P, S, Mg, Mn, Cu, and Zn in *S. bicolor* rhizosphere soil were lower than that in *L. multiflorum* rhizosphere soil; root exudates may have changed metal speciation resulting in increased plant uptake or immobilization in soil (Bais et al., 2006). In the biosolids+sawdust treatment, the concentration of Ca $(NO_3)_2$extractable nutrients was similar under *S. bicolor* and *L. multiflorum*. Cébron et al. (2015) reported that as sawdust is a good source of available C, blending them with biosolids attracted heterotrophic bacteria which consumed root exudates and available nutrients in soil as well as stimulated the rhizosphere microbial biomass.

5.4.4 NO_3^- leaching

Applying biosolids and biosolids and sawdust mixture did not significantly affect the leaching of NO_3^- in *B. napus*, *S. bicolor*, and *L. multiflorum*. This was unexpected as of the high carbon: nitrogen ratio in the fresh sawdust should immobilise mineral N in biosolids (**see Appendix A)**. Peter et al. (2013) reported that mixing fresh sawdust with other N source material such as pig manure, reduced the nutrient mobility in soil. However, the present study shows that NO_3^- was recovered in leachate in the biosolids and sawdust mixture treatments.

Following the application of biosolids and biosolids+sawdust, there was no significant differences of NO_3^- leaching in *P. radiata, L. scoparium,* and *K. robusta* (**Figure 5.6**). Especially in the biosolids+sawdust treatment, it is suspected sawdust played an important role in immobilizing organic N in biosolids as well as increased the C:N ratio, thus less N leaching into soil profile (Bugbee, 1999b; Paramashivam, 2015b). At the end of the experiment, soil-N under *P. radiata, L. scoparium,* and *K.*

robusta significantly increased up to 686 kg ha^{-1}, 1602 kg ha^{-1} and 1449 kg ha^{-1}, respectively following the application of both biosolids and biosolids+sawdust (**Figure 5.6b**).

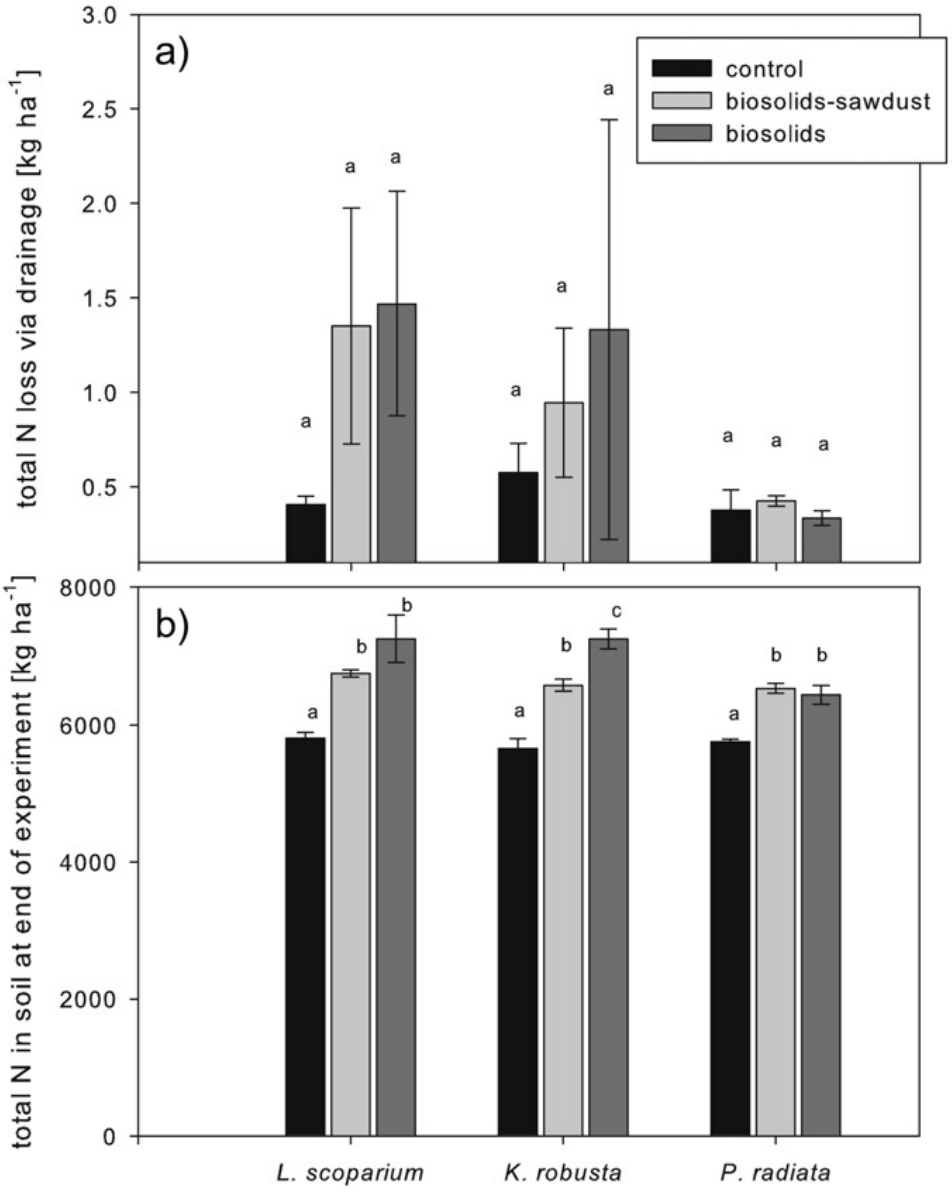


Figure 5. 6 Total N loss via NO_3^- leaching (a) and total N in soil (b) at end of the experiment [kg ha–1] after a growing period of 18 weeks in control, biosolids-sawdust and biosolids amended treatments (n = 4 ± se). Significant differences between treatments at p ≤ 0.05 are indicated by letters (a, b, c) within plant species. Esperschuetz, et al., (2017)

5.5 Conclusion

The application of high rate of 50 t ha^{-1} biosolids (equivalent of 1250 kg N ha^{-1}) to low-fertility soil supplied sufficient certain essential nutrients including P, Cu, Zn, Mn, Fe, and S for the growth of *L. multiflorum, S. bicolor,* and *B.* napus. Although blending biosolids with fresh sawdust resulted in lower available certain nutrients including N, it still could provide potential agriculture benefit in reducing

the uptake of contaminant such as Cd, Cr, and Ni into the leaves of *L. multiflorum, S. bicolor,* and *B. napus*, or Cd. However, since it is varied strongly depending on plant species, the use of sawdust in these scenarios must be implemented on a case-by case basis depending on the required outcome. In brief, applying high rates biosolids onto low-fertility soil has future potential benefit as a substitute fertilizer without significantly elevating contaminants in the plant biomass. However, leaching contaminants in to the surface and ground water body should be carefully monitored. It is recommended that a future field study to reveal the effect of sawdust decomposition on the long-term fertility of soils amended with a mixture of biowastes.

Chapter 6

The response of manuka (*Leptospermum scoparium* J.R Forst) and kanuka (*Kunzea robusta* De Lange & Toelken), and other New Zealand native plants to treated municipal wastewater

6.1 Introduction

6.1.1 Background

The reuse of Treated Municipal Wastewater (TMW) for land application has several benefits over discharging it into waterways (Angelakis et al., 1999; Coppola et al., 2004; Jiménez-Cisneros, 1995; Mohammad and Mazahreh, 2003; Mohammad Rusan et al., 2007; Oron et al., 1995). In addition to its role as irrigation water, TMW contains elevated concentrations of plant nutrients, including N, P, K, and S. (Coppola et al., 2004; Jiménez-Cisneros, 1995; Mohammad and Mazahreh, 2003; Oron et al., 1995; Toze, 2006; Vogel et al., 2015).

The long-term disposal of TMW into waterways, such as Akaroa Harbour, can have demonstrable negative environment impacts due to the increased concentration of plant Nutrients and Contaminants Associated with Biowastes (NCAB) (Bedbabis et al., 2014; Mohammad and Mazahreh, 2003; Mohammad Rusan et al., 2007; Tarchouna et al., 2010; Toze, 2006; Yadav et al., 2002).

Land application of TMW may cause dispersion of clays in the soil, resulting in runoff which may eventually pollute waterways (Magesan et al., 2000). Wastewater irrigation can increase the level of soil salinity due to the wastewater salt content (Mohammad Rusan et al., 2007). The long-term effect of treated wastewater application is Na accumulation, which could cause unstable aggregates of soil (Crescimanno et al., 1995; Kaewmano et al., 2009; Tisdall and Oades, 1982). Higher Na results in excessive swelling of the soil, which may result in the collapse of soil aggregates, making the soil prone to waterlogging, thus reducing root penetration into the soil (Kaewmano et al., 2009). Continuous land application of TMW can lead to excessive amounts of NCAB in soil (Dodds and Welch, 2000), leading to increased water contamination through leaching and runoff (Magesan et al., 2000). Too many nutrients in the wastewater, for example N and P, may cause eutrophication (Smith, 2003). Eutrophication reduces water quality and alters the ecological structure and function of freshwaters (Carpenter et al., 1998; Gong and Xie, 2001). Eutrophication from N and P can result in the mass proliferation of algae, including cyanobacteria, which may be toxic to humans and animals (Bowling

and Baker, 1996). Bowling and Baker (1996) found that eutrophication caused a bloom of cyanobacteria during a drought in Murray-Darling River, Australia, which resulted in the death of livestock. Some algae pose a significant health risk to humans using the water, causing gastroenteritis and skin irritations. Therefore, wastewater nutrient content, crop nutrient requirements, soil nutrient content and other soil fertility parameters should be considered when applying wastewater (Dodds and Welch, 2000). In addition, TMW application may leach plant nutrients and contaminants into surface and ground water (Xu et al., 2009). TMW contains human pathogens, as well as a number of organic xenobiotic compounds, such as Endocrine Disrupting Compounds (EDCs) and various pharmaceuticals (Griffin and Harrahy, 2014; Lado and Ben-Hur, 2009; Ternes, 1998; Ternes et al., 2004).

The soils of Akaroa Harbour in Banks Peninsula, Canterbury are derived from the igneous bedrock overlaid with a thick layer of loess. Given the steep landform, the erodible nature of loess, and variable climate, soil cover in this region is vulnerable to erosion (Harris and Harris, 1939).

Supporting plant growth with the application of TMW may be beneficial for the chosen species. The challenge is that the application of TMW to the land does not always positively affect plant growth. For example, a high proportion of the P present in TMW could be a limiting factor for plant growth (Iskandar and Syers, 1980). This is presumably due to the low capacity of soil to sorb P, thus the soil has a limited ability to transform into available P for plant uptake (Iskandar and Syers, 1980). Devitt et al. (2003) reported that treated wastewater caused diffuse damage to ornamental plants and trees of *Quercus virginiana, Chilopsis linearis, Prunus cerasifera* and *Pistacia chinensis*. Foliar damage increased as the Ca and Na in leaf tissue increased and the SO_4^{2-} concentration decreased (Devitt et al., 2003). This concurs with the study of (Ehlig and Bernstein, 1959), who found that foliar chlorophyll of fruit trees decreased as the absorption of Na increased. Hoffman et al. (1989) and Mantel et al. (1989) suggested that Cl, or a combination of Na and Cl, are the primary ions causing foliar damage. Although Wu et al. (1998) noted that higher tissue concentrations of Ca were positively correlated with plant tolerance to Cl, Bernstein and Francois (1975) reported that burned leaves contained higher levels of Cl, Na and Ca than unburned leaves. Hence, choosing the best suited plant and/or crop is crucial when applying TMW to land.

Several of New Zealand's more environmentally-tolerant native plants are known to respond positively to elevated nutrient levels (Stephens et al., 2005). Grown in conjunction with the use of TMW, they could be used to promote sustainable restoration (Thomas et al., 2014). Manuka (*Leptospermum scoparium*) and kanuka (*Kunzea robusta*) for instance, responded positively to the application of biosolids and Dairy Shed Effluent (DSE). Franklin et al. (2015) found that increased soil

N concentrations resulted in increased foliar N in native plants. Another study found that *K. robusta* reduced N_2O emissions following the application of DSE (Franklin et al., 2017). Several native monocotyledons, including *Phormium tenax* and *Carex virgata*, were found to have potential in the reduction of NO_3^- leaching (Franklin et al., 2015). Some of NZ's native plants are well adapted to low fertility soils and may not respond positively to high nutrient levels. Selecting native plant species to deal with this specific, is not only beneficial to the environment, but could add economic value to the land. For example, manuka and kanuka species can potentially be used to produce high value honey and essential oils and reduce erosion.

I hypothesized that there will be a distinctly different response to plant growth, elevated plant nutrients as well as trace element uptake between native plants species receiving TMW and the control.

6.1.2 Aims

The main aim was to determine how manuka (*L. scoparium*), kanuka (*K. robusta*), akiraho (*Olearia paniculata* (J.R.Forst. & G.Forst.) Druce), kiramu (*Coprosma robusta* Raoul), totara (*Podocarpus cunninghamii* G.Benn. ex D.Don), kapuka (*Grisilinea littoralis* Raoul), puahou (*Pseudopanax arboreus* (L.f.) Philipson), harakeke (*P. tenax* J.R.Forst.. & G.Forst.), whaririki (*Phormium cookianum* Le Jol), *tī kōuka* (*Cordyline australis* (Forst. f.) Hook. f.), and tarata (*Pittosporum eugenioides* A.Cunn., 1840) respond to the application of TMW.

6.2 Methods

6.2.1 Experimental site and duration

A field trial was conducted between May 2015 (planting) and May 2017 (collection of soil and leaf samples). It was part of a longer (four year) field experiment. The trial was conducted at Duvauchelle, Robinsons Bay (43° 45'08.7" S 176 56 35.7 E, elevation 5 m above sea level), Akaroa, about 75 km east of Christchurch, New Zealand (**Figure 6.1**).

Figure 6. 1 A map of Duvauchelle field trial, Robinsons Bay, Akaroa (about 75 km east of Christchurch, New Zealand).

The annual mean temperature is 11.8°C and annual precipitation is 985mm. It is located in a temperate zone with a sub humid continental climate. The field trial area was about 2000 m^2. The soil type is Pawson Silt Loam (Harris and Harris, 1939). The physical and chemical properties are shown in **Table 6.1**.

Table 6. 1 Properties of soil at experimental site, Pawson Silt Loam, Duvauchelle (43°44'53.06"S, 172°55'41.44"E). Values in brackets represent the standard error of the mean (n=65).

Properties	concentration
pH	5.2 (0.1)
NH_4^+- N (mg kg^{-1} d.w)	25 (4)
NO_3^- - N (mg kg^{-1} d.w)	48 (0.4)
Total C (%)	4.6 (0.1)
Total N (%)	0.4 (0.1)
Al (mg kg^{-1} d.w)	24735 (286)
B (mg kg^{-1} d.w)	2.4 (0.1)
Ca (mg kg^{-1} d.w)	4945(83)
Cu (mg kg^{-1} d.w)	11 (0.4)
Cd (mg kg^{-1} d.w)	0.6 (0.0)
Fe (mg kg^{-1} d.w)	22641 (386)
K (mg kg^{-1} d.w)	1729 (37)
Mg (mg kg^{-1} d.w)	3267 (26)
Mn (mg kg^{-1} d.w)	560 (15)
Na (mg kg^{-1} d.w)	302 (11)
P (mg kg^{-1} d.w)	1501 (68)
S (mg kg^{-1} d.w)	514 (9)
Zn (mg kg^{-1} d.w)	92 (3)

6.2.2 Experimental setup

Species selection

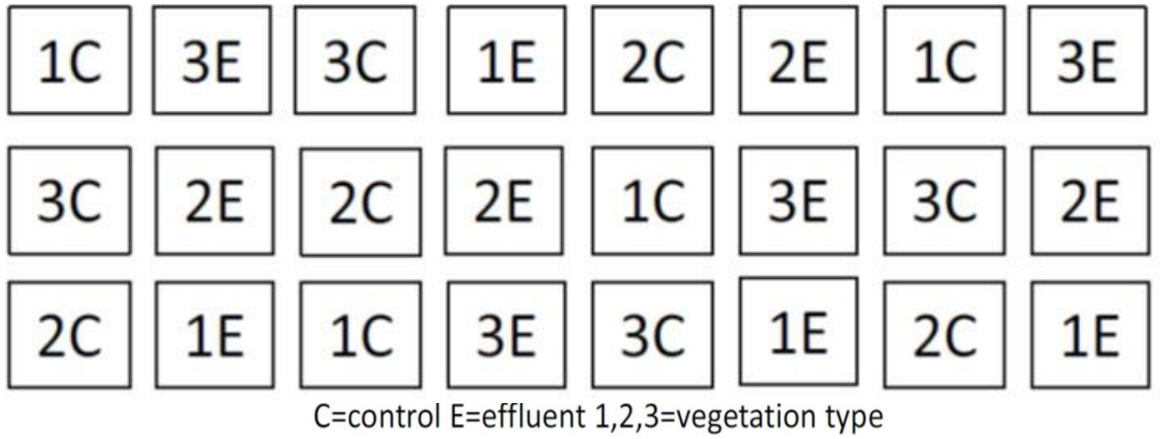
Eleven NZ native species were selected for the field trial, namely *L. scoparium*, *K. robusta*, *O. paniculata*, *C. robusta*, *P. cunninghamii*, *G. littoralis*, *P. arboreus*, *P. tenax*, *P. cookianum*, *C. australis*, and *P. eugenoides*. These species have a natural distribution in the surrounding area, are inexpensive and hardy. In addition to their environmental benefits, *L. scoparium* and *K. robusta* could provide commercial benefits through the production of essential oils and/or honey. *L. scoparium* was shown to kill soil-borne pathogens (Prosser et al., 2016) and reduce NO_3^- leaching (Esperschuetz et al., 2017). *P. tenax* is used for fibre production, and *G*. *littoralis* may be a nutritious grazing animal supplement (Dickinson et al., 2015).

To keep experimental variables consistent within and between species, seedlings of between 30 cm and 35 cm were selected for planting. Seedlings were sourced from Motukarara Native Plant Nursery (Waihora Park Motukarara, Christchurch 7672). Two-year-old seedlings were transplanted in May of 2015. Plant guards were installed to protect the plants from herbivores.

Plot trial design and treatment

Figure 6.2 shows the plant species which comprised each of the three treatments. Each 5 x 5 m plot was planted with 50 plants from the treatment group, spaced at approximately 0.5 x 0.5 m intervals. There were four replicates of each treatment, with a total of 12 plots irrigated with TMW and 12 control plots, which received rainwater only. **Plate 6.1** shows the site shortly after planting. In January

2016, TMW was applied at the level of 200 kg N ha^{-1}. Species were grouped according to their natural associations as occurs on Banks Peninsula. Individual species in each sub plot were planted regularly throughout the plot. The number of individual plants varied between species. Vegetation type 1 consisted of 25 of *L. scoparium* and 25 of *K. robusta*. Vegetation type 2 consisted of 13, 13, 12, and 12 of *O. paniculata*, *C. robusta*, *P. cunninghamii*, *and P. arboreus*, respectively. Whereas vegetation type 3 consisted of 13, 13, 12, 6, and 6 of *P. tenax*, *P. cookianum*, *C. australis*, *P. arboreus*, and *P. eugenoides,* respectively.



Vegetation type 1 = *L. scoparium and K. robusta*
Vegetation type 2 = *O. paniculata, C. robusta, P. cunninghamii,* and *P. arboreus*
Vegetation type 3 = *G. littoralis, P. tenax, P. cookianum, C. australis,* and *P. eueugenioides*

Figure 6. 2 The experiment layout of Duvauchelle field trial, Akaroa, New Zealand

Plate 6. 1 An initial view of plot trial few days after planting, Duvauchelle field trail, Robinsons Bay, Akaroa

Municipal wastewater irrigation system

TMW was obtained from the Akaroa Wastewater Treatment Plant, sited about 500m from the field site. The wastewater received secondary treatment before being applied to the plots. It was pumped to the plots using an automated drip irrigation system. From January 2016 to April 2017, each plant in the TMW treatment received wastewater at a rate of 500 mm, a rate similar to that used on an irrigated dairy farm in Canterbury. Control plots received rainwater only.

Weed control and plant measurement

A lawnmower was used to cut grass outside the plots. In March 2016, the inside part of the plots was sprayed with herbicide to control weed growth. On May 6, 2017 (2 years after planting), the survival rate and canopy volume were recorded. Canopy volume components were measured by taking the height and diameter reading at 50% of the individual plants height (Mark et al., 2002). Plant height was defined as the distance from the base of the main stem to the tallest extent of photosynthetically active plant material. Diameter reading was defined at the widest extent of photosynthetically active plant material that intersected a plane passing horizontally through the plant at 50% of the plant height. Plant height and crown diameter were measured using a wooden ruler. Plant canopy volume was estimated by applying the height and diameter measurement to a derivative of the basic ellipsoid volume formula as follows:

$$\textbf{Canopy Volume} = 0.5 * 3.14 * (r^2) * h$$

6.2.3 Sample collection and analysis

In May 2017, above ground plant parts were harvested using non-destructive sampling methods. Five plants were chosen from each of the 11 species in both the control and TMW treatment. Plant parts were cut-off from each plant and kept in labelled paper envelopes for biomass and total element analysis. They were then dried at 70°C for at least a week, ground to powder and stored in 30 ml plastic containers for further analysis of total elements. Dried leaves were then separated from branches (**Plate 6.2a** and **6.2b)**, ground using a Retch ZM200 grinder (**Plate 6.2c** and **6.2d)** and stored in sealed plastic bags. For total N, 0.2g of fine samples were transferred into crucibles before running using Flow Injection Analysis (FIA) method.

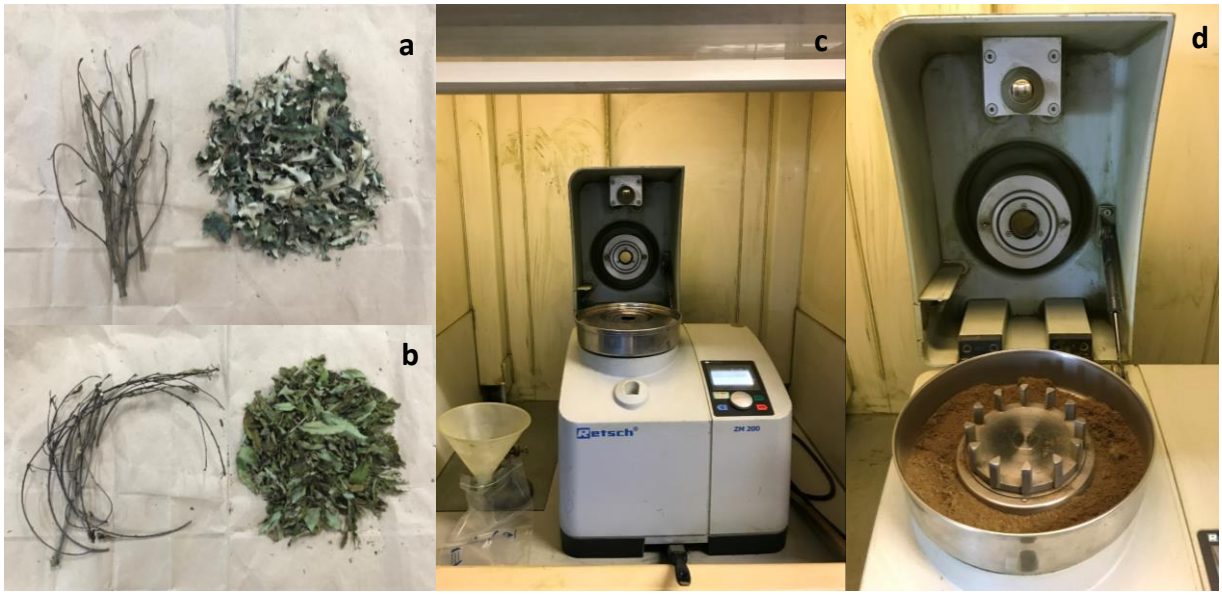


Plate 6. 2 Dried leaves separated from branches (a, b) and grinder Retch ZM200 for grinding the samples (c, d)

Rhizosphere soil was sampled from both control and TMW plots (total 24 plots). Five soil samples were taken from each plot (**Figure 6.2)** up to 15-cm deep and 5 cm in diameter of soil cores. Soil samples were sieved using a 2mm nylon sieve then kept in the fridge for further analysis.

Soil pH was determined using 10 g of soil and 25 mL of deionised water (18.2 MΩ resistivity; Heal Force® SMART Series, SPW Ultra-pure Water system, Model-PWUV) at a soil and water ratio of 1:2.5. The mixture was then shaken for an hour and left to equilibrate for 24h before measurement. Each mixture was shaken before measuring soil pH using a pH meter (Mettler Toledo Seven Easy) (Blakemore et al., 1987).

