The potential of reusing NZ'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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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, with 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

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- 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. *J. Environ. Qual.* 45:360–367. doi: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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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, Lopes et al., 2011, Lagae et al., 2009, Veeken and Hamelers, 2002, Bruun et al., 2006, Hargreaves et al., 2008, Hawke and Summers, 2006, Mohammad Rusan et al., 2007, Minhas et al., 2015, Basta et al., 2015). 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 (Di et al., 1998, Hawke and Summers, 2006, Agopsowicz et al., 2008, Krogmann et al., 1997, Lavado et al., 2005, Singh and Agrawal, 2008, Stoven and Schnug, 2009, Qiang et al., 2004, Vogeler et al., 2006, Mohammad Rusan et al., 2007, Correa et al., 2006). Excessive NO_3^- leaching can contribute to the eutrophication of lakes and rivers as well as contaminate groundwater (Davis, 2014, Fowler et al., 2013, De Vries et al., 2013), detrimentally affecting human health (McFarland et al., 2013, Galbally et al., 2013, Agopsowicz et al., 2008). 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 (Robinson et al., 2009, Wang and Jia, 2010, Robinson et al., 2007, McCutcheon and Schnoor, 2004, Lomonte et al., 2010, Prosser, 2011, Galbally et al., 2013, Chague-Goff, 2005, Tanner, 2001, Domínguez et al., 2008). 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 (Hedley et al., 2013b, Fitzgerald, 2012) and kill pathogens in biosolids-amended soil (Prosser, 2011, Fitzgerald, 2012). *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 (Wardle, 1991, Stephens et al., 2005, Bertin et al., 2003b).

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 (Trotter et al., 2005, Scott et al., 2000). *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 (Turan and Esringu, 2007, Licht and Schnoor, 1993, Pilipovic et al., 2006, Wang et al., 2009, Barceló and Poschenrieder, 2003).

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 (Daniels et al., 2001, Schmidt et al., 2001, Bugbee, 1999b). 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 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)

The 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-microbes 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 (Harvey et al., 2002, Clothier and Green, 1997). 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 reduce 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 (Robinson et al., 2007, Grifoll and Cohen, 1996, Robinson et al., 2006) and is strongly influenced by plant species and climate (Robinson et al., 2007, McCulley et al., 2004, Allen et al., 1998). 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 Serenium-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).

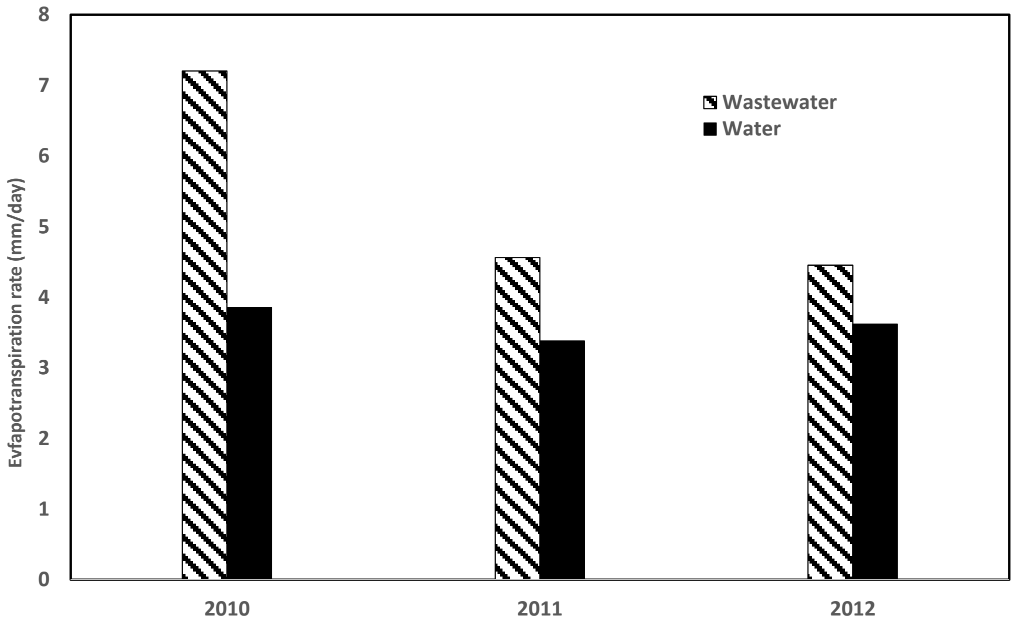


Figure 2. 1 Evapotranspiration rates (mm day^{-1}) of willow trees (*Salix* spp.) treated with different wastewater effluent during the 2010, 2011 and 2012 growing seasons. Data adapted from (Curneen and Gill, 2014).

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 (Ola et al., 2015, Li et al., 2014). Together with the chemical and biological activity in the rhizosphere, the physical action of the roots contributes to establishment of macrospores (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 (Germann et al., 2012, Jørgensen et al., 2002, Baets et al., 2007). 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 (Mosaddeghi et al., 2009, Głąb, 2013) 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 (Clothier and Green, 1997, Bertin et al., 2003a) 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 make contact with 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, Schnoor et al., 1995, Erenoglu et al., 2011). 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 (Curneen and Gill, 2014, Wang and Oyaizu, 2009, Robinson et al., 2007, Białowiec et al., 2007, Martin and Stephens, 2006). 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^{o}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 (Courty et al., 2015, Lambers et al., 2009, Hartmann et al., 2009, Adesemoye and Kloepper, 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	(Wang et al., 2005, Antolín et al., 2005, Knowles et al., 2011, Mok et al., 2013, Paramashivam, 2015b, Smith and Tibbett, 2004, Fijalkowski et al., 2011)	7.3-8.2	(Di et al., 1998, Zaman et al., 2002, Zaman et al., 1999a)	7.3	(Mohammad Rusan et al., 2007)	4.3-5.7	5.5	(Bugbee, 1999a, 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, 1999a, 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., 2002, Zaman et al., 1999a)	0.002-0.01	(Curneen and Gill, 2014, Gersberg et al., 1986, Monnet et al., 2002)	0.06-0.1	0.03	(Bugbee, 1999a, Paramashivam, 2015b)
Total P (mg kg^{-1})	3900- 6600	(Antolín et al., 2005, Knowles et al., 2011, Paramashivam, 2015b, Wang et al., 2005, Fijalkowski et al., 2011, Mok et al., 2013)	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	(Knowles et al., 2011, Paramashivam, 2015b, Wang et al., 2005, Antolín et al., 2005, Fijalkowski et al., 2011, Mok et al., 2013)	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, Mok et al., 2013, Wang et al., 2005, Knowles et al., 2011)	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., 2003a, Kozdrój and van Elsas, 2000, Walker et al., 2003, Hodge and Millard, 1998). 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 (Dakora and Phillips, 2002, Bertin et al., 2003a, 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 (modified and adopted from Dakora et al. 2004 and 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, chemoattractants, 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., 2003a, Dakora and Phillips, 2002, Kozdrój and van Elsas, 2000, Walker et al., 2003, Degryse et al., 2008, Koo et al., 2010). Organic acids are the main compounds of root exudates, including oxalic, tartaric, succinic, and the most important for solubilisation and mobilization of plant nutrients and metals (Jones and Darrah, 1993, Chang et al., 2002, Koo et al., 2010). They assist in nutrient uptake by increasing the availability the of P and micronutrients including Fe and Zn (Gerke et al., 2000, Gerke, 2000, Hinsinger, 2001b, Keller and Romer, 2001, Römheld and Marschner, 1990, Schilling et al., 1998, Hopkins et al., 1998, Jones, 1998, Ryan et al., 2001). 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 formation. 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 (Treeby et al., 1989, Zhang et al., 1991, Mench et al., 1988).

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 (Ishikawa et al., 2003, Galloway et al., 2008) 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, Fowler et al., 2013, De Vries 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 (Tanaka et al., 2010, Qiao et al., 2015, Ishikawa et al., 2003, Gopalakrishnan et al., 2007). 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, Tanaka et al., 2010, Ishikawa et al., 2003), 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 (Hartmann et al., 2009, Jane et al., 1996, Lipton et al., 1987, Neumann et al., 1999, Ahonen-Jonnarth et al., 2000). For example, alfalfa (*Medicago sative*) 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 axalate, malate, and citrate) of root exudates from *Pinus sylvestris* increased significantly in soils containing Al (Ahonen-Jonnarth et al., 2000).

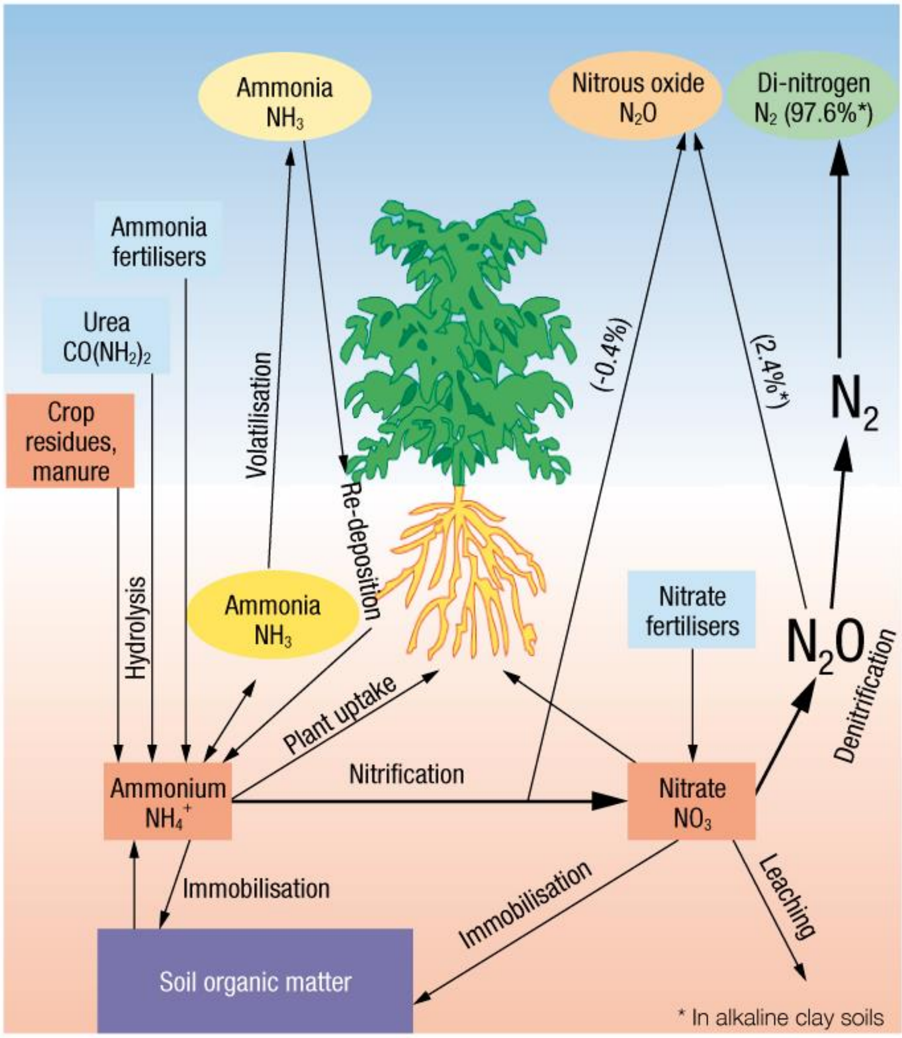


Figure 2. 2 The nitrogen cycle. Adapted from (Dixon, 2014).

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 (Stottmeister et al., 2003, Mukerji et al., 2006). 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 (Morikawa and Erkin, 2003, Harvey et al., 2002, Cohen et al., 2004, Khan, 2006, 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 (Stottmeister et al., 2003, Mukerji et al., 2006). 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 (Pulford and Watson, 2003, Mukhopadhyay and Joy, 2010, 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 (Wang et al., 2014, Cleveland et al., 2002, Wieder et al., 2008, Wurzburger and Hendrick, 2009, Bowman et al., 2004) 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 (Wang et al., 2014, Brady, 2008, Villalobos-Vega et al., 2011). These particular interactions can then have further significant effects on the fluxes of nutrients and contaminants associated with biowastes (Kozdrój and van Elsas, 2000, Cohen et al., 2004). 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 auriculiformes*) 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 (Rogers and Smith, 2007, Singh and Agrawal, 2008, Cytryn et al., 2011).

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 (Weber et al., 2007, Brady, 2008, Antolín et al., 2005). 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 (Wardle, 1991, Stephens et al., 2005). *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 (Trotter et al., 2005, Scott et al., 2000). 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).

a b

Figure 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 (Perry et al., 1997a, Perry et al., 1997b, Lis-Balchin and Hart, 1998, Lis-Balchin et al., 2000).

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) (Hedley et al., 2013b, Fitzgerald, 2012) and killing pathogens in biosolids-amended soil (Prosser, 2011, Fitzgerald, 2012). 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 kilo (MinistryforPrimaryIndustries, 2014) due to the perceived health benefits resulting from phenolic compounds such as trimethoxybenzoic acid, methylglyoxal, and 2-methoxybenzoic (Weston et al., 1999, Stephens et al., 2010). 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 (MinistryforPrimaryIndustries, 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).

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 (Mhando et al., 1993, Ringrose and Neilsen, 2005, Xu et al., 2002, Albaugh et al., 2015, Cromer et al., 1993, Hunter, 2001, Judd et al., 1996, Messina, 1992, Carlson et al., 2001, Campion et al., 2006, Weggier et al., 2008, Bennett et al., 1996, Pankaj et al., 2008). 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 (Mhando et al., 1993, Ringrose and Neilsen, 2005, Xu et al., 2002, Albaugh et al., 2015, Cromer et al., 1993, Hunter, 2001, Judd et al., 1996, Messina, 1992, Carlson et al., 2001, Campion et al., 2006, Weggier et al., 2008, Bennett et al., 1996, Pankaj et al., 2008) 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 (Xu et al., 2002, Carlson et al., 2001). 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 26^{th} to November 26^{th}, 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, 2cm 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 (**Figure 3.3b**).

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

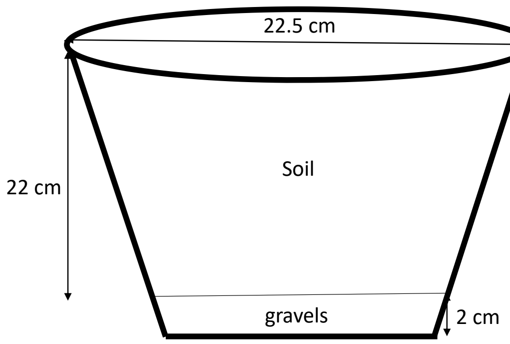


Figure 3. 2 Pot design used in the experiment

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. 3 (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, 2001b) 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 70^0 C 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.

Significant differences (α=0.05) between treatments were determined by analysis of variance. 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 3.4 shows above ground biomass of *K. robusta* in combination with different macronutrient treatments.

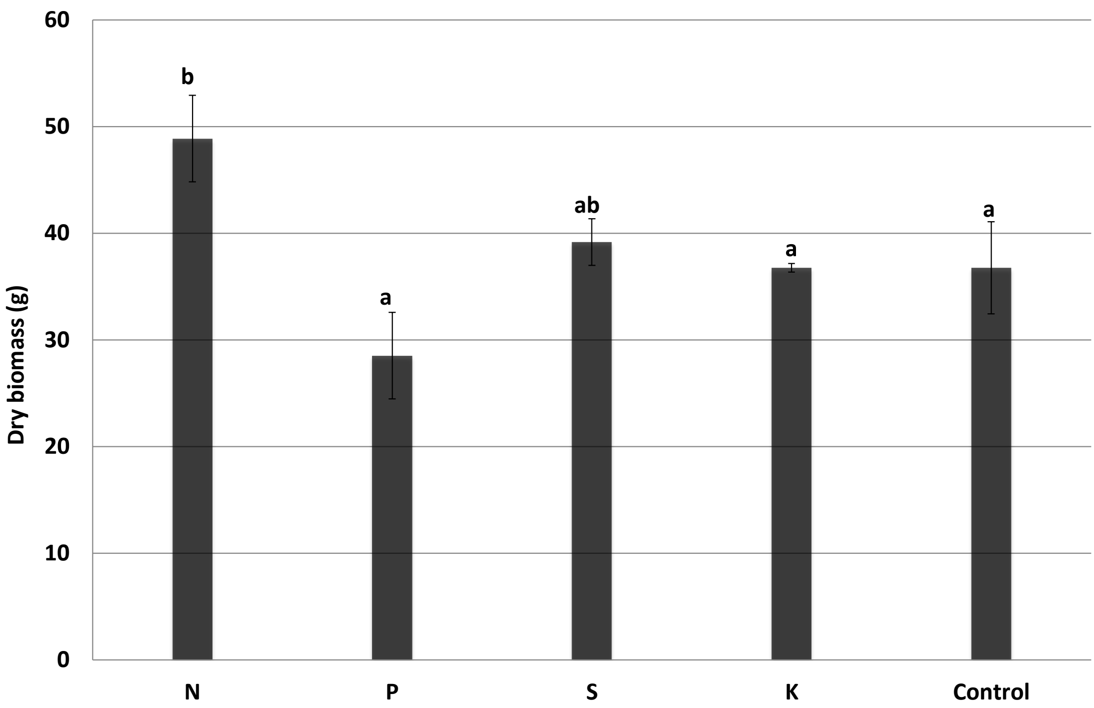


Figure 3. 4 Above ground biomass of *K. robusta* in combination with different macronutrient treatments (n=5). Treatments that share letters have means that do not differ significantly.

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 3.4)**. 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 ton ha^{-1}), which is 33% higher than the control (**Figure 3.4)**.

Figure 3.4 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 3.5**).

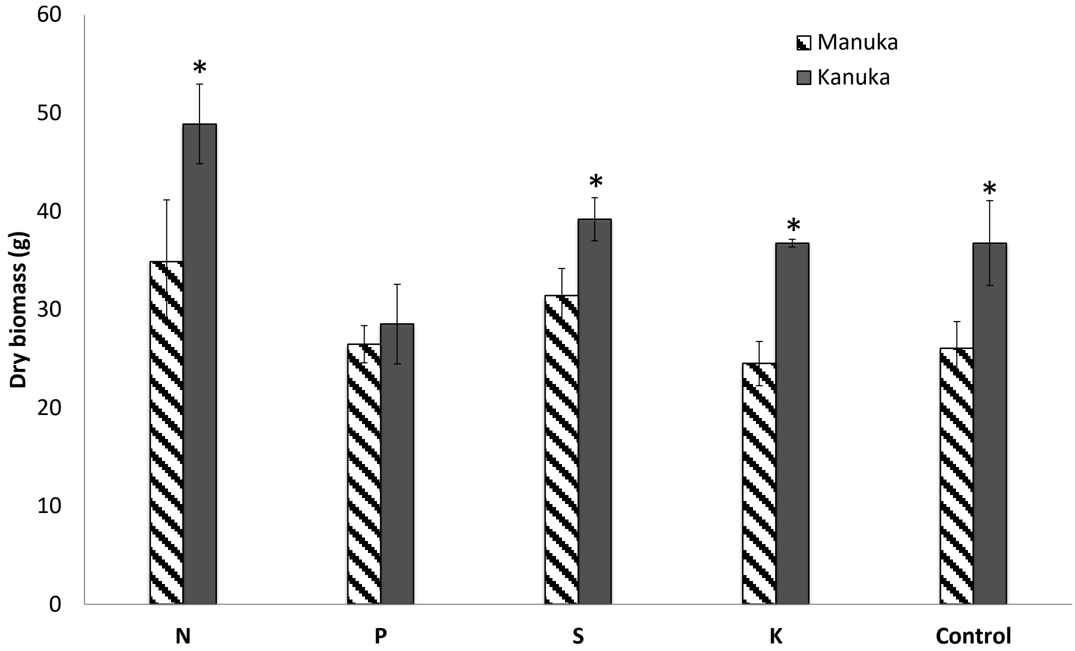


Figure 3. 5 Comparison of above ground biomass of *L. scoparium* and *K. robusta i*n combination with different macronutrient treatment (n=5). Asterisks (*) signify significant differences between species at *p≤0.05*.

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 3.6**)*. K. robusta* responded positively to the application of 100 kg/ha of P (in KH_2PO_4 form) by showing the highest root to shoot ratio value of 0.6 (**Figure 3.6**).

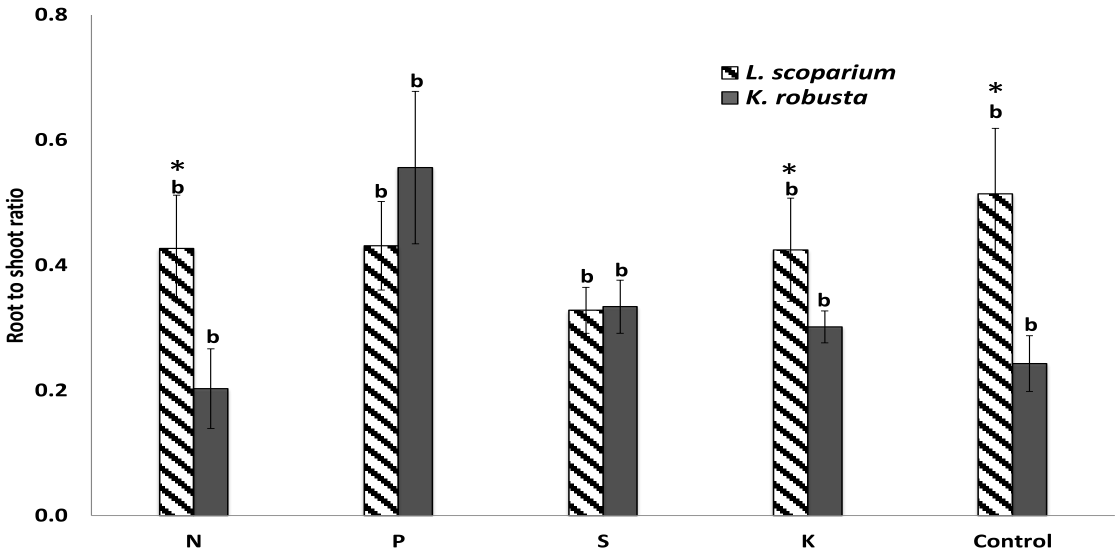


Figure 3. 6 Root-to-shoot ratio in combination with different macronutrient treatments (n=5). Treatments that share letters have means that do not differ significantly. Asterisks (*) signify significant differences between species at *p≤0.05*.

3.3.2 Foliar nutrients concentration

Nitrogen

Figure 3.7 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 3.7**). 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 3.7**).

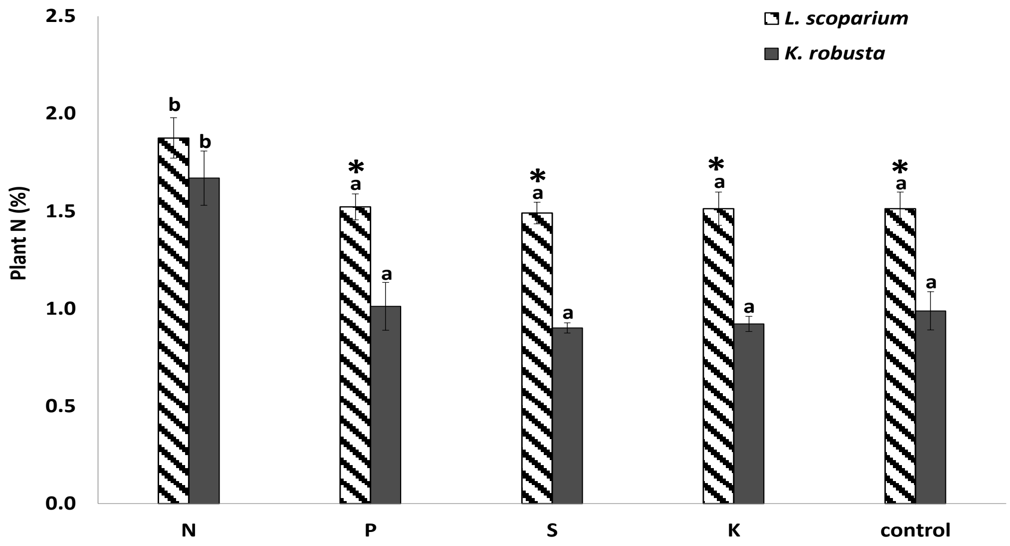


Figure 3. 7 N concentration in the leaves in combination with macronutrient treatments (n=5). Treatments that share letters have means that do not differ significantly (*p≤0.05*). Asterisks (*) signify significant differences between species at *p≤0.05*.

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

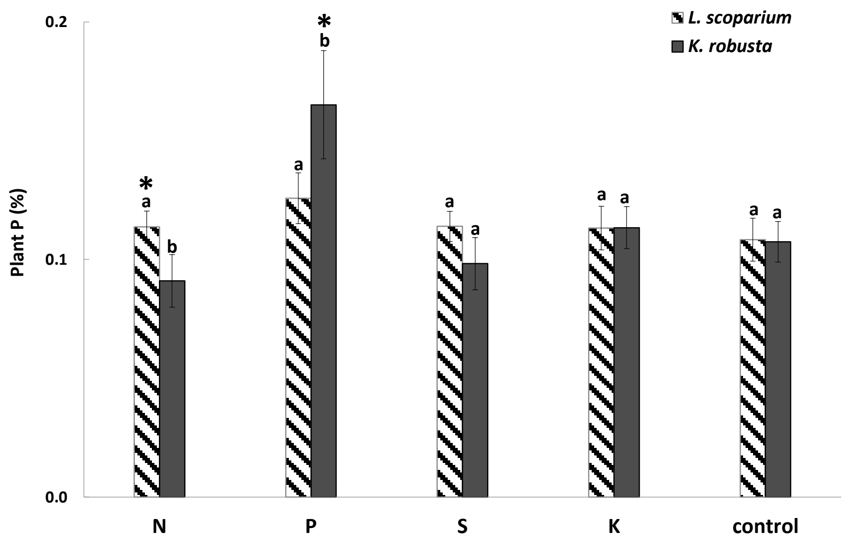


Figure 3. 8 Leaf P concentration in combination with macronutrient treatment (n=5). Treatments that share letters have means that do not differ significantly (*p≤0.05*). Asterisks (*) signify significant differences between species at *p≤0.05*.

Figure 3.8 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 3.8,** 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.

Potassium (K)

In combination with *K. robusta,* the application of all individual fertilizers (N, P, K, and S) increased significantly ($p \leq 0.05$) foliar K compared to the control (**Figure 3.10**). 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 3.10**). On the other hand, amending low fertility soil with macronutrients did not significantly affect the accumulation of foliar K in *L. scoparium.*

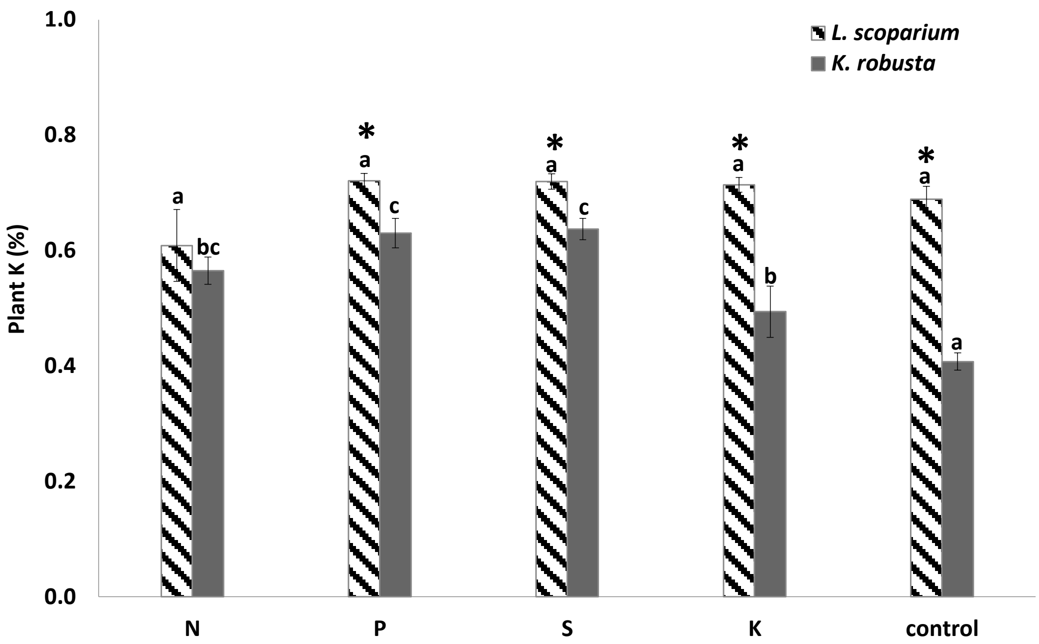


Figure 3. 9 Leaf K concentration in combination with macronutrient treatment (n=5). Treatments that share letters have means that do not differ significantly (*p≤0.05*). Asterisks (*) signify significant differences between species at *p≤0.05*.

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 3.9** 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 3.9**).

3.3.3 Rhizosphere soil nutrient concentration

Figures 3.10, 3.11, 3.12 and 3.13 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 3.10**).

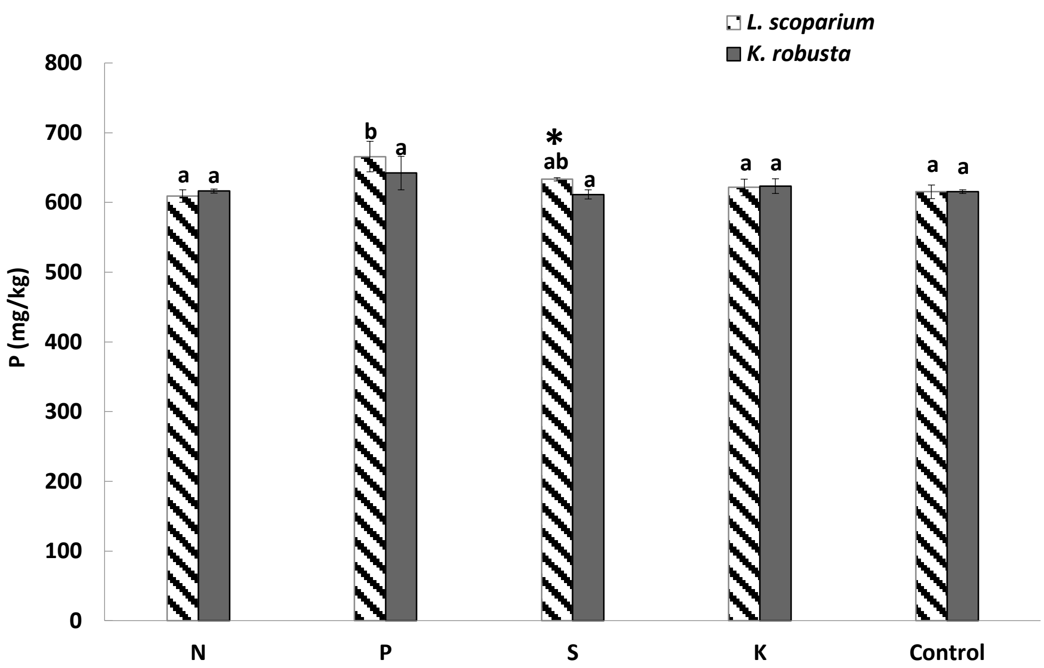


Figure 3. 10 Total soil P concentration in combination with macronutrient treatment (n=3). Treatments that share letters have means that do not differ significantly (*p≤0.05*). Asterisks (*) signify significant differences between species at *p≤0.05*.

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 3.10**). 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 3.11**).

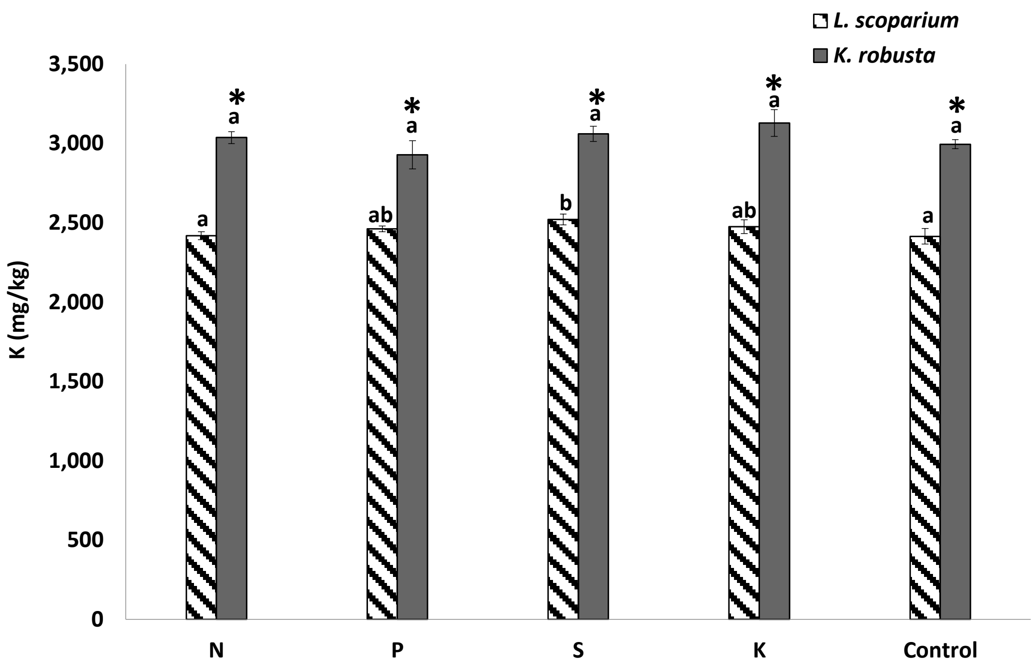


Figure 3. 11 Total soil K concentration in combination with macronutrient treatment (n=3). Treatments that share letters have means that do not differ significantly (*p≤0.05*). Asterisks (*) signify significant differences between species at *p≤0.05*.

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 3.12**). 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 3.12**).

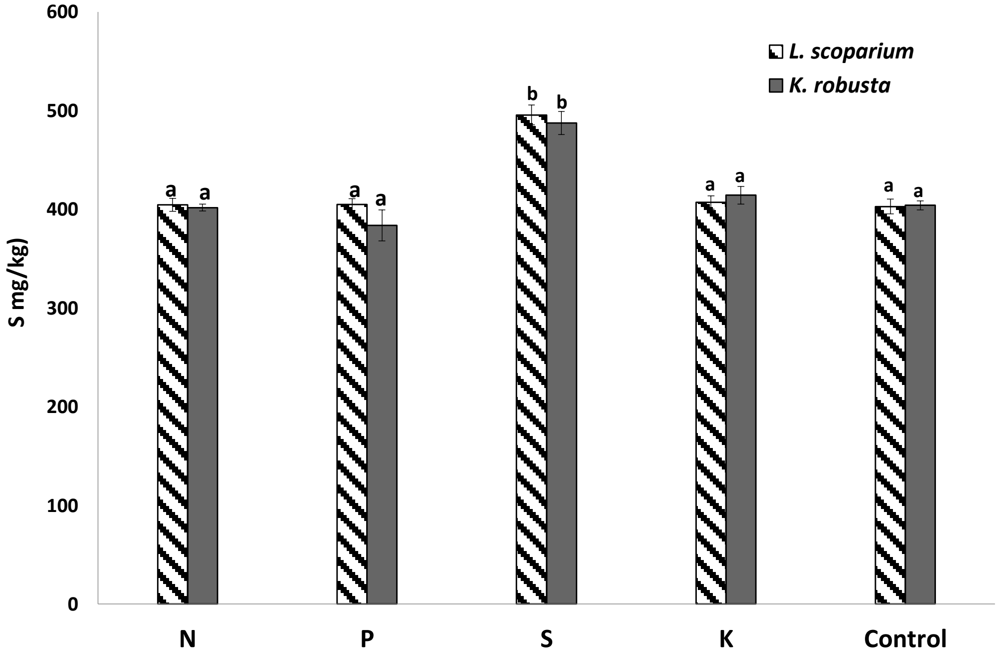


Figure 3. 12 Total soil S concentration in combination with macronutrient treatment (n=3). Treatments that share letters have means that do not differ significantly (*p≤0.05*).

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 3.13**).

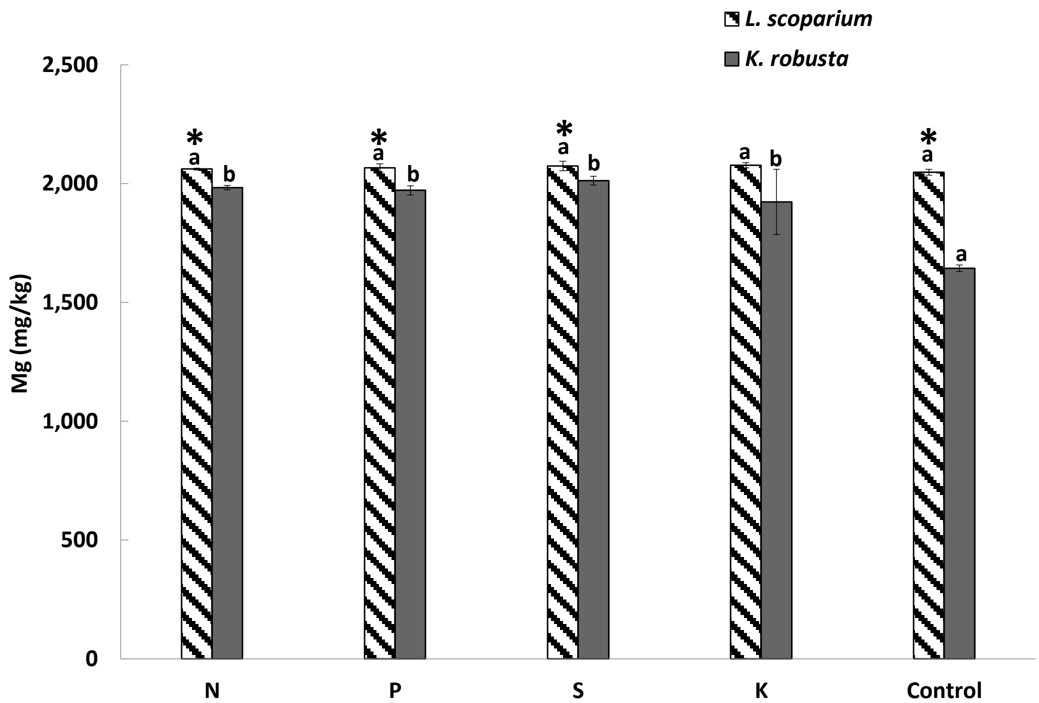


Figure 3. 13 Total soil Mg concentration in combination with macronutrient treatment (n=3). Treatments that share letters have means that do not differ significantly (*p≤0.05*). Asterisks (*) signify significant differences between species at *p≤0.05*.

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 14**). 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**.**

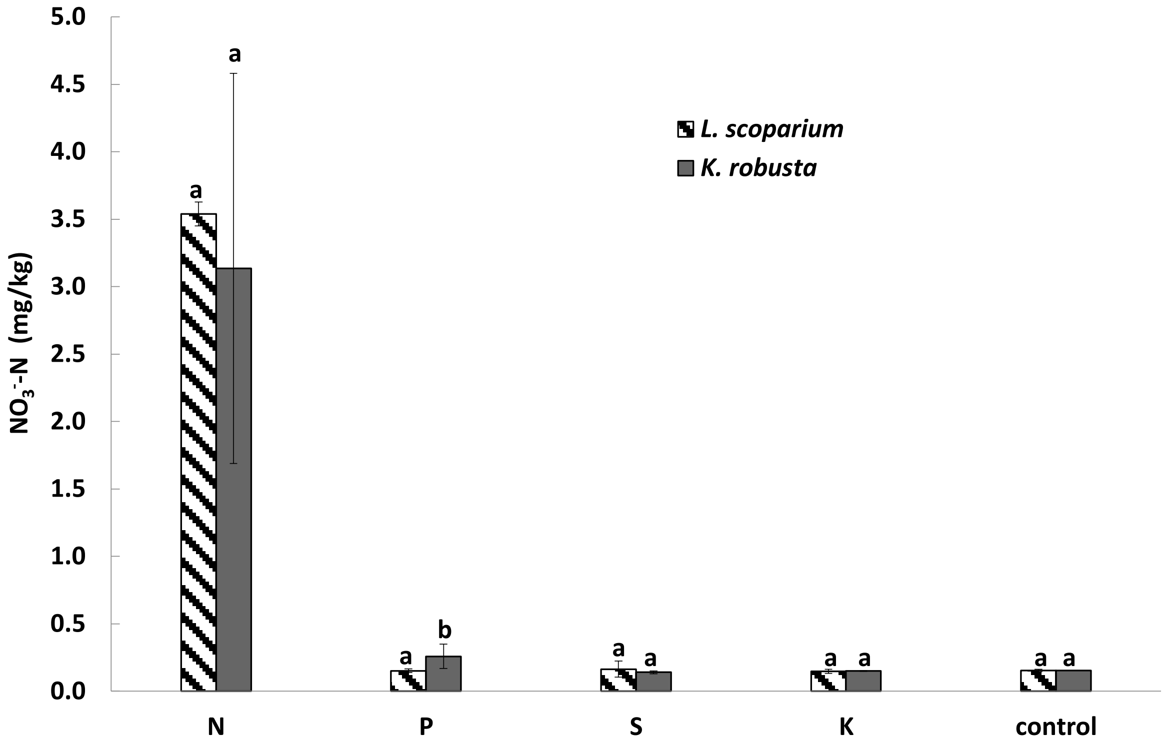


Figure 3. 14 Soil NO_3^--N in combination with macronutrient treatment (n=3). Treatments that share letters have means that do not differ significantly (*p≤0.05*).

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., 2003b). 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 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**

K. 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 *L. scoparium* and *K. robusta* 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., 2008a, Bai et al., 2013a, Bai et al., 2013b, Di et al., 1998, Hawke and Summers, 2006, Moir et al., 2013, Hedley et al., 2013a, Zaman et al., 2002, Bright and Healey, 2003, Haynes et al., 2009, Paramashivam, 2015b, Cogger et al., 2013). 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, Powlson et al., 2012, Murphy et al., 2007). 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 (Wang and Jia, 2010, Zaman et al., 2002, Kimberley et al., 2004, Singh and Agrawal, 2008). The application of biosolids provides nutrients, increases organic matter, improves soil structure, enhances nutrient absorption by plants (Weber et al., 2007, Singh and Agrawal, 2008, Morera et al., 2002, Antolín et al., 2005, Freeman and Cawthon, 1999), as well as increase the number and activities of soil microbes (Rogers and Smith, 2007, Singh and Agrawal, 2008, Cytryn et al., 2011). 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 (Singh and Agrawal, 2008, Lteif et al., 2007). 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.

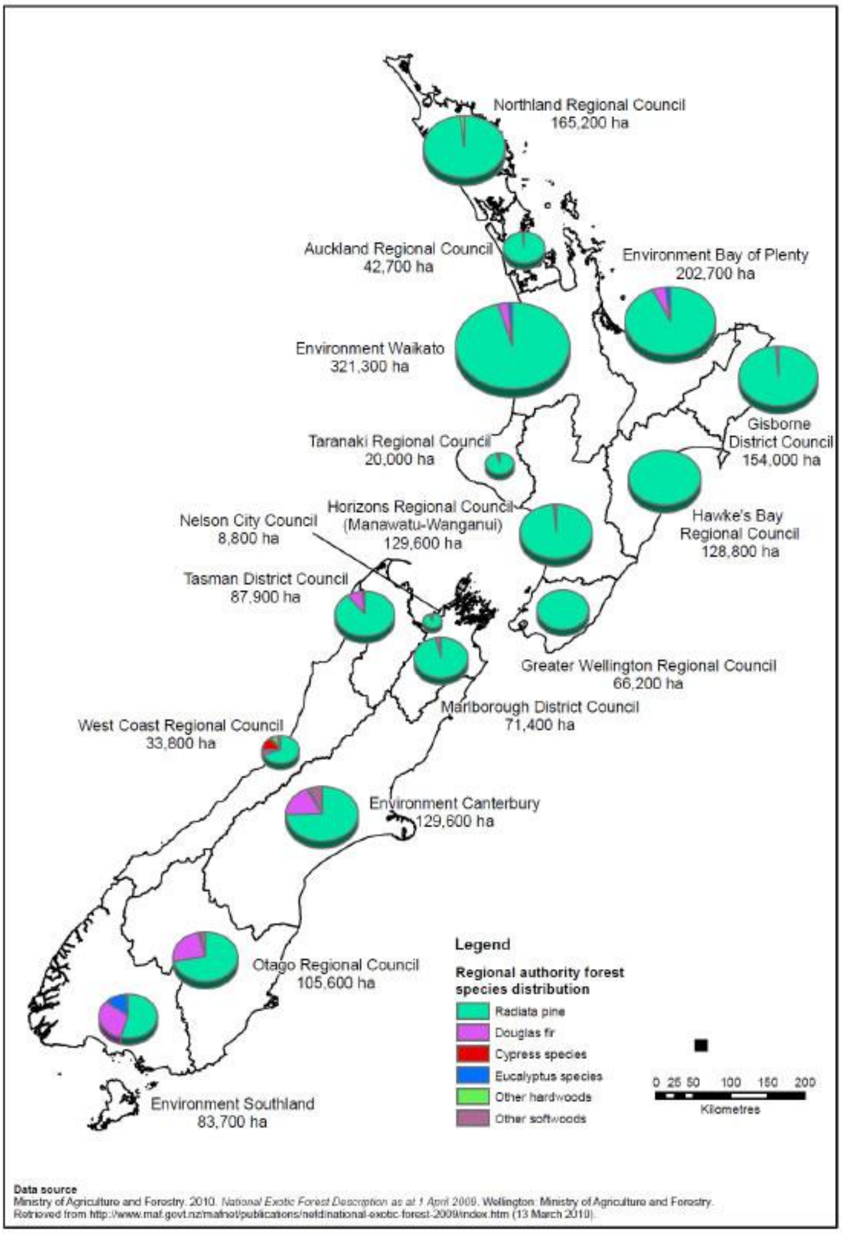


Figure 4. 1 Distribution of commercial forest species by region in New Zealand (MAF, 2010)

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* did not significantly affect its growth, the application of macronutrients of significantly increased N uptake. I hypothesize 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 (Kimberley et al., 2004, Antolín et al., 2005, Bradley, 2011, Hawke and Summers, 2006, Zaman et al., 2002, Singh and Agrawal, 2008, Wang et al., 2009). Further, I hypothesise 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 (**Figure 2A** - 43^0 25’19” S, 172^0 15’52”E), New Zealand, was used as planting medium. Fresh Dairy Shed Effluent (DSE) was collected from Lincoln University Dairy Farm, New Zealand (**Figure 2B** - 43°38’40"S, 172°26’ 32” E; 17 m asl) in January 2015. Biosolids were obtained from the Kaikoura Wastewater Treatment Plant, New Zealand (**Figure 2C** - 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.

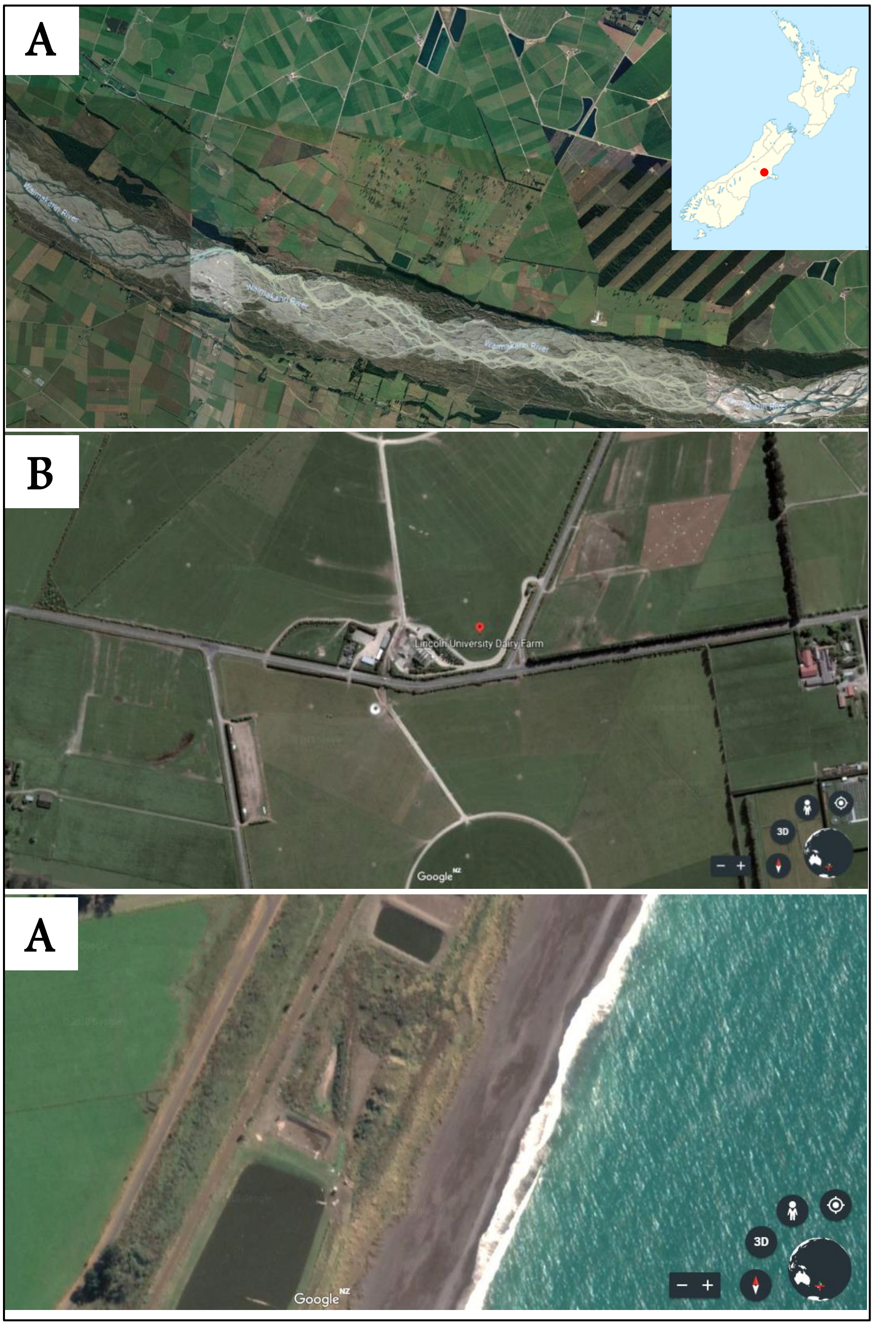


Figure 4. 2 (A) a former pine plantation of Eyrewell for obtaining soil medium; (B) Kaikoura Wastewater Treatment Plant for collecting biosolids; (C) Lincoln University Dairy Farm, New Zealand for sourcing DSE (Google Earth).