Total C and N were detected by Flow Injection Analysis (FIA) method using 0.5 g of oven-dried soil samples. An Elementar Vario-Max CN Elementar analyser (Elementar ®, Germany) was used to analyse the total C and N content in the soil and plant samples. The analysis was conducted by adding 40 mL of a 2M KCl reagent to 4.0 g of fresh soil, the solution was then shaken on an end-over-end shaker for 1h, centrifuged at 2000 rpm for 10 min and subsequently filtered through Whatman 41 filter paper (Blakemore et al., 1987). Extracted solutions were kept at -20°C until analysed. Ammonium-N (NH_4^+-N) and nitrate-N (NO_3^--N) were determined using a flow injection analyser (FIA FS3000 twin channel analyser, Alpkem, USA).

Soils were digested using a microwave digester (the CEM MARS Xpress - CEM Corporation, Matthew, PO Box 200 North Carolina, 28106-0200, USA), using 0.2 g of sample in 8 mL of AristarTM nitric acid (± 69%) and filtered by means of Whatman 52 filter paper (pore size 7 µm) after dilution with milliQ

water to a volume of 10 mL. Certified Reference Materials (CRMs) for soil (International Soil analytical Exchange - ISE 921) and plant samples (International Plant analytical Exchange IPE 100) from Wageningen University, The Netherlands, were digested. Concentrations of Cd, B, Ca, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, P, S and Zn of both plants and soil were determined using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES Varian 720 ES - The Varian 720 ICP-OES - Varian Australia Pty Ltd, 679 Springvale Road, Melbourne in soils (Kovács et al., 2000; Simmler et al., 2013; Valentinuzzi et al., 2015). Extraction and digestion solution and method blanks were analysed in triplicate as part of standard quality control procedure for the analysis and were below the ICP-OES's detection limit for all metals. Recoverable concentrations of the CRMs were within 93% - 110% of the certified values.

6.2.4 Data analysis

Significant differences ($\alpha=0.05$) between control and TMW treatments of each species were determined by Independent-Sample t-test. Percentage data were transformed using Arcsin Transformation prior to the t-test. The analyses was performed using SPSS v.23 (Meyers, 2013).

6.3 Results

6.3.1 TMW characteristics

Table 6.3 shows the characteristics of TMW used in the experiment. As shown in Table 6.3, TMW possesses considerable amounts of N, P, K, Ca, Mg, and S, which are considered essential nutrients for improving plant growth and soil fertility and productivity levels. However, over a longer period, several elements such NO_3^-, P, and S could potentially stimulate the mass proliferation of algae, including cyanobacteria, which may be toxic to humans and animals, thus damaging fisheries and tourism industries. **Table 6.3** shows that the Sodium Adsorption Ratio (SAR) of TMW was above the threshold for crop irrigation purposes (FAO, 2018). This indicates that long term application of TMW may result in aggregate instability (dispersion of clay colloids) in soil, resulting in a breakdown in soil structure and consequent problems with infiltration, aeration, and drainage (FAO, 2018). Amending soil with a high alkaline material such as gypsum, dolomite, or lime could be an alternative option for maintaining soil quality (FAO, 2018).

Table 6. 2 Characteristics of TMW and mass plant macro and micro-nutrients added through irrigating treated municipal wastewater at a rate of 500 mm per year. Values in brackets represent standard deviation of mean (n=54).

Properties	Concentration	Mass added (kg ha^{-1} yr^{-1})
N (mg L^{-1})	18 (7.5)	90
P (mg L^{-1})	11 (5)	55
K (mg L^{-1})	22 (5)	110
S (mg L^{-1})	25 (11)	125
Mg (mg L^{-1})	19 (5.5)	95
Ca (mg L^{-1})	59 (12)	295
Al (mg L^{-1})	0.43 (0.11)	2.15
B (mg L^{-1})	0.1 (0.04)	0.5
Na (mg L^{-1})	95 (21)	475
Pb (mg L^{-1})	<0.01 (0.00)	0.05
Cr (mg L^{-1})	<0.01 (0.00)	0.05
Cu (mg L^{-1})	0.04 (0.03)	0.2
Zn (mg L^{-1})	0.17 (0.11)	0.85
Mn (mg L^{-1})	0.06 (0.03)	0.3
Fe (mg L^{-1})	0.96 (0.25)	4.8
Cd (mg L^{-1})	<0.01 (0.00)	0.05
pH	7.5 (0.6)	-
EC (dS/m)	423 (40)	-
NO_3^- - N (mg L^{-1})	18 (7.5)	-
Sodium Accumulation Ratio (SAR)	15 (2.6)	-

Table 6.3 shows that the concentrations of trace elements in the TMW were relatively low and meet the standards for wastewater reuse in irrigation (FAO, 2018). Given the fact that these metals could accumulate in soil and plants with continuous use of TMW as irrigation, monitoring should be an important component of wastewater management. In addition, the annual mass of N added per hectare is approximately almost half of the maximum rate permitted in most agriculture threshold of 200 kg ha^{-1} yr^{-1}. Phosphorus and K are within the ranges that these nutrients would be added to maintain an intensively grazed pasture (DairyNZ, 2018). However, TMW contains more than double the amount (20 – 50 kg ha^{-1} yr^{-1}) of S, which is likely to leach as S is poorly retained by most NZ soils, including the Banks Peninsula loess.

6.3.2 Growth parameter

Plant survival

Figure 6.3 shows that there were no significant (*p>0.05*) differences between the TMW-irrigated and non-irrigated plots. With the exception of *P. arboreus,* all indicator plants had more than a 60% survival rate. Over all, the survival rate of plants watered with TMW was apparently higher than that of the plants in the control. The results show that seven species, namely *C. robusta*, *G. littoralis*, *C. australis*, *P. cunninghamii*, and *P. eugenoides* had more than a 90% survival rate in five months after

receiving TMW. On the other hand, *P. arboreus* had less than a 50% survival rate (**Figure 6.3**). *L. scoparium* and *K. robusta*) had fair survival rates of more than 70%.

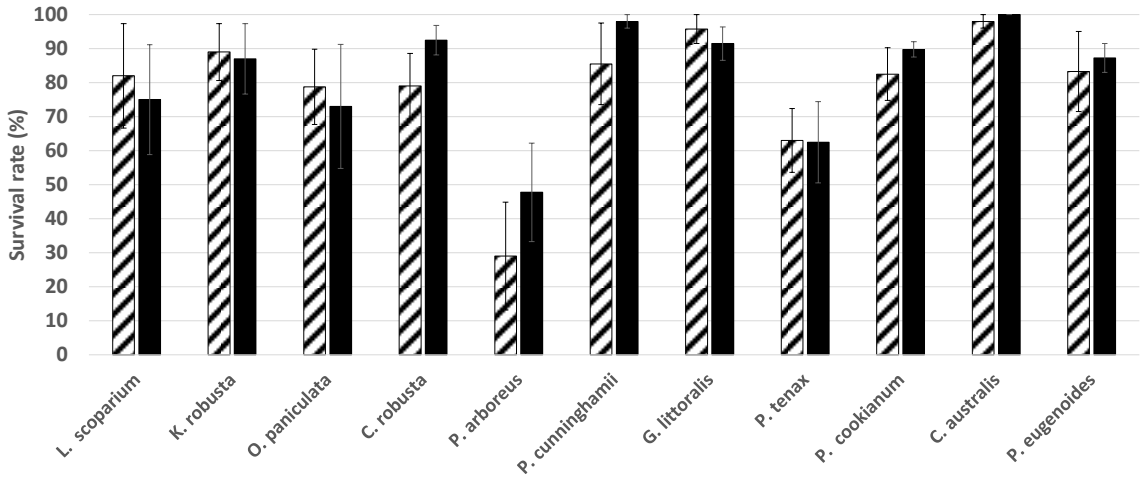


Fig. 6. Survival rate (%) of of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean (n=4). There were no significant differences between the controls (striped bars) and treatments (solid bars) at *p≤0.05*.

Crown volume

In general, plants growing in the TMW-treated plots were visibly larger than the control plots (**Plate 6.3)**. This was confirmed by crown volume measurements, which showed that of the 11 species, crown volume was significantly increased in 8 species, compared to the controls.

Plate 6. 3 Plant condition of Duvauchelle field trial, Akaroa (June 2017)

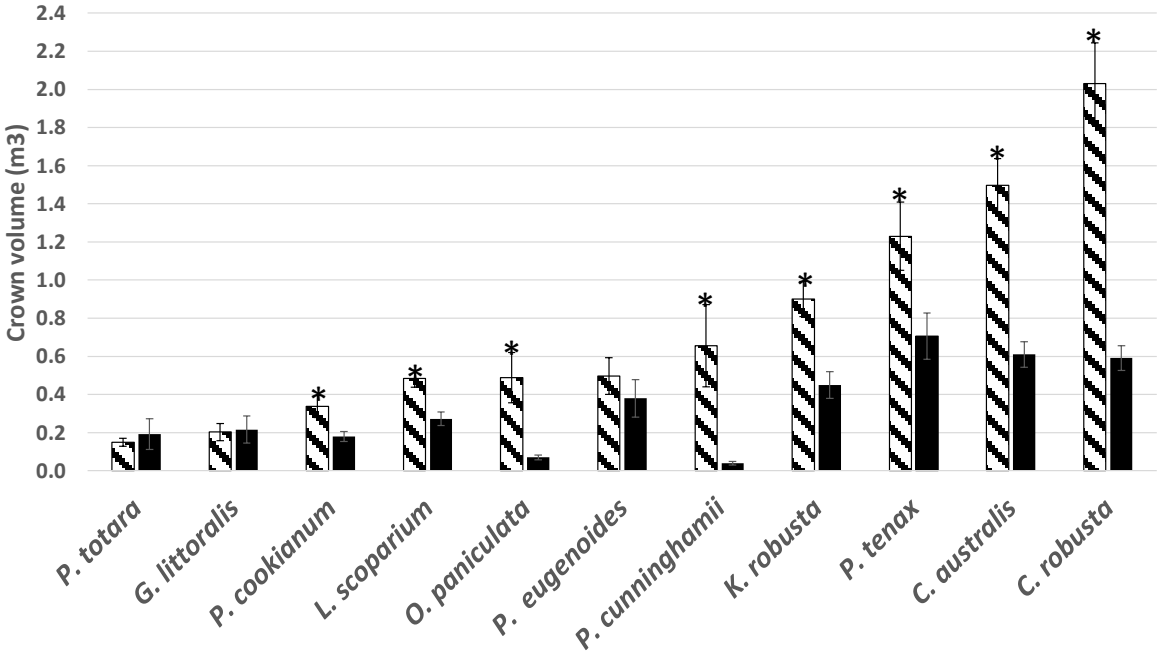


Figure 6. 4 Crown volume (m^3) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean. Asterisks (*) signify significant differences between the effluents (striped bars) and controls (solid bars) at *p≤0.05*

Application of TMW significantly increased (*p<0.05*) the studied vegetative growth parameter (crown volume) compared with control (receiving none). Generally, the crown volume of each species grown in the TMW treatments was significantly higher than those found of the control. With the exception of *P. cunninghamii*, *G. littoralis*, and *P. eugenoides*, independent t-test analysis proved that the application of TMW significantly (*p≤0.05*) increased the canopy volume of tested species (**Figure 6.4).** The increased rates of canopy volume vary amongst species tested. Irrigating TMW in to soils increased approximately fifteen times of above ground part (canopy volume) of *P. arboreus*, whereas the percent increase of canopy volume of *P. tenax* was only 74%.

6.3.3 Nutrient uptake

Macronutrients

Results showed that, in general, most species tested responded positively to the application of TMW. In general, irrigation with TMW gave significantly higher concentrations of N, P, K, S, Mg, and Na in the leaves of certain NZ native plants compared with the non-irrigated treatment. Compared to controls - with the exception of *P. tenax*, *G. littoralis*, and *P. cunninghamii* - most species accumulated significantly higher N in their leaves (**Figure 6.5).** *L. scoparium*, *K. robusta*, and *C. robusta* were the top

three species with the greatest concentration of foliar N. In contrast, *P. cunninghamii* accumulated the lowest concentration of foliar N (**Figure 6.5).**

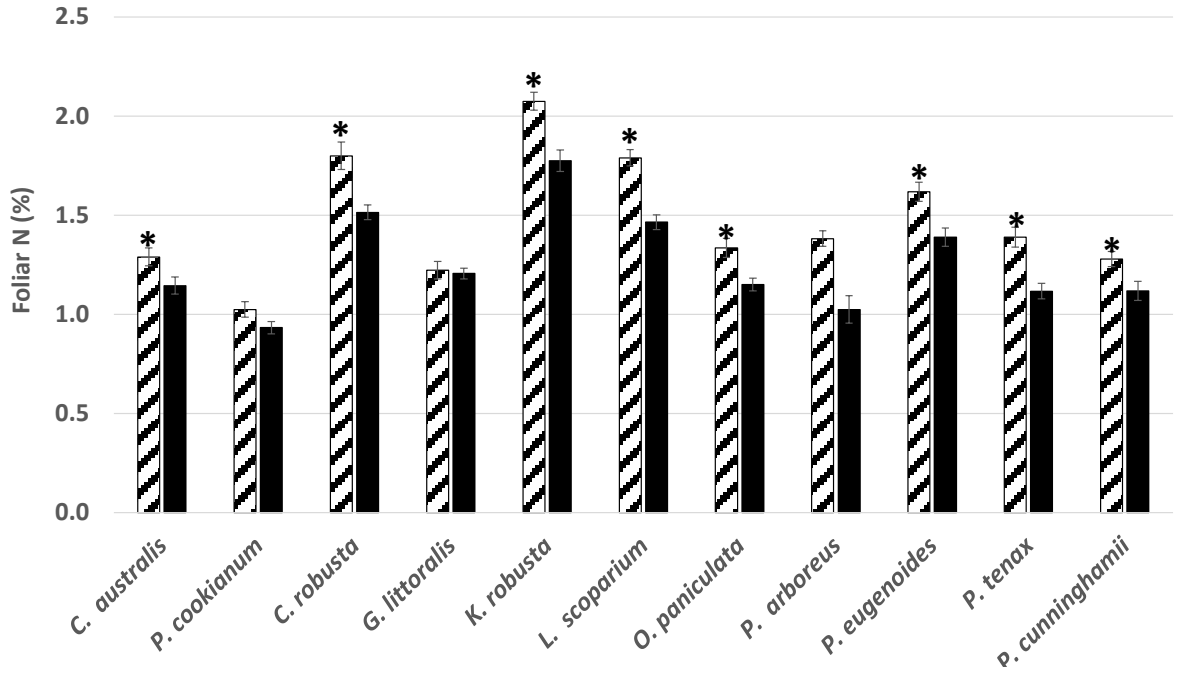


Figure 6. 5 Total concentration of foliar N (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean. Asterisks (*) signify significant differences between the effluents (striped bars) and controls (solid bars) at *p≤0.05*

This study showed that adding 500 mm of TMW to soils over an 18-month experimental period significantly increased the total concentration of foliar N of nine species tested from 13% (*C. australis*) to 24% (*P. tenax*). Species with greater canopy volumes (>1m^3), including *C. robusta*, *C. australis*, and *P. tenax*, accumulated higher N in their leaves. Remarkably, two important New Zealand native species, *L. scoparium* and *K. robusta*, which were irrigated with TMW and have relatively smaller canopy volume accumulated reasonably large amounts of N in their leaf tissue, 22% and 17%, respectively (**Figure 6.5**).

Figures 6.6 and **6.7** show that irrigating the plots with TMW resulted in significantly different concentration levels of foliar P and K of certain species in this study. Irrigating 500 mm of TMW on to the soils increased the accumulation of both foliar P and K of *L. scoparium* by 16%. The application of TMW significantly increased the accumulation of K in the leaves of *C. robusta* and *K. robusta* by 48% and 17% respectively. The present study indicates that compared to the control, seven plants which were irrigated with TMW accumulated significantly higher concentrations of other macronutrients including S in their leaves (**Figure 6.8)**. After 18 months of the experimental period, *L. scoparium*

increased its foliar S concentration by 82% (the largest increase) compared to the control, whereas *C. australis* increased foliar S by 21% (the lowest increase) (**Figure 6.8**).

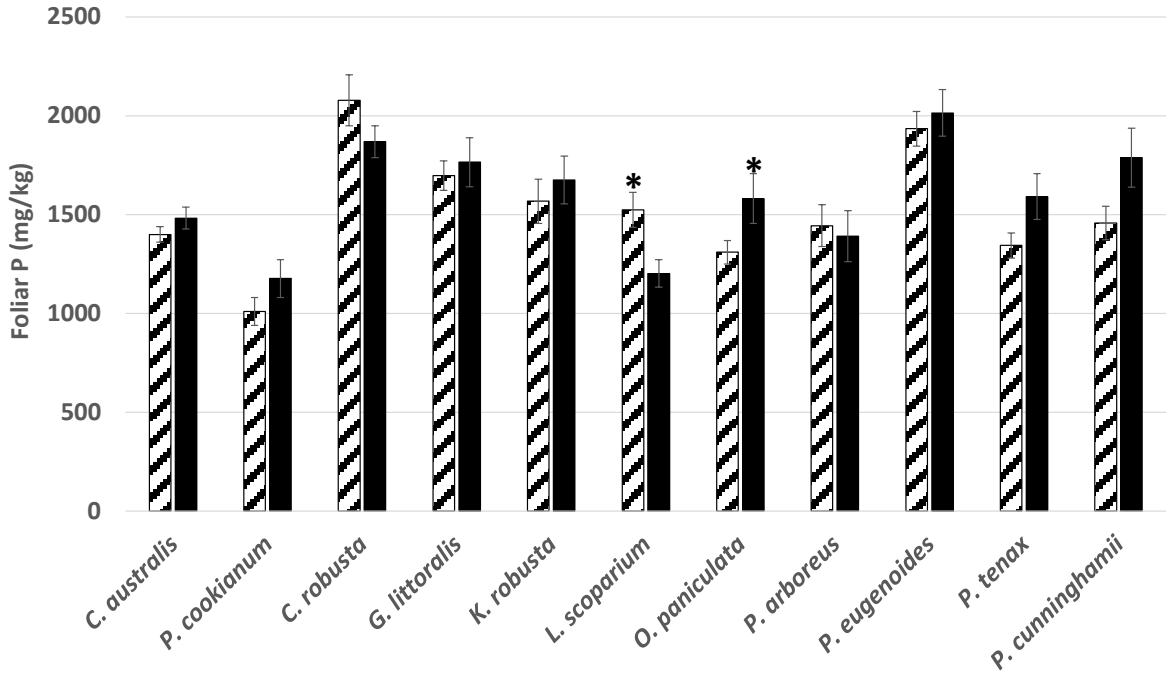


Figure 6. 6 Total concentration of foliar P (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean. Asterisks (*) signify significant differences between the effluents (striped bars) and controls (solid bars) at *p≤0.05*.

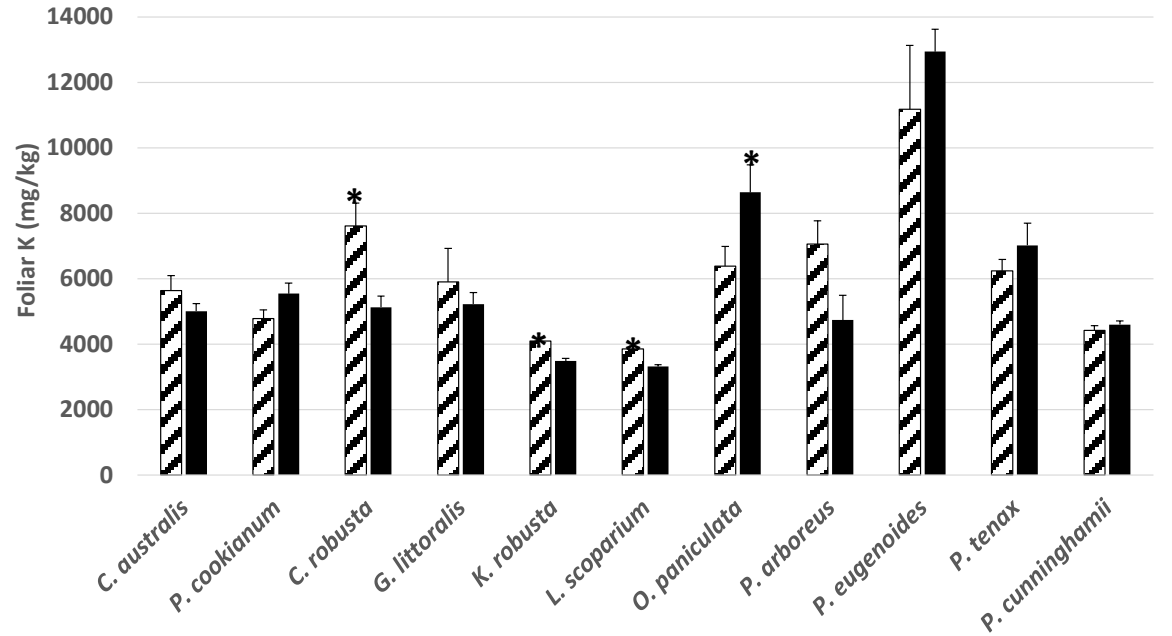


Fig. 11. Total concentration of foliar K (mg/kg) of of each species in response to

Figure 6. 7 Total concentration of foliar K (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean. Asterisks (*) signify significant differences between the effluents (striped bar) and controls (solid bars) at *p≤0.05*.

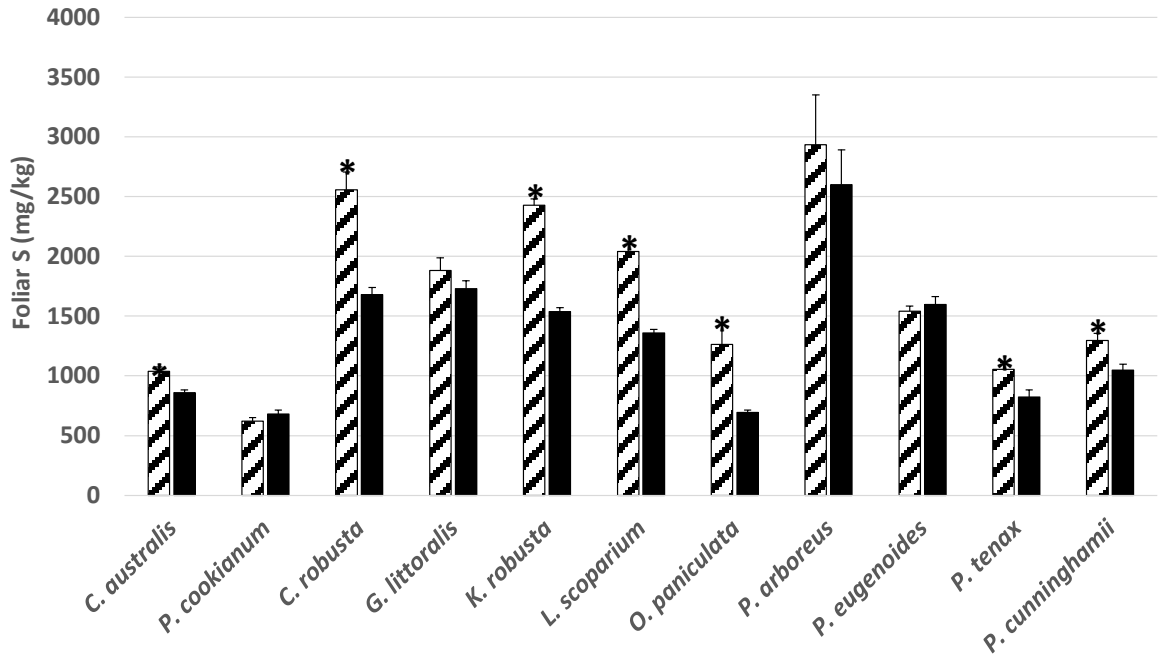


Figure 6. 8 Total concentration of foliar S (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean. Asterisks (*) signify significant differences between the effluents (striped bars) and controls (solid bars) at *p≤0.05*.

Five TMW-treated species, namely *L. scoparium*, *K. robusta*, *P. cookianum*, *O. paniculata*, and *P. arboreus* accumulated significantly higher Na by 22%, 25%, 69%, 110%, and 291%, respectively than that of the control (**Figure 6.9**).

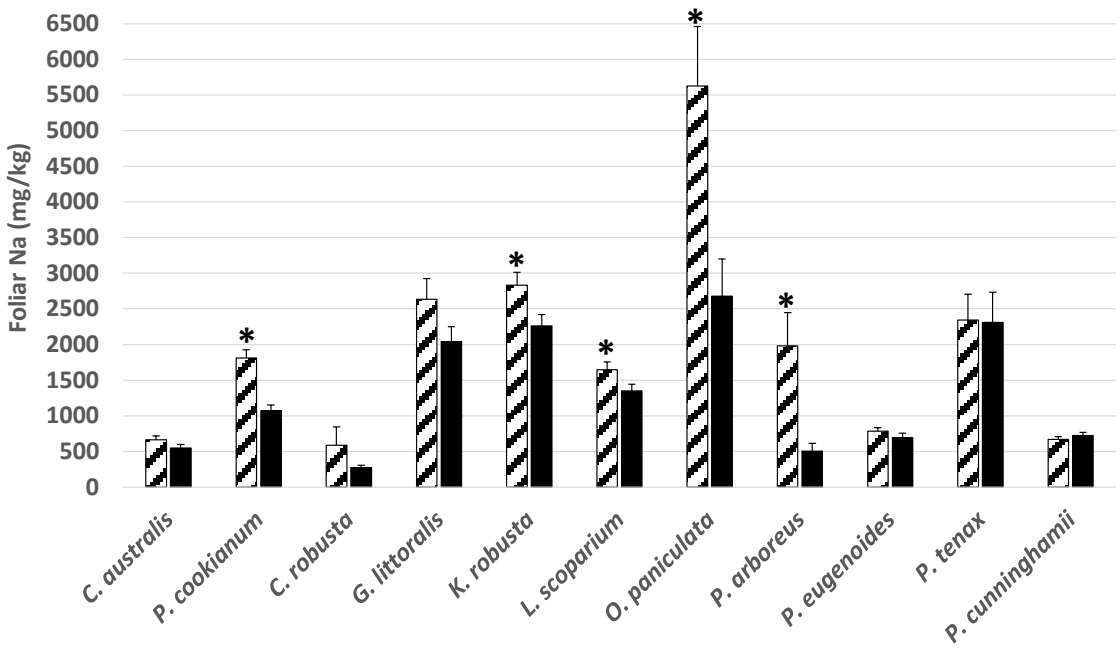


Figure 6. 9 Total concentration of foliar Na (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean. Asterisks (*) signify significant differences between the effluents (striped bass) and controls (solid bars) at *p≤0.05*.

Irrigating soil with TMW reduced the level of accumulation of certain macro-elements in the leaves of the species tested. Adding TMW to soils lowered the concentration level of both foliar P and K of *O. paniculata* by 26%. A similar trend can be seen on the concentration of foliar Mg of certain species after 18 months of experimental period. In the TMW treatment, the accumulation of Mg on the leaves of *C. robusta*, *K. robusta*, and *P. cunninghamii* was significantly lower than those in the control (**Figure 6.10**).

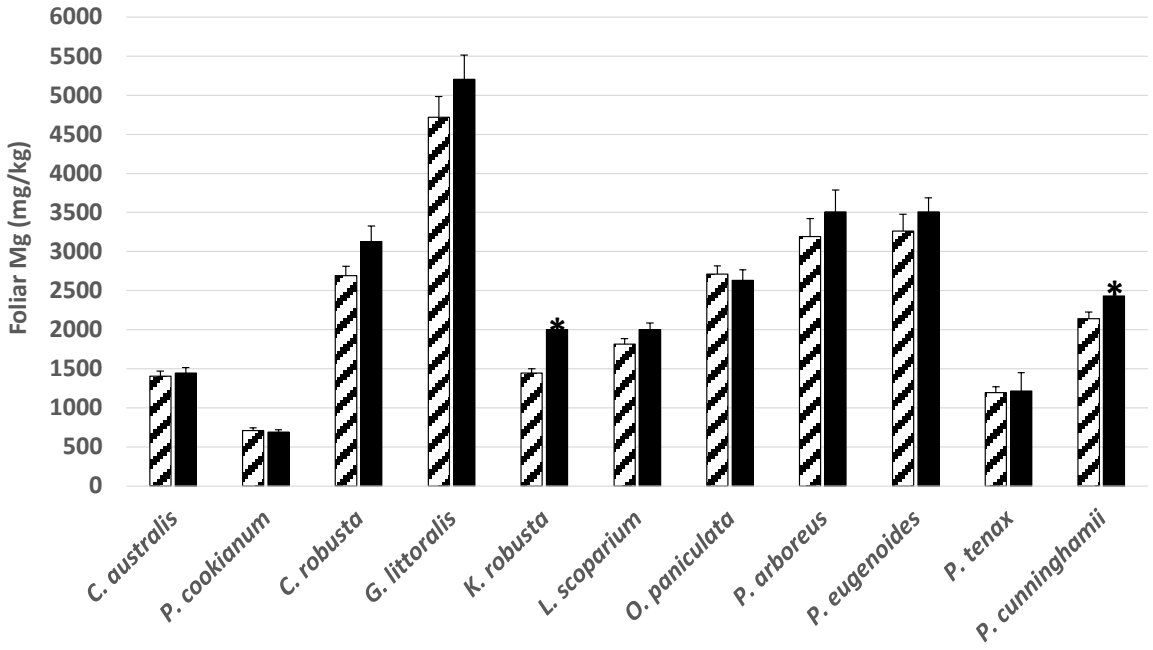


Figure 6. 10 Total concentration of foliar Mg (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean. Asterisks (*) signify significant differences between the effluents (striped bars) and controls (solid bars) at *p≤0.05*.

Micronutrients

The results indicated that the application of TMW generally led to changes in the physicochemical characteristics of soil and consequently significant (p≤0.05) differences in the uptake of some micronutrients by certain plants tested. **Figures 6.11, 6.12, and 6.13** show that irrigation of soils with TMW significantly altered the concentration level of foliar Fe, Mn, and Cd in *L. scoparium*, *P. tenax*, *K. robusta*, and *P. cunninghamii*. Irrigation with TMW lowered the concentration level of foliar Fe and Mn in *L. scoparium* by 40% and 29%, respectively (**Figures 6.11** and **6.12**). A similar trend was observed by TMW-treated *K. robusta*, and *P. cunninghamii,* which accumulated significantly higher foliar Mn by 45% and 33%, respectively, than the control. In contrast, the application of TMW significantly elevated the concentration of foliar Fe in *P. tenax* by 36% (**Figure 6.11**). In addition, *L. scoparium* and *P. cunninghamii* significantly reduced the concentration of foliar Cd (**Figure 6.13**).

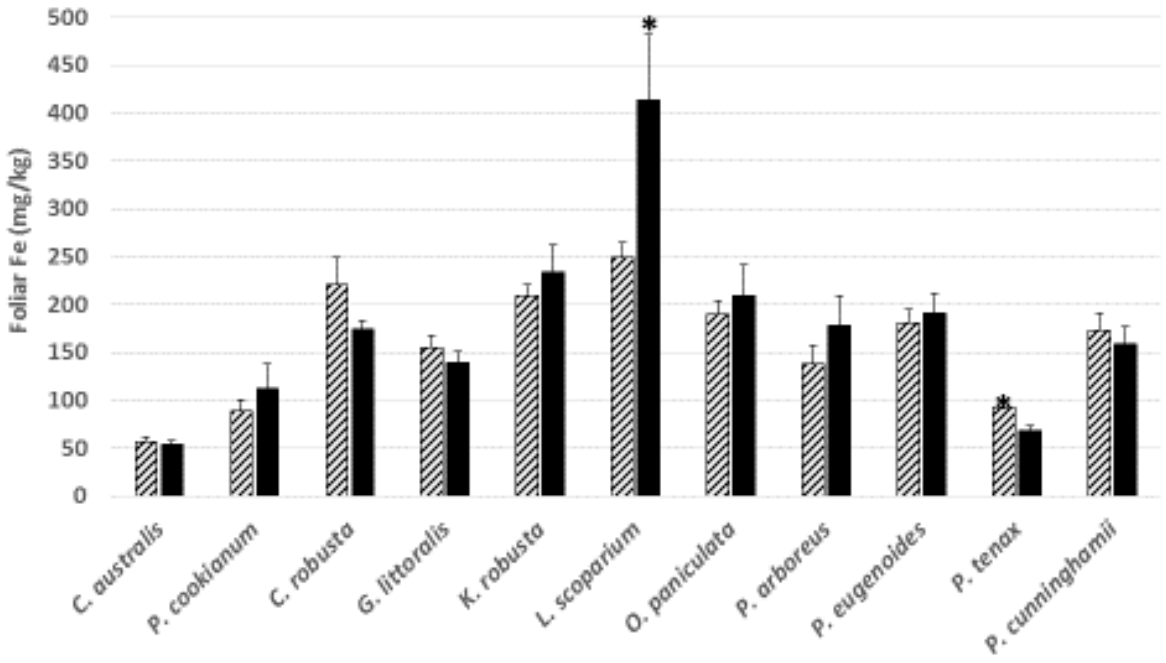


Figure 6. 11 Total concentration of foliar Fe (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean. Asterisks (*) signify significant differences between the effluents (striped bars) and controls (solid bars) at *p≤0.05*.

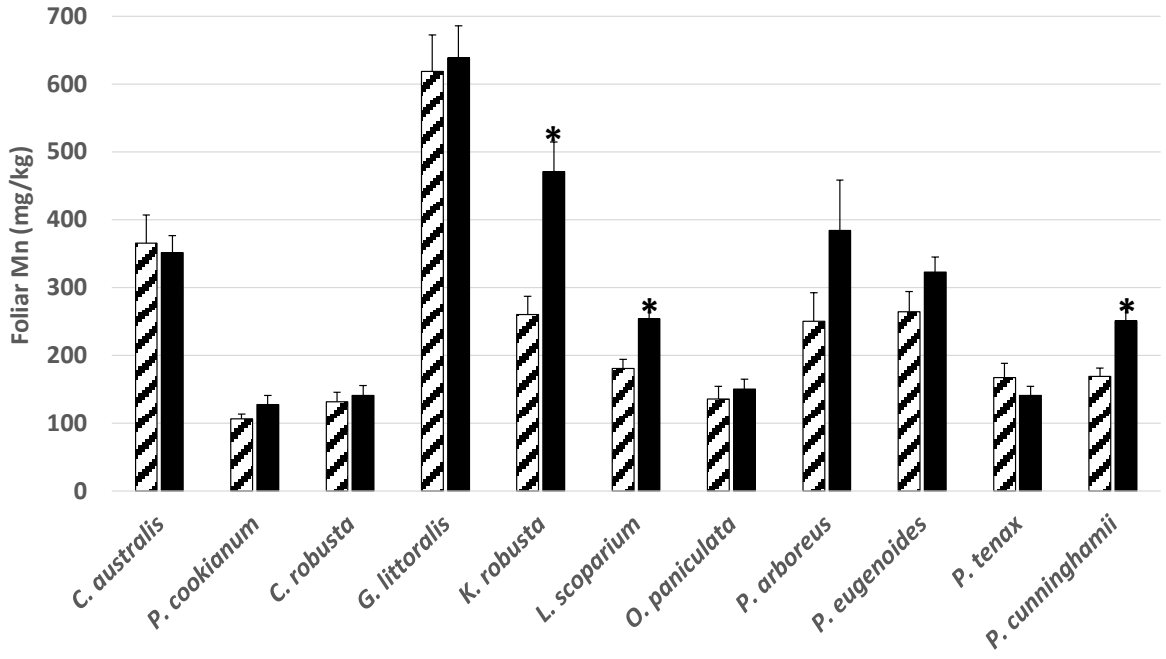


Figure 6. 12 Total concentration of foliar Mn (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean. Asterisks (*) signify significant differences between the effluents (striped bars) and controls (solid bars) at *p≤0.05*.

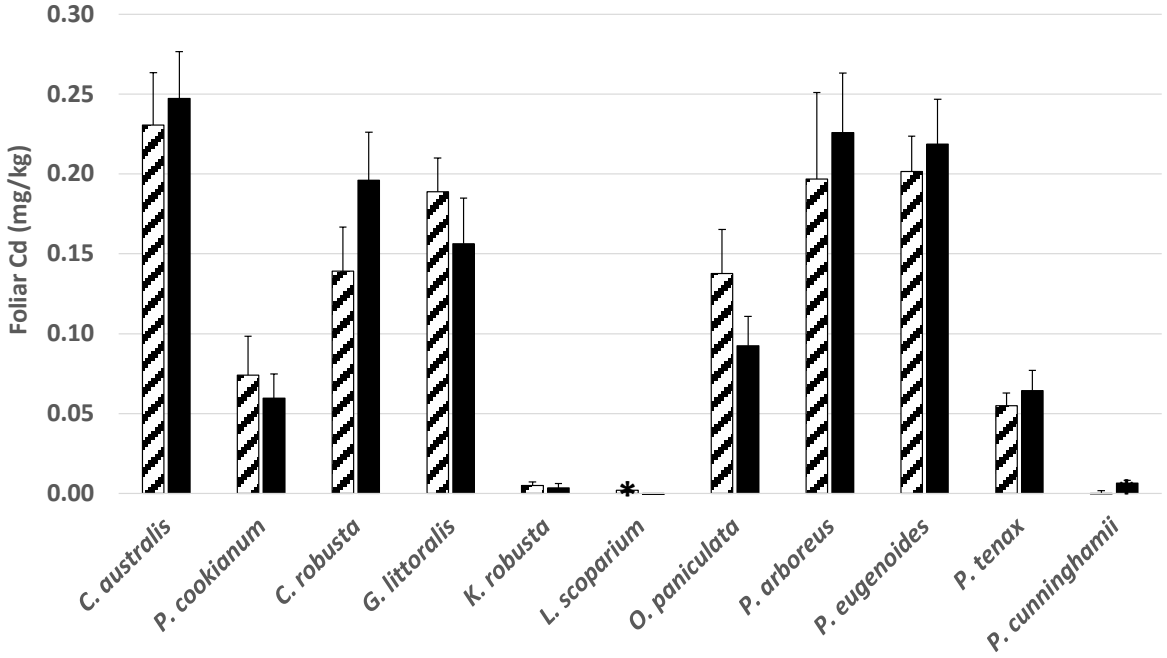


Figure 6. 13 Total concentration of foliar Cd (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean. Asterisks (*) signify significant differences between the effluents (striped bass) and controls (solid bars) at *p≤0.05*.

6.3.4 Total element concentrations in soil

Soil pH and EC

The irrigation with TMW resulted in significant changes in soil chemical properties. **Figure 6.14** shows that there was a significant increase of pH throughout the irrigated plots. Results indicated that the application TMW increased soil pH by 6-10%. A similar trend can be seen on EC values. Irrigation of soils with 500 mm of TMW resulted in higher EC throughout the experimental plots. After 18 months of irrigation, the EC values of soil under three different vegetation types increased between 43% and 86% (**Figure 6.14**).

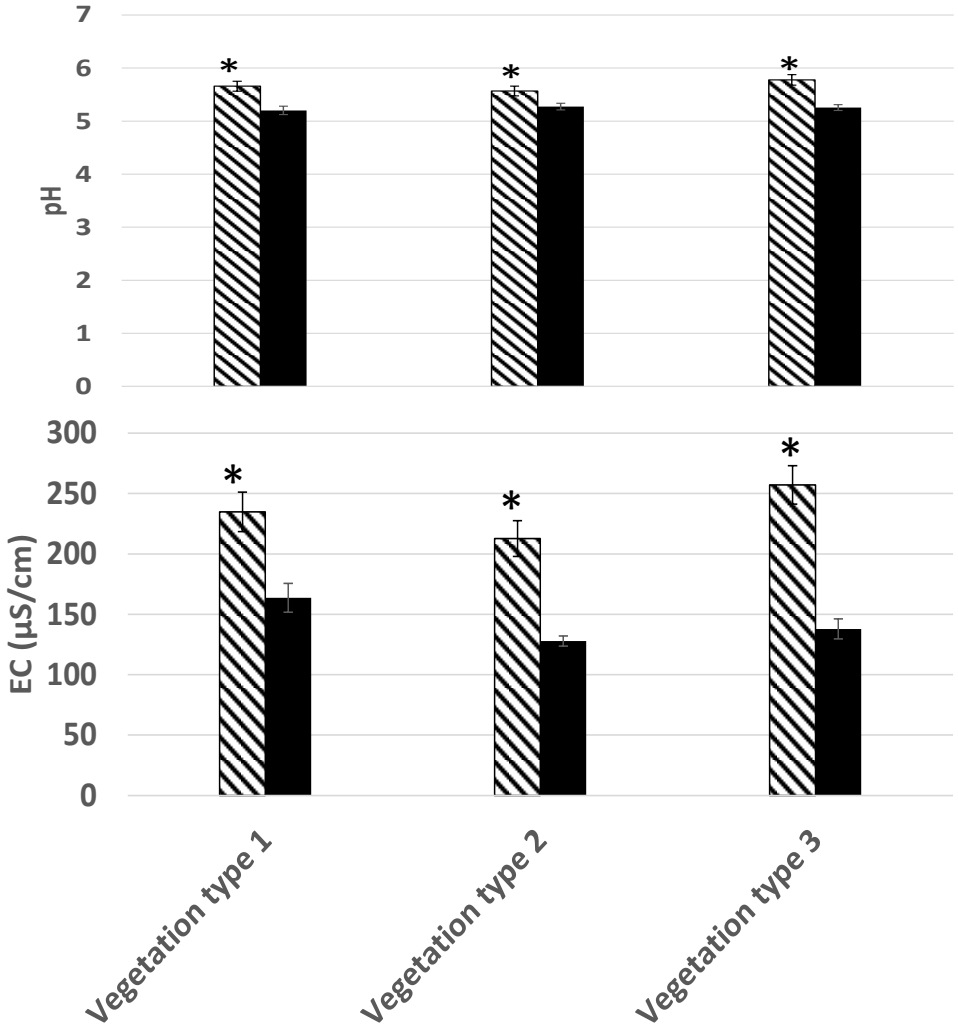


Figure 6. 14 Soil pH and EC of each vegetation type in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean (n=20). Asterisks (*) signify significant differences between the effluents (striped bass) and controls (black bars) at *p≤0.05*.

Total N and C

Results indicated that the application of TMW to soils significantly increased the concentration of soil C and N (**Figures 6.15** and **6.16**). As shown in **Figure 6.15,** after 18 months of regular irrigation of TMW, the soil C concentration increased by 12% and 13% in vegetation type 1 and 2 respectively. A similar trend was observed by soil N, which increased by 13% and 15% in combination with vegetation type 2 and 3, respectively.

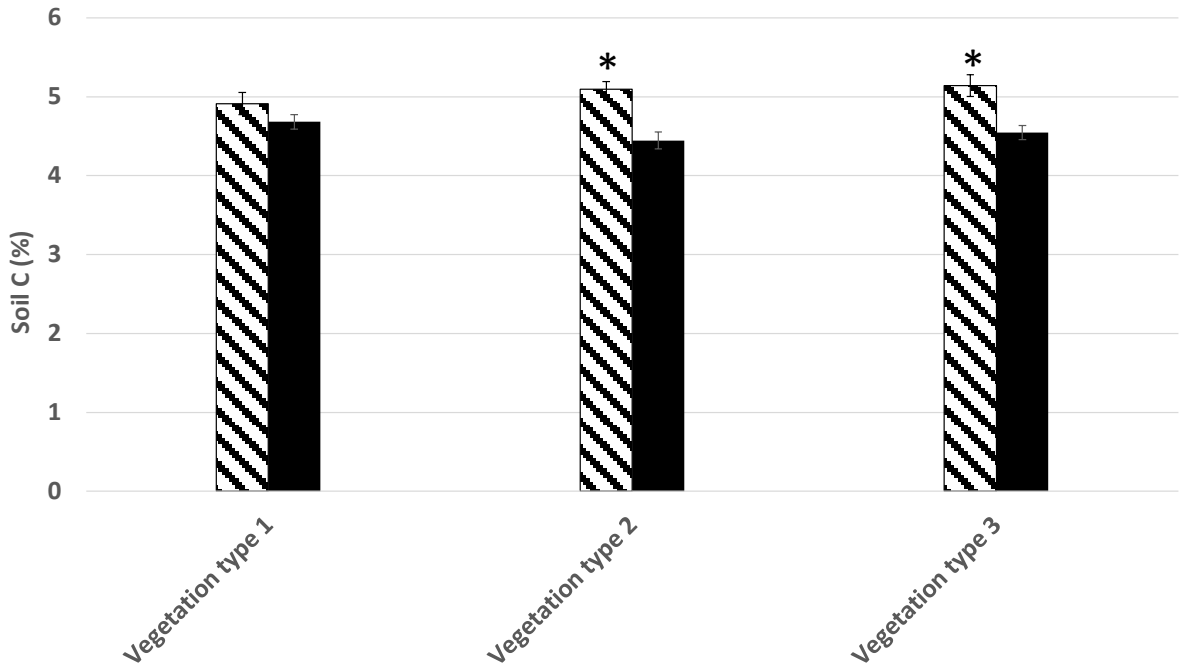


Figure 6. 15 Total concentration of soil C (%) of each vegetation type in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean (n=4). Asterisks (*) signify significant differences between the effluents (striped bass) and controls (black bars) at *p≤0.05*.

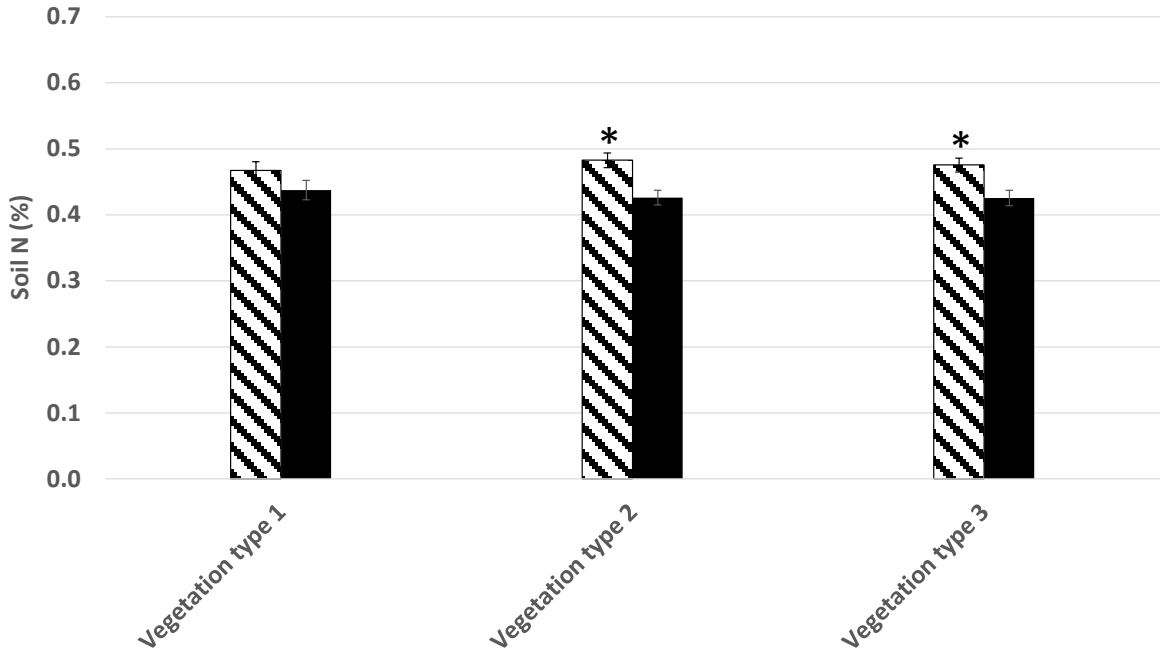


Fig. 20 Total concentration of soil N (%) of of each vegetation type in response to

Figure 6. 16 Total concentration of soil N (%) of each vegetation type in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean (n=4). Asterisks (*) signify significant differences between the effluents (striped bar) and controls (black bars) at *p≤0.05*

Mineral Nitrogen

Figures 6.17 and **6.18** show that TMW-treated soil increased the amount of NH_4^+ - N and $N0_3^-$ - N stored in the soil profile.