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 4.3**). The treatments contained 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 DSE and biosolids were first homogenised thoroughly using a 100 L plastic tank and black tarpaulin respectively (**Plate 4.1).** DSE then further stored in the fridge for further application in the greenhouse. The biosolids were mixing 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 using a randomized block design.

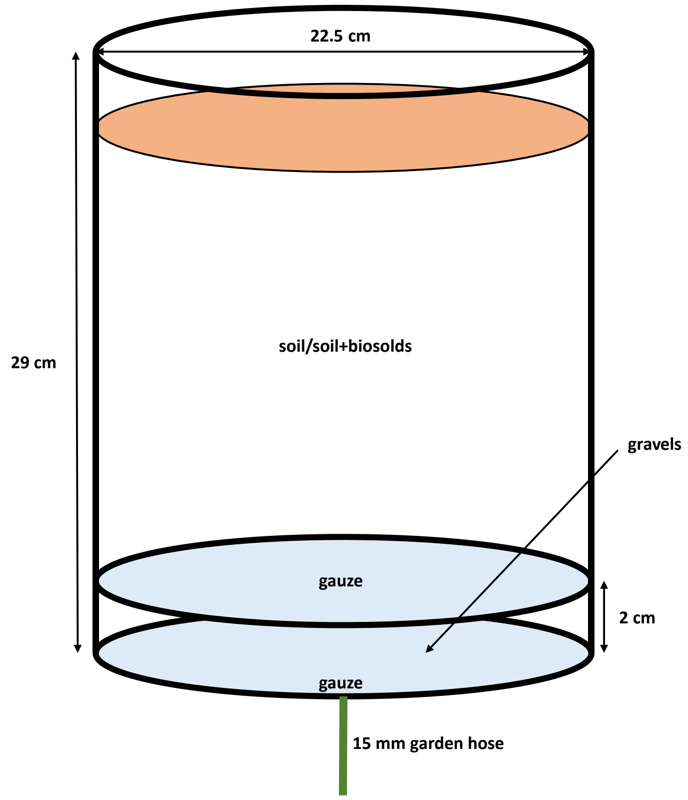


Figure 4. 3 Pot design used in the present study

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 $70^{0}C$ until constant weight was obtained and ground using a Retch ZM200 grinder.

a b

Plate 4. 1 (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

Significant differences (α=0.05) between treatments were determined by analysis of variance, 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.

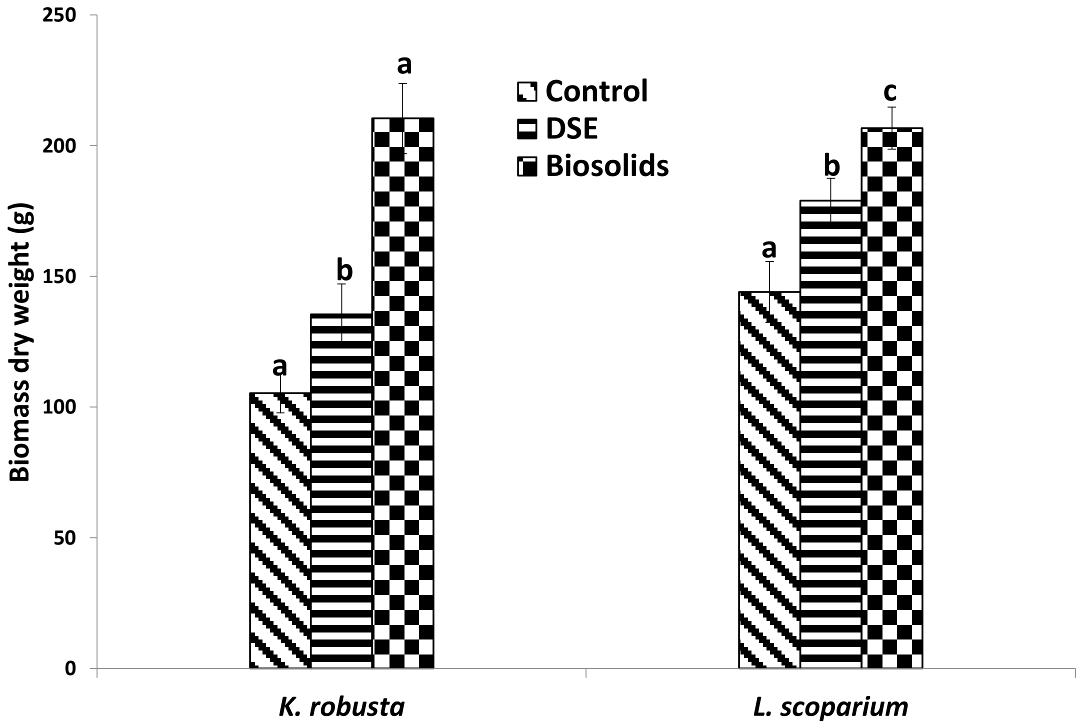


Figure 4. 4 Cumulative above ground biomass of *L. scoparium, K. robusta, and L. perenne* in combination with DSE, biosolids, and the control (n=4). Treatments that share letters have means that do not differ significantly (*p≤0.05*).

Figure 4.4 shows that with the exception combination of DSE and *L. multiflorum*, compared to the control, the addition of 2600 kg N ha^{-1} equiv. of biosolids and 200 kg N ha^{-1} equiv. 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 4.4** and **4.5**).

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}.

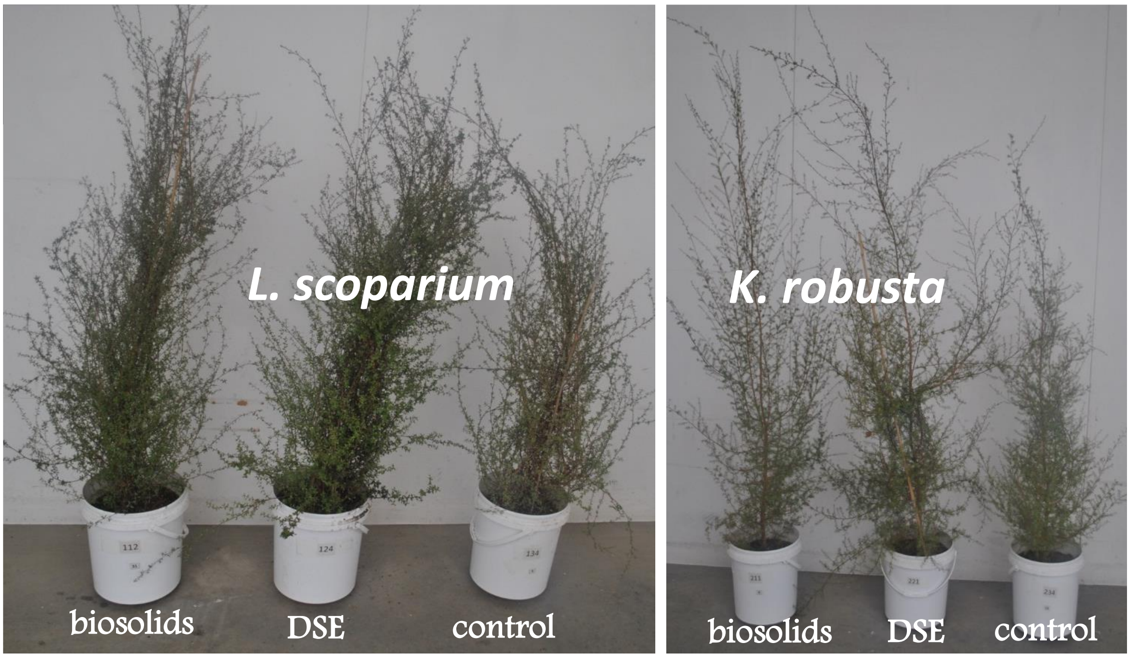


Figure 4. 5 Plant growth responses under different treatments of 12 weeks experiment period under Eyrewell soil medium.

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 4.6**). In combination with DSE, *L. scoparium* produced 25% higher above ground dried biomass than that of in *K. robusta.*

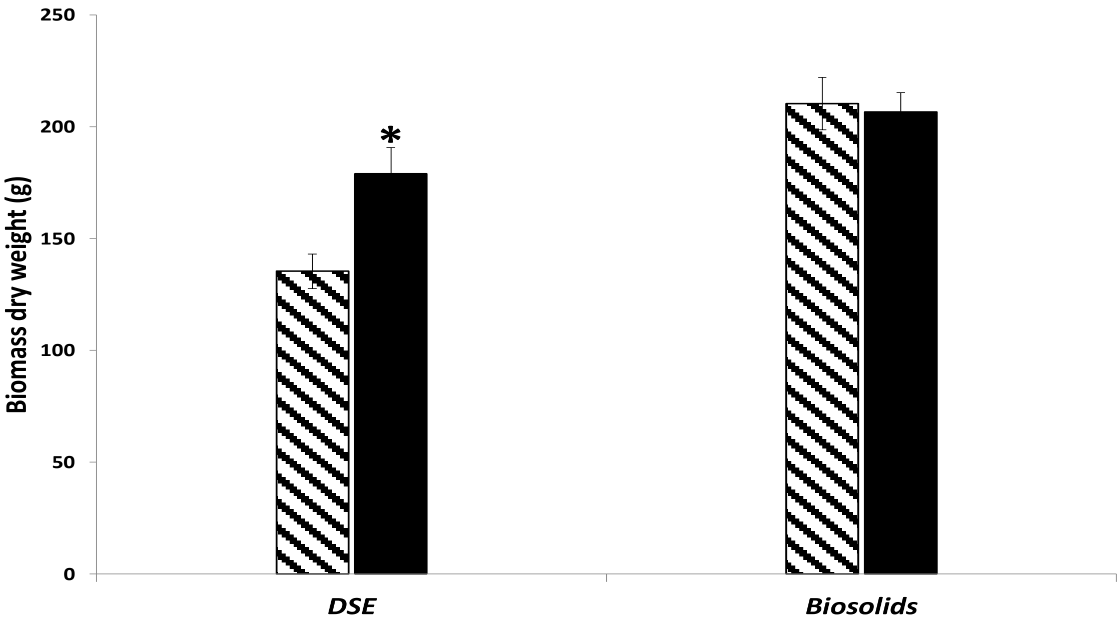


Figure 4. 6 Comparison cumulative above ground biomass of *L. scoparium* and *K. robusta* combination with DSE and biosolids (n=4). Asterisks (*) signify significant differences between *K. robusta* (striped bars) and *L. scoparium* (solid bars) at *p≤0.05*.

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 4.7** and **4.8.** 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 4.7**). 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 4.7**).

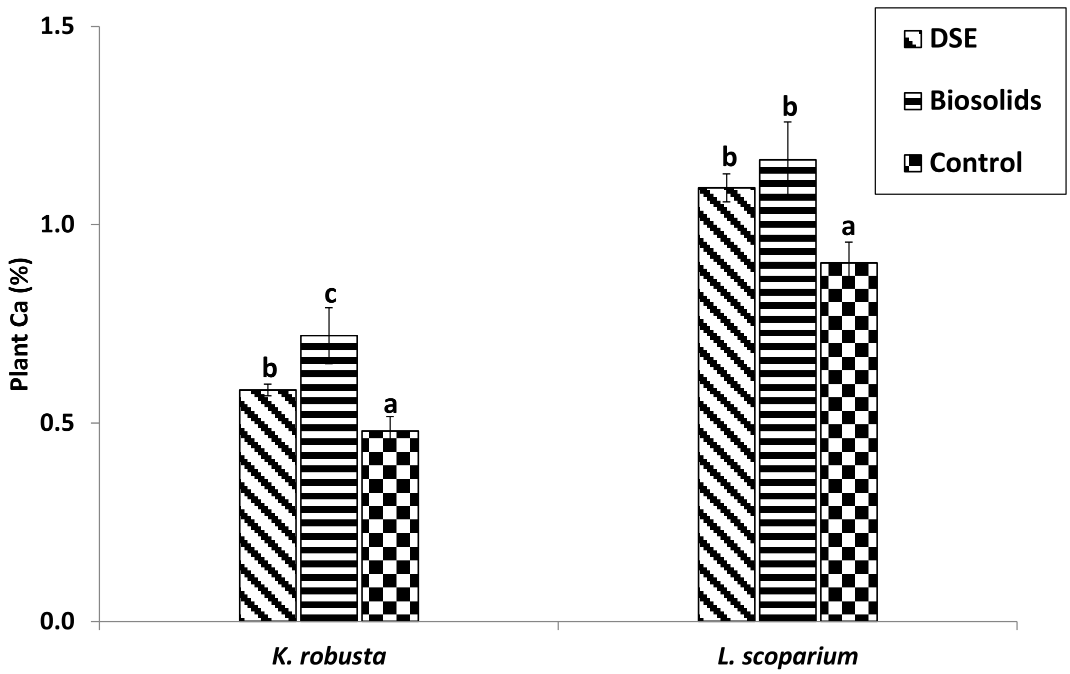


Figure 4.7 Total concentrations of foliar Ca (%) of *L. scoparium* and *K. robusta* measured at the end of experiment. Error bars represent the standard error of the mean. Treatment that share letters have means that do not differ significantly (p<0.05).

In combination with biosolids, *K. robusta* accumulated 32% higher foliar S concentration than the control (**Figure 4.8B**). In contrast, **Figure 4.8B** 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 4.8A**).

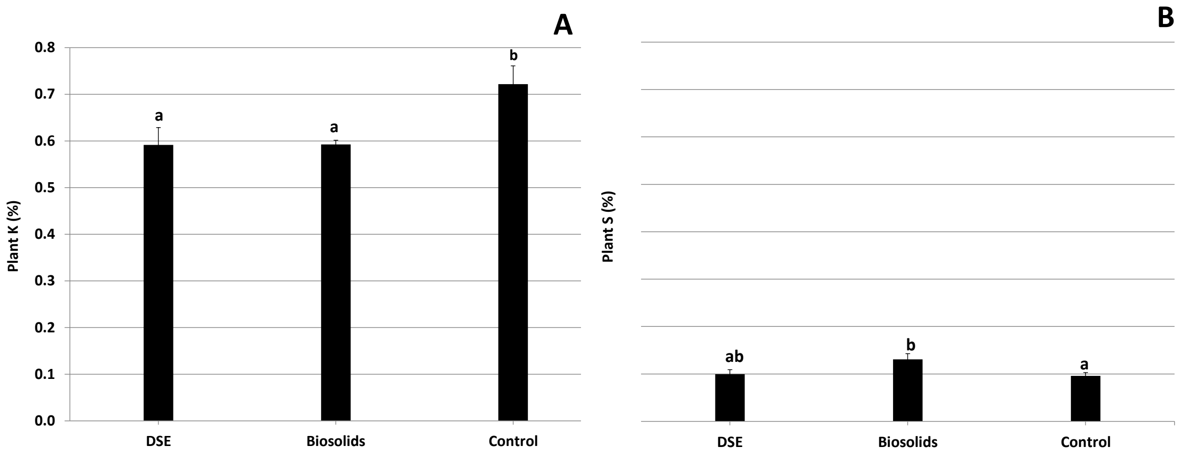


Figure 4. 8 Total concentrations of foliar (A) K and (B) S (%) of *K. robusta* measured at the end of experiment. Error bars represent the standard error of the mean. Treatment that share letters have means that do not differ significantly (p<0.05).

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 4.9)**. 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*.

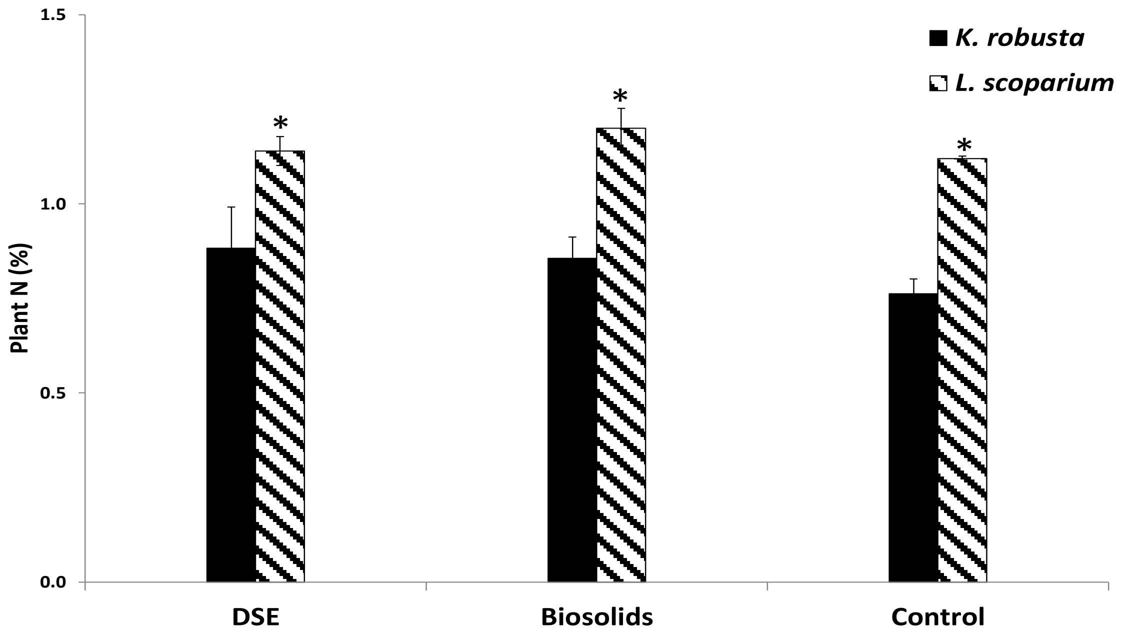


Figure 4. 9 Total concentrations of foliar N (%) of *L. scoparium* and *K. robusta* measured at the end of experiment. Error bars represent the standard error of the mean. Asterisks (*) signify significant differences between the effluents (striped bars) and controls (solid bars) at *p≤0.05*.

Micronutrients

Figure 4.10 shows total concentrations of foliar micronutrients (mg/kg) of *L. scoparium* and *K. robusta* measured at the end of experiment.

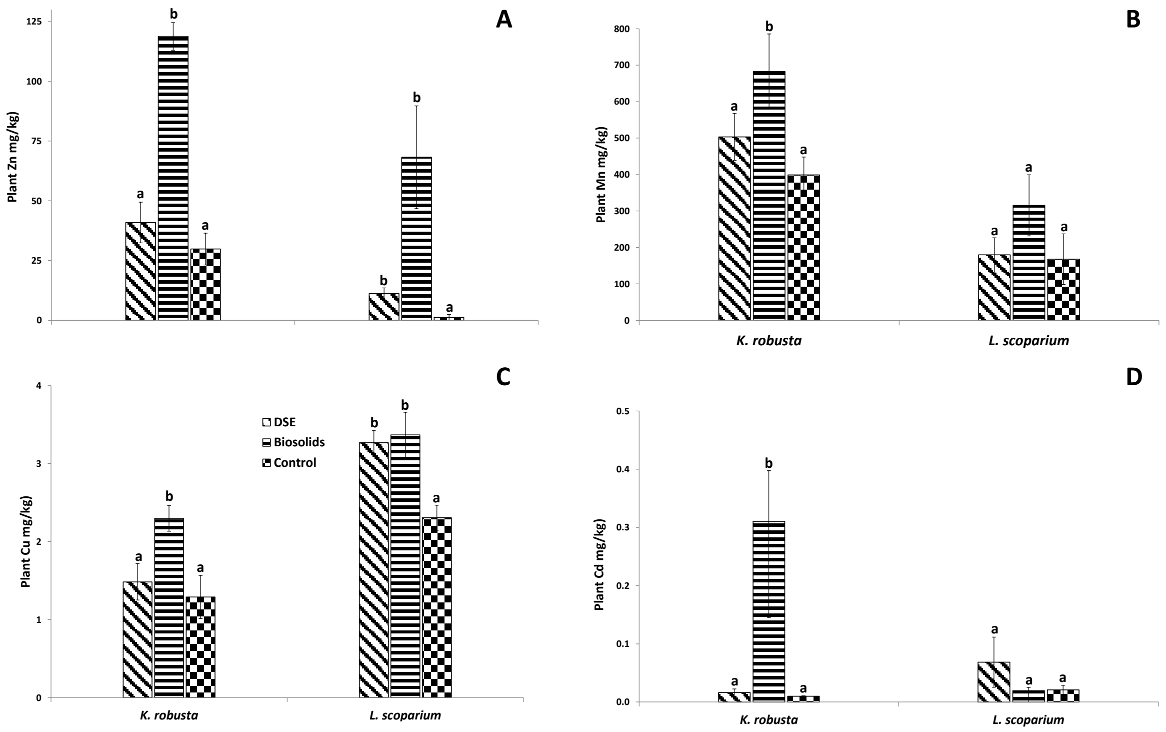


Figure 4. 10 Total concentrations of foliar micronutrients (mg/kg) of *L. scoparium* and *K. robusta* measured at the end of experiment. Error bars represent the standard error of the mean. Treatment that share letters have means that do not differ significantly (p<0.05)

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 4.10B**). Biosolids significantly increased the uptake of Zn by both *L. scoparium* and *K. robusta* by 569% and 298% respectively (Figure 11). 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 4.10A**).

K. robusta accumulated significantly ($p \leq 0.05$) higher Cd in the biosolids treatment (**Figure 4.10D).** In contrast, **Figure 4.10D** 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 ($p \leq 0.05$) the uptake of Mn (**Figure 4.10B**). The application of biosolids increased the concentration of foliar Mn in *K. robusta* by 71% compared to the control. In contrast, **Figure 4.10B** 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 4.10B** and **4.10D**).

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 (Weber et al., 2007, Brady, 2008, Antolín et al., 2005), contributed in retaining nutrients and making them available to plants (Garcıa-Gil et al., 2004, Kaur et al., 2008, Wong et al., 2001, Delibacak et al., 2009). 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. (2016a) 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 Ca, K, S, Cu, Zn, and Mn to plants. The biowastes may have increased nutrient cycling, making more nutrients available (Murphy et al., 2007, Antolín et al., 2005, Morera et al., 2002, 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., 2003b), particularly elevating the availability of Zn (Keller and Römer, 2001a, Hinsinger, 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 of 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 of *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 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 our finding. In the present study, the DSE and biosolids contained somewhat lower concentrations of Cu and Zn the experimental period was shorted. 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 (Broadley et al., 2007, Alloway, 2013). 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 (Esperschuetz et al., 2016a, Alloway, 2013). 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 *L. scoparium, K. robusta, P. radiata, L. multiflorum*, *B. napus*, and *S. bicolor* 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 have co-authored of 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 *L. multiflorum* (Santibanez et al., 2008, Ahumada et al., 2009, Bai et al., 2013b). 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 (*Brassica 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 or preventing nutrient leaching into waterways (Turan and Esringu, 2007, Licht and Schnoor, 1993, Pilipovic et al., 2006, Wang et al., 2009, Barceló and Poschenrieder, 2003). 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 (Daniels et al., 2001, Schmidt et al., 2001, Bugbee, 1999b). 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. radiate, 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, Esperschütz et al., 2016). 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, 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 w 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., 2016a). The lysimeters were weeded fortnightly.

5.3.2 Analyses and measurements

A final destructive harvest of all lysimeters was performed after 18 w. 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 Esperschütz et al. (2016); Esperschuetz et al. (2016a); Esperschuetz et al. (2016b); 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 w 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**).