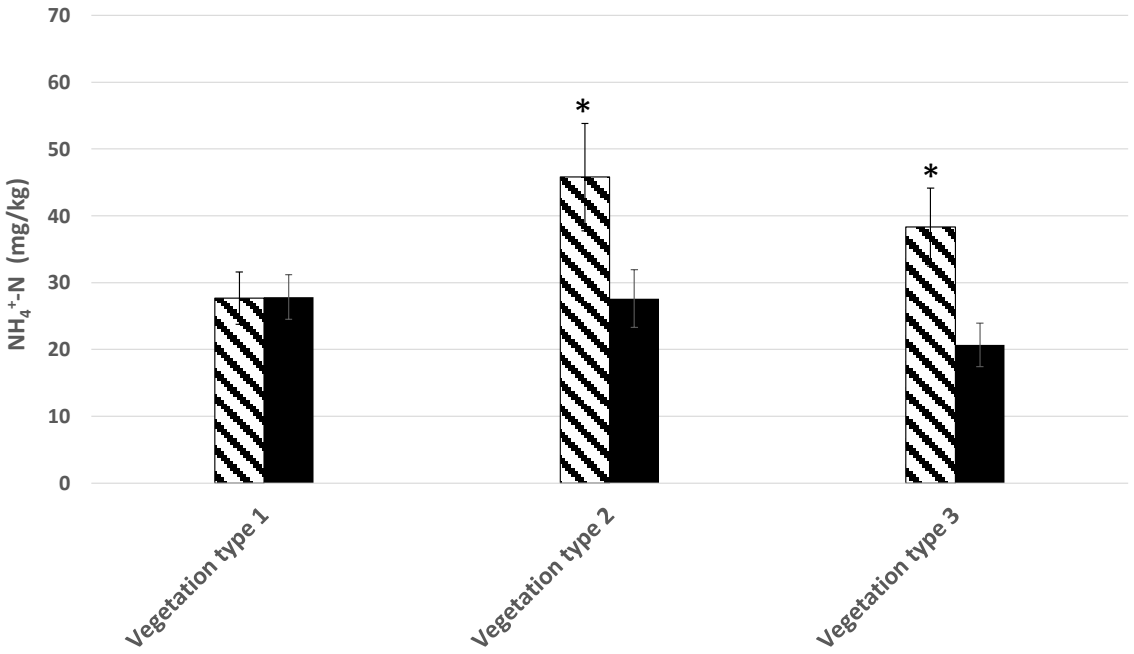


Figure 6. 17 Concentration of NH_4^+-N (mg/kg) of each vegetation type in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean (n=20). Asterisks (*) signify significant differences between the effluents (striped bars) and controls (black bars) at *p≤0.05*

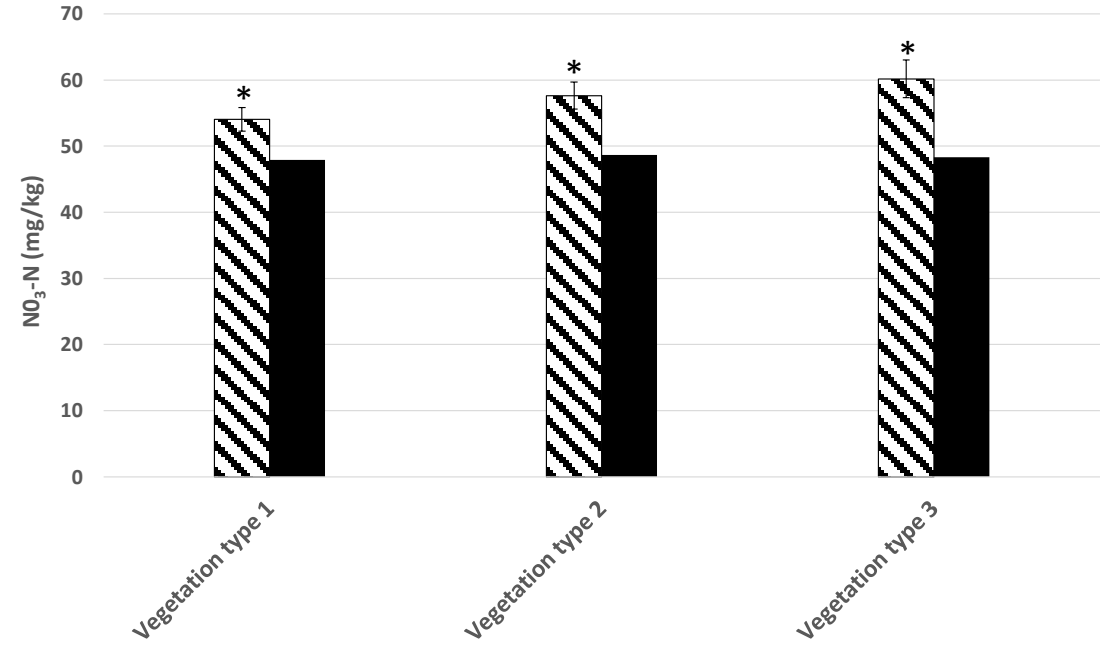


Figure 6. 18 Concentration of $N0_3^-$-N (mg/kg) of each vegetation type in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean (n=20). Asterisks (*) signify significant differences between the effluents (striped bars) and controls (black bars) at *p≤0.05*

Figures 6.17 and **6.18** show that with the exception of the concentration of NH_4^+-N under vegetation type 1, irrigation with TMW over the 18-months of the experimental period s significantly increased the amount of NH_4^+-N and NO_3^- -N in the top soil.

Other elements

Irrigating TMW on to soils significantly altered the concentrations of certain macro- and micro-elements in the top soil (**Figures 6.19, 6.20, 6.21, 6.22, 6.23** and **6.24**). The results show that the three different types of vegetation (type 1, 2, and 3) respond differently to the application of TMW in regard to the concentration level of macro and micro elements. Depending on the vegetation type, this study found that the concentration of certain soil elements which was irrigated with TMW can be (1) significantly higher; (2) significantly lower; and (3) either significantly lower or higher than that of the control.

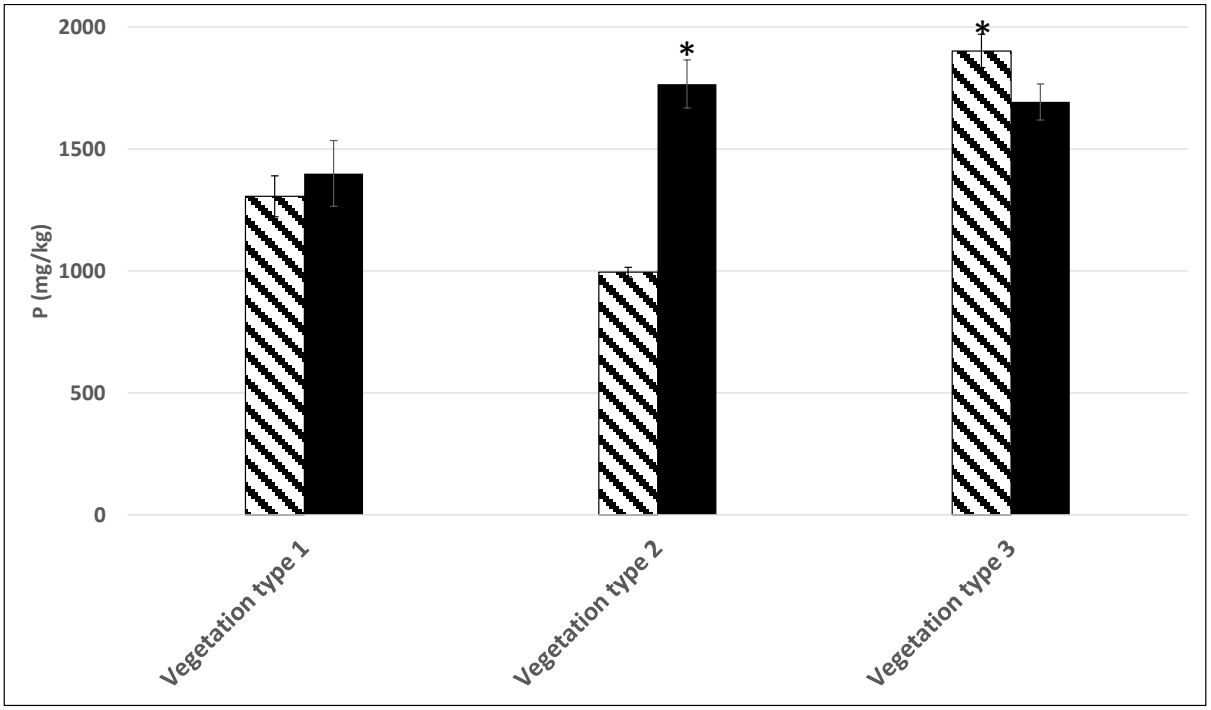


Fig. 23 Total concentration of soil P (mg/kg) of each vegetation type in response to

Figure 6. 19 Total concentration of soil P (mg/kg) of each vegetation type in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean (n=20). Asterisks (*) signify significant differences between the effluents (striped bars) and controls (solid bars) at *p≤0.05*

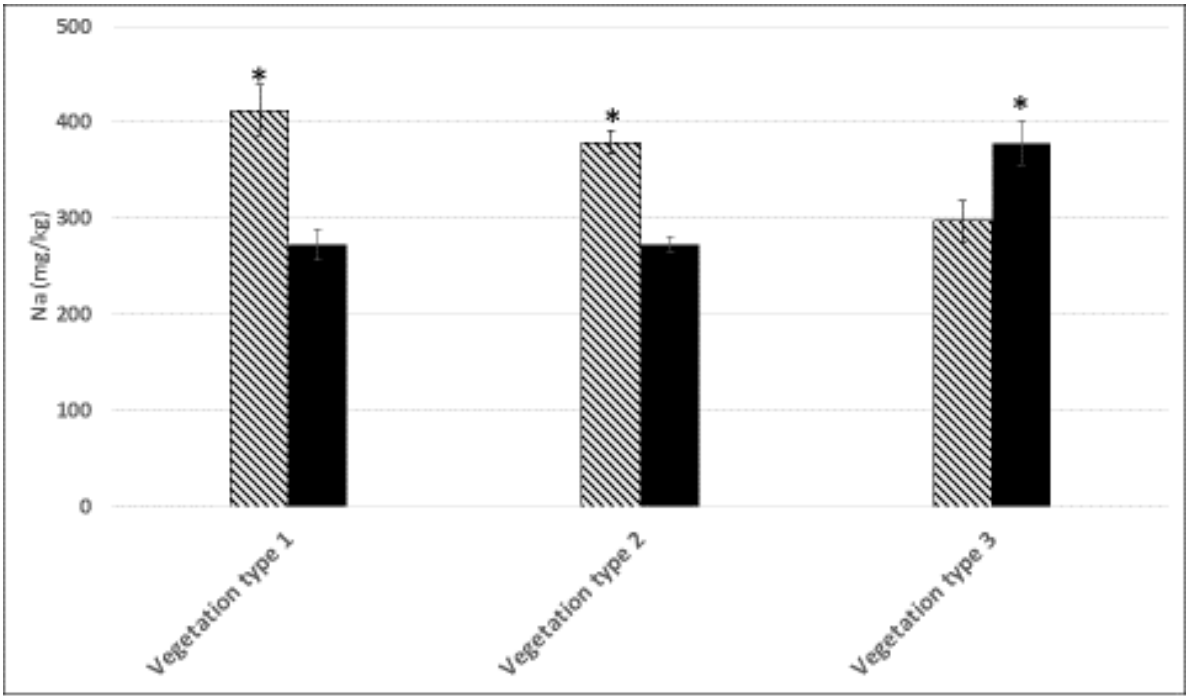


Figure 6. 20 Total concentration of soil Na (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean (n=20). Asterisks (*) signify significant differences between the effluents (striped bars) and controls (solid bars) at *p≤0.05*

Results showed that irrigating soil with TMW significantly affected the concentration level of certain macronutrients. **Figures 6.19 and 6.20** show that TMW significantly elevated the total soil P under vegetation type 3, and Na in combination with vegetation types 1 and 2. In contrast, the concentration of P in the soil significantly declined (by approximately 40%) under vegetation type 2 (**Figure 6.19)**. A similar trend can be seen in the concentration of soil Na in combination with TMW treatment which was significantly lower than that of the control on vegetation type 3 plots (**Figure 6.20**). The study found that adding TMW to soil significantly increased the concentration of the soil K on vegetation type 2 plots, soil S on vegetation type 1 and 2 plots, and soil Mg when combined with vegetation type 3 (**Figures 6.21, 6.22** and **6.23)**.

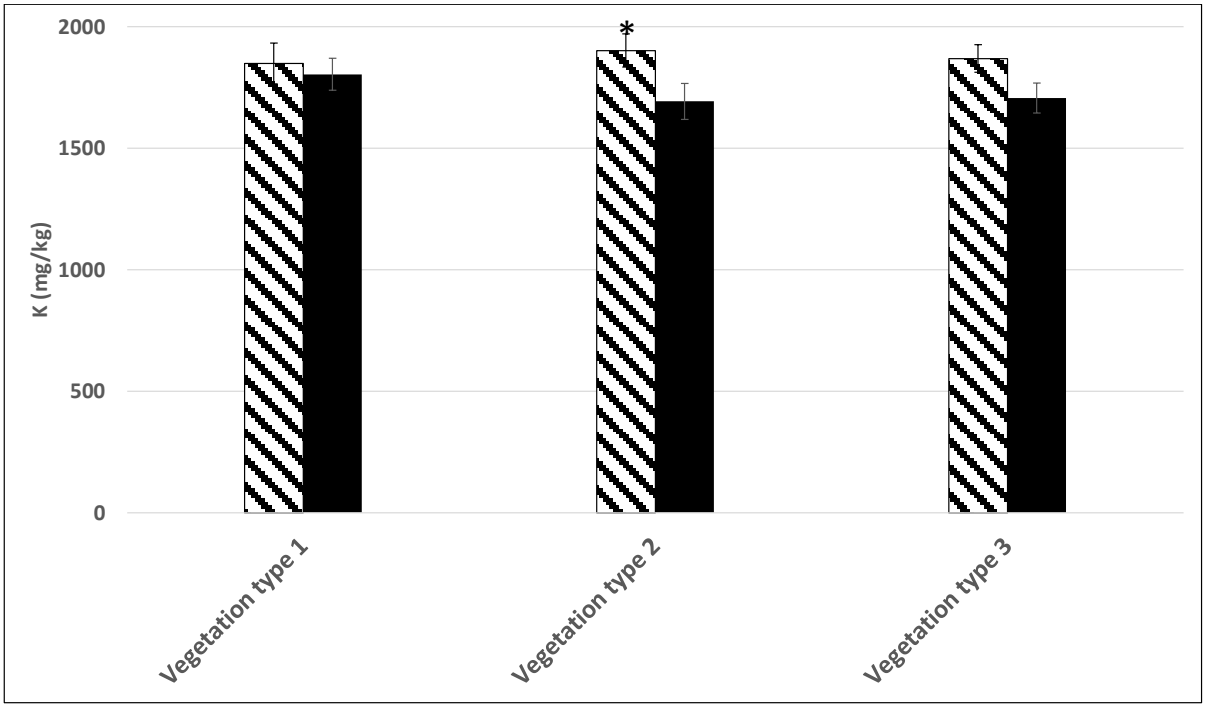


Fig. 25. Total concentration of soil K (mg/kg) of of each species in response to Treated

Figure 6. 21 Total concentration of soil K (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean (n=20). Asterisks (*) signify significant differences between the effluents (striped bars) and controls (solid bars) at *p≤0.05*

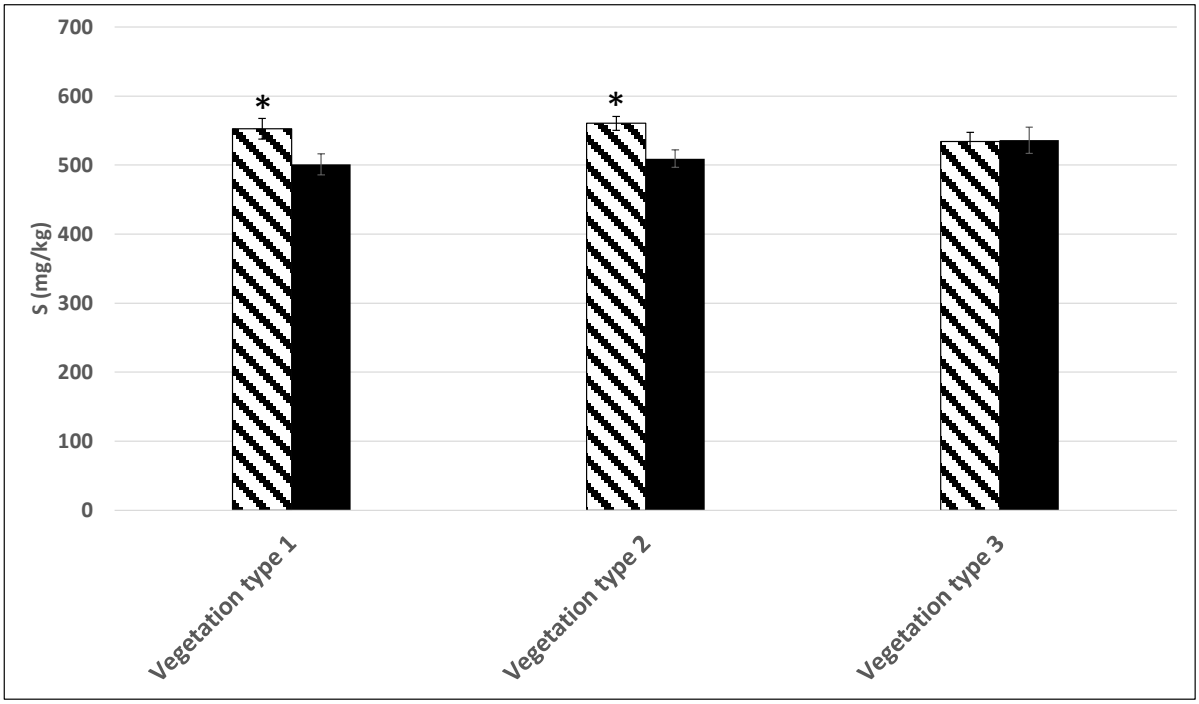


Fig. 26. Total concentration of soil S (mg/kg) of of each species in response to Treated

Figure 6. 22 Total concentration of soil S (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean (n=20). Asterisks (*) signify significant differences between the effluents (striped bars) and controls (solid bars) at *p≤0.05*

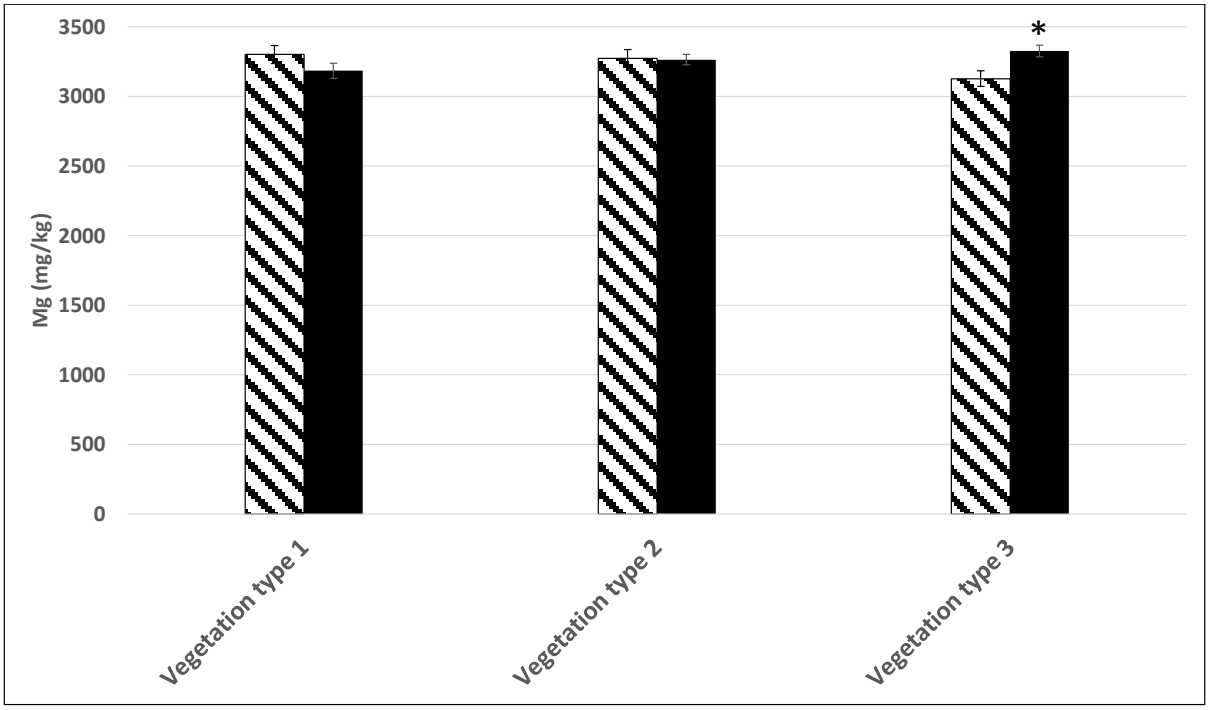


Fig. 27 Total concentration of soil Mg (mg/kg) of of each species in response to

Figure 6. 23 Total concentration of soil Mg (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean (n=20). Asterisks (*) signify significant differences between the effluents (striped bars) and controls (solid bars) at *p≤0.05*.

With the exception of B, the concentration of some trace elements including Cd, Cu, Fe, Ni, and Zn in the TMW treatment plots was significantly lower than that of controls (**Table 6.4**). Results indicated that the concentration of some trace elements including Cu, Fe, Ni, and Zn were significantly lower on TMW treatment in combination with vegetation type 3 compared to that of the control. The concentrations of Ni, Zn, Fe, and Cu were significantly decreased by 8%, 19% 22%, and 28%, respectively, following irrigation with TMW compared to the controls. This study indicates that in combination with vegetation types 2 and 3, the application of TMW significantly decreased the concentration of soil Cd compared to the control (**Table 6.4**). In combination with vegetation types 2 and 3, TMW application significantly reduced soil Cd by 24% and 49%, respectively, compared to the controls.

Table 6. 3 Total trace elements (mg/kg) of each vegetation type in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Values in brackets represent the standard error of the mean (n=20). Treatments share same letter do not significant at *p≤0.05*.

	Treatment	Vegetation type					
		1		2		3	
Cu	TMW	11	(0.4)[a]	10	(0.4)[a]	9	(0.2)[a]
	Control	11	(1)[a]	11	(0.4)[a]	12.3	(0.4)[b]
Cd	TMW	0.5	(0.1)[a]	0.45	(0.06)[a]	0.33	(0.2)[a]
	Control	0.5	(0.1)[a]	0.59	(0.02)[b]	0.7	(0.01)[b]
Fe	TMW	21954	(732)[a]	21189	(710)[a]	18799	(426)[a]
	Control	21434	(781)[a]	22598	(794)[a]	24152	(403)[b]
Pb	TMW	23	(2)[a]	23	(4)[a]	21	(2.3)[a]
	Control	23	(2)[a]	28	(4)[a]	22	(2)[a]
Ni	TMW	8	(1)[a]	7	(0.1)[a]	6	(0.2)[a]
	Control	7	(0.1)[a]	7	(0.1)[a]	7	(0.1)[b]
Mn	TMW	526	(25)[a]	593	(19)[a]	591	(37) [a]
	Control	584	(30)[a]	531	(25)[a]	568	(22) [a]
Zn	TMW	102	(21)[a]	91	(6)[a]	81	(5)[a]
	Control	90	(5)[a]	90	(5)[a]	99	(4)[b]

6.4 Discussion

6.4.1 Characteristics of TMW

The TMW used in this experiment contains essential nutrients for improving plant growth and soil fertility and productivity levels (**Table 6.5**). The TMW pH is 7.5, which is within the acceptable interval for agriculture irrigation, which ranges from 6.5 to 8.4 (FAO, 2018; Pescod et al., 1992).

The TMW used in this study contained low concentration of NO_3^--N when compared to other studies (**Table 6.5**) (Bedbabis et al., 2014; Mohammad Rusan et al., 2007; Parveen et al., 2013; Tarchouna et al., 2010). In contrast, the value of NH_4^+-N was far lower than wastewater used in the previous studies (Bedbabis et al., 2014; Parveen et al., 2013; Tarchouna et al., 2010). The concentrations of micronutrients and heavy metals in the wastewater were relatively low and meet the standards for wastewater reuse in irrigation (Pescod et al., 1992).

Table 6. 4 Characteristics of TMW used in the experiment versus characteristics of other municipal wastewater used in previous trials.

Properties	TMW	A	B	C	D	E	F	G	H
pH	7.5	7.3	7.85	7.19	-		7.135	7.6	6.5-8.40
NO_3^- (mg L^{-1})	18	30	20.7	-	-		1.5	15.9	-
NH_4^+ (mg L^{-1})	0.5		55.6	-	-		74	37.9	-
Na (mg L^{-1})	95		131	14	-		331.5	470	-
K (mg L^{-1})	22	333	39	0.3	-	3.28	23.5	38	-
Mg (mg L^{-1})	19		43	2.8	-	17.25	62	83.8	-
Ca (mg L^{-1})	59		324	3.1	-		117.5	95.8	-
P (mg L^{-1})	11	15.5	-	-	-	4.43	6.1	10.3	-
Pb (mg L^{-1})	<0.01	0.77	2.8	0.3	<0.01	-	-	<0.01	5.0
Cr (mg L^{-1})	<0.01	-	0.16		0.02	-	0.02	-	-
Cu (mg L^{-1})	0.04	0.01	6.1	0.2	0.06	-	0.01	-	0.2
Zn (mg L^{-1})	0.17	0.19	-	-	0.07	-	0.06	0.4	2.0
Mn (mg L^{-1})	0.06	0.07	-	-	0.12	-	0.03	0.5	0.2
Fe (mg L^{-1})	0.96	0.87	-	-	0.37	-	0.13	-	5.0
Cd (mg L^{-1})	<0.01	0.02	0.276	0.1	0.01	-	<0.01	<0.01	0.01

A (Mohammad Rusan et al., 2007), B (Tarchouna et al., 2010), C (Ali et al., 2011), D (Smith et al., 1996), E (Selahvarzi and Hosseini, 2012), F (Parveen et al., 2013), G (Bedbabis et al., 2014), H (Pescod et al., 1992).