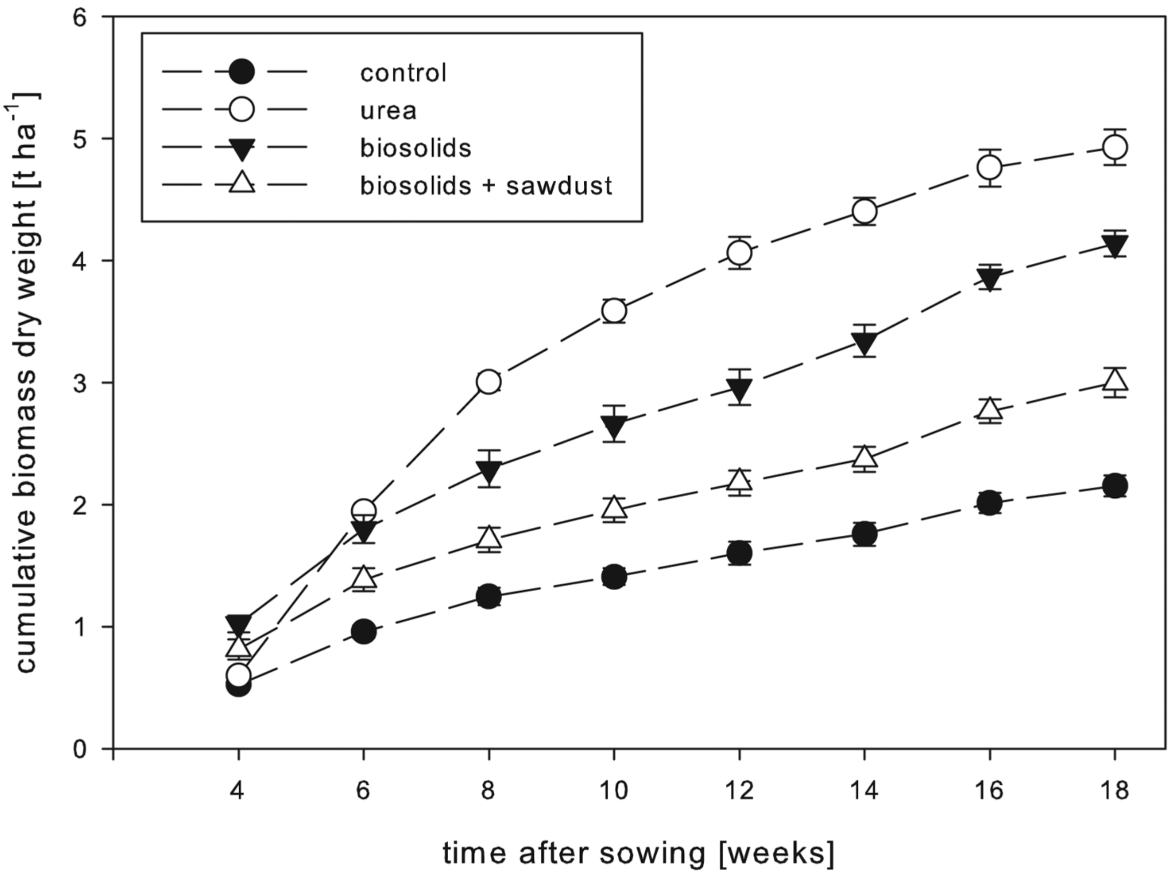


Figure 5. 1 Cumulative biomass (dry weight) in t ha^{-1} equivalent during the 18-wk experimental period. Each point is the average of six replicates with bars representing the standard error of the mean. Non-overlapping bars indicate significant differences (*p* ≤ 0.05). (Esperschuetz, et al., 2016)

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, 1999a).

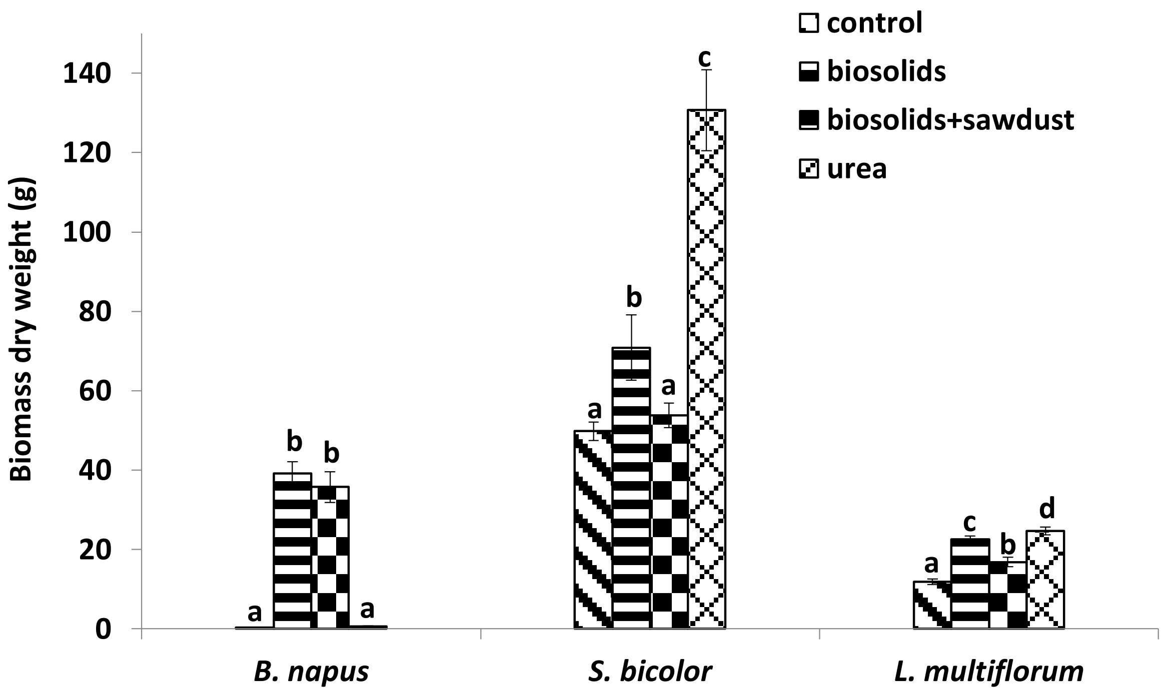


Figure 5. 2 Total aboveground plant biomass of *B. napus, S. bicolor* and *L. multiflorum* at the end of the experiment. Significant differences ($p \leq 0.05$) are represented by lowercase letters. (Esperschuetz, et al., 2016b)

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.

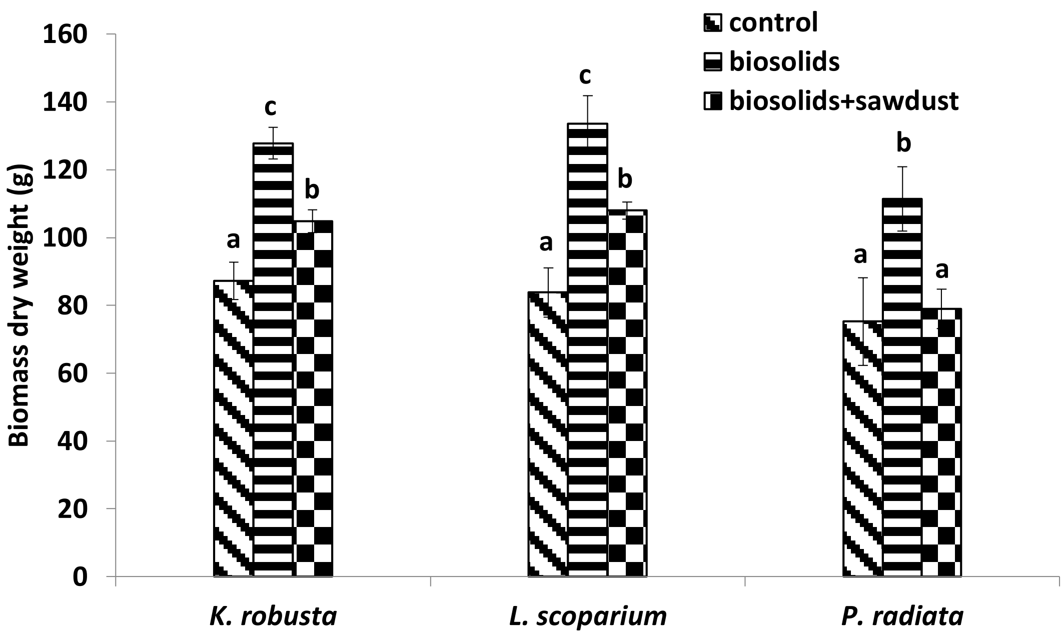


Figure 5. 3 Aboveground plant biomass [g DW] after a growing period of 18 weeks in control, biosolids and biosolids+sawdust treatments (n=4±se). Significant differences between treatments at p ≤ 0.05 are indicated by letters (a, b, c) within plant species. Esperschuetz, et al., (2017)

P. 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 (Whiteside et al., 2012, Smith et al., 2011). 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). Esperschütz et al. (2016).

	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 micronutrients 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). Esperschütz et al. (2016).

	Control	Urea	biosolids	Biosolids+sawdust
		mg kg^{-1} dry wt		
N	11.4 (1.0)^{b}	8.9 (0.3)^{a}	10.5 (0.3)^{ab}	9.9 (0.8)^{ab}
P	5.9 (0.1)^{a}	6.0 (0.2)^{a}	10.3 (0.6)^{c}	8.7 (0.4)^{b}
K	21.6 (2.3)^{a}	19.8 (0.7)^{a}	150.4 (8.3)^{c}	91.7 (3.7)^{b}
S	37.4 (1.0)^{a}	35.2 (0.8)^{a}	60.2 (1.7)^{c}	51.0 (2.4)^{b}
Ca	96.0 (3.9)^{a}	105.8 (13.6)^{a}	118.7 (14.4)^{a}	105.5 (7.1)^{a}
Mg	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).

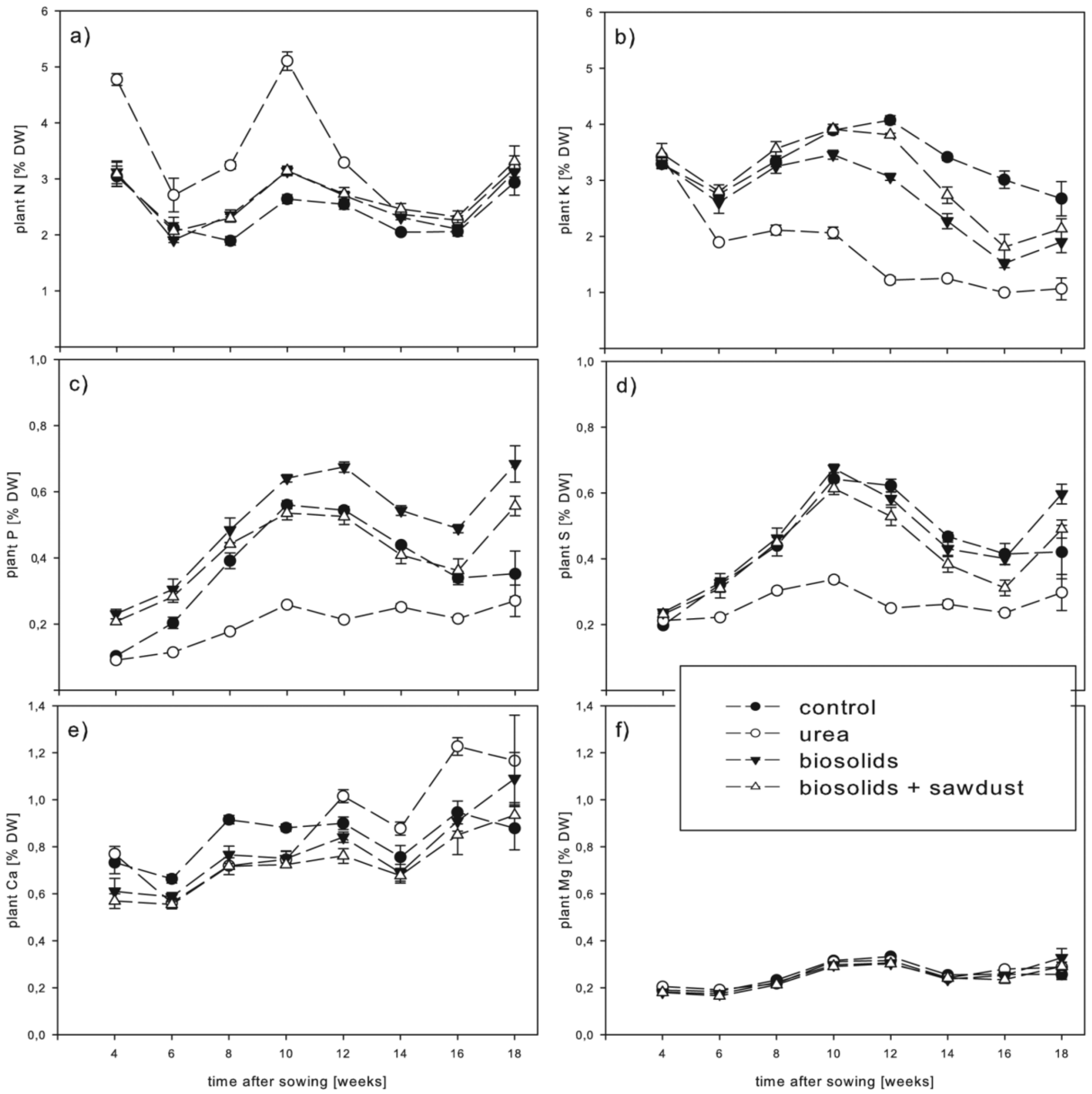


Figure 5. 4 Average concentrations of macro elements over the experimental period. Error bars represent the standard error of the mean. Non-overlapping error bars indicate significant difference between means (*p* ≤ 0.05). Adopted from Esperschuetz, et al., (2016)

L. 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 (Oliver et al., 2005, Khoshgoftar et al., 2004). 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 (Antoniadis et al., 2008a, Ahumada et al., 2009, 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* (Antoniadis et al., 2008a, Ahumada et al., 2009, 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 $p \leq 0.05$. Esperschütz et al. (2016)

	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 micronutrient 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 micronutrients in *B. napus* and *S. bicolor* biomass in response to different soil amendments. Different lowercase letters indicate significant differences between treatments at *p* ≤ 0.05. Esperschütz et al. (2016)

	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* ≥ 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.

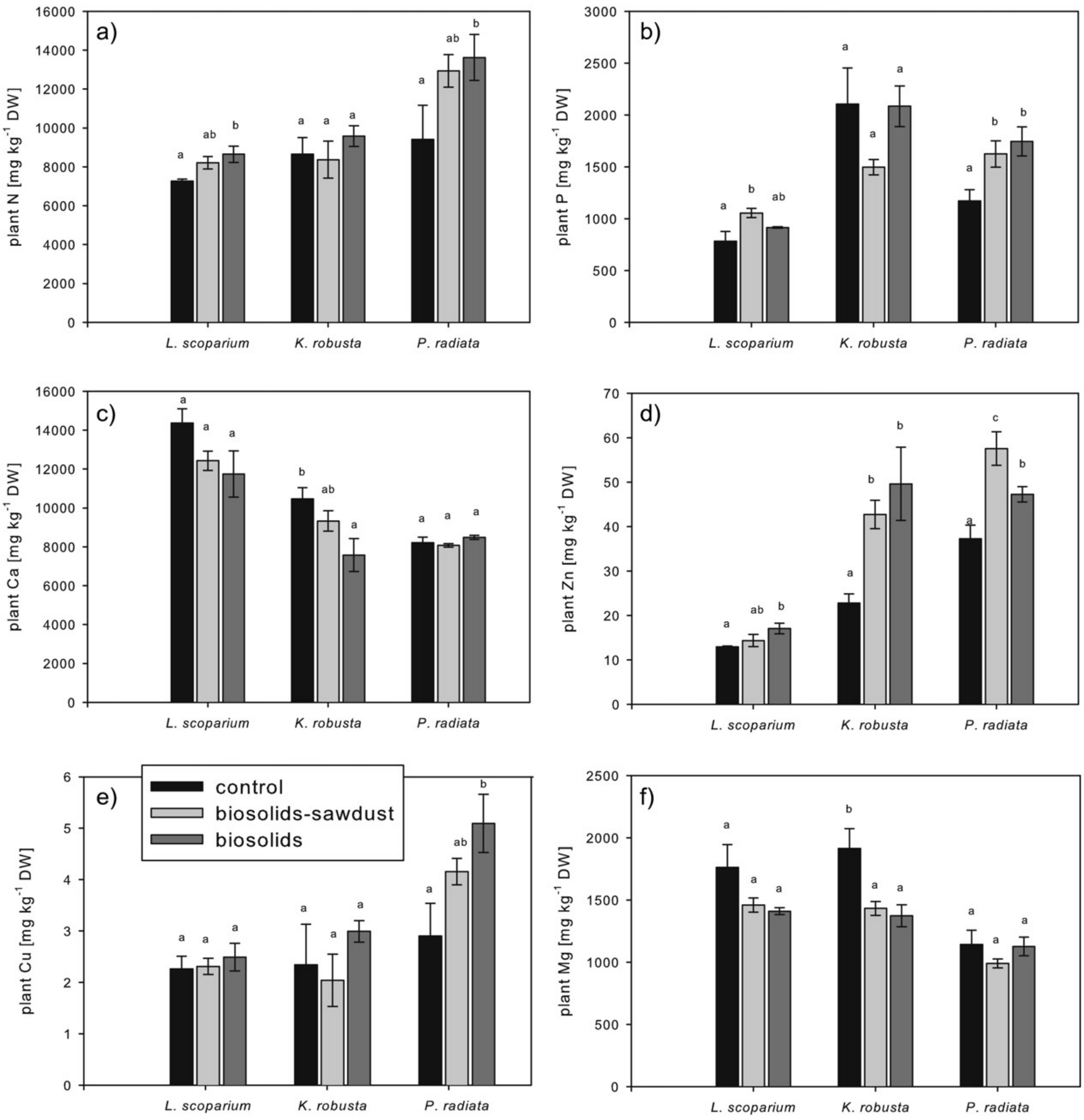


Figure 5. 5 Concentration of selected macro- and micronutrients in plant leaves [mg kg−1 DW] after a growing period of 18 weeks in control, biosolids-sawdust and biosolids amended treatments (n=4±se). Significant differences between treatments at p ≤ 0.05 are indicated by letters (a, b, c) within plant 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 (Koo et al., 2013, Bertin et al., 2003b). 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 $p \leq 0.05$. Esperschütz et al. (2016)

	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	00a 0.01 ± 0.00a↓
Fe	1.10 ± 0.09a	2.15 ± 0.32 b	1.01 ± 0.05a	09a 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 (Hedmark and Scholz, 2008, Keeling and Bohlmann, 2006, Sanati, 2005, Hall, 2007). 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 N 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, 1999a, 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**).

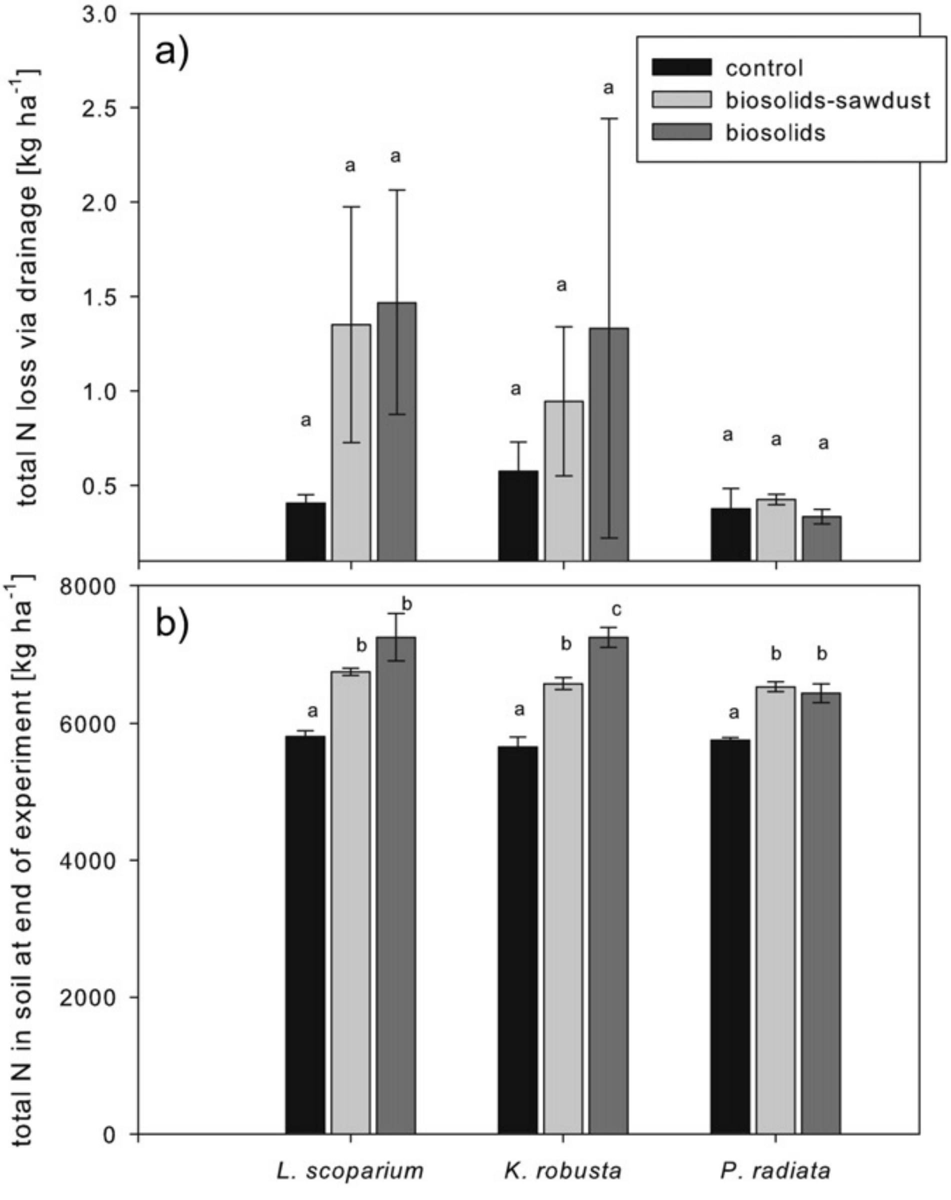


Figure 5. 6 Total N loss via NO_3^- leaching (a) and total N in soil (b) at end of the experiment [kg ha−1] after a growing period of 18 weeks in control, biosolids-sawdust and biosolids amended treatments (n = 4 ± se). Significant differences between treatments at p ≤ 0.05 are indicated by letters (a, 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. (Mohammad and Mazahreh, 2003, Vogel et al., 2015, Oron et al., 1995, Jiménez-Cisneros, 1995, Coppola et al., 2004, Toze, 2006).

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) (Mohammad Rusan et al., 2007, Mohammad and Mazahreh, 2003, Bedbabis et al., 2014, Tarchouna et al., 2010, Yadav et al., 2002, Toze, 2006).

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 (Kaewmano et al., 2009, Tisdall and Oades, 1982, Crescimanno et al., 1995). 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 (Lado and Ben-Hur, 2009, Ternes, 1998, Ternes et al., 2004, Griffin and Harrahy, 2014).

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 hypothesised 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.), wharariki (*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})	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

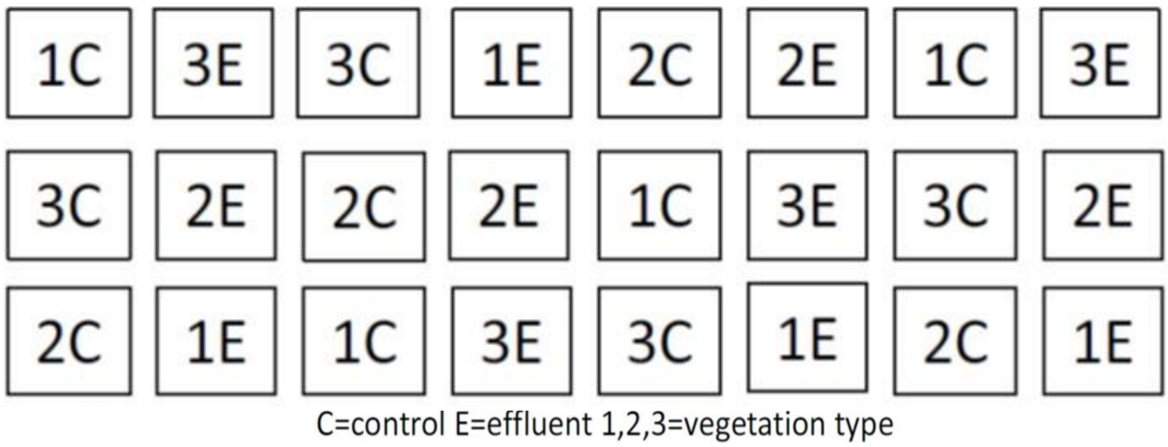
Species selection

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 due to tannins and trace elements (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. Figure 3 shows the site shortly after planting. In January 2016, TMW was applied at the level of 200 kg N ha^{-1}.



Vegetation type 1 = *L. scoparium and K. robusta*
Vegetation type 2 = *O. paniculata, C. robusta, P. cunninghamii,* and *P. arbore*us
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:

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

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.

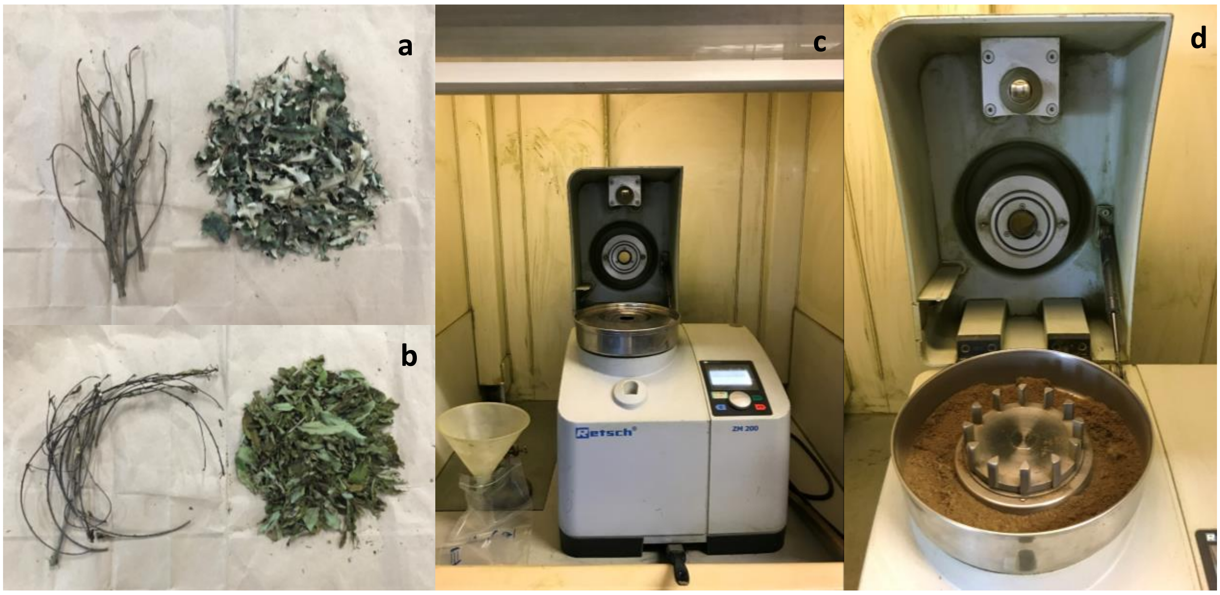


Plate 6. 2 Dried leaves separated from branches (a,b) and grinder Retch ZM200 for grinding the samples (c,d)

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 (*α=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 2 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%.

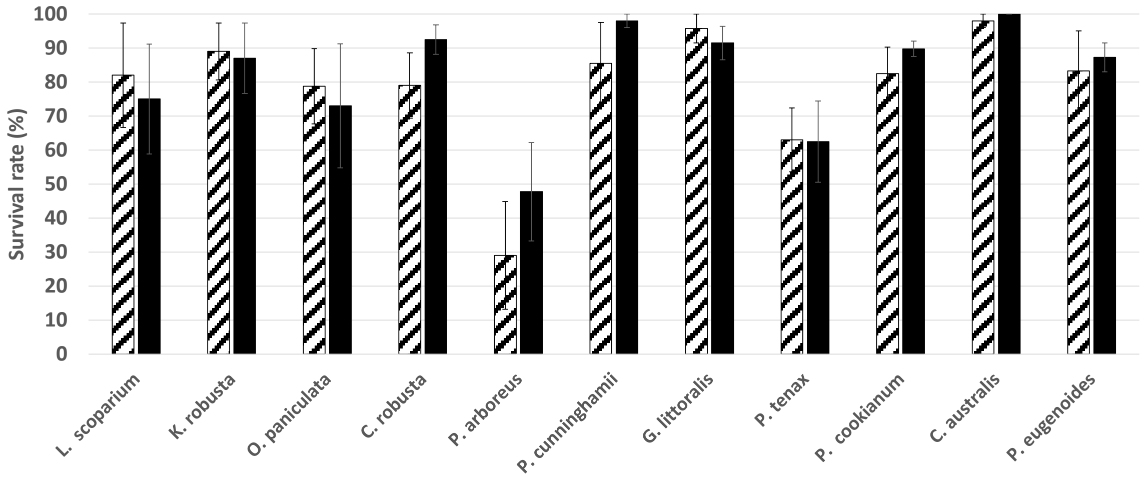


Figure 6. 3 Survival rate (%) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean (n=4). There were no significant differences between the controls (striped bars) and 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, compared to the controls.

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

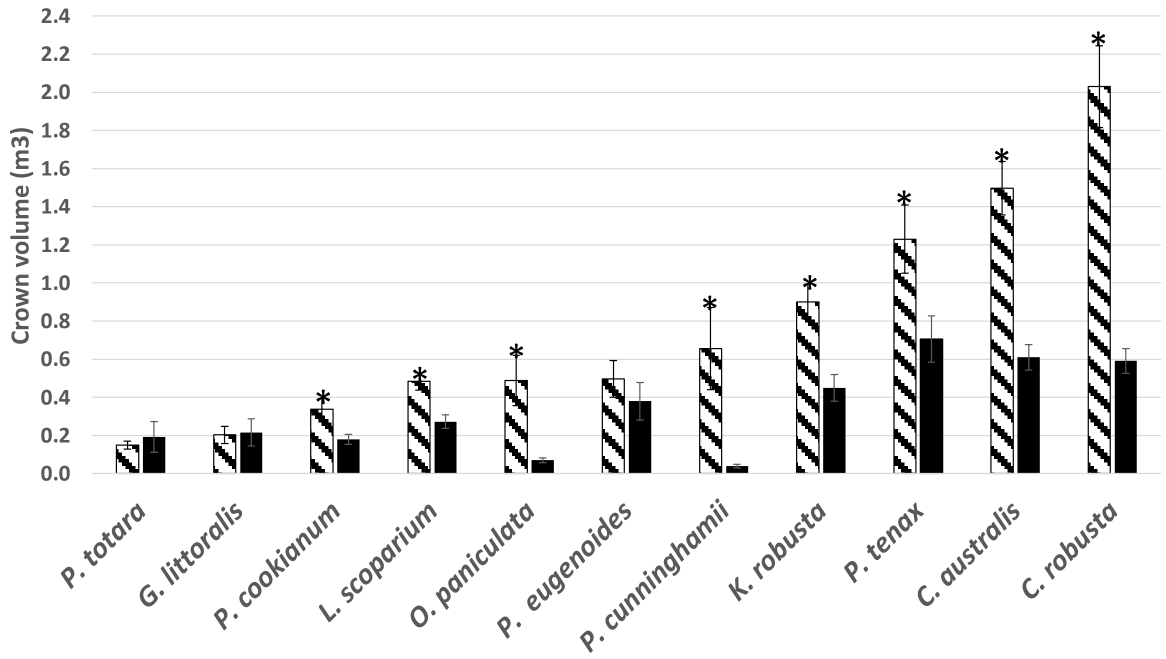


Figure 6. 4 Crown volume (m^3) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean. Asterisks (*) signify significant differences between the effluents (striped bars)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

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 ($p \leq 0.05$) 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).**

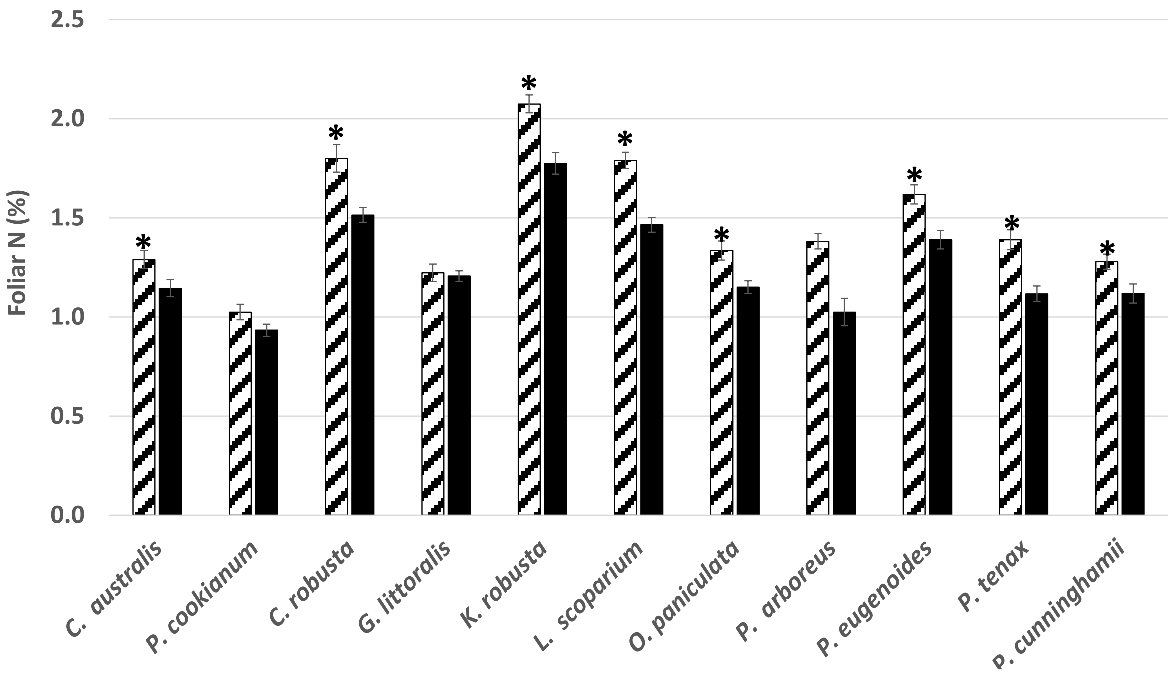


Figure 6. 5 Total concentration of foliar N (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean. Asterisks (*) signify significant differences between the effluents (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 6.7**).

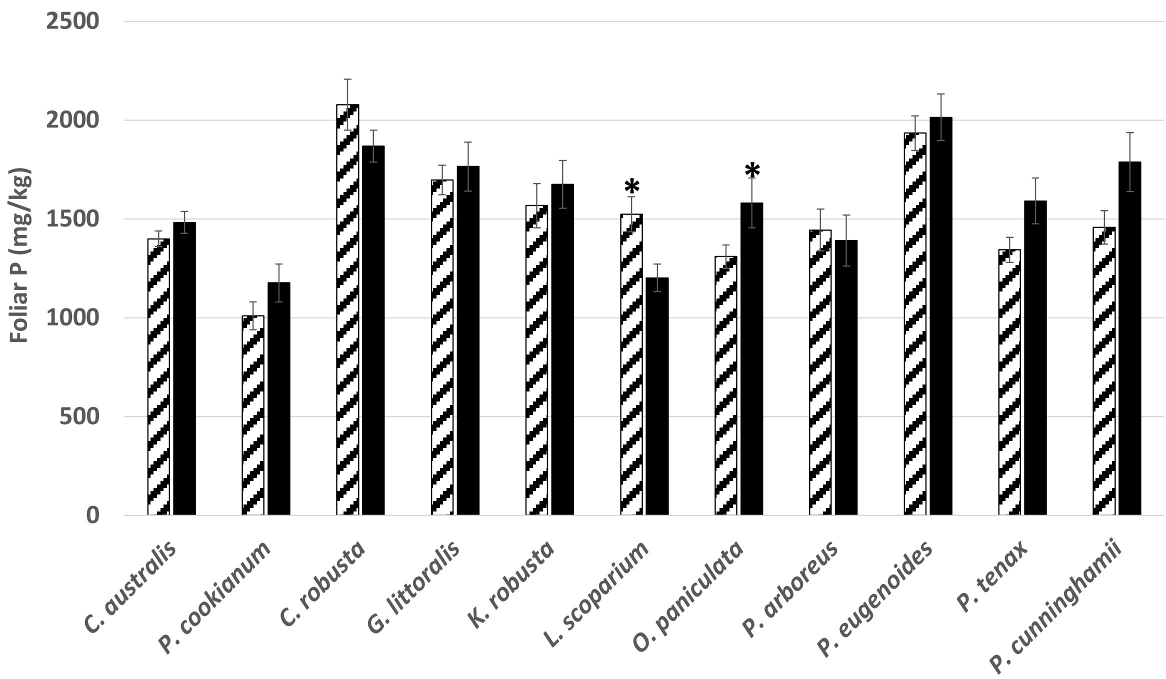


Figure 6. 6 Total concentration of foliar P (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean. Asterisks (*) signify significant differences between the effluents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

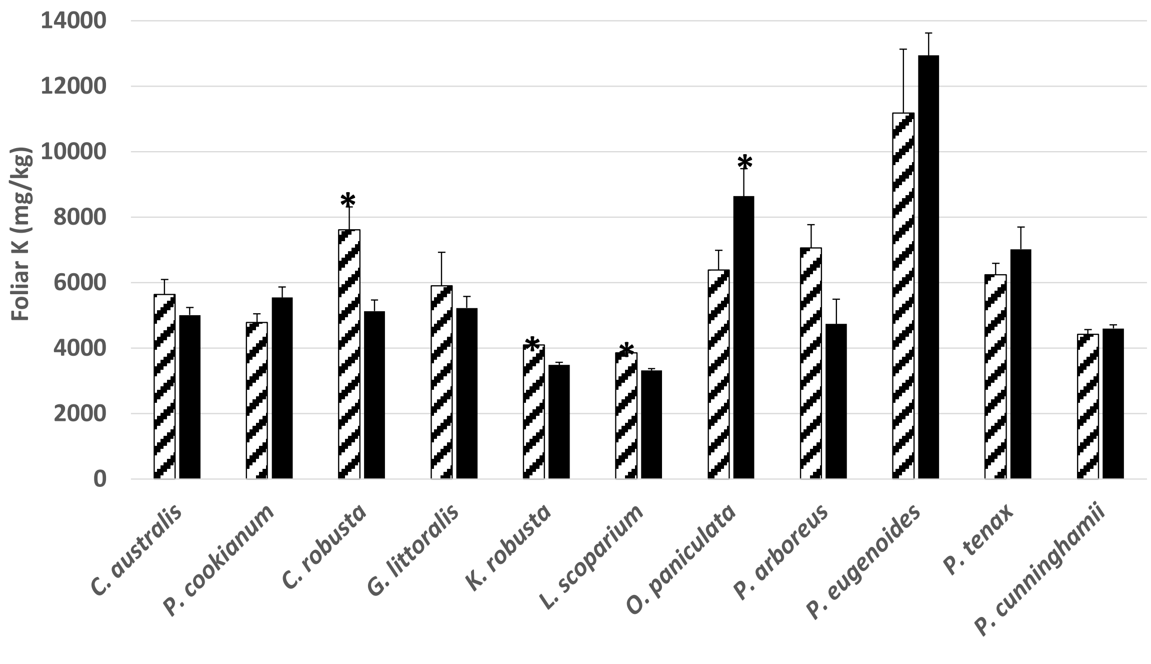


Figure 6. 7 Total concentration of foliar K (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean. Asterisks (*) signify significant differences between the effluents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

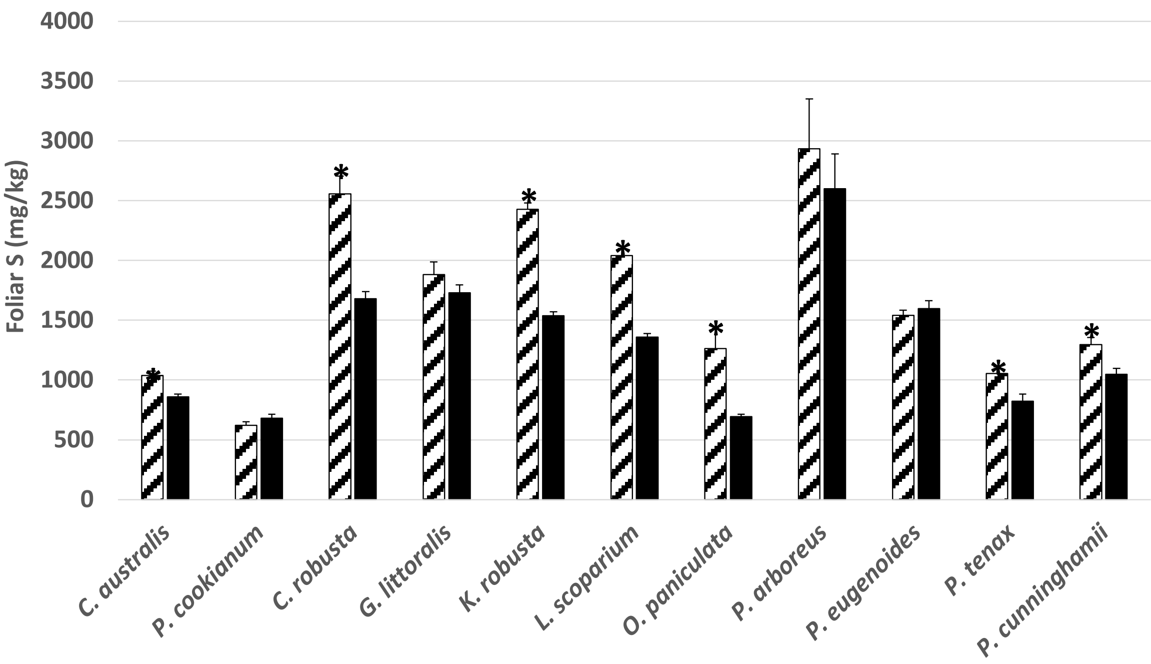


Figure 6. 8 Total concentration of foliar S (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean. Asterisks (*) signify significant differences between the effluents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

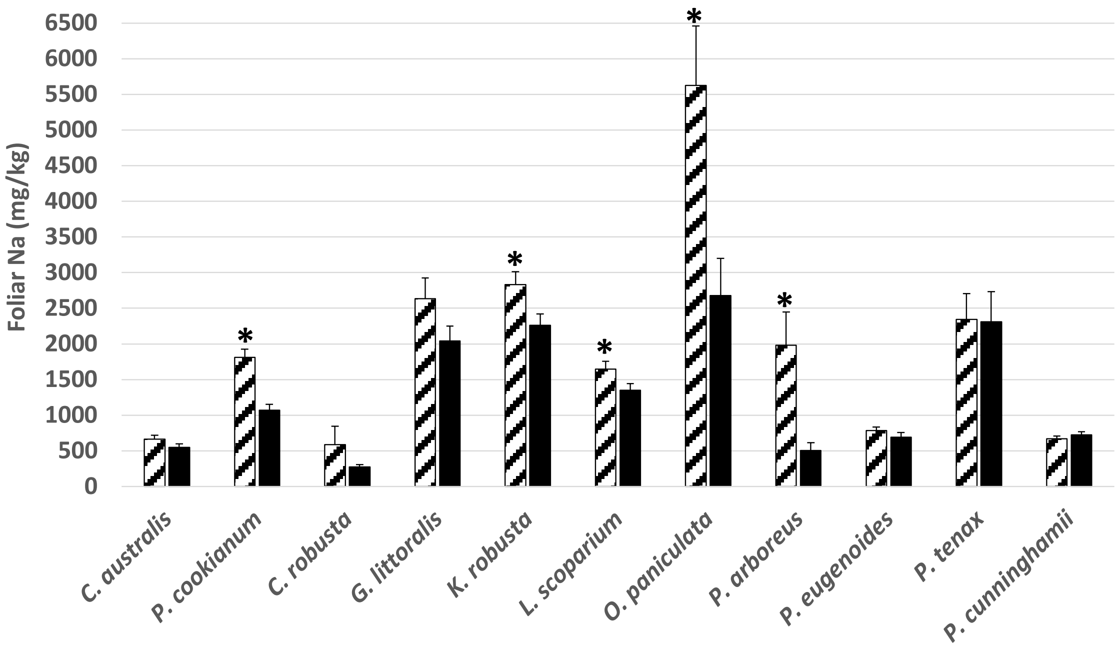


Figure 6. 9 Total concentration of foliar Na (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean. Asterisks (*) signify significant differences between the effluents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

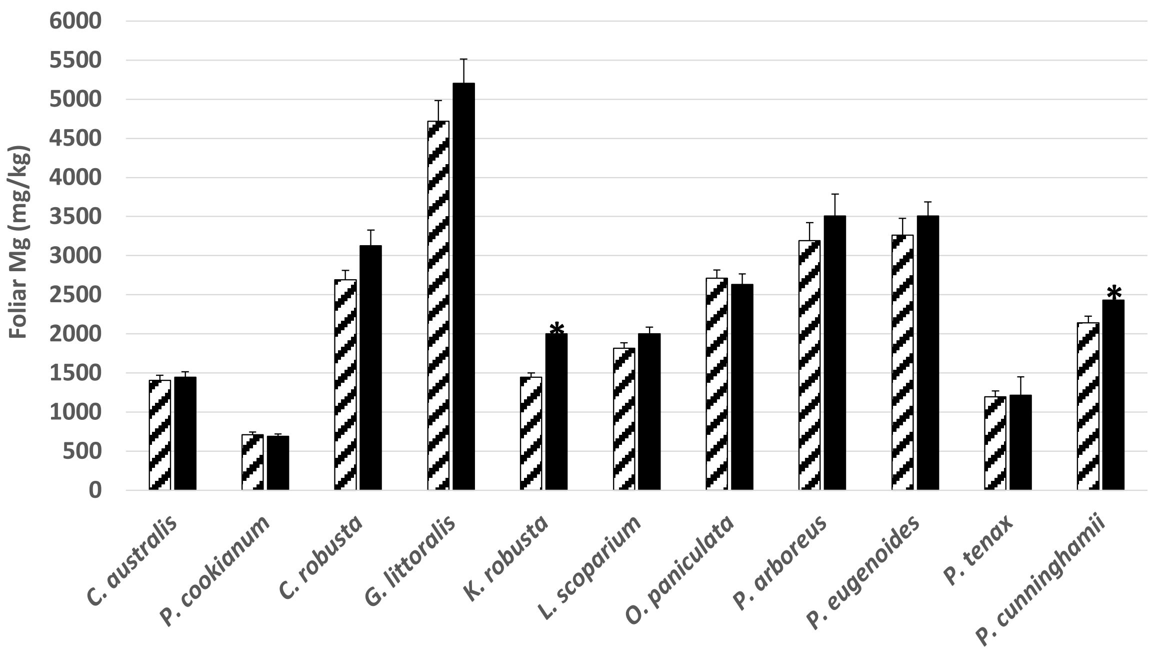


Figure 6. 10 Total concentration of foliar Mg (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean. Asterisks (*) signify significant differences between the effluents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

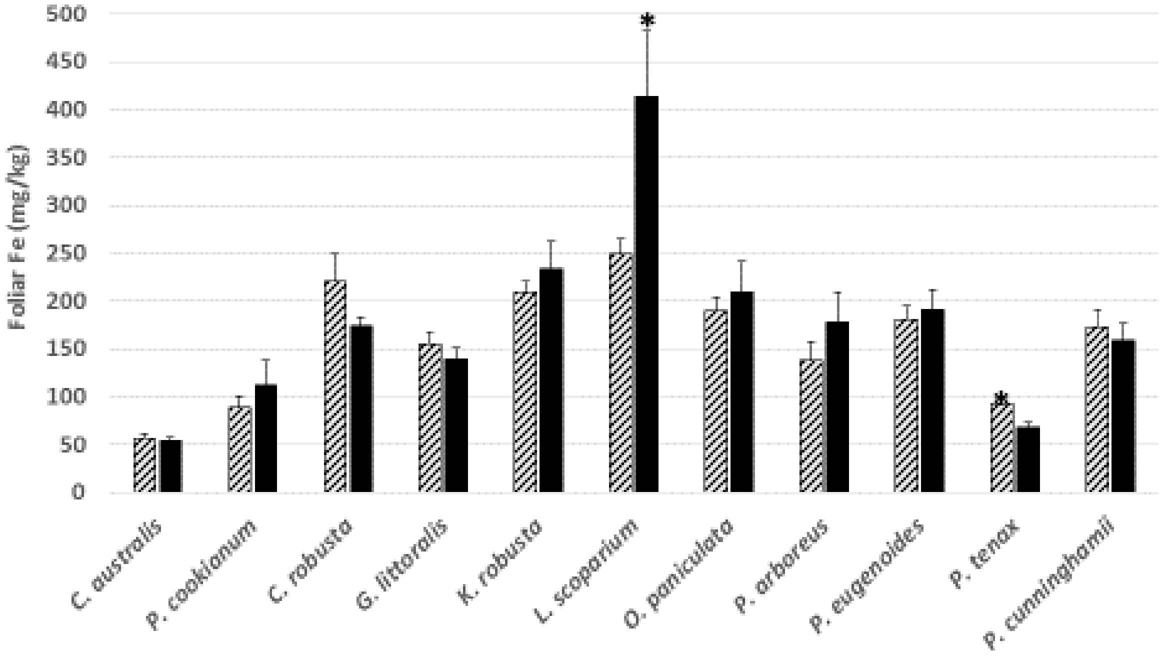


Figure 6. 11 Total concentration of foliar Fe (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean. Asterisks (*) signify significant differences between the effluents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