Although the TMW in the present study contained essential nutrients for improving plant growth and rebuilding low fertility soil, NO_3^-, P, and S could potentially stimulate algal blooms, thus threatening fisheries and tourism industries in the long run. Since the SAR of TMW was above the threshold for crop irrigation purposes (FAO, 2018), use of TMW may end up affecting soil aggregate instability resulting in a breakdown in soil structure and consequent problems with infiltration, aeration, and drainage (FAO, 2018). Therefore maintaining soil quality by adding high alkaline material, such as gypsum, dolomite, or lime, is necessary (FAO, 2018). In addition, although trace elements in the TMW were within the threshold for wastewater reuse in agriculture irrigation (FAO, 2018), periodic monitoring of these elements is needed for long term used of TMW.

6.4.2 Survival rate

The survival rate of the plants was affected by TMW. The survival rate indicated that the 11 species tested in this research tolerated the application of TMW. The survival rate of species treated with TMW was between 60% - 90%, this was comparable with previous studies (Selahvarzi and Hosseini, 2012; Stewart and Flinn, 1984; Stewart et al., 1990). Stewart and Flinn (1984) found that southern mahogany (*Eucalyptus botryoides*), river red gum (*E. camadulensis*), southern blue gum (*E. globulus*), flooded gum (*E. grandis*), Sydney blue gum (*E. saligna*), swamp mahogany (*E. robusta*), yellow stringy bark (*E. muellerata*), spotted gum (*E. maculata*), river she oak (*Casuarina cunninghamiana*), slash pine (*Pinus elliottii*) and hoop pine (*Araucaria cunninghamii*) treated with 1130 – 1150mm of TMW per year during a 1 year treatment period, resulted in survival rates of between 59 – 93%. Selahvarzi and

Hosseini (2012) found that the seedlings of *Fraxinus excelsior* grown under irrigation of about 200 mL per day with TMW, resulted in a maximum survival rate of about 95%. In contrast, the survival rate of this study is lower than that reported by Kanekar et al. (1993). Their study had a 100% survival rate for *Acasia nilotica* and *Casuarina equisetifolia* watered with 2 L per week of treated wastewater. Kanekar et al. (1993) found that there was no significant difference in the survival rate of these species irrigated with treated wastewater compared to the control (tap water). Stewart et al. (1990) reported that during a 6-month experimental period, seven species (namely river red gum (*Eucalyptus camadulensis*), flooded gum (*E. grandis*), blue gum (*E. saligna*), river she-oak (*Casuarina cunninghamiana*), radiata pine (*Pinus radiata*), poplar clone 70/51 (*Populus deltoides*), and poplar clone 70/51 (*Poplar deltoides* x *P. nigra*) received 1171-1792mm per annum of secondary-treated municipal effluent resulting in a 83-100% survival rate, lower than this study.

6.4.3 Plant growth

Researchers posit that TMW has a stimulatory effect on the vegetative growth of trees through the provision of water, plant nutrients and organic matter, and improvement of the physical characteristics of soil, by enhancing cell elongation and division (Ali et al., 2012; Ali et al., 2011; Bhati and Singh, 2003; Gerhart et al., 2006; Guo and Sims, 2000; Guo et al., 2012; Hassan, 1996; Hopmans et al., 1990; Kanekar et al., 1993; Ogbonnaya and Kinako, 1993; Ostos et al., 2008; Selahvarzi and Hosseini, 2012; Singh and Bhati, 2005; Stewart et al., 1990). The authors stated that various species had different responses to irrigation with TMW. The height of seven species that were irrigated with municipal effluent for four years had significant differences (Hopmans et al., 1990). Treatment of *Eucalyptus grandis* with municipal effluent resulted in doubled tree growth rate when compared to *E. grandis* trees grown in a rainfed site for four years (Stewart and Flinn, 1984). The study of Ogbonnaya and Kinako (1993) suggested that the seedlings of *Eucalyptus globules* irrigated with sewage water had a greater growth rate than non-irrigated seedlings. Similar results were reported by Gerhart et al. (2006) who investigated the effects of irrigating with industrial saline wastewater on the growth of nine species (three desert legume trees, three xeric-adapted shrubs and three groundcovers). In this study, municipal effluent irrigation resulted in stimulation of tree growth and increased biomass production. In addition, Ostos et al. (2008) reported similar results with *Pistacia lentiscus*. My results are similar to that of Kanekar et al. (1993). Their study reported that there was a significant difference in plant height of *Casuarina equisetifolia* watered with treated wastewater compared to the control (tap water) treatment during a 5-month trial. Kanekar et al. (1993) found that the application of 2 L $week^{-1}$ of treated wastewater significantly increased the plant height of *Casuarina equisetifolia.* However, there was no significant difference in plant height between *Acasia nilotica* irrigated with treated wastewater compared to the control (Kanekar et al., 1993).

The greater growth production (canopy volume) of the plants irrigated with TMW may be due to sufficient availability of water and essential elements (Guo and Sims, 2000; Ostos et al., 2008). The previous studies attributed growth increase to organic matter and macro- and micronutrient concentrations in the wastewater applied (Guo and Sims, 2000; Ostos et al., 2008). Effluent contains considerable amounts of NO_3^-, PO_4^{3-} and K, which are considered essential nutrients for improving plant growth and soil fertility (Guo and Sims, 2000; Ostos et al., 2008; Selahvarzi and Hosseini, 2012). Irrigation with TMW increased of the growth and production of *F. excelsior* (Selahvarzi and Hosseini, 2012). A pot experiment conducted by Ali et al. (2011) to study the effect of primary and secondary sewage effluent treatments and tap water on the growth of seedlings of mahogany (*Switenia mahogani*) found that the effects of sewage effluent on growth parameters were more pronounced as water treatments were used for a long period. In addition, bald cypress (*Taxodium distichum*) seedlings which were planted in effluent marsh experienced greater basal diameter growth compared to those in the control (Lundberg et al., 2011).

Although *L. scoparium* and *K. robusta* species are naturally adapted to low fertility soil, their growth can be increased by adding an N-source. Adding TMW to soil significantly increased EC**,** thus altering the soil physical properties and stimulating soil microbial activity, particularly mycorrhiza, in soil and stimulated the growth of *L. scoparium* and *K. robusta,* presumably due to higher porosity of the soil compared to non-effluent treatment. This is in agreement with Smith et al. (2011), Haynes and Goh (1987) and Watson and Mardern (2004) who found that applying N-source biowaste (biosolids) resulted in higher porosity of the growth media, hence the increased root biomass of *K. robusta*. Moyersoen and Fitter (1999), Weijtmans et al. (2007), and Walbert et al. (2010) found that ectomycorrhizal were associated with the growth of *L. scoparium* and *K. robusta.* Arbuscular mycorrhiza played an important role in promoting growth following the application of biosolids and sawdust mixture (Smith et al., 2011; Whiteside et al., 2012). It is supported by Moyersoen and Fitter (1999) and Weijtmans et al. (2007) who found that both ectomycorrhizal and arbuscular mycorrhiza colonisation were observed in *K. robusta* and *L. scoparium*.

6.4.4 Nutrient accumulation

The nutrient concentrations of the eleven species tested reflect differences in biomass accumulation and, to a greater degree, differences in nutrient concentrations in the leaves. Differences between irrigated and non-irrigated plots in accumulation of N, P, K, S, Mg, and Na in the above ground biomass of certain NZ native species were significant. The data showed that high rates of canopy volume are not necessarily associated with large accumulations of nutrients. *L. scoparium* and *K. robusta*, for example, ranked eighth and fourth out of eleven species for canopy volume, yet accumulated more

N, K and Ca than any of the other species, presumably because of their extensive root system. Utilization of TMW increased foliar N, P K, and S and at the same time decreased foliar Mg and Mn concentration of some species tested in the present study. This study found that different species had different responses to the application of TMW in accumulating specific elements. The results showed that some species irrigated with TMW, took up significantly higher amounts of macronutrients but accumulated significantly lower amounts of micronutrients. For instance, *L. scoparium* and *K. robusta* irrigated with TMW, accumulated significantly higher S than those grown on non-irrigated plots. However, these species accumulated significantly lower Fe and Mn respectively compared to those of the control.

The increase of N, P, K, S, Mg, Na, Fe and Mn in plant parts might be attributed to an increase in the occupancy root zone by applying TMW that reflected on their uptake by roots. The results of this study agree with previous findings (Alghobar and Suresha, 2017; Ali et al., 2011; Balkhair and Ashraf, 2016; Minogue et al., 2012; Mohammad and Ayadi, 2004; Parveen et al., 2013; Parveen et al., 2014; Singh and Bhati, 2005; Singh and Agrawal, 2010; Walia and Goyal, 2010). Singh and Bhati (2005) and Ali et al. (2011) found that concentrations of N, P, K, Mg, Cu, Fe, Mn, and Zn were greater in seedlings of *Dalbergia sissoo* and *Swietenia mahogani* which were irrigated with municipal effluent than the non-irrigated treatment. Similar results were reported by Minogue et al. (2012) who found that the two-year application of tertiary treated wastewater containing 2.73mg L^{-1} NO_3^- - N and 0.30 mg L^{-1} total P increased the total foliage N and P by 44% and 36%, respectively of *Populus deltoids.* The nutrient concentrations of the above-ground biomass of the 11 NZ species which were irrigated with TMW, was comparable to other species irrigated with similar kinds of effluent (Minogue et al., 2012; Parveen et al., 2013; Varkey et al., 2015). For *L. scoparium* and *K. robusta* in particular, adding TMW significantly increased the accumulation of foliar N, P, K, and S in *L. scoparium*, whereas *K. robusta* uptake foliar N, K, S, and Mg. This was presumably due to the variation in exudate composition between species which influenced the capability to transform nutrients into bio-available form (Esperschuetz et al., 2017; Walker et al., 2003). As exudate composition varies greatly between plant species (Walker et al., 2003), this can lead to contrasting plant responses in terms of nutrient and contaminant uptake, and may explain the differences in nutrient concentration increases observed between plant species in this study.

6.4.5 Effects on soils

Soil pH increased by at least 0.5 units from around 5.2 to 5.7 as a result of irrigation with TMW. Alteration of soil pH under irrigation with TMW was previously reported by several authors (Ghosh et al., 2012; Mancino and Pepper, 1992; Singh et al., 2012; Varkey et al., 2015). Mancino and Pepper

(1992) found, for instance, that compared with irrigation with drinking water, irrigation with TMW raised the soil pH under Bermuda grass *(Cynodon dactylon* L.) by 0.1 to 0.2 units over the 3-year experimental period. They attributed such a pH rise to (i), the high content of basic cations such as Na^{+}, Ca^{2+} and Mg^{2+} of the TMW, which raised the alkaline reserve of the soil, and (ii), an increased rate of denitrification that produced hydroxyl ions. Whereas Singh et al. (2012) and (Varkey et al., 2015) reported that the soil pH under vegetables, cotton, maize, sugarcane, pulses vegetation decreased by one to half unit after the application of domestic sewage water over four decades. Singh et al. (2012) reported that soil pH decreased from 7.9 to the range of 7.52–7.63 under several wheat plants irrigated with domestic wastewater. Irrigating the soils with 500 mm of TMW resulted in higher EC throughout the experimental plots. This finding was in agreement with previous work by (Morugán-Coronado et al., 2011; Saffari and Saffari, 2013), that the application of sewage water would be expected to increase soil EC. Morugán-Coronado et al. (2011) found that the application of treated waste water to grapes (*Vitis labrusca*), increased the EC after 2-years.

The results in this present study show a highly significant increase in the concentration of C and N in soils treated with TMW, compared to non-TMW. This is due to the TMW containing high concentrations of total N and C. This finding, especially total C, agree with Varkey et al. (2015) who reported that there was an increase of one-and-a-half to two times in organic C content, available N, P, K and S, in the sewage-irrigated soils compared to soils not irrigated with sewage. In contrast, Azouzi et al. (2015) found that the average percentage of total organic C in isohumic soil which was irrigated by TMW (1.07%) was lower than in control soil (1.34%). Another study found no changes to total C and N after 2 years of application of treated wastewater to the soil (Mohammad Rusan et al., 2007).

Irrigating the soils with TMW significantly affected the concentrations of both macro and micronutrients in soils. In combination with flax-dominant species (vegetation type 3) and *L.* scoparium/*K. robusta* (vegetation type 1)*,* adding TMW significantly elevated the total soil P and Ca, respectively. In combination with *L. scoparium*/*K. robusta* (vegetation type 1) and *Olearia*-dominant species (vegetation type 2), TMW application increased Na concentrations in rhizosphere soil. On the other hand, the concentration of P in the soil significantly declined, by approximately 40%, on *Olearia*-dominant species (vegetation type 2) plots. A similar trend can be seen on the concentration of soil Ca under the combination of TMW treatment and flax-dominant species (vegetation type 3). The study found that adding TMW to soil significantly increased the concentration of soil K on vegetation type 2 plots, soil S on vegetation type 1 and 2 plots, and soil Mg on vegetation type 3 plots. The present study suggested that flax-dominant species (vegetation type 3) successfully reduced the concentration level of Cd, Cu, Fe, Ni, and Zn in the soil. Particularly related to soil salinity, although in combination with

all vegetation types, the application of 500 mm of TMW increased the level of EC of soil, ranging between 0.23 and 0.27 dS/m, still within the range of permissible limit for crops and trees (<0.7 dS/m) (FAO, 2018).

Table 6. 5 Summary of the concentrations of macro and micro elements of each vegetation type in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017).

Element	Veg type 1	Veg type 2	Veg type 3
P	NS	decreased	increased
K	NS	increased	NS
Mg	NS	NS	increased
S	increased	increased	NS
Ca	increased	NS	decreased
Na	increased	increased	decreased
B	NS	increased	increased
Cd	NS	decreased	decreased
Cu	NS	NS	decreased
Fe	NS	NS	decreased
Ni	NS	NS	decreased
Zn	NS	NS	decreased

NS = not significant different ($p \leq 0.05$)

6.5 Conclusions

The results of this 18-month study showed that *L. scoparium*, *K. robusta*, *O. paniculata*, *C. robusta*, *P. cunninghamii*, *G. littoralis*, *P. arboreus*, *P. tenax*, *P. cookianum*, *C. australis*, and *P. eugenoides* responded positively to the application of TMW. There were positive effects of the irrigation with TMW on plant growth parameters. Plant survival and canopy volume were significantly affected by TMW treatment. Plant survival rate was more than 60% after 18 months of TMW irrigation. In number, the survival rate of plants irrigated with TMW was higher than that of the plants in the control, but there was statistically non-significance between each of the species tested. The application of TMW significantly increased the canopy volume of eight species, but not *P. cunninghamii*, *G. littoralis*, *P. eugenoides*. Also, adding TMW to soil increased foliar N, P K, Na, S, and Fe, whereas foliar Mg and Mn of certain species decreased. This study found that different species had different responses to the application of TMW in accumulating specific elements.

Soil parameters were significantly affected by TMW irrigation. TMW irrigation improved chemical properties and fertility status of soils by elevating the concentrations of total C and N, EC, and pH. Total P and Na were higher under flax-dominant species (vegetation type 3), *L. scoparium* and *K. robusta* species (vegetation type 1), and under both *L. scoparium* and *K. robusta* and Olearia-dominant

species (vegetation type 1 and 2) respectively. Amending soil with TMW significantly increased the concentration of soil K concentration on Olearia-dominant species (vegetation type 2) plots, soil S concentration on *L. scoparium*/*K. robusta* and Olearia-dominant species (vegetation type 1 and 2) plots, and soil Mg concentration on flax-dominant species (vegetation type 3) plots. In contrast, the concentration of these macro elements in the soil was lower on *Olearia*-dominant species (vegetation type 2) plots as well as the concentration of soil Ca and Na concentration on flax-dominant species (vegetation type 3) plots. This study indicates that flax dominant species (vegetation type 3) successfully reduced the concentrations level of soil Cd, Cu, Fe, Ni, and Zn.

Chapter 7

The response of *Leptospermum scoparium* and *Kunzea serotina* to compost and mixed of sawdust and dairy shed effluent (Eyrewell field trial)

7.1 Introduction

7.1.1 Background

The former Eyrewell forest comprised a large area of land (6,764 ha) which was planted as production pine forest in the early 1930's, mainly *P. radiata* (Wilson, 2014). In 2000,purchased the Eyrewell Forest (Te Whenua Hou) and have converted the land to predominately irrigated dairy pasture (Wilson, 2014). A collaboration between Lincoln University, New Zealand and Ngai Tahu was established to reduce the environmental impact of dairy conversion. Therefore, approximately 150 hectares is already set-aside for Biodiversity and Restoration Program, which was aimed to protect and expand vegetation remnants within the farms and enhance the future trajectory of the ecological restoration (Dollery, 2017). This project provides a template for establishment, monitoring and enhancement of native habitats, focusing on the ecological and environmental benefits of restoration planting. Given the existing low fertile soil of the site, with a varying mixture of gravels with finer stones (65-85%), sands, and silts intimately mixed and low N (**Table 6.2**) and organic C ranged from 2.7 – 3.4% on 0 – 20 cm depth (Cameron et al., 1994), this particular biodiversity restoration program requires judicious species selection. Factor such as the ability of species to adapt the existing site condition including poor soil quality, must be carefully considered. Hence, using indigenous species of New Zealand, which were previously grown in this region is highly recommended for this specific purpose. New Zealand's native plant species such as *L. scoparium* and *K. serotina* to deal with this specific issue not only beneficial to the environmental but could add economic value to the land through the production of honey or essential oils (Ronghua et al., 1984; Stephens et al., 2005).

7.1.2 Rationale of study

Historically, before converted into production pine forest, former Eyrewell forest was relatively unproductive due to the dry soils and mainly used for sheep farming. They are contained approximately 6-25% New Zealand native species of kānuka (*Kunzea robusta* and *K. serotina*), with additional 1-5% of up to 30ft tall of mānuka (*Leptospermum scoparium*) species (McGlone et al., 2001;

Meurk et al., 1995; Wendelken, 1966). These species were found associated with an understory of prickly mingmingi (*Leptecophylla juniperina)* (Wardle, 1991).

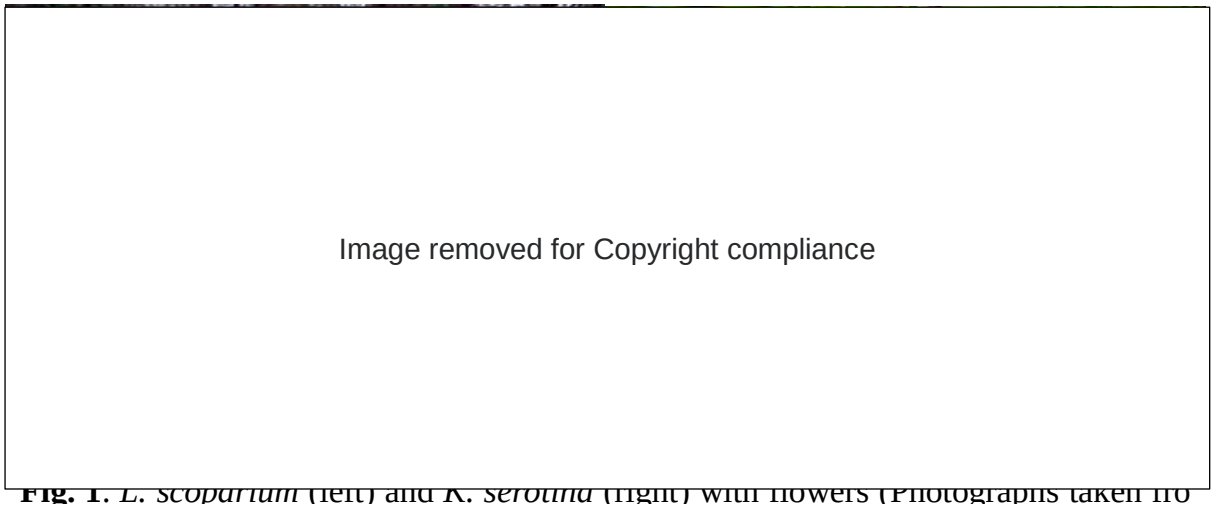


Fig. 1. *L. scoparium* (left) and *K. serotina* (right) with flowers (Photographs taken fro

Plate 7. 1 *L. scoparium* (left) and *K. serotina* (right) with flowers (Photographs taken from: http://www.bushmansfriend.co.nz/xurl/PageID/9165/ArticleID/-14073/function/moreinfo/content.html).

Both species are known as fast growing species, preferring drier, free draining soils, and commonly found in degraded environments and low fertility soil in New Zealand (Burrows, 1973; Stephens et al., 2005). In particular *Kunzea serotina*, referred to as plains kānuka, has been found in areas of stony soils that are frost-prone from 30-2000 m a.s.l. North and South Islands, from the central volcanic plateau in the north to central Otago in the south are the main habitat of this species (Dollery, 2017). Hence, these two New Zealand native species had been the appropriate species to be planted in this specific restoration areas.

Previous experiments (**Chapter 3 - 6**) of this thesis found that *L. scoparium* and *K. robusta* gave positive response in combination with biowastes on low fertility soil. The results showed that amending low fertility soil with biosolids and dairy shed effluent improved the growth and increased the uptake of certain essential nutrients and contaminants associated with biowastes (NCAB) below threshold concentration level of both *L. scoparium* and *K. robusta*. Several authors found that adding fresh sawdust only into the soil did not significantly affect plant growth (Bugbee, 1999b; Dania et al., 2012; Shaheen et al., 2017). Dania et al. (2012) reported that amending sawdust into soils did not significantly affect the plant height of maize (*Zea mays*). Similar findings were reported by who discovered that the application of fresh sawdust (contains equal to 100 kg N ha^{-1}) did not significantly affect the growth parameters (plant height and shoot dry weight) of soybean (*Glycine max*). This suggests that the sawdust reduced the availability of some nutrients (Bugbee, 1999b)

Sawdust and compost are inexpensive and readily available in the Canterbury region, New Zealand. The timber industry produces large volumes of wood waste, including sawdust, which is often inappropriately disposed of in wood waste piles (Robinson et al., 2007; Tao et al., 2005). However, adding such biowastes to soil may have negative consequences. Sawdust, for instance, may inhibit plant growth by immobilising plant-available nitrogen (Brady, 2008) and releasing phytotoxic tannins (Davey, 1953). .

Therefore, I hypothesized that although *L. scoparium* and *K. serotina* are pioneer species and can tolerate poor environments condition such as low fertility soil, adding sawdust and DSE mixture (SD+DSE) and compost will enhance the soil quality and nutrients uptake, thereby increased growth. I also hypothesized that amending soil with such biowastes will lead to increased concentrations of nutrients and contaminants associated with biowastes (NCAB) in both the aerial portions and soil.

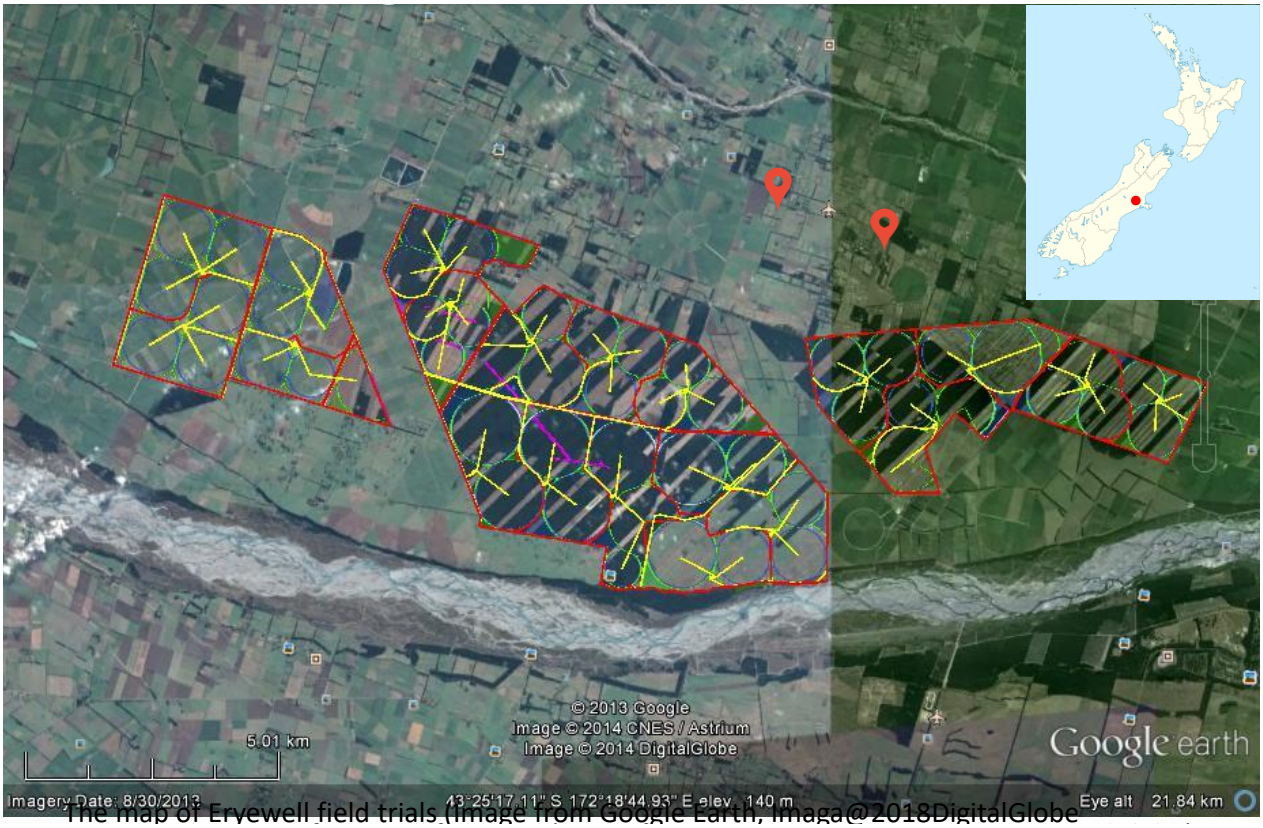
7.1.3 Aims

The aim of this research was to investigate the growth, nutrients uptake, and soil quality following the application of SD+DSE and compost on to low fertility soil in combination with *L. scoparium* and *K. serotina*.