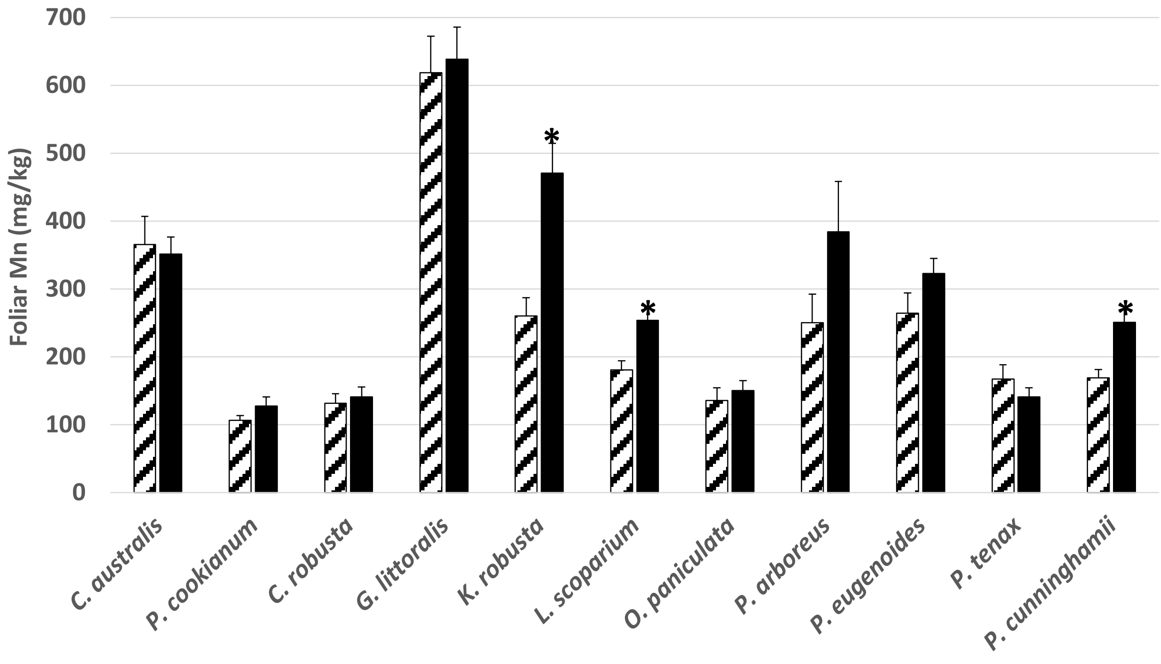


Figure 6. 12 Total concentration of foliar Mn (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean. Asterisks (*) signify significant differences between the effluents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

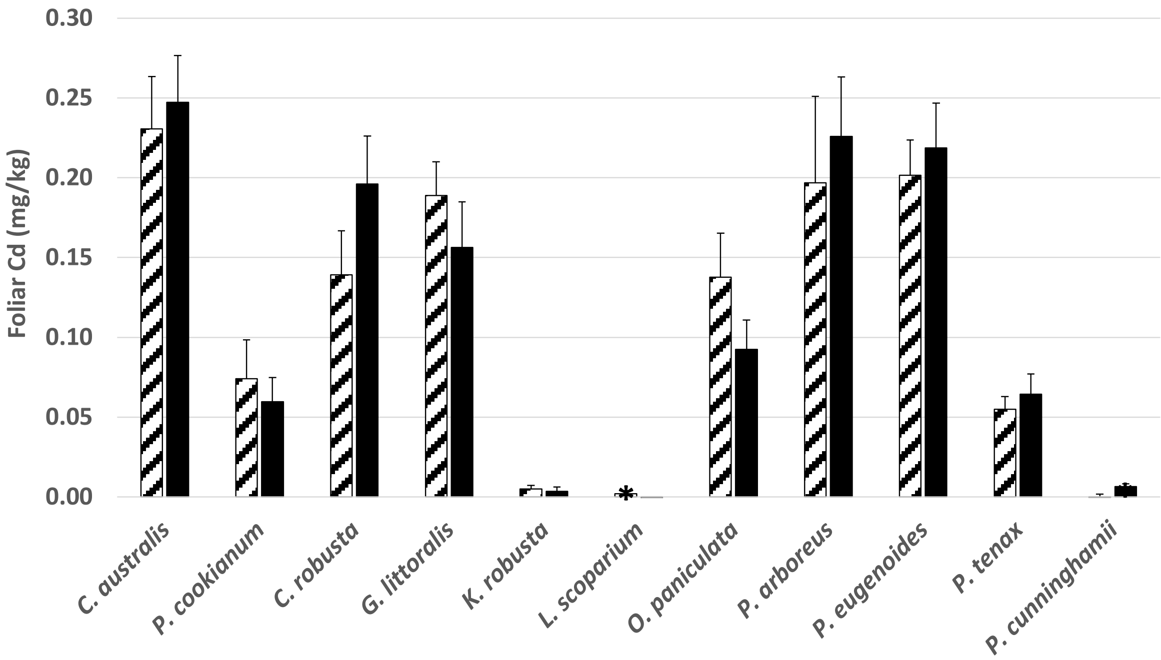


Figure 6. 13 Total concentration of foliar Cd (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean. Asterisks (*) signify significant differences between the effluents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

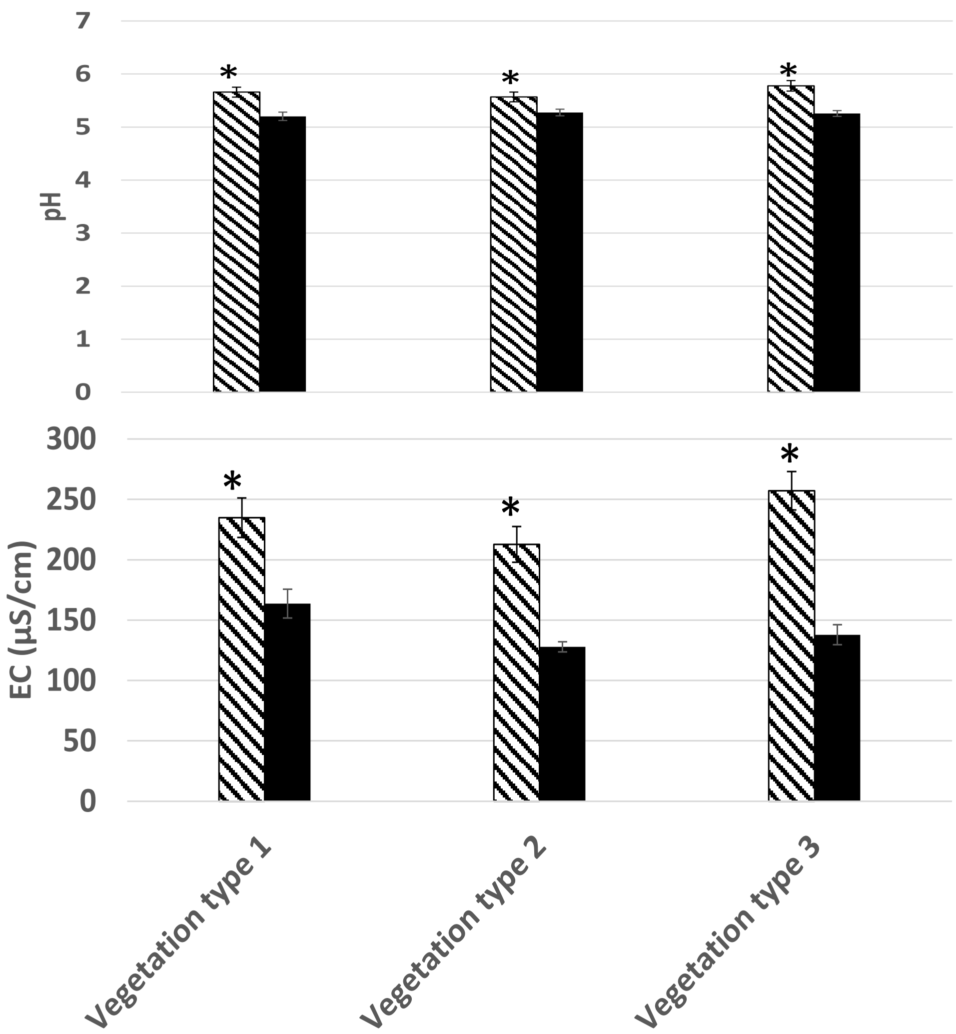


Figure 6. 14 Soil pH and EC of each vegetation type in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean (n=20). Asterisks (*) signify significant differences between the effluents (striped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

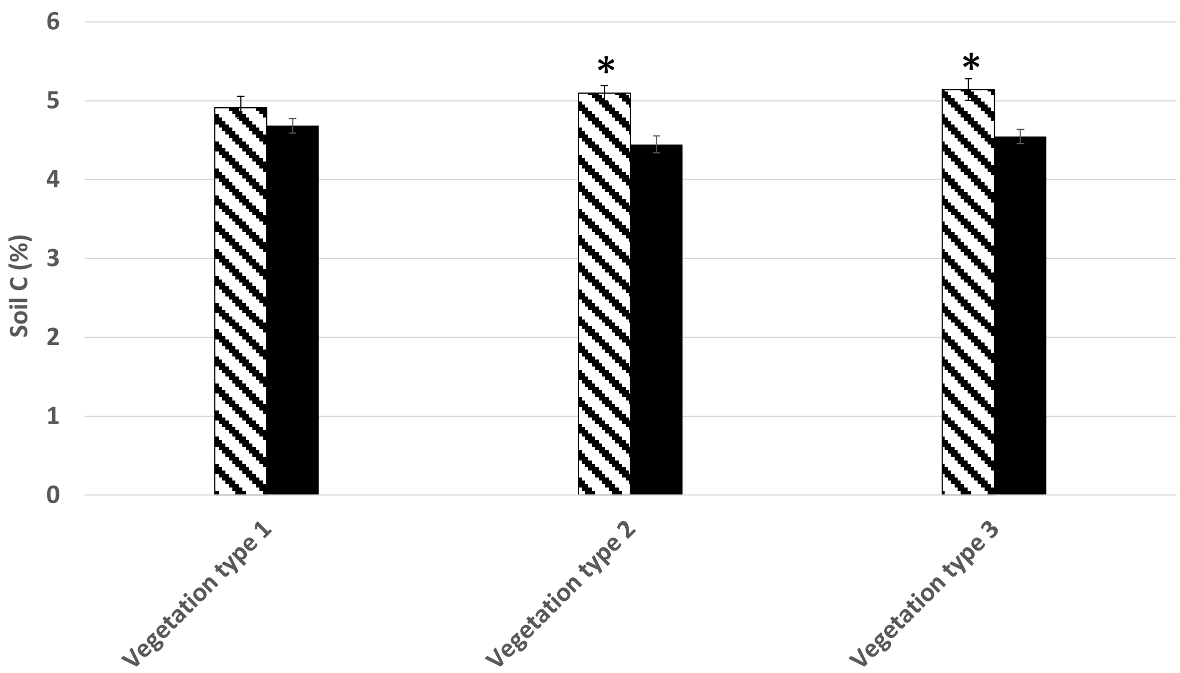


Figure 6. 15 Total concentration of soil C (%) of each vegetation type in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean (n=4). Asterisks (*) signify significant differences between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

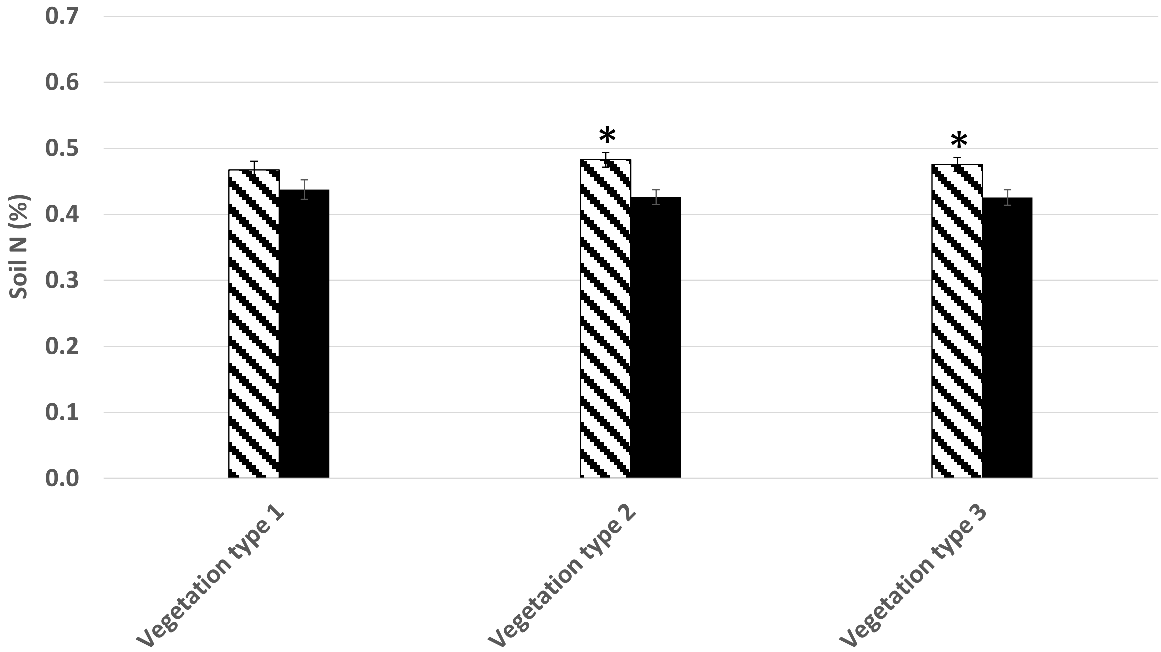


Figure 6. 16 Total concentration of soil N (%) of each vegetation type in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean (n=4). Asterisks (*) signify significant differences between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

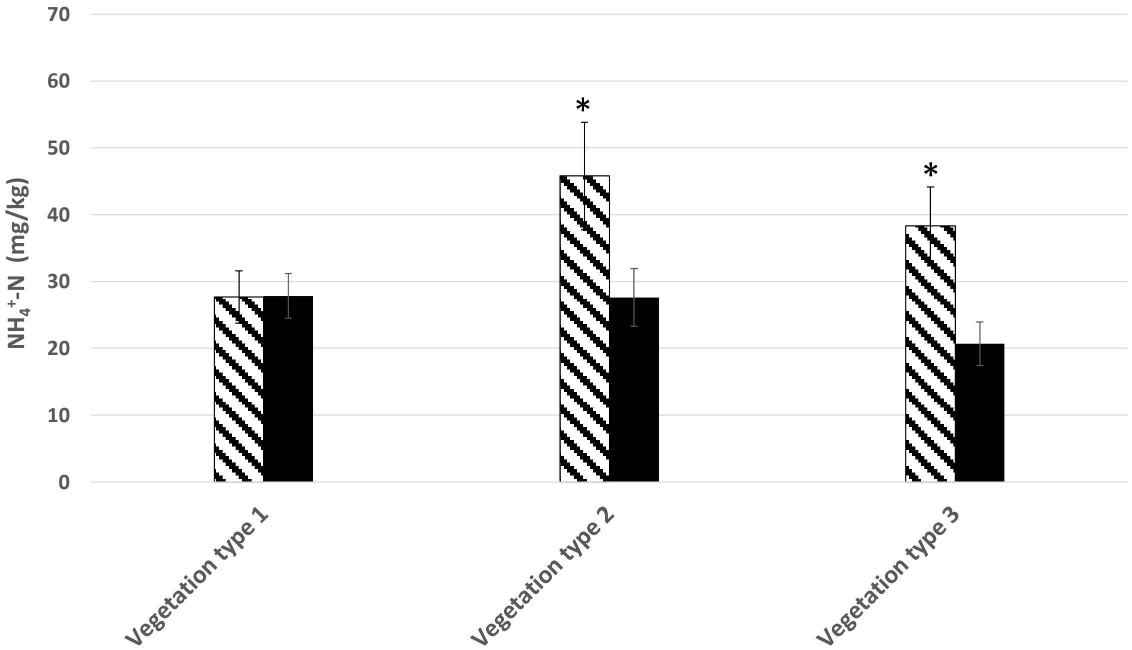


Figure 6. 17 Concentration of NH_4^+-N (mg/kg) of each vegetation type in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean (n=20). Asterisks (*) signify significant differences between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

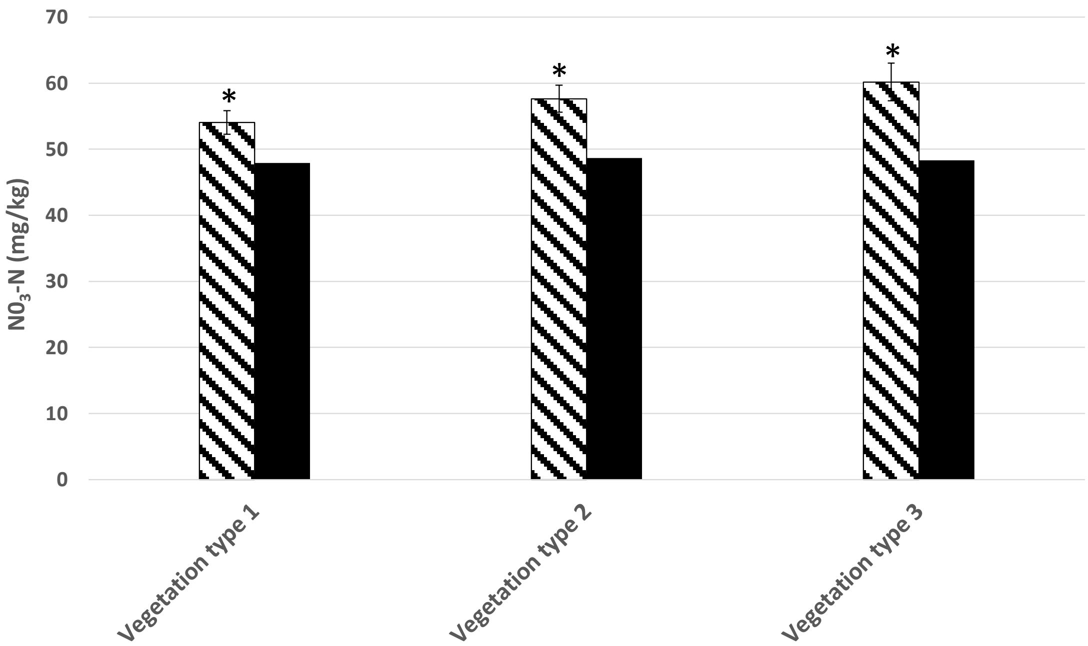


Figure 6. 18 Concentration of $N0_3^-$-N (mg/kg) of each vegetation type in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean (n=20). Asterisks (*) signify significant differences between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

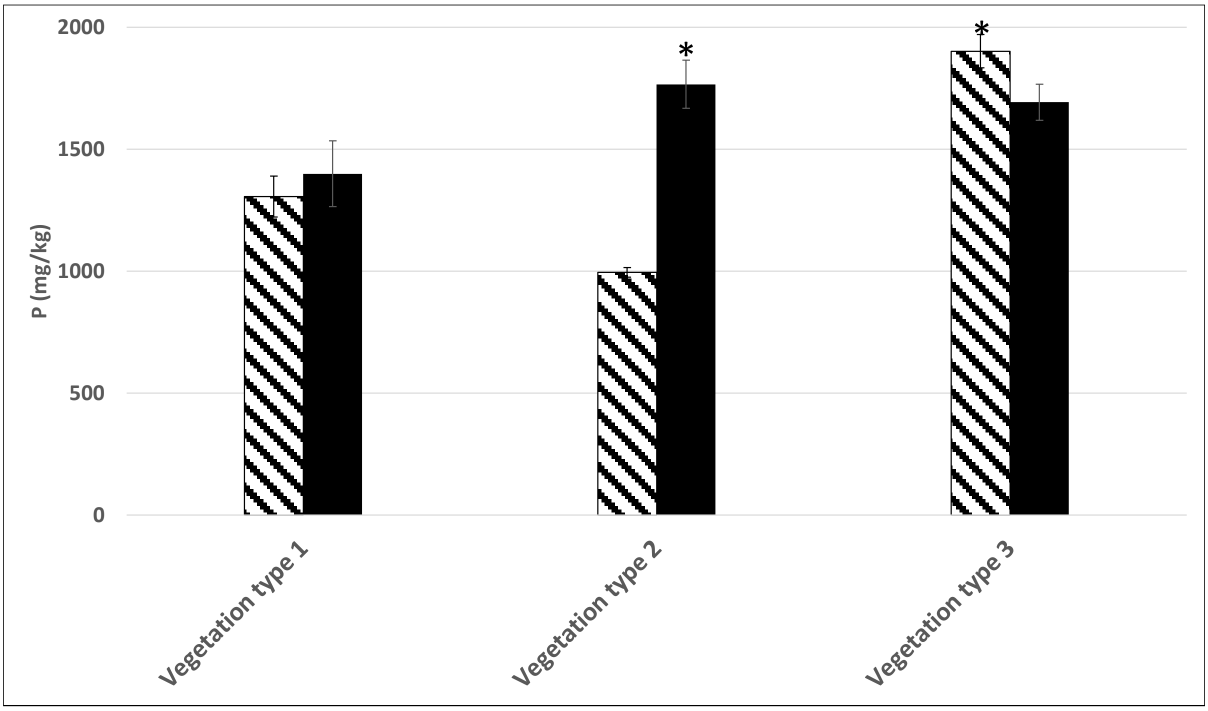


Figure 6. 19 Total concentration of soil P (mg/kg) of each vegetation type in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean (n=20). Asterisks (*) signify significant differences between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

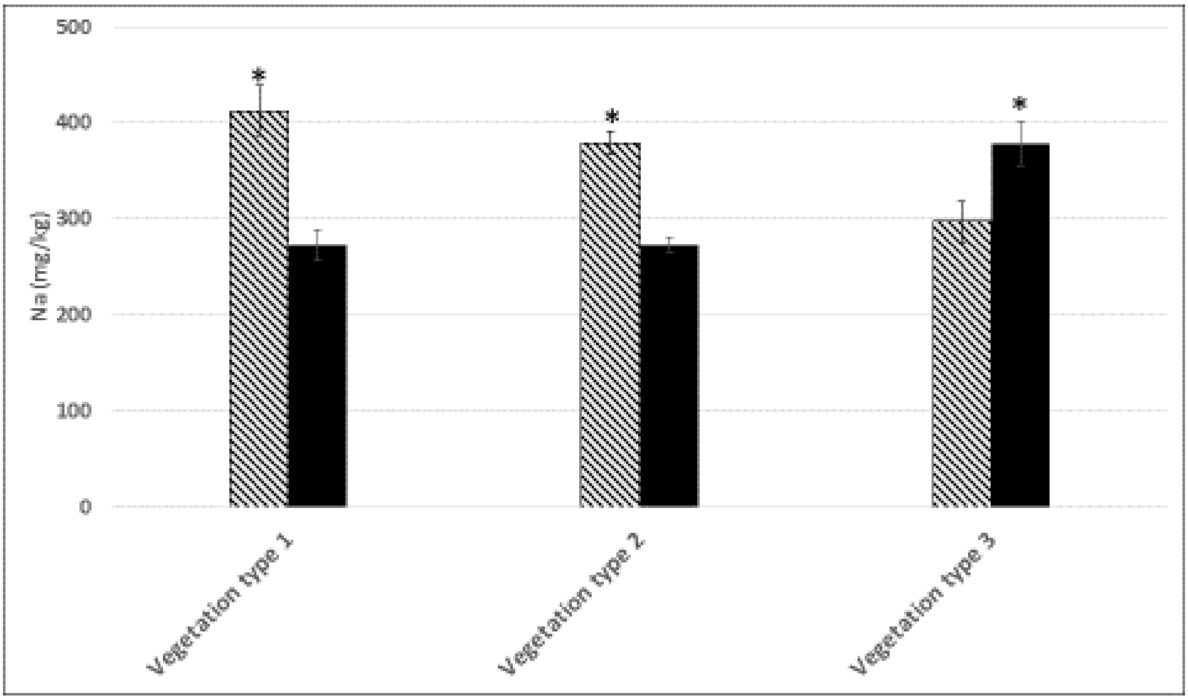


Figure 6. 20 Total concentration of soil Na (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean (n=20). Asterisks (*) signify significant differences between the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

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)**.

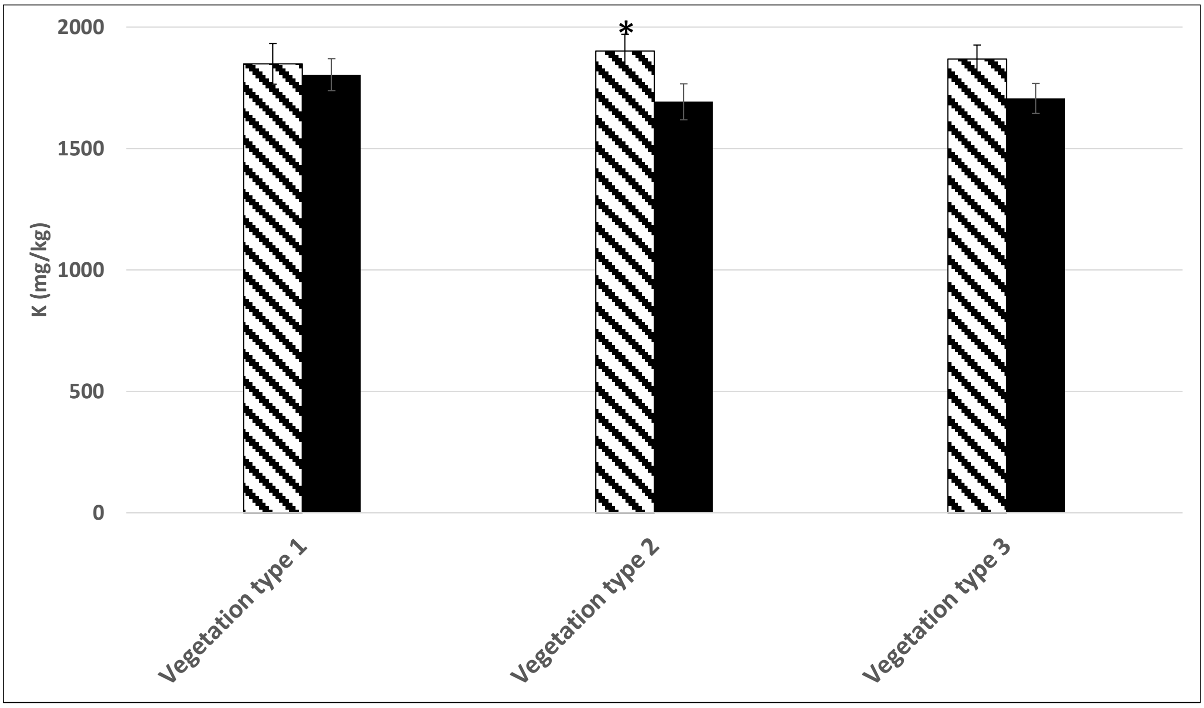


Figure 6. 21 Total concentration of soil K (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean (n=20). Asterisks (*) signify significant differences between the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

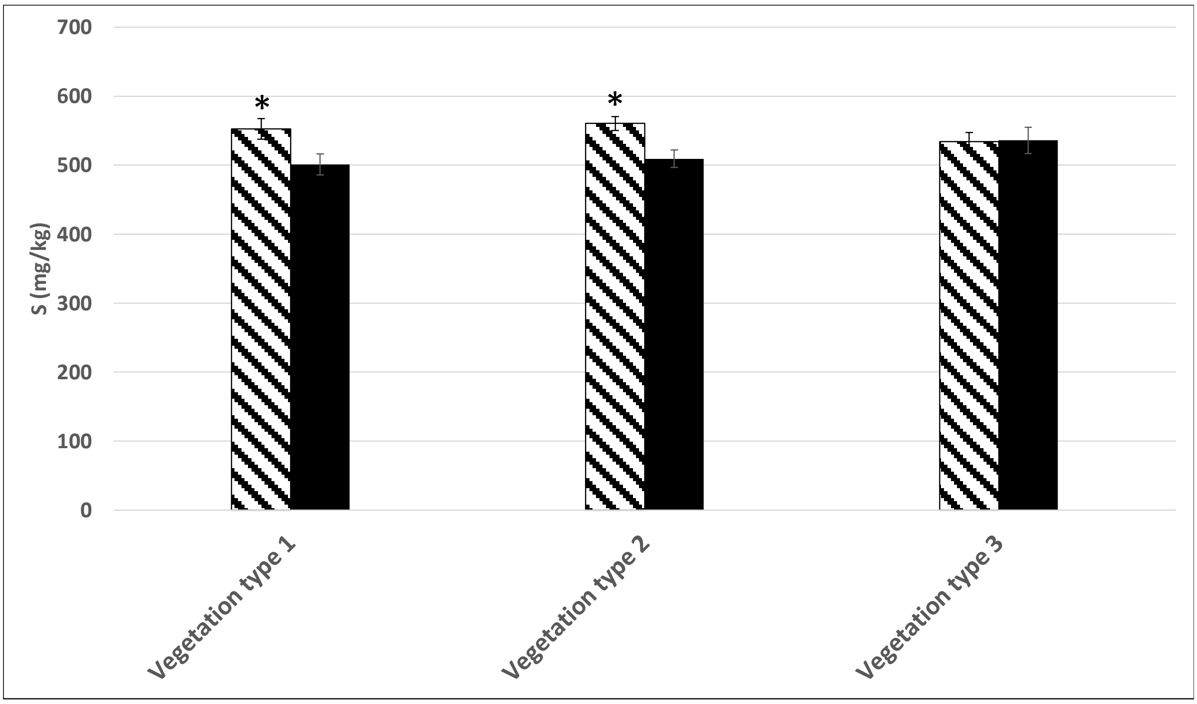


Figure 6. 22 Total concentration of soil S (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean (n=20). Asterisks (*) signify significant differences between the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

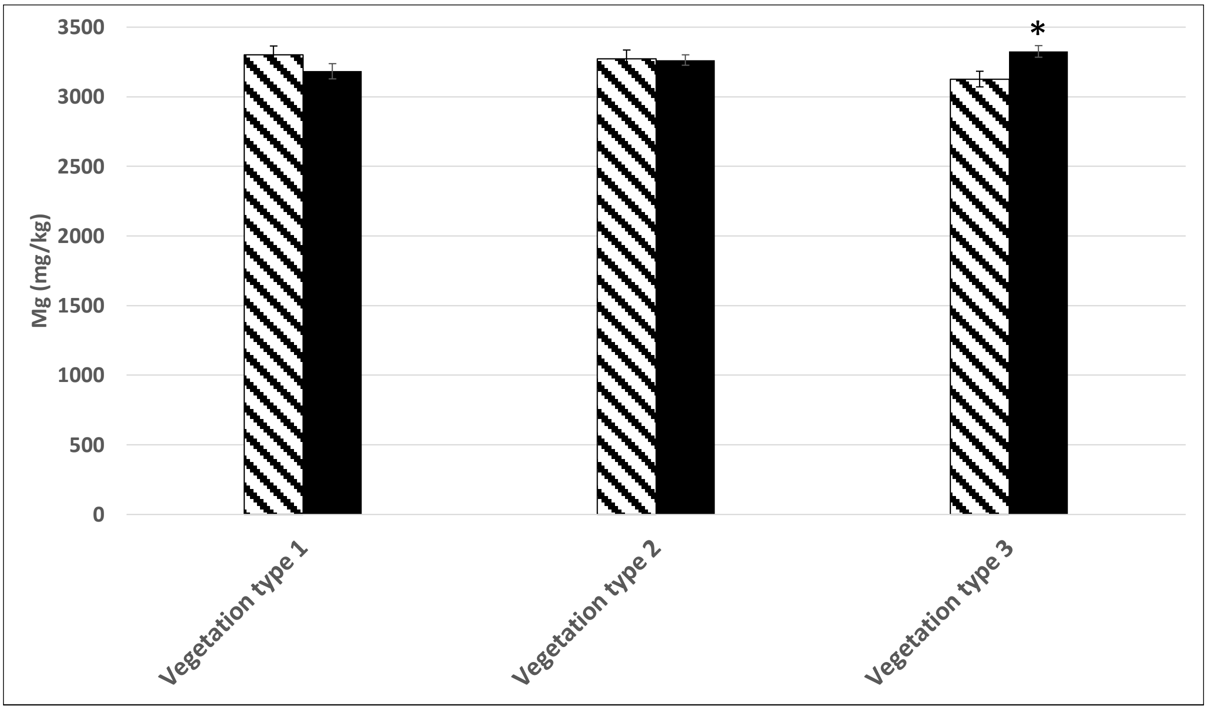


Figure 6. 23 Total concentration of soil Mg (mg/kg) of each species in response to Treated Municipal Wastewater (TMW) treatment 18 months after treatment application (End of May, 2017). Error bars represent the standard error of the mean (n=20). Asterisks (*) signify significant differences between the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

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 (Pescod et al., 1992, FAO, 2018).

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 (Stewart and Flinn, 1984, Stewart et al., 1990, Selahvarzi and Hosseini, 2012). 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 (Selahvarzi and Hosseini, 2012, Hopmans et al., 1990, Stewart et al., 1990, Ogbonnaya and Kinako, 1993, Gerhart et al., 2006, Guo et al., 2012, Ostos et al., 2008, Guo and Sims, 2000, Kanekar et al., 1993, Bhati and Singh, 2003, Hassan, 1996, Singh and Bhati, 2005, Ali et al., 2012, Ali et al., 2011). 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 (Ostos et al., 2008, Guo and Sims, 2000). 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 (Whiteside et al., 2012, Smith et al., 2011). 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 (Singh and Bhati, 2005, Balkhair and Ashraf, 2016, Alghobar and Suresha, 2017, Minogue et al., 2012, Parveen et al., 2014, Parveen et al., 2013, Walia and Goyal, 2010, Singh and Agrawal, 2010, Ali et al., 2011, Mohammad and Ayadi, 2004). 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, Varkey et al., 2015, Parveen et al., 2013). 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 (Walker et al., 2003, Esperschuetz et al., 2017). 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 (Varkey et al., 2015, Mancino and Pepper, 1992, Singh et al., 2012, Ghosh et al., 2012). 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 (Saffari and Saffari, 2013, Morugán-Coronado et al., 2011), 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≤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, Ngāi Tahu Property of South Island 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. serotine*), with additional 1-5% of up to 30ft tall of mānuka (*Leptospermum scoparium*) species (Meurk et al., 1995, McGlone et al., 2001, Wendelken, 1966). These species were found associated with an understory of prickly mingmingi (*Leptecophylla juniperina)* (Wardle, 1991).

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, 1999a, 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, 1999a)

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. serotine* 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 (**Figure 7.1)** 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).

Figure 7. 1 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 7.2**). 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.

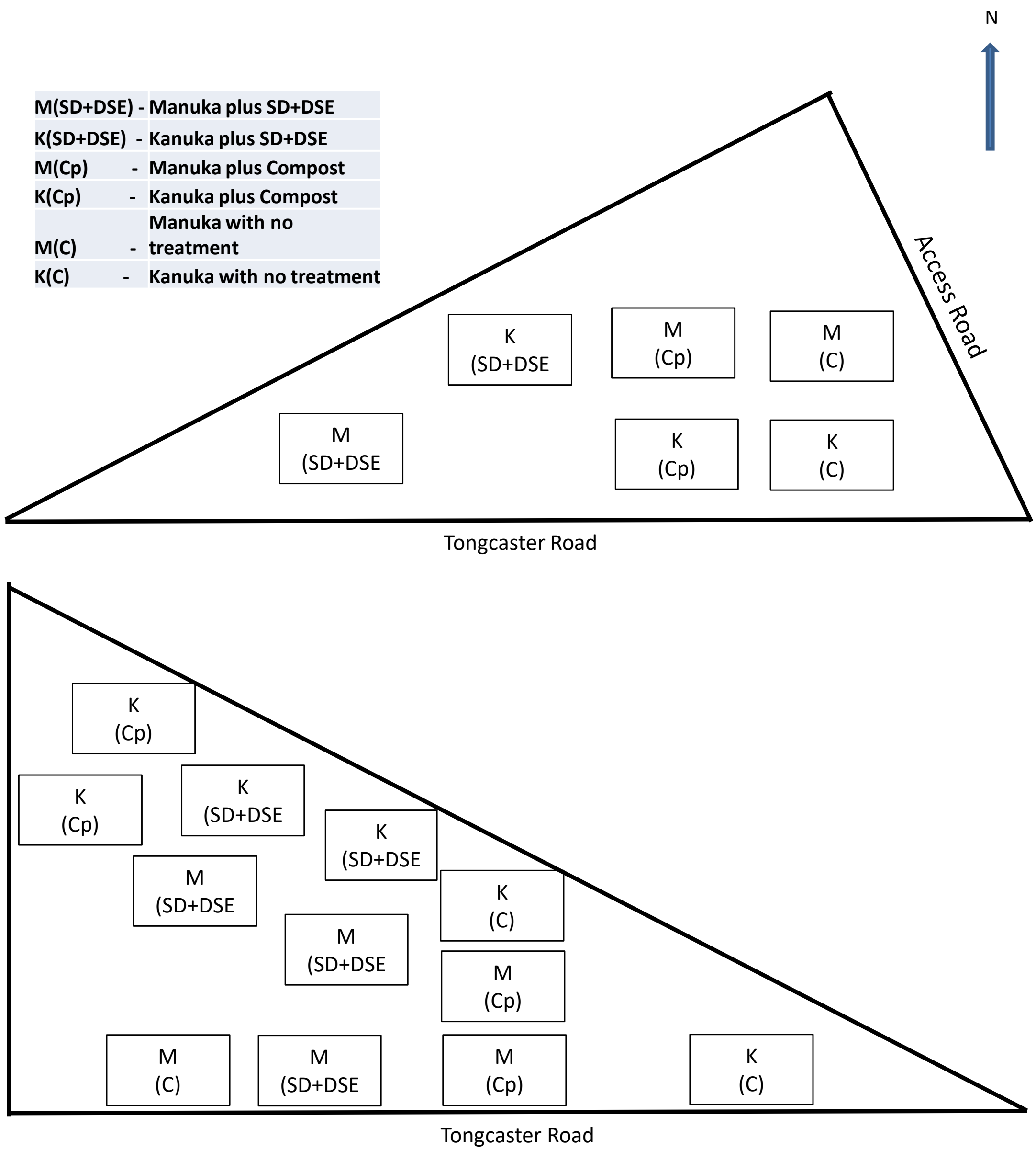


Figure 7. 2 Plots design of Eyrewell field trial

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 (**Figure 7.3**). Seedlings of *L. scoparium* and *K. serotina* and plant guards were sourced from Native Solution Ltd., P.O. Box 631, Rangiora North Canterbury 7400.

Plate 7. 2 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. 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.

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	3,200	1,200
P	480	270
K	810	820
S	320	130
Ca	3,000	690
Mg	640	270

Compost and sawdust were applied by spreading on entire plot with approximately 10 cm in thick (**Plate 7.3**).

a b

Plate 7. 3 SD+DSE (a) and compost (b) application of Eyrewell field trial

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. serotine* were then separated from branches, ground using a Retch ZM200 grinder (**Plate 7.4**), 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 7.5)**, and then kept in the fridge for further analysis of soil pH, EC and mineral N and total elements.

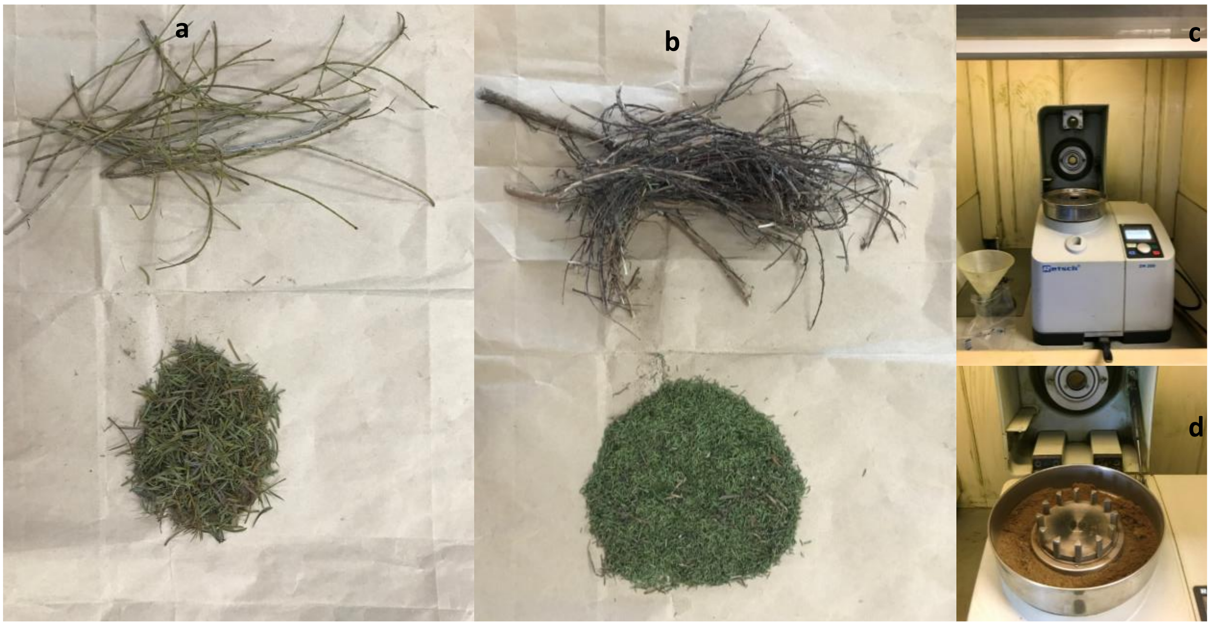


Plate 7. 4 Dried leaves of L. scoparium (a) and K. serotine (b) separated from branches and grinder Retch ZM200 for grinding the samples (c, d)

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. 5 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 7.4** 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. serotine,* 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*.

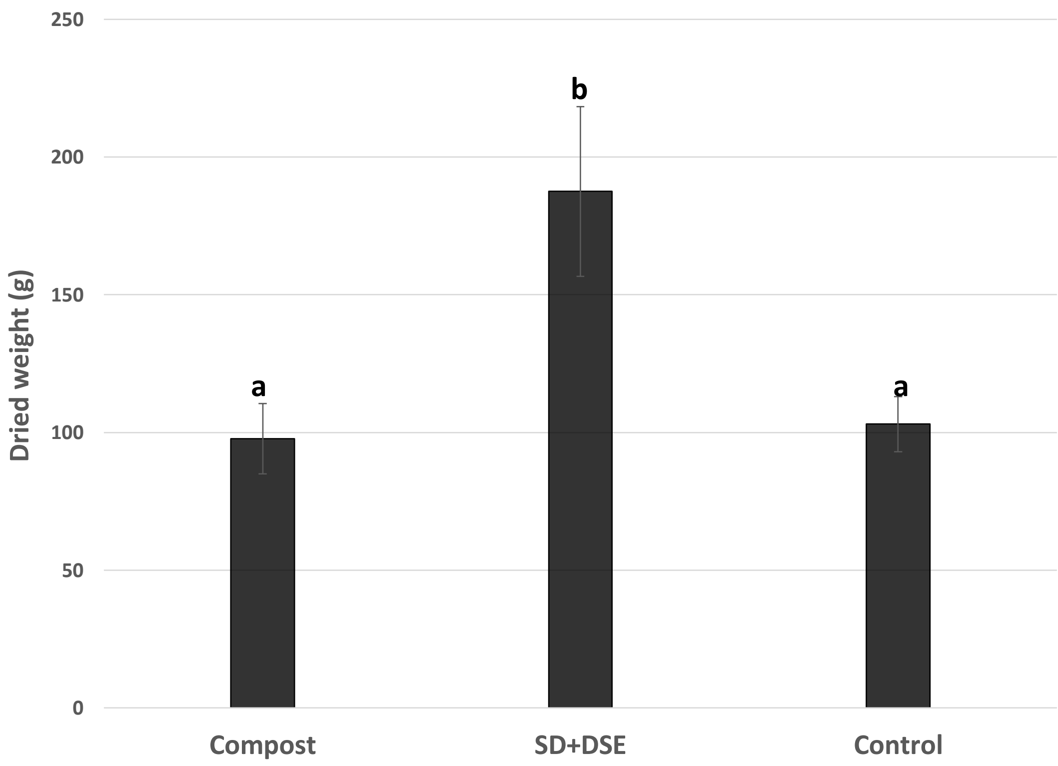


Figure 7. 3 Above ground dried weight (g) of *K. serotina* in response to Sawdust plus Dairy Shed Effluent (SD+DSE) and Compost treatment. Error bars represent the standard error of the mean (n=15, 15 and 10). Bars with the same letters are not significantly different (*p≤0.05*).

7.3.3 Effect of treatments on the nutrient uptake

Macronutrients

Figure 7.4 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 7.4A)**. However, **Figure 7.4A)** 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 7.4A**).

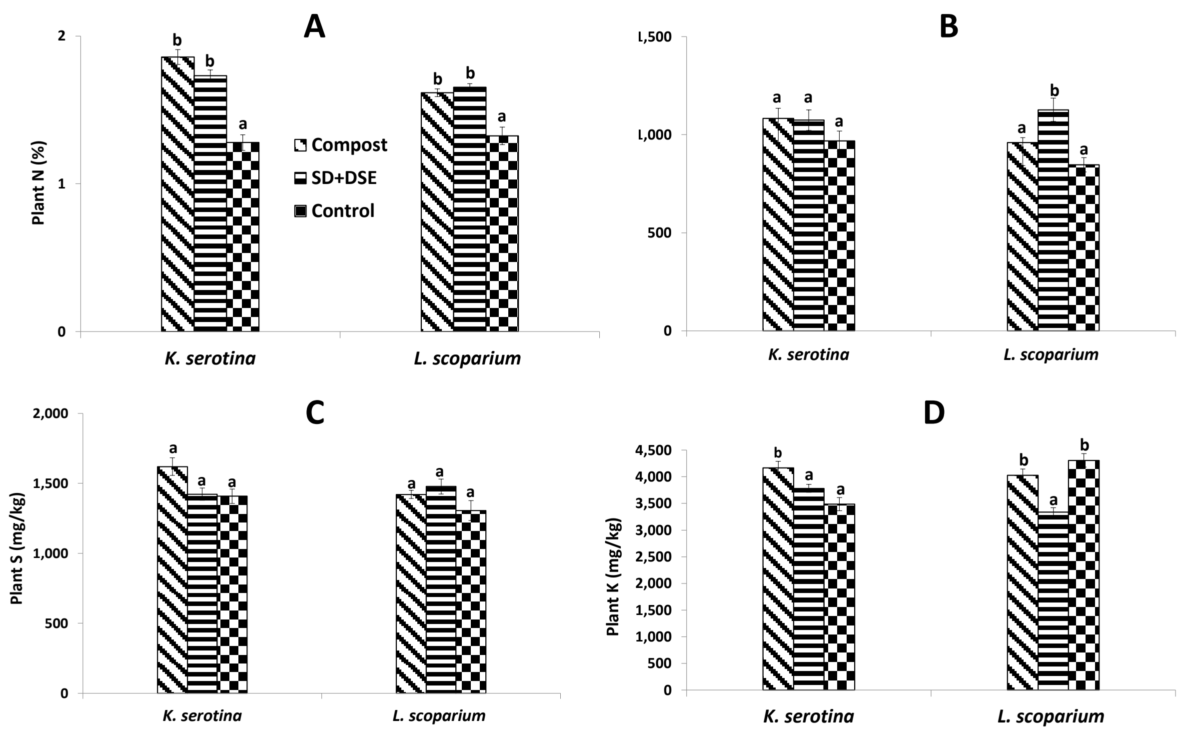


Figure 7. 4 Total concentration of foliar N, P, K, and S (%) of *K. serotina* and *L. scoparium* in response to SD+DSE and Compost treatment. Error bars represent the standard error of the mean (n=15, 10, and 14). Bars with the same letters are not significantly different (*p≤0.05*).

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 7.4B - 7.4D**). 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 7.4C** and **7.4D**). *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 7.4B**). 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 7.4C**). 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 7.4D**). 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%.

1.1.1.1 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.

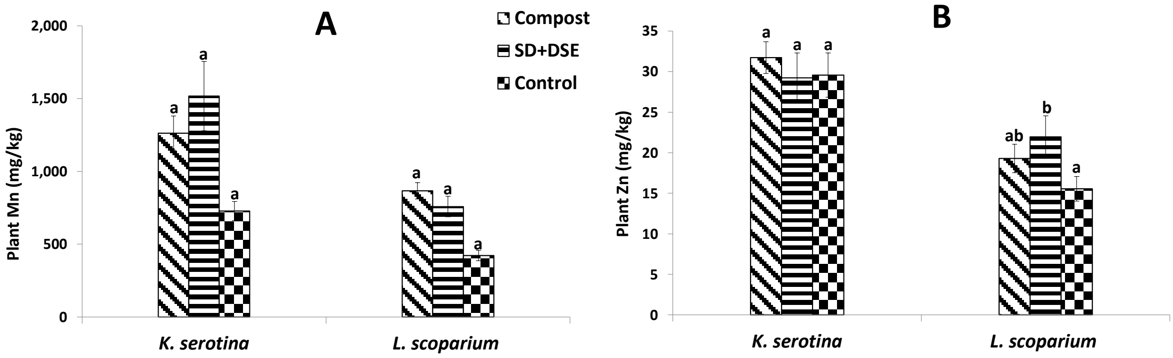


Figure 7. 5 Total concentration of foliar Mn and Zn (mg/kg) of *K. serotina* and *L. scoparium* in response to SD+DSE and Compost treatment. Error bars represent the standard error of the mean (n=15, 10 and 14). Bars with the same letters are not significantly different (*p≤0.05*).

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 7.5**). 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 7.5A**). 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

1.1.1.2 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 7.6A**). Adding SD+DSE did not signify (P>0.05) the concentration of NH_4^+-N in underlying soil of both *L. scoparium* and *K. serotina* (**Figure 7.6A**).