7.2 Methods

7.2.1 Site description

The experimental plots (**Plate 7.2)** are in former Eyrewell State Forest, Canterbury Plains, which are the largest alluvial plains in New Zealand, consisting of a series of gently sloping fans built up by four major rivers (Molloy, 1988). They are approximately 60 km north of Christchurch (43^0 43’21.04” S, 172^0 33’39.46”E, about 158 m above sea level). The climate of the region is dry with a prevalence of strong north-westerly föhn winds, warm summers, cool winters and low rainfall (800 mm yr^{-1}) leading to low humidity and high evapotranspiration rates (Dollery, 2017).



The map of Eryewell field trials (image from Google Earth, Imaga@2018DigitalGlobe

Plate 7. 2 The map of Eyrewell field trials (Image from Google Earth, Imaga@2018DigitalGlobe

The soil is a Lismore soil (Pallic Firm Brown Soils, Hewitt 1998) developed from alluvium which is one of the most fertile, agriculturally important soils in the Canterbury region, covering 10% of the intermediate terraces on the plains (Molloy, 1988). The soils are yellow-grey earths, mostly classified as Lismore stony silt-loam derived from Greywacke gravels and thin loess deposits.

7.2.2 Experimental set up

Experimental design

The field trial consisted of small and large restoration plot (**Figure 7.1**). The mall restoration contained six 6 x 9 m experimental plots, whereas the large restoration plot consisted of twelve 6 x 9 m experimental plots. The treatments were assigned to give a randomized block design with three replications of each compost and mixture of SD+DSE treatment. Each experimental plot contained 54 plants with 1 m distance between plants.

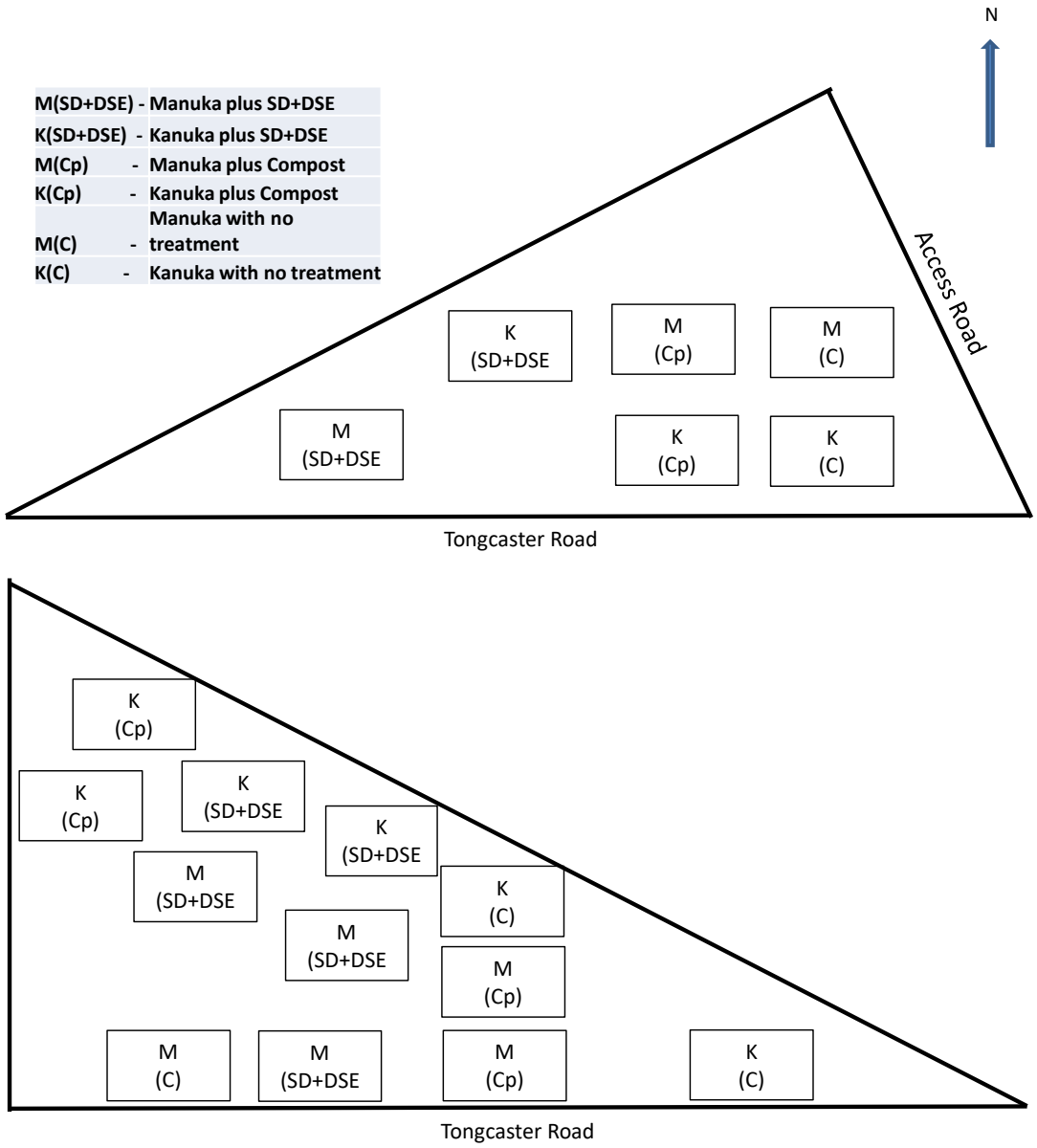


Figure 7. 1 Plots design of Eyrewell field trial

Species and the timing of the planting

One-year-old seedlings were transplanted in August 2014. To protect from herbivores, plant guards were installed on each single plant (**Plate 7.3**). Seedlings of *L. scoparium* and *K. serotina* and plant guards were sourced from Native Solution Ltd., P.O. Box 631, and Rangiora North Canterbury 7400.

Plate 7. 3 Plant guards to protect plants from herbivores at Eyrewell field trial

Treatment and treatment application

The trial involved the application of SD+DSE and compost treatments. They were applied by spreading on entire plot to a depth of 10 cm (**Plate 7.4**). The SD+DSE application rate was the equivalent of 138 t ha^{-1} dry weight, providing 1200 kg N ha^{-1} and the equivalent of 120 t ha^{-1} dry weight, which contains 2400 kg N ha^{-1} equivalent of compost. DSE was mixed with sawdust by simply spraying over the pile of sawdust (done by Ngāi Tahu). **Table 7.1** shows the detail of treatment applied on Eyrewell field trial.

a b

Plate 7. 4 SD+DSE (a) and compost (b) application of Eyrewell field trial

Table 7. 1 Treatment application details of Eyrewell trial

Plant	Reserve	Treatment	Rep	Date Treatment added
L. scoparium/K. serotina	Small	Control	2	NA
L. scoparium/K. serotina	Small	SD+DSE	2	24.03.16
L. scoparium/K. serotina	Small	Compost	2	24.03.16
L. scoparium/K. serotina	Large	Control	4	NA
L. scoparium/K. serotina	Large	SD+DSE	4	17.07.16
L. scoparium/K. serotina	Large	Compost	4	03.06.16

In the small reserve, three plots of *L. scoparium* and three plots of *K. serotina* (**Figure 7.3**). While the large reserve contains six *L. scoparium* and six *K. serotina* plots. Four *L. scoparium* plots and four *K. serotina* plots received SD+DSE and compost treatment respectively, whereas two *L. scoparium* plots and two *K. serotina* plots received neither a mixture of SD+DSE nor compost (control). In brief, the design therefore provided three replicates of each treatment (**Table 7.1**).

Table 7.2 shows mass plant macro and micro-nutrients added through compost and SD+DSE treatments.

Table 7. 2 Mass plant macro-nutrients added through compost and SD+DSE treatments.

	Mass added (kg ha^{-1})	
	Compost	**SD+DSE**
N	2,400	1,200
P	480	270
K	810	820
S	320	130
Ca	3,000	690
Mg	640	270

Sawdust was sourced Calving sheds of Ngai Tahu Farms. It was brought in as untreated wood chips by Ngai Tahu Farms. Compost was collected from Selwyn District Council municipal green waste compost. There was no additional watering of the plants throughout the growth period. The chemical properties of soil, SD+DSE, and compost used in this field trial are listed in **Table 7.3.**

Table 7. 3 Concentration of nutrients, trace elements and contaminants in soils, S+DSE, and compost used in the present study. Values represent the mean (*n* = 5). Values in parentheses are the standard error.

Properties	Soil		S+DSE		compost	
C/N ratio	25.3	(0.3)	40.4	(1.2)	11.9	(0.6)
C [%]	4.3	(0.4)	38.1	(0.8)	23.5	(1.8)
N [%]	0.17	(0.02)	0.9	(0.0)	2.0	(0.1)
P [%]	0.05	(0.00)	0.2	0.01	0.3	(0.0)
K [%]	0.2	(0.01)	0.6	(0.2)	0.5	(0.1)
S [%]	0.03	(0.00)	0.1	(0.0)	0.2	(0.0)
Ca [%]	0.2	(0.01)	0.5	(0.0)	1.9	(0.1)
Mg [%]	0.3	(0.00)	0.2	(0.0)	0.4	(0.0)
B [mg kg^{-1}]	5.0	(0.3)	7.9	(0.3)	36.5	(3.8)
Cu [mg kg^{-1}]	4.1	(0.2)	6.9	(0.4)	25.3	(0.6)
Zn [mg kg^{-1}]	72	(1.5)	51.5	(1.7)	134.6	(5.9)
Mn [mg kg^{-1}]	265	(15)	151.1	(15.5)	347.6	(10.1)
Fe [mg kg^{-1}]	21121	(291)	3083	(215)	7315	(2474)
Cd [mg kg^{-1}]	0.2	(0.01)	0.02	(0.0)	0.6	(1.6)

Plant measurement, sample collection/preparation, and analysis

After 12 months of treatment application (end of summer, in March 2017), five plants from every plot were harvested. The aboveground portions were excised and kept in labelled paper envelopes for biomass and total element analysis. Fresh weight was recorded before oven drying at ± 70^{o}C for at least one week or until a constant weight was achieved; the dry weight was measured. Dried leaves of *L. scoparium* and *K. serotina* were then separated from branches, ground using a Retch ZM200 grinder (**Plate 7.5**), and stored in the sealed plastic bag for further analysis. For total N, 0.1920-0220g of ground sample was weighed into crucibles before running N total analysis using Rapid Max-N Exceed (EAS REGAINER® technology).

Rhizosphere soil samples from each selected plant were collected, sieved using 2 mm nylon sieve, stored in seal plastic bag (**Plate 7.6)**, and then kept in the fridge for further analysis of soil pH, EC and mineral N and total elements.

Plate 7. 5 Dried leaves of *L. scoparium* (a) and *K. serotina* (b) separated from branches and grinder Retch ZM200 for grinding the samples (c, d)

10 g of soil and 25 mL of deionised water (18.2 MΩ resistivity; Heal Force® SMART Series, SPW Ultra-pure Water system, Model-PWUV) Soil pH was determined using at a soil and water ratio of 1:2.5.

The mixture was then shaken for an hour and left to equilibrate for 24h before measurement. Each mixture was shaken before detecting soil pH using a pH meter (Mettler Toledo Seven Easy) (Blakemore et al., 1987). Total C and N were detected by an Elementar Vario-Max CN Elementar analyser (Elementar ®, Germany) using 0.5g of oven-dried soil samples was used to analyse the total carbon and nitrogen content in the soil and plant samples. Whereas mineral N (NH_4^+-N and NO_3^--N) were determined using 2M KCL extraction method using 4.0g fresh soil (Blakemore et al., 1987). The analysis was carried out by mixing 4.0g of fresh soil and 40 mL of a 2M KCl reagent. The solution was then shaken on an end-over-end shaker for 1h, centrifuged at 2000 rpm for 10 min and subsequently filtered through Whatman 41 filter paper. Extracted solutions were kept at -20°C until analysed. Ammonium-N (NH_4^+-N) and nitrate-N (NO_3^--N) were determined using a flow injection analyser (FIA FS3000 twin channel analyser, Alpkem, USA).

For total element, soil and plant samples were digested using a microwave digester (The CEM MARS Xpress - CEM Corporation, Matthew, PO Box 200 North Carolina, 28106-0200, USA) of 0.2 g of sample in 8 mL of AristarTM nitric acid (± 69%) and filtered by means of Whatman no. 52 filter paper (pore size 7 µm) after dilution with milliQ water to a volume of 10 mL. Certified Reference Materials (CRMs) for soil (International Soil analytical Exchange - ISE 921) and plant samples (International Plant analytical Exchange IPE 100) from Wageningen University, The Netherlands, were digested.

Total foliar and soil P, K, S, Mg, Ca, Mn, and Cd were then determined using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES Varian 720 ES - The Varian 720 ICP-OES - Varian Australia Pty Ltd, 679 Springvale Road, Melbourne in soils (Kovács et al., 2000) and (Simmler et al., 2013; Valentinuzzi et al., 2015). Extraction and digestion solution and method blanks were analysed in triplicate as part of standard quality control procedure for the analysis and were as below the ICP-OES's detection limit (<0.1 mg/kg) for all metals. Recoverable concentrations of the CRMs were within 93% - 110% of the certified values.

Plate 7. 6 Harvesting rhizosphere soil from each selected plant samples of Eyrewell field trial

7.2.3 Statistical analysis

An analysis of variance (ANOVA) was carried out to determine the treatment effects on the measured parameters. Duncan post-hoc test at P≤0.05 was employed when the treatment effect was found to be significant. Statistical analysis of the data was conducted using standard analysis of variance procedures using SPSS IBM SPSS v.**22** (International Business Machines Corp., New Orchard Road, Armonk, New York 10504 914-499-1900).

7.3 Results

7.3.1 Plant survival

Table 7.4 shows the effect of application of SD+DSE and compost on survival rate (%) of plants after 12-month trial period.

Table 7. 4 Effect of application of SD+DSE and compost on survival rate of plants (%). Values in parentheses represent the standard error of the average survival rate of each species throughout the experiment (*n* = 4). Values with the same letter are not significantly different.

Species	Treatment		
	Compost	SD+DSE	Control
L. scoparium	97 (10) [a]	88 (12) [a]	88 (2) [a]
K. serotina	88 (12) [a]	90 (5) [a]	87 (2) [a]

† Different lowercase letters indicate significant differences between treatments at $p \leq 0.05$, using One-Way ANOVA followed by Duncan Post Hoc Tests

Table 7.4 shows that adding mixture of SD+DSE and compost on to the soils did not significantly (*p>0.05*) affect the survival rate of either *L. scoparium* or *K. serotina* during the 12-month experimental period. **Table 7.4** indicates that the highest mortality rate occurred for *K. serotina* in the compost treatment plots (12%) which is similar to that of the control plots (13%). Similarly, 12% of *L. scoparium* in the SD+ DSE died after 12-month of experimental period. The same mortality rate (12%) of *L. scoparium* occurred in control plots. Just 3% of *L. scoparium* on compost treatment plots died after 12-month of treatment application.

7.3.2 Vegetative growth

The application of SD+DSE and compost did not positively affect the plant height of *L. scoparium* and *K. serotina* compared to the control. **Figure 7.2** shows that unlike *L. scoparium*, *K. serotina* responded positively to the application of SD+DSE by producing significantly ($p < 0.05$) higher above ground dried biomass. Compared to the control, in combination with *K. serotina,* the application of SD+DSE increased the dried biomass by 82% (up to 187.5 g per plant, equivalent to 38 t ha^{-1}). Adding compost did not significantly ($p > 0.05$) affect the shoot development of *K. serotina*.

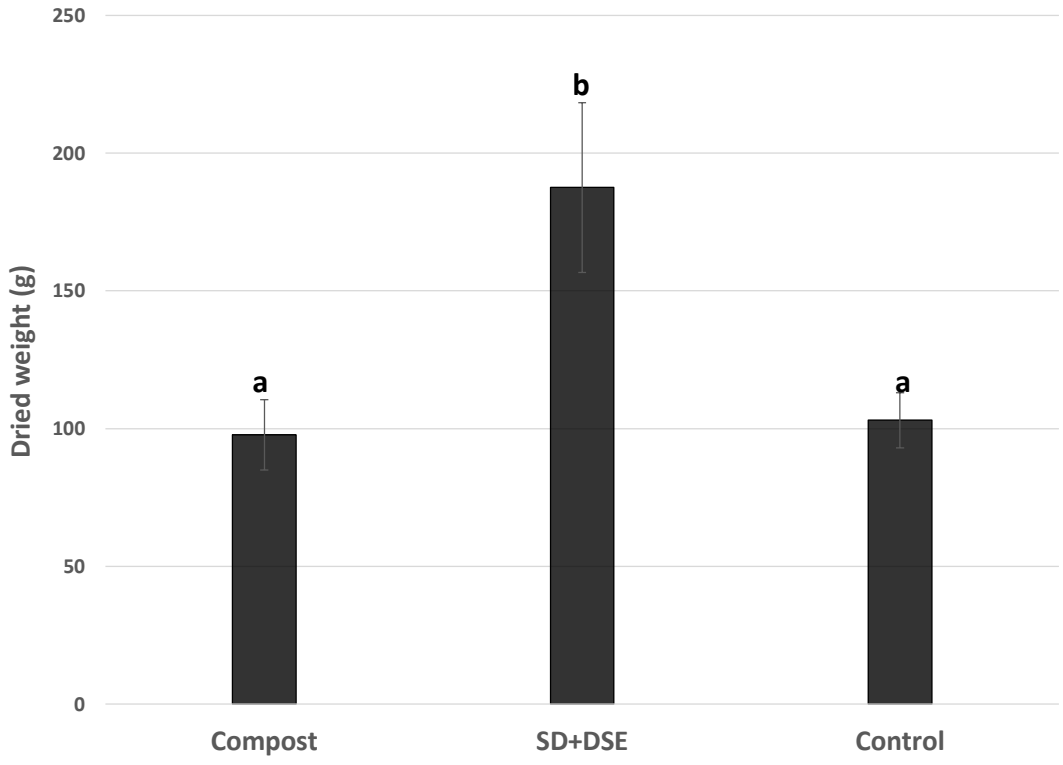


Figure 7. 2 Above ground dried weight (g) of *K. serotina* in response to Sawdust plus Dairy Shed Effluent (SD+DSE) and Compost treatment. Error bars represent the standard error of the mean (n=15, 15 and 10). Bars with the same letters are not significantly different (*p≤0.05*).

7.3.3 Effect of treatments on the nutrient uptake

Macronutrients

Figure 7.3 shows the total concentrations of the foliar macronutrients of *L. scoparium* and *K. serotina* measured at the end of experimental period. Both *L. scoparium* and *K. serotina* accumulated significantly (*p≤0.05*) higher concentrations of foliar N than the control (**Figure 7.3A)**. However, **Figure 7.3A)** indicates that there was no significant (*p>0.05*) difference in foliar N concentration between SD+DSE and compost treatments for both species. In the compost treatment, the concentration of N in the leaves of both *L. scoparium* and *K. serotina* increased by 22% and 47%, respectively, whereas amending SD+DSE increased the concentration of foliar N of *L. scoparium* and *K. serotina* species by 25% and 37% respectively (**Figure 7.3A**).

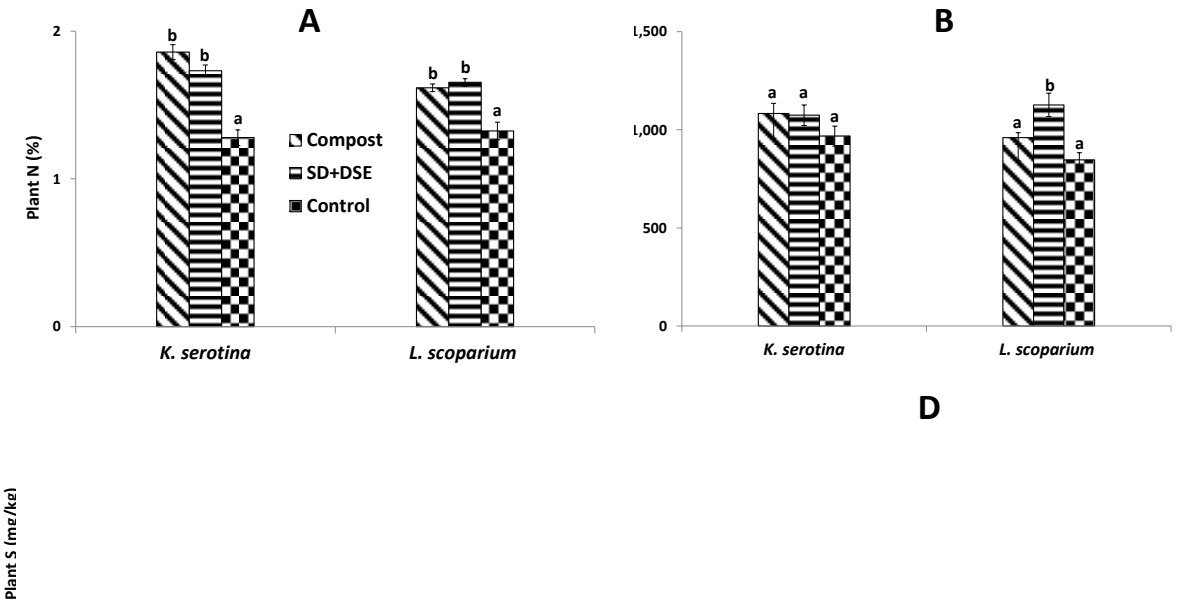


Figure 7. 3 Total concentration of foliar N, P, K, and S (%) of *K. serotina* and *L. scoparium* in response to SD+DSE and Compost treatment. Error bars represent the standard error of the mean (n=15, 10, and 14). Bars with the same letters are not significantly different (*p≤0.05*).

The results indicate that the addition of mixture SD+DSE and compost significantly (*p≤0.05*) increased the concentration of foliar P, K, and S of *L. scoparium* compared to that of in the control treatment (**Figures 7.3B - 7.3D**). On the other hand, compared to the control, *K. serotina* accumulated significantly (*p≤0.05*) higher foliar concentrations of only on K and S in response of both SD+DSE and compost treatment (**Figures 7.3C** and **7.3D**). *L. scoparium* took up significantly more P in the SD+DSE treatments. In contrast, amending compost did not significantly (*p>0.05*) alter the concentration of foliar P of this species (**Figure 7.3B**). Adding SD+DSE and compost on to the soils significantly (*p≤0.05*) increased the concentration of foliar K on *K. serotina*. Compared to the control, the application of SD+DSE and compost increased the concentration of foliar K of *K. serotina* by 8% and 19%, respectively**.** In contrast, *L. scoparium* responded differently to the application of biowastes in related to the uptake of *K. serotina*. The amending of both SD+DSE and compost significantly (*p≤0.05*) lowered the concentration of foliar K of *L. scoparium* by 18% (**Figure 7.3C**). Compared to the control, the application SD+DSE resulted in significantly (*p≤0.05*) higher accumulation of foliar S of *L. scoparium*, but not in the compost treatment (**Figure 7.3D**). In contrast**,** compared to the control, *K. serotina* took up significantly *p≤0.05*) higher S in the compost treatment, but not in the SD+DSE plots. Adding SD+DSE increased the concentration of foliar S of *L. scoparium* by 13%**,** whereas, amending compost elevated the level concentration of foliar S of *K. serotina* by 15%.

7.1.1.1. Micronutrients

Figure 6.5 shows the total concentration of foliar Mn and Zn (mg/kg) of *K. serotina* and *L. scoparium* in response to SD+DSE and Compost treatment.

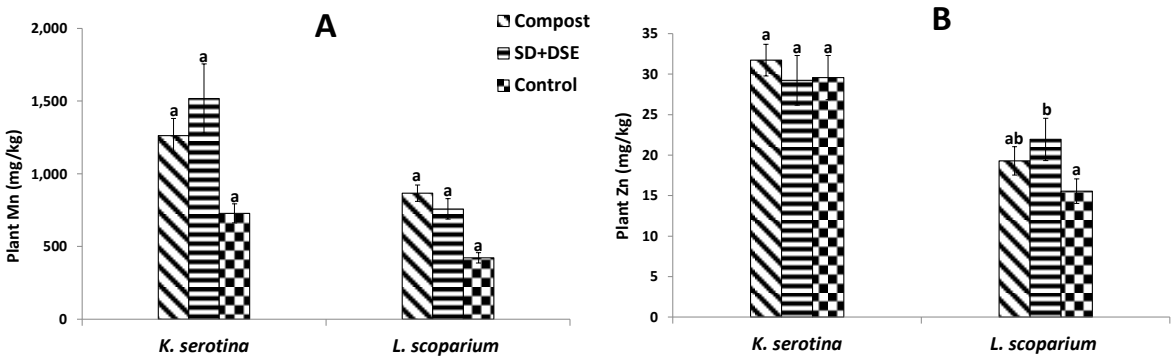


Figure 7. 4 Total concentration of foliar Mn and Zn (mg/kg) of *K. serotina* and *L. scoparium* in response to SD+DSE and Compost treatment. Error bars represent the standard error of the mean (n=15, 10 and 14). Bars with the same letters are not significantly different (*p≤0.05*).

In both SD+DSE and compost treatments, *L. scoparium* accumulated significantly (*p≤0.05*) higher foliar concentrations of Mn and Zn than in the control (**Figure 7.4**). The addition SD+DSE and compost has elevated the concentration of foliar Mn of *L. scoparium* by 80% and 106%, respectively. In contrast, *K. serotina* accumulated significantly (*p≤0.05*) higher concentration of Mn only (**Figure 7.4A**). Similar to *L. scoparium*, *K. serotina* uptake significantly higher foliar Mn in both the SD+DSE and compost treatments by 108% and 74%, respectively.

7.3.4 Element concentrations in rhizosphere soil

Total C, pH, and EC

The application of SD+DSE and compost did not significantly (P > 0.05) increase total soil C in the underlying soil compared to the control in both the *L. scoparium* and *K. serotina* plots (**Table 7.5**). Results indicate that in combination with *L. scoparium* and *K. serotina,* SD+DSE and compost application did not give significant effect to the concentration level of C in the rhizosphere soil (underlying soil). Adding both SD+DSE and compost significantly reduced the EC of rhizosphere soil under *L. scoparium* and *K. serotina* (**Table 7.5**). In combination with *L. scoparium,* compost application significantly increased the pH of rhizosphere soil, where the pH was significantly reduced in combination with *K. serotina* following the application of both SD+DSE and compost treatment.

Table 7. 5 Total C, pH, and EC of soil) of *L. scoparium and K. serotina* in response to SD+DSE and Compost treatment. Values in bracket represent Standard error of mean.

	L. scoparium			*K. serotina*		
	SD+DSE	**Compost**	**Control**	**SD+DSE**	**Compost**	**Control**
C [%]	4.7 (0.5)[a]	5.9 (0.7)[a]	4.6 (0.5[a]	4.9 (0.4)[a]	4.0 (0.2)[a]	4.1 (0.2)[a]
pH	4.6 (0.1)[b]	4.3 (0.1)[a]	4.3 (0.1)[a]	4.5 (0.0)[ab]	4.3 (0.0)[a]	4.6 (0.1)[b]
EC [dS/cm]	159 (12)[a]	173 (17)[a]	234 (20)[b]	123 (7)[a]	127 (6)[a]	164 (25)[b]

† Different lowercase letters indicate significant differences between treatments at $p \leq 0.05$, using One-Way ANOVA followed by Duncan Post Hoc Tests

Ammonium and nitrate

Results show that after 18 months of treatment applications the NH_4^+ - N concentrations exhibited significant (P<0.05) differences on both *L. scoparium* and *K. serotina* plots. After 18-month experimental period, the highest amount of NH_4^+ - N was found in the compost treated soils compared to the control (**Figure 7.5A**). Adding SD+DSE did not significantly (P>0.05) effect the concentration of NH_4^+-N in underlying soil of both *L. scoparium* and *K. serotina* (**Figure 7.5A**).

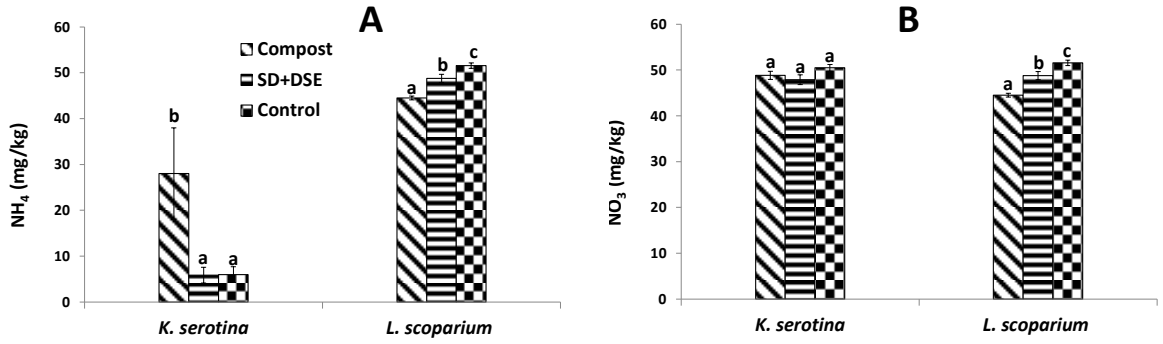


Figure 7. 5 Concentration of NH_4^+-N and of NO_3^--N (mg/kg) of (A) *L. scoparium* and (B) *K. serotina* in response to SD+DSE and Compost treatment. Error bars represent the standard error of the mean (n=14, 10 and 15). Bars with the same letters are not significantly different (*p≤0.05*).

The results indicate that in combination with *L. scoparium*, the application of both SD+DSE and compost significantly (P<0.05) decreased the NO_3^- −N concentration (**Figure 7.5B**). Both SD+DSE and compost reduced the concentration of NO_3^- by 14% and 5% respectively. In contrast, adding these two biowastes on the *K. serotina* plots did not significantly (P>0.05) alter the concentration of NO_3^- −N in the underlying soil.

Macronutrients

Figure 7.6 shows total concentration of soil macronutrients of *L. scoparium* and *K. serotina* in response to SD+DSE and compost treatments during the18-months experimental period.

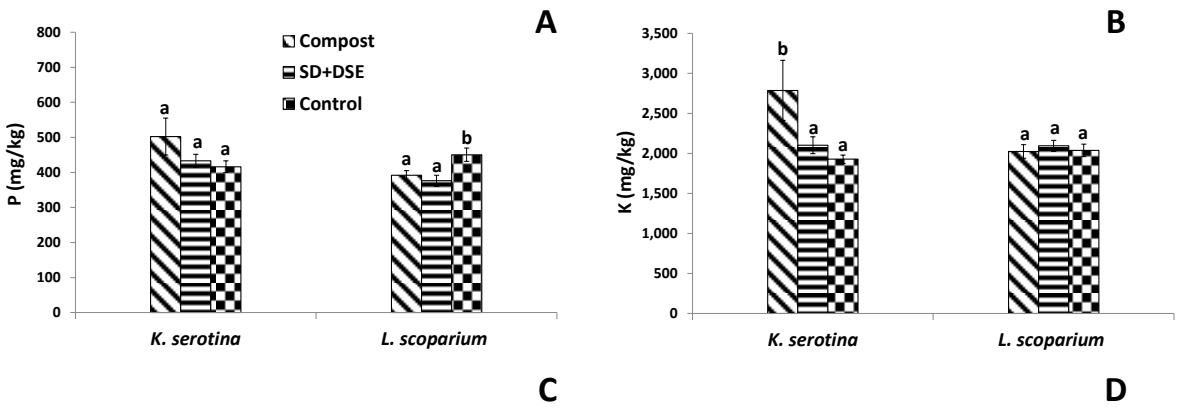


Figure 7. 6 Concentration of soil macronutrients (mg/kg) of *L. scoparium* and *K. serotina* in response to SD+DSE and Compost treatment. Error bars represent the standard error of the mean (n=14, 10 and 15). Bars with the same letters are not significantly different (*p≤0.05*).

In combination with *L. scoparium,* SD+DSE and compost treatments decreased the soil P and Mg (**Figure 7.6A, 7.6D**). Soil P was decreased by 17% and 4% following the application of compost and SD+DSE, respectively in combination with *L. scoparium*. Mg was significantly decreased after the application of compost and SD+DSE and by 16% and 34%, respectively, in combination with *K. serotina*. Compared to the control, there was no significant difference between the Mg concentrations in combination with *K. serotina,* following the application of compost. There was no significant difference in the concentration of soil P and Mg between SD+DSE and compost treatments in combination with both *L. scoparium* and *K. serotina*. In contrast, the application of both SD+DSE and compost significantly increased the concentration of soil K and Ca under of *K. serotina*. **Figure 7.6B** and **7.6C** show that in combination with *K. serotina*, there was a significant difference in the concentration of K following the application of compost compared to the control. In combination with *K. serotina*, the application of SD+DSE and compost increased the concentration of K by 8% and 31%, respectively (**Figure 7.6B)**. However, there was no significant difference in K concentrations between compost and SD+DSE treatment in combination with *K. serotina.* In combination with *K. serotina*, the application of compost increased the concentration of Ca by 24% (**Figure 7.6C)**.

Trace Elements

Figure 7.7 shows total concentration of soil trace elements of *L. scoparium* and *K. serotina* in response to the SD+DSE and compost treatments during the18-month experimental period.

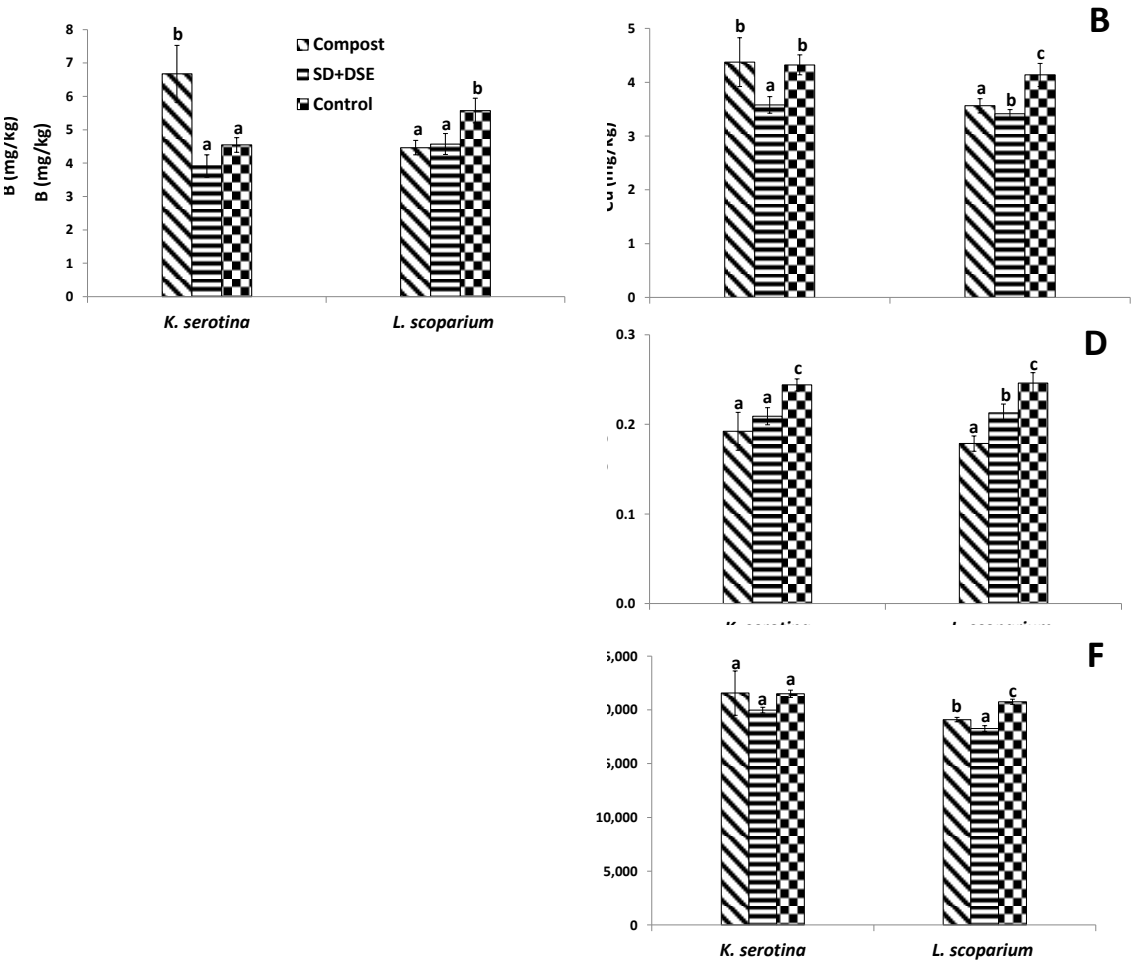


Figure 7. 7 Concentration of soil trace elements (mg/kg) of *L. scoparium* and *K. serotina* in response to SD+DSE and Compost treatment. Error bars represent the standard error of the mean. Bars with the same letters are not significantly different (*p≤0.05*).

The results indicate that in combination with *L. scoparium,* both SD+DSE and compost treatments significantly decreased the concentrations of B, Cu, Cd, Zn, and Fe in the rhizosphere soil compared to the control (**Figure 7.7A, 7.7B, 7.7D, 7.7E, 7.7F**). For *L. scoparium*, the SD+DSE treatment significantly reduced the concentration of rhizosphere soil B, Cu, Zn, and Fe concentrations by 25%, 16%, 2%, and 9%, respectively. The results indicate that in combination with *K. serotina,* the application of SD+DSE significantly reduced the concentration of rhizosphere soil Cu concentration by 21% (**Figure 7.7B**). In contrast, in combination with *K. serotina*, compost application significantly elevated the concentration of soil B and Mn by 32% and 25%, respectively (**Figure 7.7A** and **7.7C**). Both *L. scoparium* and *K. serotina* responded positively to the application of SD+DSE and compost treatment by significantly

reducing the concentration of Cd in rhizosphere soil (**Figure 7.7D**). In combination with compost treatment, *K. serotina* and *L. scoparium* declined the concentration of Cd in underlying soil by 27 and 38%, respectively.

7.4 Discussion

7.4.1 Effect on plant growth parameters

The present study found that mixing sawdust with N-rich material including DSE increased the growth of *K. serotina* by 82% (from 118 to 187 per plant equiv. to from 21 to 38 t ha^{-1}). Presumably, blending sawdust with other N-rich biowastes can undergo its decomposition process. Applying high C-source material including sawdust and compost into soil or blending them with other biowastes, for example biosolids, may reduce plant growth as the high C/N ratio of sawdust would have resulted in the immobilization of available N (Haynes and Goh, 1987; Smith et al., 2011). Esperschuetz et al. (2017) reported that blending biosolids with sawdust did not negatively affect the above ground biomass production of *K. robusta*. Presumably, *K. serotina* benefitted from other macro- and micronutrients aside from N, which are applied with SD+DSE (Anderson et al., 2012; Antoniadis et al., 2008a).

7.4.2 Nutrient accumulation

This study is in agreement with previous studies, which have shown that the application of biowastes increased the uptake of certain macro and micronutrients (Bugbee, 1999b; Esperschuetz et al., 2016b; Esperschuetz et al., 2017; Haynes and Swift, 1986; Nishanth and Biswas, 2008; Olayinka and Adebayo, 1985; Olayinka and Adebayo, 1989; Schmidt et al., 2014; Shaheen et al., 2017). Esperschuetz et al. (2017) reported that in combination with *L. scoparium*, biosolids application increased plant N and Zn, whereas *K. robusta* accumulated significantly higher plant Zn only when combining with biosolids application. Biosolids application to *P. radiata* significantly increased plant N, Zn, and Cu concentration compared to control (Esperschuetz et al., 2017). Shaheen et al. (2017) found that the application of poultry manure nitrogen increased the stalk N of soybean (*Glycine max*) from 62% to 82%. Root exudates may have played an important role in creating and supporting the accumulation of certain macro and micro elements. This in agreement with Koo et al. (2013) and Bertin et al. (2003), who reported that applying biowastes including biosolids could have stimulated root exudation, such as organics acids, which in turn are responsible for nutrients solubilisation and mobilization. Similarly, Hinsinger (2001b) and Keller and Römer (2001a) found that organics acids can increased the availability of P and Zn. Olayinka and Adebayo (1989) reported that blending sawdust with cow dung (incubated for 0, 2 and 4 weeks before application) in the ratio of 1:3 significantly increased the uptake of N and P. of maize (*Zea mays*) during 6-week experimental period.

L. scoparium responded positively to the application of SD+DSE by accumulating significantly higher concentration of almost all essential elements for its growth compared to control. On the other hand, in combination with *K. serotina,* SD+DSE application increased the foliar N and Mn only. Compared to control, both *L. scoparium* and *K. serotina* gave almost similar response by accumulating significantly higher concentration of macro and microelements. Presumably, due different type of root exudates with plant species (Walker et al., 2003), which can lead to different plant responses with regard to the accumulation of nutrients and contaminants associated with biowastes (NCAB). Esperschuetz et al. (2017) reported that myrtaceae family species *K. robusta*, may have mobilized Zn only and Zn and N in *L. scoparium* after application of biosolids. On the other hand, *P. radiata* accumulated significantly higher foliar N, P, Cu, and Zn (Esperschuetz et al., 2017). In combination with *L. scoparium,* both SD+DSE and compost application decreased of plant Ca. Since plants uptake Ca mainly through root tips (White and Broadley, 2003), the application of these biowastes have may altered the of root growth and or chemical composition of available nutrients in rhizosphere part, thus creating unfavourable condition for uptake this particular essential element into plant parts.

In addition, mixing the biowastes including DSE with fresh sawdust may have limited the effect of SD+DSE application in combination with *K. serotina*. This is in agreement with Esperschuetz et al. (2017) who reported that with the exception of Zn, in combination with both *L. scoparium* and *K. robusta*, mixing biosolids with fresh sawdust did not result in significantly different element concentrations compared to biosolids. Olayinka and Adebayo (1989) reported that blending sawdust with cow dung (incubated for 0,2 and 4 weeks before application) in the ratio of 1:3 significantly increased the uptake of N and P but did not affect the uptake of K, Ca, Mg, and Na of maize (*Zea mays*) during 6-week experimental period. The high C/N ratio of sawdust would have resulted in the immobilization of available nutrients in biowastes including biosolids (Haynes and Goh, 1987). In addition, potential NCAB such as Cd, Cr, Ni, and Pb and as, were detected in plant leaves only in low concentrations, and were not significantly increased by either SD+DSE or compost application compared to the controls.

7.4.3 Effect on soil quality

In combination with both *L. scoparium* and *K. serotina*, the application of SD+DSE and compost significantly reduced the Electrical Conductivity (EC) of rhizosphere soil. The condition may have resulted in less available plant nutrients to be uptake into plant parts (Bernstein, 1975; De Kreij and Van Den Berg, 1990; Samarakoon et al., 2006). Samarakoon et al. (2006) reported that uptake N, P, K and Ca significantly increased with the increasing EC.

The present study found that *L. scoparium* and *K. serotina* not only responded positively by enhancing their growth parameters but also effectively reduced soil's NCAB. It seems that SD+DSE not only improved plant growth but also reduced certain trace elements in soil. This result is in agreement with previous investigator who found that the application of mixture sawdust with other N-rich biowaste (biosolids) improved plant growth and reduced concentration of NCAB in soil (Ajmal et al., 1998; Bugbee, 1999b; Marchetti et al., 2000; Yu et al., 2000). In addition to reducing NO_3^- leaching, wood waste, which can be expensive and environmentally damaging to dispose of (Robinson et al., 2007), can effectively immobilized metals such as Cd, Cr, Cu, Ni, Pb and Zn from industrial effluents (Ajmal et al., 1998; Marchetti et al., 2000; Yu et al., 2000). The level of sorption of individual metals can be vary depending on the affinity of each element to the proteins, carbohydrates, and phenolic compounds in the sawdust (Bulut and Tez, 2007). Blending N-rich with sawdust can stimulate the decomposition processes which increase the cation exchange capacity of the sawdust, as more functional groups form on the surface of the sawdust particles (Jokova et al., 1997). Thus, it is likely that the sorption of metals by sawdust will increase, at least temporarily, as it decomposes (Esperschuetz et al., 2016b). Previous studies have reported that mixing sawdust with other biowastes has altered the availability of certain soil nutrients such as P and S by exerting effect of microbial activity due to leaching of organic compound including phenols, tannins, lignin, and terpenes (Hall, 2007; Hedmark and Scholz, 2008; Keeling and Bohlmann, 2006; Sanati, 2005). The lower concentrations of certain trace elements such as Cu, Zn, Fe, and Cd indicate that the application of SD+DSE and compost in combination with *L. scoparium* and *K. serotina* is still an ideal rate. The present study shows that *L. scoparium* and *K. serotina* utilised different way in exerting the macro- and micronutrients in soil probably due the root exudation and growth (Do Nascimento and Xing, 2006). For example, the concentrations of available P, S, Mg, Mn, Cu, and Zn rhizosphere soil were *K. serotina* higher than that of in *L. scoparium*. Presumably due to root exudates, which played an important role for metal complexation and uptake into plants or immobilization in soil (Bais et al., 2006). In the SD+DSE treatment, the concentration of available nutrients was no different between *L. scoparium* and *K. serotina*. Since sawdust is a good source of available C (Cébron et al., 2015), blending them with other biowastes could have attracted heterotrophic bacteria which consumed root exudates and available nutrients in soil as well as stimulated the rhizosphere microbial biomass.

7.5 Conclusion

L. scoparium and *K. serotina* responded positively to the application of 138 t ha^{-1} dry weight of SD+DSE providing 1200 kg N ha^{-1} and 120 t ha^{-1} dry weight, which contains 2400 kg N ha^{-1} equivalent of compost in low-fertility soil. In addition to the improvement of plant growth, in combination with *L. scoparium*

and *K. serotina*, the amendment of these two biowastes has some benefits in terms of enhancing nutrients uptake, stimulating N mineralization potential, as well as reducing nutrients and contaminants associated with biowaste (NCAB) in soils, therefore proper use of these biowastes may be an important management strategy for sustainable forest and or agriculture production systems. Considering their chemical composition, these biowastes constitutes an excellent source of major and minor nutrient elements and is therefore of interest in correcting certain nutrient deficiencies in soils.

Chapter 8

General discussion and conclusions

The broad aim of this thesis was to determine the effect of biowastes on the growth of the plants and to investigate how New Zealand native and exotic vegetation play role in reducing the negative effect of (NCAB). Chapters 4 – 7 have demonstrated that a range of contrasting biowastes, including biosolids, TMW, municipal compost and DSE, increase the growth of most, but not all, NZ native species and all exotic species. Wood waste, which does not contain significant concentrations of plant nutrients, tended to offset the growth benefits of the biosolids when applied in combination. These effects were measured on distinct soil types, namely Orthic Brown, Pawson Silt Loam, and Lismore Stony Silt Loam.

A single large application of biosolids or compost to a low-fertility soil, dramatically improved plant growth while maintaining soil and foliar contaminant concentrations within acceptable limits. Similarly, continual application of DSE (Chapter 4) and TMW (Chapter 6) improved growth without causing nutrient imbalances or unacceptable uptake of contaminants. The experiments in this theses used young seedlings of tree species (*L. scoparium, K. robusta, K. serotina* and *P. radiata*), which would represent the field situation when biowastes would be used to re-establish vegetation on low-fertility or degraded soil. These results cannot be extrapolated to mature vegetation, which may also receive biowastes due to morphological and physiological changes in the plant as it develops.

The thesis shows that there is a significant economic and environmental opportunity to reuse biowastes that may otherwise be disposed of into water bodies or landfill at a significant cost. In New Zealand, the cost is approximately NZ$200-250 per tonne, excluding transport costs, with an average annual cost of NZ$ 33×10^6 per year (WCC, 2008). Discharge of TMW into waterways and the application of excess DSE onto pastures are partly responsible for the widespread degradation of NZ's freshwater resources. Instead, the biowastes could produce valuable native or exotic crops. Recent media reports (https://www.tvnz.co.nz/one-news/new-zealand/lot-blood-sweat-and-tears-east-coast-company-cutting-bees-make-most-manuka-plantation) have shown that manuka oil production can produce a gross return of ($100k ha-1 yr-1) compared to and beef ($4k h1-1 yr-1). Biosolids, DSE, TMW, and compost increased the growth of *L. scoparium* by 30% – 60%, which could significantly improve profits. However, further research is needed to demonstrate the quality of the oil or honey is not adversely affected by the biowastes. Oil quality may be detrimentally affected by

contaminates if they are concentrated in the oil fraction (not measured in this study) or whether the active ingredients in the oil are reduced when biowastes are added.

There were some indications (Chapter 5), that *L. scoparium* and *K. robusta* reduce N mobility in soil. This warrants further investigation, in particular, the effect of these species on a range of nitrifying bacteria and archaea under contrasting geochemical conditions. Similarly, the chemistry of the rhizosphere could be further investigated relating to allochemicals that may be exuded by the roots or even localised changes in pH that may reduce nitrification.

Recent reports by Drinnan and Carrucan (2005) and Stephens et al. (2005) have shown that there is considerable genetic diversity in members of the genus *Leptospermum* and *Kunzea*. Therefore, my findings may not be applicable to all ecotypes or subspecies.

The ecological effects of long-term biowaste addition should be elucidated. It is well known that the addition of high N-containing materials to soil can inhibit the growth of P-fixing mycorrhizal fungi (Grant et al., 2005). If the biowastes are used for ecological restoration, then a full survey of the effects of the biowastes on the invertebrate populations should be carried out. NZ-native vegetation that is re-established using biowastes is likely to have different characteristics to vegetation that occurs spontaneously on degraded or low-fertility soils, since the biowastes may represent a shortcut to near-climax vegetation. This research demonstrated that, in many cases, exotic species had a greater growth response than NZ-native species when biowastes were applied. This may result in excessive competition from weeds in the field situation.

In 2002, the New Zealand government aimed to reuse 95% of the biosolids produced in this country (MfE, 2010). As an alternative to landfilling and ocean disposal, application of biosolids to farmland (both agricultural and forestry land) is becoming increasingly popular. By 2010, New Zealand had approximately 2.5 million ha of land in exotic forest in which *Pinus radiata* are the fastest growing commercial plantations. Several thousands of hectares of these lands are classified as low-fertility soils, which contain low organic matter and are acidic and thus have low nutrients contents. Hence, these kind of lands can be an appropriate alternative for biowastes addition as the contaminants associated with biowastes are less to enter the food chain. The findings of the present research have relevance to assessing the potential role of native species including *L. scoparium* and *K. robusta* to mitigate negative environmentally impact following the application of biowastes. Information regarding the performance of native plants in high N environments will facilitate the strategic incorporation of these species into farming systems. Native species like *L. scoparium* and *K. robusta* species, for instance, are shown to be tolerant to elevated soil N and are suitable for planting on N-

loaded soils. In above particular program, the application of this research can play an important role in minimizing the negative impact of excessive nutrients and contaminants associated with biowaste (NCAB). In addition, the findings of the present study could benefit and applicable to support the valuable manuka honey and essential oils industry of New Zealand. The present study has proved that the application of high rates of either single or mixing biowastes, for example, biosolids and biosolids and sawdust mixture improved the growth of *L. scoparium* and *K. robusta* improved growth rate, elevated macro- and micronutrients uptake, and increased soil quality without reaching threshold levels for food crops for both human and animal health.

Appendix A

Supplementary information to Chapter 3

Table A. 1 Total above ground dried biomass (g) of *L. scoparium* and *K. robusta* in the different macronutrient treatment (n=5) Values in brackets represent the standard error of the average concentration per pot throughout the experiment (n=5).

Treatment	*L. scoparium*			*K. robusta*		
	Dry biomass		% increased	Dry biomass		% increased
N	34.8	(8.0)	34	48.9	(5.2)	33
P	26.4	(2.8)	2	28.5	(2.8)	-22
S	31.4	(2.4)	21	39.2	(5.5)	7
K	24.5	(3.5)	-6	36.8	(0.5)	0
Control	26.0	(3.5)	-	36.8	(0.5)	-

Table A. 2 Total concentration (% d.w) of macronutrients in the leaves of *L. scoparium* measured at the end of the experiment. Values in brackets represent the standard error of the average concentration per pot throughout the experiment (n=5). % inc. indicates the percentage increase relative to the control.

	Treatment													
	N			P			S			K			Control	
	conc		% inc.	conc		% inc.	conc		% inc.	conc		% inc.	conc	
N	1.9	(0,1)	19	1.5	(0.1)	-6	1.5	(0.1)	-6	1.5	(0,1)	-6	1.6	(0.1)
P	0.1	(0,0)	0	0.1	(0.0)	0	0.1	(0.0)	0	0.1	(0,0)	0	0.1	(0.0)
K	0.6	(0,0)	-14	0.7	(0.0)	0	0.7	(0.0)	0	0.7	(0,0)	0	0.7	(0.0)
S	0.2	(0,0)	0	0.2	(0.0)	0	0.1	(0.0)	0	0.1	(0,0)	0	0.2	(0.0)
Ca	1.5	(0,2)	25	1.4	(0.1)	17	1.4	(0.1)	17	1.2	(0,1)	0	1.2	(0.0)
Mg	0.2	(0,0)	0	0.2	(0.0)	0	0.2	(0.0)	0	0.2	(0,0)	0	0.2	(0.0)

Table A. 3 Total concentration (% d.w) of macronutrients in kanuka leaves measured at the end of experiment. Values in brackets represent the standard error of the average concentration per pot throughout the experiment (n=5).

	Treatment													
	N			P			S			K			Control	
	conc		% inc.	conc		% inc.	conc		% inc.	conc		% inc.	conc	
N	1.6	(0,1)	78	1.0	(0.1)	11	0.9	(0.0)	0	0.9	(0,0)	0	0.9	(0.1)
P	0.1	(0,0)	0	0.2	(0.0)	100	0.1	(0.0)	0	0.1	(0,0)	0	0.1	(0.0)
S	0.1	(0,0)	0	0.1	(0.0)	0	0.1	(0.0)	0	0.1	(0,0)	0	0.1	(0.0)
K	0.6	(0,0)	50	0.6	(0.0)	50	0.6	(0.0)	50	0.5	(0,1)	25	0.4	(0.0)
Ca	0.5	(0,0)	-38	0.6	(0.1)	-14	0.5	(0.0)	-29	0.8	(0,0)	14	0.7	(0.0)
Mg	0.1	(0,0)	-50	0.2	(0.0)	100	0.1	(0.0)	0	0.2	(0,0)	100	0.1	(0.0)

Table A. 4 Total concentration (%) of macronutrients in the rhizosphere soil of *L. scoparium* over the experimental period. Values in brackets represent the standard error of the average concentration per pot throughout the experiment (n=3).

	Treatment														
	N			P			S			K			Control		
	conc		% inc.	conc		% inc.	conc		% inc.	conc		% inc.	conc		
P	0.06	(0.0)	-2	0.07	(0.0)	15	0.06	(0.0)	3	0.06	(0.0)	1.6	0.06	(0.0)	
S	0.04	(0.0)	0	0.04	(0.0)	0	0.05	(0.0)	23	0.04	(0.0)	2.5	0.04	(0.0)	
K	0.24	(9.4)	0	0.25	(0.0)	4	0.25	(0.0)	5	0.25	(0.0)	2.5	0.24	(0.0)	
Ca	0.31	(0.0)	1	0.30	(0.0)	-2	0.32	(0.0)	3	0.31	(0.0)	0.3	0.31	(0.0)	
Mg	0.21	(0.0)	2	0.21	(0.0)	2	0.21	(0.0)	1	0.21	(0.0)	1.5	0.21	(0.0)	

Table A. 5 Total concentration (%) of macronutrients in *K. robusta* rhizosphere soil measured at the end of the experiment. Values in brackets represent the standard error of the average concentration per pot throughout the experiment (n=3).

	Treatment														
	N			P			S			K			Control		
	conc		% inc.	conc		% inc.	conc		% inc.	conc		% inc.	conc		
P	0.06	(0,0)	2	0.07	(0.0)	7	0.06	(0.0)	0	0.06	(0.0)	2	0.06	(0.0)	
S	0.04	(0.0)	-1	0.04	(0.0)	-5	0.05	(0.0)	21	0.04	(0.0)	3	0.04	(0.0)	
K	0.04	(0,0)	0	0.04	(0.0)	-5	0.05	(0.0)	23	0.04	(0.0)	3	0.04	(0.0)	
Ca	0.30	(0.0)	2	0.29	(0.0)	-2	0.31	(0.0)	2	0.31	(0.0)	5	0.30	(0.0)	
Mg	0.20	(0,0)	21	0.20	(0.0)	20	0.20	(0.0)	23	0.19	(0.01)	17	0.16	(0.0)	

Table A. 6 Mineral N concentration (mg/L) in *L. scoparium* and *K. robusta* rhizosphere soil measured at the end of the experiment. Values in brackets represent the standard error of the average concentration per pot throughout the experiment (n=3).

	L. scoparium						*K. robusta*					
	NH_4^+-N			NO_3^--N			NH_4^+-N			NO_3^--N		
	conc		% inc.	conc		% inc.	conc		% inc.	conc		% inc.
N	0.2	(0.0)	0	3.5	(1.7)	1650	0.2	(0.2)	0	3.1	(1.5)	1450
P	0.2	(0.0)	0	0.2	(0.1)	0	0.2	(0.0)	0	0.3	(0.1)	50
S	0.2	(0.1)	0	0.2	(0.1)	0	0.2	(0.0)	0	0.1	(0.0)	-50
K	0.1	(0.0)	-50	0.2	(0.0)	0	0.2	(0.0)	0	0.2	(0.0)	0
Control	0.2	(0.0)	-	0.2	(0.0)	-	0.2	(0.0)	-	0.2	(0.0)	-

Appendix B

Supplementary information to Chapter 4

Table B. 1 Cumulative above ground dried biomass (g) of *L. scoparium* and *K. robusta* in the DSE, biosolids, and the control treatment (n=4). Values in brackets represent the standard error of the average concentration per pot throughout the experiment (n=4).

Treatment	*L. scoparium*		*K. robusta*	
	Dry Biomass	% increase	Dry biomass	% increase
DSE	179 (8.5)	24	135 (11.7)	29
Biosolids	207 (8.1)	44	210 (13.5)	100
Control	144 (11.7)	-	105 (7.7)	-

*after six weeks of experiment

Table B. 2 Total concentrations of macronutrients of above ground of *L. scoparium* (%) measured at the end of experimental period. Values in bracket represent Standard error of mean.

Element		DSE	Biosolids	Control
N	Mean	1.1 (0.0)	1.2 (0.1)	1.1 (0.0)
	% increased	1.8	7.1	-
Ca	Mean	1.1 (0.0)	1.2 (0.1)	0.9 (0.1)
	% increased	20.9	28.7	
K	Mean	0.7 (0.0)	0.6 (0.0)	0.6 (0.1)
	% increased	11.6	-2.8	-
Mg	Mean	0.3 (0.0)	0.3 (0.0)	0.3 (0.0)
	% increased	9.5	15.6	-
P	Mean	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)
	% increased	19.7	44.5	-
S	Mean	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)
	% increased	-0.8	-3.5	-

Table B. 3 Total macronutrients concentration (%) of above ground of *K. robusta* in the Eyrewell soil medium measured at the end of experimental period. Values in bracket represent Standard error of mean.

Element		DSE	Biosolids	Control
N	Mean	0.9 (0.1)	0.9 (0,1)	0.8 (0,1
	% increased	15.7	12.3	-
Ca	Mean	0,6 (0.0)	0,7 (0,1)	0.5 (0,0)
	% increased	21.9	50.7	-
K	Mean	0,6 (0.0)	0.6 (0,0)	0.7 (0,0)
	% increased	-18.1	-17.9	-
Mg	Mean	0.2 (0,02)	0.2 (0,0)	0.2 (0,0)
	% increased	2.,8	3.2	-
P	Mean	0.2 (0.0)	0.2 (0,0)	0.2 (0,0)
	% increased	9.8	14.8	-
S	Mean	0.1 (0.0)	0,1 (0,0)	0.1 (0,0)
	% increased	-3.3	31.7	-

Table B. 4 Total concentrations of micronutrients of above ground of *L. scoparium* (mg/kg) measured after 12 wk of the experimental period. Values in bracket represent Standard error of mean

Element		DSE		Biosolids		Control	
B	Mean	50.3	(7.8)	54.5	(11.9)	39.0	(3.5)
	% increased	29.0		39.7		-	
Cd	Mean	0.0	(0.0)	0.1	(0.0)	0.02	(0.0)
	% increased	-6.0		228.5		-	
Cu	Mean	3.3	(0.2)	3.4	(0.3)	2.3	(0.2)
	% increased	41.6		46.0		-	
Fe	Mean	60.2	(12.4)	71.4	(19.3)	43.9	(1.9)
	% increased	37.0		62.7		-	
Mn	Mean	179.7	(46.9)	315.3	(83.9)	167.9	(69.2)
	% increased	7.0		87.8		-	
Zn	Mean	11.2	(2.3)	68.2	(21.5)	1.2	(69.2)
	% increased	9.8		569.1		-	

Table B. 5 Total concentrations of micronutrients of above ground of *K. robusta* (mg/kg) measured after 12 wk of the experimental period. Values in bracket represent Standard error of mean

Element		DSE		Biosolids		Control	
B	Mean	50.8	(5.6)	32.5	(4.2)	49.2	(7.2)
	% increased	3.3		-34.0		-	
Cd	mean	0.02	(0.01)	0.3	(0.1)	0.01	(0.0)
	% increased	67.4		3078.9		-	
Cu	mean	1.5	(0.2)	2.3	(0.2)	1.3	(0.3)
	% increased	15.0		78.0		-	
Fe	mean	71.9	(15.9)	47.0	(5)	95.4	(41.6)
	% increased	-24.6		-50.7		-	
Mn	mean	503.4	(64.3)	683	(102)	398.9	(49.2)
	% increased	26.2		71.2		-	
Zn	mean	40.9	(8.5)	118.8	(5.8)	29.8	(6.7)
	% increased	37.2		298.6		-	

Appendix C

Supplementary information to Chapter 6

Table C. 1 Total concentrations of foliar N (%) of species tested which are significant different to the control measured at the end of experimental period. Values in bracket represent Standard error of mean.

Species		Treatment	
		TMW	Control
C. australis	Mean	1.3 (0.1)	1.2 (0.0)
	% increased	13	-
C. robusta	Mean	1.8 (0.1)	1.5 (0.0)
	% increased	19	-
K. robusta	Mean	2.1 (0.1)	1.8 (0.1)
	% increased	17	-
L. scoparium	Mean	1.8 (0.0)	1.5 (0.0)
	% increased	22	-
O. paniculata	Mean	1.3 (0.1)	1.2 (0.0)
	% increased	16	-
P. eugenoides	Mean	1.6 (0.1)	1.4 (0.0)
	% increased	16	-
P. tenax	Mean	1.4 (0.1)	1.1 (0.0)
	% increased	24	-
P. cunninghamii	Mean	1.2 (0.0)	1.1 (0.1)
	% increased	14	-

Table C. 2 Total concentrations of foliar P (mg/kg) of species tested which are significant different to the control measured at the end of experimental period. Values in bracket represent Standard error of mean.

Species		Treatment			
		TMW		Control	
L. scoparium	Mean	1524	(89)[b]	1202	(69)a
	% increased	16		-	
O. paniculata	Mean	1310	(106)[a]	1581	(129)[b]
	% increased	-26		-	

Table C. 3 Total concentrations of foliar K (mg/kg) of species tested which are significant different to the control measured at the end of experimental period. Values in bracket represent Standard error of mean.

Species		Treatment			
		TMW		Control	
C. robusta	Mean	7617	(700)[b]	5131	(338)[a]
	% increased	48		-	
K. robusta	Mean	4093	(120)[b]	3484	(76)[a]
	% increased	17		-	
L. scoparium	Mean	3858	(82)[b]	3315	(58)[a]
	% increased	16		-	
O. paniculata	Mean	6380	(609)[a]	8641	(839)[b]
	% increased	-26		-	

Table C. 4 Total concentrations of foliar S (mg/kg) of species tested which are significant different to the control measured at the end of experimental period. Values in bracket represent Standard error of mean.

Species		Treatment	
		TMW	Control
C. australis	Mean	1039 (36)[b]	859 (25)[a]
	% increased	21	-
C. robusta	Mean	2556 (149)[b]	1679 (61)[a]
	% increased	52	-
K. robusta	Mean	2428 (53)[b]	1538 (32)[a]
	% increased	58	-
L. scoparium	Mean	2042 (57)[b]	1357 (32)[a]
	% increased	50	-
O. paniculata	Mean	1261 (170)[a]	693 (21)[b]
	% increased	82	-
P. eugenoides	Mean	1054 (58)[b]	824 (58)[a]
	% increased	28	-
P. tenax	Mean	1296 (59)[b]	1049 (49)[a]
	% increased	24	-

Table C. 5 Total concentrations of foliar Mg (mg/kg) of species tested which are significant different to the control measured at the end of experimental period. Values in bracket represent Standard error of mean.

Species		Treatment	
		TMW	Control
K. robusta	Mean	1448 (51)[a]	2001 (72)[b]
	% increased	-28	-
P. cunninghamii	Mean	2141 (86)[a]	2430 (95)[b]
	% increased	-12	-

Table C. 6 Total concentrations of foliar Ca (mg/kg) of species tested which are significant different to the control measured at the end of experimental period. Values in bracket represent Standard error of mean.

Species		Treatment	
		TMW	Control
C. robusta	Mean	21043 (1209)[a]	22002 (643)[b]
	% increased	-4	-
K. robusta	Mean	4655 (193)[a]	5869 (288)[b]
	% increased	-21	-
P. cunninghamii	Mean	9421 (454)[a]	10329 (340)[b]
	% increased	-9	-

Table C. 7 Total concentrations of foliar Fe (mg/kg) of species tested which are significant different to the control measured at the end of experimental period. Values in bracket represent Standard error of mean.

Species		Treatment	
		TMW	Control
L. scoparium	Mean	249 (16)[a]	413 (69)[b]
	% increased	-40	-
P. tenax	Mean	93 (8)[b]	69 (5)[a]
	% increased	36	-

Table C. 8 Total concentrations of foliar Mn (mg/kg) of species tested which are significant different to the control measured at the end of experimental period. Values in bracket represent Standard error of mean.

Species		Treatment	
		TMW	Control
L. scoparium	Mean	181 (14)[a]	254 (19)b
	% increased	-29	-
K. robusta	Mean	260 (27)[a]	471 (43)[b]
	% increased	-45	-
P. cunninghamii	Mean	169 (21)[a]	251 (14)[b]
	% increased	-33	-

Table C. 9 Total soil N (%) of different vegetation type measured at the end of experimental period. Values in bracket represent Standard error of mean.

Vegetation type		Treatment	
		TMW	Control
1	Mean	0.47 (0.0) [a]	0.44 (0.0) [a]
	% increased	12	-
2	Mean	0.48 (0.0) [b]	0.43 (0.0) [a]
	% increased	13	-
3	Mean	0.48 (0.0) [b]	0.43 (0.0) [a]
	% increased	12	-

Table C. 10 Total soil C (%) of different vegetation type measured at the end of experimental period. Values in bracket represent Standard error of mean.

Vegetation type		Treatment	
		TMW	Control
1	Mean	4.9 (0.0) [a]	4.7 (0.2) [a]
	% increased	14	
2	Mean	5.1 (0.0) [b]	4.4 (0.1) [a]
	% increased	15	
3	Mean	5.1 (0.0 [b]	4.5 (0.1) [a]
	% increased	13	

Appendix D

Supplementary information to Chapter 7

Table D. 1 Effect of the application of mixture sawdust+DSE and compost on plant height (cm). Values in parentheses represent the standard error of the average survival rate of each species throughout the experiment (*n* = 3).

Species	Treatment					
	Compost		SD+DSE		Control	
L. scoparium	74.9	(7.2)[a]	73.6	(8.1)[a]	84.0	(11.1)[a]
K. serotina	61.3	(7.3)[a]	57.4	(5.4)[a]	84.2	(0.8)[a]

† Different lowercase letters indicate significant differences between treatments at $p \leq 0.05$, using One-Way ANOVA followed by Duncan Post Hoc Tests

Table D. 2 Effect of the application of mixed sawdust and DSE and compost on the dried weight of above plant part (g). Values in parentheses represent the standard error of the average survival rate of each species throughout the experiment (*n* = 3).

Treatment	*L. scoparium*			*K. serotina*		
	Dried weight		% increased	Dried weight		% increased
Control	141.7	(24.7)[a]		103.0	(10.0)[a]	-
Compost	154.9	(11.1)[a]	11.0	97.7	(12.8)[a]	-5.2
Sawdust+DSE	118.1	(23.2)[a]	-1.0	187.5	(30.8)[b]	82.0

† Different lowercase letters indicate significant differences between treatments at $p \leq 0.05$, using One-Way ANOVA followed by Duncan Post Hoc Tests.

Table D. 3 Total concentrations of foliar macronutrients of *L. scoparium* measured at the end of experimental period. Values in bracket represent Standard error of mean.

Element		Treatment					
		Compost		SD+DSE		Control	
N (%)	Mean	1.6	(0.0)	1.7	(0.1)	1.3	(0.1)
	% increased	22		25			-
P (mg/kg)	Mean	959	(24)	1127	(60)	847	(35)
	% increased	13		33			-
K (mg/kg)	Mean	4029	(112)	3336	(81)	4306	(122)
	% increased	-6		-25			-
S (mg/kg)	Mean	1421	(30)	1477	(53)	1305	(69
	% increased	9		13			-
Ca (mg/kg)	Mean	6166	(366)	6701.5	(370)	7375	(345)
	% increased	-16		-9			-
Mg (mg/kg)	Mean	1805	(68)	2141	(110)	1843	(66)
	% increased	-2		16			-

Table D. 4 Total concentrations of foliar macronutrients of *K. serotina* measured at the end of experimental period. Values in bracket represent Standard error of mean.

Element		Treatment					
		Compost		SD+DSE		Control	
N (%)	Mean	1.9	(0.1)	1.7	(0.1)	1.3	(0.1)
	% increased	47		37		-	
P (mg/kg)	Mean	1083	(53)	1074	(53)	968	(52)
	% increased	12		11		-	
K (mg/kg)	Mean	4165	(125)	3782	(76)	3487	(124)
	% increased	19		8		-	
S (mg/kg)	Mean	1620	(62)	1423	(44)	1408	(51)
	% increased	15.1		1		-	
Ca (mg/kg)	Mean	6264	(371)	7258	(436)	7421	(348)
	% increased	-16				-	
Mg (mg/kg)	Mean	1908	(87)	1863	(92)	2049	(109)
	% increased	-7		-9		-	

Table D. 5 Total concentrations of foliar micronutrients of *L. scoparium* measured at the end of experimental period. Values in bracket represent Standard error of mean.

Element		Treatment					
		Compost		SD+DSE		Control	
Cu (mg/kg)	Mean	2.6	(0.3)	2.9	(0.2)	2.9	(0.2)
	% increased	-12		-2		-	
Fe (mg/kg)	Mean	448.1	(96.4)	272.0	(48.9)	346.8	(76.3)
	% increased	29		-22		-	
Mn (mg/kg)	Mean	866.9	(56.3)	758.5	(70.4)	421.6	(34.3)
	% increased	106		80		-	
Ni (mg/kg)	Mean	0.7	(0.1)	0.6	(0.1)	4.3	(2.4)
	% increased	-84		-86		-	
Zn (mg/kg)	Mean	19.3	(1.7)	21.9	(2.6)	15.5	(1.5)
	% increased	24		41		-	

Table D. 6 Total concentrations of foliar micronutrients of *K. serotina* measured at the end of experimental period. Values in bracket represent Standard error of mean.

Element		Treatment					
		Compost		SD+DSE		Control	
Cu (mg/kg)	Mean	2.7	(0.2)	2.6	(0.3)	2.7	(0.2)
	% increased	0		-5		-	
Fe (mg/kg)	Mean	286.0	(25.6)	265.8	(28.8)	332.0	(34.5)
	% increased	-14		-20		-	
Mn (mg/kg)	Mean	1263.3	(116.5)	1517.3	(238.0)	727.8	(65.4)
	% increased	74		108		-	
Ni (mg/kg)	Mean	2.3	(0.2)	2.1	(0.2)	3.2	(0.4)
	% increased	-29		-36		-	
Zn (mg/kg)	Mean	31.7	(1.9)	29.2	(3.1)	29.6	(2.7)
	% increased	7		-1			

Table D. 7 NH_4^+-N and NO_3^--N concentrations of *L. scoparium* after 18 months applications of the mixture sawdust and DSE and compost (mg/kg). Values in bracket represent Standard error of mean.

Element		Treatment					
		Compost		SD+DSE		Control	
NH_4^+-N	Mean	47.7	(0.0) [b]	15.0	(0.0) [a]	0.2	(0.0) [a]
	% increased	568		0.2		-	
NO_3^+-N	Mean	44	(0.0) [a]	0.2	(0.0) [b]	0.2	(0.0) [c]
	% increased	-14		-5		-	

Table D. 8 NH_4^+-N and NO_3^--N concentrations of *K. serotina* after 18 months applications of the mixture sawdust and DSE and compost (mg/kg). Values in bracket represent Standard error of mean.

Element		Treatment					
		Compost		SD+DSE		Control	
NH_4^+-N	Mean	28	(0.0) [b]	0.2	(0.0) [a]	0.2	(0.0) [a]
	% increased	370		-2		-	
NO_3^--N	Mean	49	(0.0) [a]	0.2	(0.0) [a]	0.2	(0.0) [a]
	% increased	-3		-5		-	

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