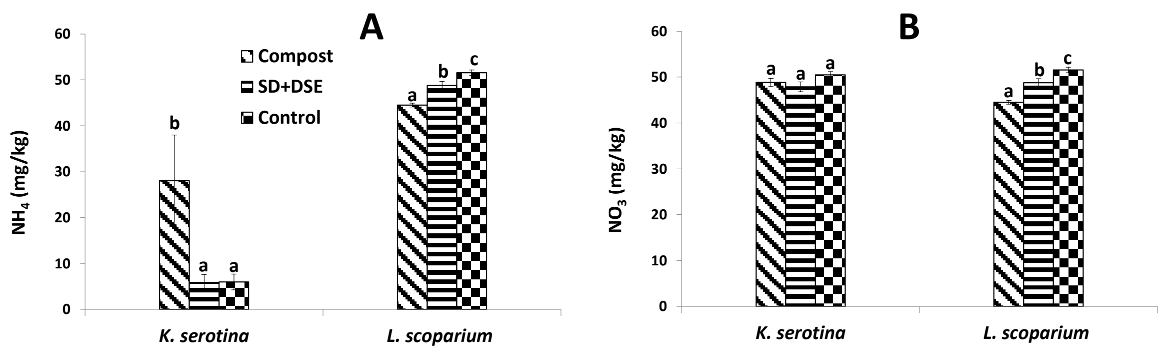


Figure 7. 6 Concentration of NH_4^+-N and of NO_3^- -N (mg/kg) of (A) *L. scoparium* and (B) *K. serotine* in response to SD+DSE and Compost treatment. Error bars represent the standard error of the mean (n=14, 10 and 15). Bars with the same letters are not significantly different (*p≤0.05*).

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 7.6B**). 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 7.7 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.

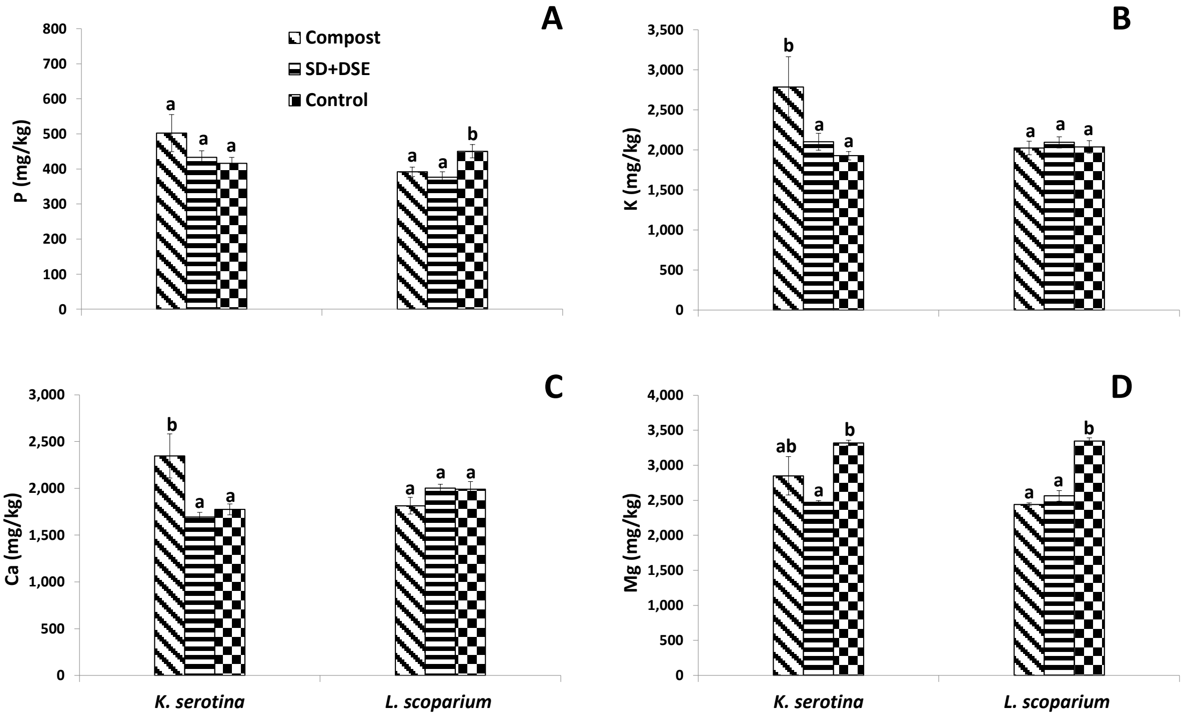


Figure 7. 7 Concentration of soil macronutrients (mg/kg) of *L. scoparium* and *K. serotine* in response to SD+DSE and Compost treatment. Error bars represent the standard error of the mean (n=14, 10 and 15). Bars with the same letters are not significantly different (*p≤0.05*).

In combination with *L. scoparium,* SD+DSE and compost treatments decreased the soil P and Mg (**Figure 7.7A, 7.7D**). 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. serotine,* 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 7.7B** and **7.7C** 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 7.7B)**. 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 7.7C)**.

1.1.1.3 Trace Elements

Figure 7.8 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.

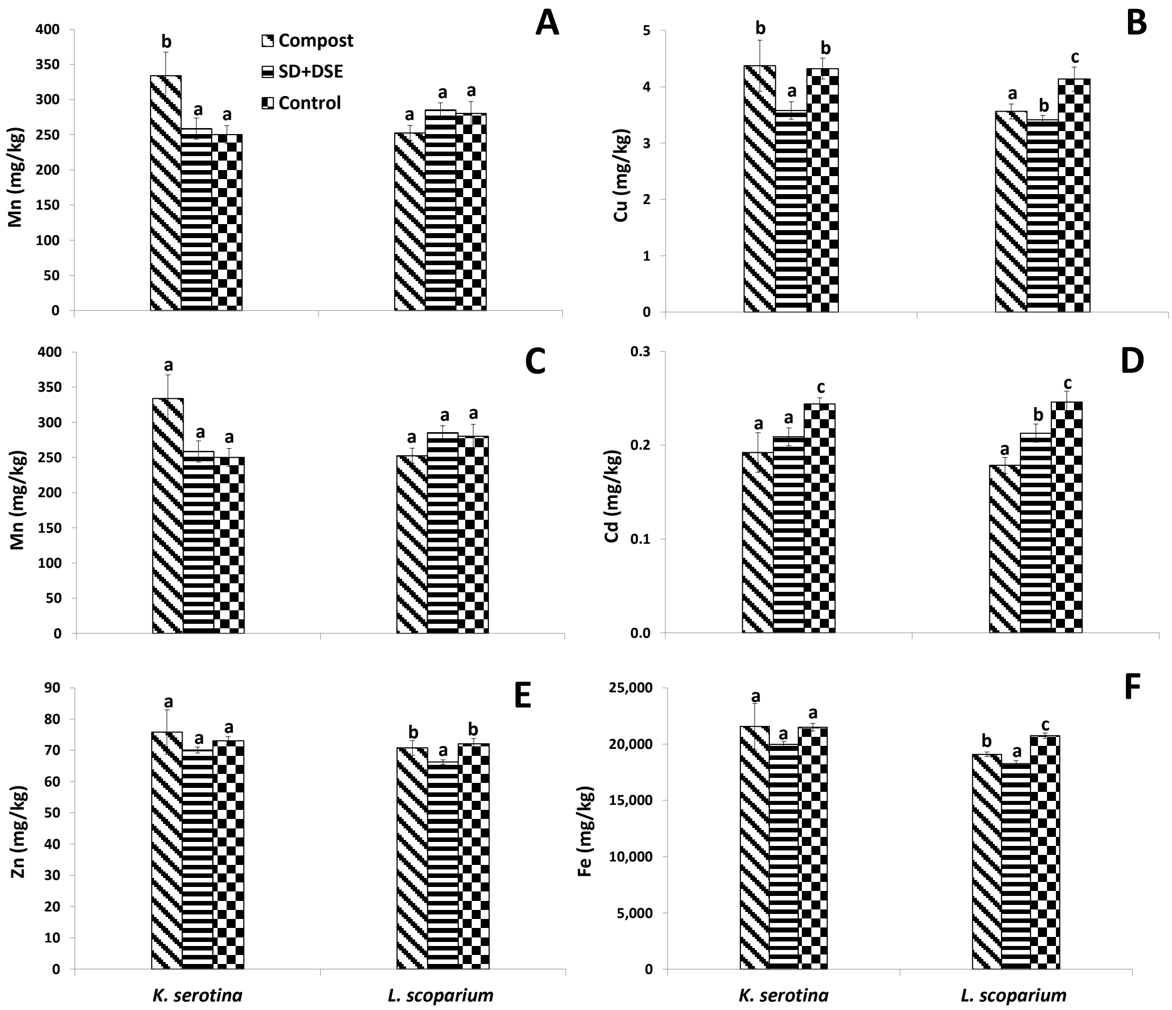


Figure 7. 8 Concentration of soil trace elements (mg/kg) of *L. scoparium* and *K. serotine* in response to SD+DSE and Compost treatment. Error bars represent the standard error of the mean. Bars with the same letters are not significantly different (*p≤0.05*).

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 7.8A, 7.9B, 7.8D, 7.8E, 7.8F**). 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. serotine,* the application of SD+DSE significantly reduced the concentration of rhizosphere soil Cu concentration by 21% (**Figure 7.8B**). In contrast, in combination with *K. serotina*, compost application significantly elevated the concentration of soil B and Mn by 32% and 25%, respectively (**Figure 7.8A** and **7.8C**). 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 7.8D**). 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 (Antoniadis et al., 2008a, Anderson et al., 2012).

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 (Olayinka and Adebayo, 1989, Haynes and Swift, 1986, Olayinka and Adebayo, 1985, Nishanth and Biswas, 2008, Bugbee, 1999a, Schmidt et al., 2014, Shaheen et al., 2017, Esperschütz et al., 2016, Esperschuetz 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. (2003b), 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 (2001a) and Keller and Römer (2001b) 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 (Samarakoon et al., 2006, De

Kreij and Van Den Berg, 1990, Bernstein, 1975). 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 (Bugbee, 1999a, Ajmal et al., 1998, 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 (Esperschütz et al., 2016). 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 (Hedmark and Scholz, 2008, Keeling and Bohlmann, 2006, Sanati, 2005, Hall, 2007). 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 Nloaded 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		-	

Appendix E

Supplementary information to Chapter 5

My role in this study was helping Dr Juergen Esperschuetz and Dharini Paramashivam with the experimental maintenance, data collection, final harvesting, soil and plant samples preparation for analysis, and some data analysis, which are parts of the following published paper

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WASTE MANAGEMENT

Effect of Pine Waste and Pine Biochar on Nitrogen Mobility in Biosolids

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Abstract

Humanity produces ~27 kg of dry matter in biosolids per person per year. Land application of biosolids can improve crop production and remediate soils but may result in excessive nitrate N (NO_3^-–N) leaching. Carbonaceous materials can reduce the environmental impact of biosolids application. We aimed to ascertain and compare the potentials for Monterey pine (*Pinus radiata* D. Don)-sawdust-derived biochars and raw sawdust to reduce NO_3^-–N leaching from biosolids. We used batch sorption experiments 1:10 ratio of material to solution (100 mg kg^{-1} of NH_4^+ or NO_3^-) and column leaching experiments with columns containing biosolids (2.7% total N, 130 mg kg^{-1} NH_4^+ and 1350 mg kg^{-1} NO_3^-) mixed with soil, biochar, or sawdust. One type of low-temperature (350°C) biochar sorbed 335 mg kg^{-1} NH_4^+, while the other biochars and sawdust sorbed <200 mg kg^{-1} NH_4^+. None of the materials sorbed NO_3^-. Biochar added at rates of 20 to 50% reduced NH_4^+–N (<1% of total N) leaching from columns by 40 to 80%. Nitrate leaching (<7% of total N) varied little with biochar form or rate but was reduced by sawdust. Incorporating dried sawdust with biosolids showed promise for mitigating NO_3^-–N leaching. This effect likely is due to sorption into the pores of the biochar combined with denitrification and immobilization of N rather than chemical sorption onto surfaces.

Core Ideas

- Dry sawdust reduced nitrate leaching from biosolids; moist sawdust was less effective.
- Biochar was ineffective in reducing nitrate leaching from aged biosolids.
- Biochar chemically sorbed significant amounts of ammonium, whereas sawdust did not.
- Neither biochar nor sawdust chemically sorbed nitrate.
- Sawdust physically sorbed both ammonium and nitrate.

Humanity produces ~27 kg of biosolids (treated sewage sludge) per person per year (Hue, 2014). Applying biosolids to productive land improves plant growth (Ronald et al., 2008) but may result in both high levels of nitrate (NO_3^-) leaching (Correa et al., 2006) and contamination of the soil and food chain. The application of biosolids to prime agricultural land is still unacceptable to many stakeholders, even though many countries have guidelines to manage their environmental impacts. As a consequence, many biosolids are disposed of in landfills, into waterways, or burned. This represents a waste of organic matter and plant nutrients.

Soil degradation is a common problem in most countries. In New Zealand, thousands of hectares of land, formally under Monterey pine (*Pinus radiata* D. Don) plantations (Ministry of Agriculture and Forestry, 2010) have both low soil organic matter levels and soil fertility (Brockerhoff et al., 2005). Similarly, land affected by open-cast mining often fails to develop a vegetative cover and requires remediation. In both cases, biosolids have been used to successfully re-establish soil fertility (Daniels et al., 2003; Novak et al., 2009). However, to achieve a meaningful increase in soil organic matter, high rates (>50 t ha^{-1}) of biosolids are required (Henry et al., 1994). Given that biosolids comprise 2 to 5% N by weight (Daniels et al., 2001), rebuilding degraded soil can result in N rates of up to 2500 kg ha^{-1}, which is well in excess of the maximum rates currently permitted (~200 kg ha^{-1} yr^{-1}) in most jurisdictions (EPA Victoria, 2004; New Zealand Waste Water Association, 2003). Most of the N in biosolids is in an organic form, and as it mineralizes, it provides a source of plant available inorganic N that promotes plant growth with minimal N leaching. However, biosolids can also contain significant amounts of inorganic N as ammonium (NH_4^+–N), which can rapidly nitrify to form NO_3^-–N. In aged biosolids, NO_3^-–N may also be present at significant levels (Smith et al., 1998). In both cases, NO_3^-–N may be leached. Excessive loadings of mineral N are associated with high levels of NO_3^-–N leaching, which can contribute to eutrophication of lakes, rivers, and groundwater (Davis, 2014) and thus, should be prevented.

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Abbreviations: CEC, cation exchange capacity; FIA, flow-injection analysis; IC, inorganic C; TC, total C; TOC, total organic C; VOC, volatile organic compound; WSC, Water-soluble C.

low-temperature biochars. A muffle furnace was used to manufacture biochars at 350°C in a low-oxygen environment (Zhang et al., 2015). Sawdust (200 g) was weighed into steel containers covered with aluminum foil. The temperature was monitored using a thermocouple to ensure the temperature of the material was maintained at 350°C. Chars were prepared with pyrolysis times of 3 and 12 h. The target temperature, 350°C, was reached at the rate of 16°C min^{-1}. Higher temperature biochars were produced using a specialized furnace (Hina et al., 2010) equipped with a rotating cylinder of 5-L capacity. Liquefied petroleum gas was used as the heat source to pyrolyze the sawdust at 400 and 550°C. The target temperatures were reached at rates of 38 and 46°C min^{-1}, respectively. Treatments were prepared with and without steam activation. Steam activation (henceforth denoted as "A") was achieved by injecting water into the pyrolysis chamber at a rate of 4 mL min^{-1} with an airflow of 10 mL min^{-1}. A further biochar was also made from pine at 350°C, as previously described by Knowles et al. (2011). This biochar contained particles sizes from <1 to 45 mm and was sieved (<4 mm) and is subsequently referred to as bulk biochar. We included this biochar because Taghizadeh-Toosi et al. (2012) and Knowles et al. (2011) demonstrated that this char affected N fluxes in soil. Table 1 and Supplemental Table S1 show the properties of the sawdust and biochars.

The pH of the materials was determined in water using a sample to water ratio (w/w) of 1:2.5 following the method of Blakemore et al. (1987). Soil carbon (C) and N concentrations were measured using an Elementar Vario MAX CN analyzer (Elementar GmbH). Actual cation exchange capacity (CEC) was measured for all materials using the method described by Blakemore et al. (1987), which uses Ag^{+} as the index cation. Extractable NH_4^{+} and NO_3^{-} concentrations in the soil and biosolids were determined using a 2 M KCl extract following the method of Blakemore et al. (1987) and Clough et al. (2001). Water-soluble C (WSC) was determined using cold (20°C) and hot (80°C) water extracts (Ghani et al., 2003). To measure WSC, 3 g of oven-dried material and 30 mL of cold distilled water were placed in polypropylene centrifuge tubes on an end-over-end shaker for 30 min and then centrifuged for 20 min at 2253 *g*. The extracts were then decanted off and filtered through 0.45-μm cellulose nitrate membrane filters. The sample remaining in the centrifuge tube had 30 mL of distilled water added before it was then placed in a hot water bath at 80°C for 16 h then centrifuged and filtered as before. Total carbon (TC), inorganic carbon (IC), and total organic carbon (TOC) concentrations of the WSC samples were measured using a TOC-5000A analyzer (Shimadzu Oceania Pty Ltd.). Total elemental concentrations were measured in acid digests using inductively coupled plasma–optical emission spectrometry (Varian 720-ES) fitted with SPS-3 auto-sampler and ultrasonic nebulizer (Simmler et al., 2013). Digests were prepared with 0.5 g of material mixed with 5 mL of HNO_3 and 1 mL of H_2O_2 (Merck hydrogen peroxide 30%). The mixtures were digested at 175°C for 20 min and diluted up to 25 mL with Milli Q (double deionized water). Wageningen reference soil (ISE 989) and plant (IPE 100) material were analyzed for quality assurance (Van Dijk and Houba, 1998). Recoveries were 95 to 108% for the elements measured.

Batch Sorption Experiments

Batch sorption experiments were performed with all individual materials (not mixtures) using an ambient solution of 0.01 M $CaCl_2$ solution containing 100 mg L^{-1} NH_4^{+} [pH 5.1 as $(NH_4)_2SO_4$] or NO_3^{-} (pH 7.0 as KNO_3) following the method of Wang et al. (2010). Samples (20 g of dry matter) were weighed into 250-mL centrifuge tubes and replicated thrice. Controls were also performed and had no sample addition. Then 200 mL of either the $(NH_4)_2SO_4$ or KNO_3 solution was added and the samples were then placed on an end-over-end shaker for 6 h. Previous experiments had indicated that this was the minimum time required for the biochar samples to equilibrate with the NH_4^{+} solution (data not shown). Harmayani and Anwar (2012) showed that equilibrium times for biochars in batch experiments varied from 1 to 96 h.

The effect of pH on sorption was determined for the bulk biochar. Batch sorption experiments used 10 g of bulk biochar and 100 mL of a 100 mg L^{-1} NH_4^{+} [as $(NH_4)_2SO_4$ in a 0.01 M $CaCl_2$ matrix]. The pH of the initial mixtures (pH = 5.1) were adjusted downward by adding 400 and 200 μL of 0.6 M HCl to give pH values of 3.4 and 4.2, respectively. The pH was adjusted upward by adding 450 μL of 0.03 M KOH or 750 μL of 0.3 M KOH to give pH values of 6.1 and 7.1, respectively. After shaking (2 h), samples were centrifuged at 2253 *g* for 10 min, filtered (Whatmann 52), then analyzed for residual NH_4^{+} and NO_3^{-} concentrations using flow-injection analysis (FIA; Alpkem FS 3000 twin channel analyzer).

The biosolids were not sterile. Thus, a test of the potential microbial activity on sorption experiment results was performed over a 48-h sorption experiment where the Lismore soil and bulk biochars were mixed with the $(NH_4)_2SO_4$ solution at a ratio of 1:10. Unsterilized and sterilized (using 1 mL of 5% v/v phenol) treatments were included. Samples were again shaken on an end-over-end shaker with subsamples collected at 10-min and 6-, 24-, and 48-h intervals, with all samples analyzed for both NH_4^{+} and NO_3^{-} concentrations.

Column Leaching Experiments

Leaching columns (4-cm height by 4-cm diam.) with an internal volume of 50.3 cm^3 were filled with mixtures of biosolids (sieved to <2 mm), pyrolyzed or unpyrolyzed (sawdust) pine wood (sieved to <4 mm), and quartz sand (<1 mm). Supplemental Table S2 lists, in detail, the treatments with the masses of each material. There were three replicates of each treatment. The total dry matter in each column was 15 g. Column bulk densities ranged between 0.5 and 1.5 g cm^{-3}. The volume of water in the columns at field capacity varied between 9.4 (sand) and 28.9 cm^3 (sawdust plus biosolids). Each column was irrigated daily with 5 mL of deionized water. The eluent was collected weekly and analyzed for both NO_3^{-}–N and NH_4^{+}–N concentrations using FIA. Columns were leached under laboratory conditions (20°C) for at least 3 mo or until the NH_4^{+}–N and NO_3^{-}–N concentrations in the eluent had stabilized at levels equal to <5% of the concentrations recorded in the initial flush.

Data were analyzed using Minitab 16 (Minitab, 2010). Data sets were analyzed using ANOVA with Fisher's LSD post hoc test to compare means. The level of significance was 0.05.

Mixing carbonaceous substances, such as sawdust or biochar, with biosolids can offset some of the negative environmental effects of biosolids addition (Knowles et al., 2011; Daniels et al., 2001; Schmidt, 2001; Simmler et al., 2013). Composting biosolids with sawdust can reduce NO_3^- leaching (Ammari et al., 2012). The timber industry produces large volumes of wood waste, including sawdust, which is often inappropriately disposed of in wood waste piles (Robinson, 2007; Wendong et al., 2005). Provided the sawdust is not contaminated with timber treatment residues, such as Cu, Cr, and As, this waste material may potentially be used to improve environmental outcomes from biosolids-amended soils. Costs would be greatly reduced if the sawdust could be incorporated with the biosolids on site rather than being composted beforehand. However, it is unclear whether uncomposted mixtures are effective in mitigating NO_3^-–N leaching. Composting of biosolids may improve quality of organic matter, which in turn may beneficial for soil (Bernal et al., 2009). Furthermore, composting can reduce amount of potentially phytotoxic compounds (Borchard et al., 2014). Thus, applying biosolids directly into soil may reduce treatment costs but may risk negative effects on soil health and crop growth that increase costs.

Potentially, NO_3^-–N leaching could also be reduced by pyrolyzing pine waste and using the resulting biochar as a biosolids amendment. The sorptive properties of biochar are profoundly affected by the source material, the pyrolysis temperature (Glaser et al., 2002), the particle size (Kwapinski et al., 2010), and the degree of weathering the biochar has undergone in the soil (Novak et al., 2009). Steam activation of biochar can change the sorptive properties of biochar (Borchard et al., 2012). Ducey et al. (2013) showed that steam activation of biochars increased the microbiological communities in the soil. Fungo et al. (2014) reported that steam activation of biochar derived from *Eucalyptus* spp. wood increased the biochar's capacity to suppress CH_4 and N_2O emissions from soil.

Amending biosolids with biochar has been shown to reduce NO_3^-–N leaching from pasture by over 50% (Knowles et al., 2011) when the biochar was made from Monterey pine pyrolyzed at 350°C. Other authors using the same biochar, have also reported lower concentrations of NO_3^-–N in pasture soils following the application of ruminant urine (Taghizadeh-Toosi et al., 2011).

Reductions in soil NO_3^-–N leaching following biochar amendment to soils have been reported to range from 10 to 96% with results varying widely because of experimental conditions, applied N form, N and biochar rates used, biochar feedstock variations, and pyrolysis temperatures (Guo et al., 2014; Knowles et al., 2011; Sika and Hardie, 2014; Troy et al., 2014). It is unclear why biochar amendment of biosolids reduced NO_3^-–N leaching, although it was speculated that biochar could adsorb NH_4^+–N or NO_3^-–N, thus rendering it less available for leaching and plant uptake or that it inhibited either the mineralization of organic-N or nitrification (Knowles et al., 2011).

We hypothesized that mixing biosolids with either pine sawdust or biochar would reduce the mobility of NO_3^-–N and NH_4^+–N. We aimed to determine the potential of Monterey pine sawdust and various sawdust-derived biochars for N immobilization in biosolids and biosolids-amended soils.

Materials and Methods

Soil (Lismore stony silt loam) was collected (0–30 cm) from the Lincoln University Ashley Dene sheep farm (43°39′05.82″ S, 172°19′41.47″ E), New Zealand. The soil is a low-fertility Lismore soil formed from gravel glacial outwash with a variable depth of silty loess deposited at the surface. The soil is well drained and has moderate to rapid permeability (Waikato Regional Council, 2011). The soil was air-dried to a gravimetric moisture content (θ_g) of 11.85% and sieved to <2 mm. Table 1 and Supplemental Table S1 give the chemical properties of the soil. Biosolids were obtained from the Kaikōura Regional treatment works (42°21′47.78″ S, 173° 41′20.32″ E), New Zealand. Approximately 160 kg of stockpiled and weathered biosolids were collected and homogenized using a concrete mixer and initially passed through a 20-mm sieve. A 2-kg subsample was passed through a 2-mm nylon sieve. Biosolids θ_g equaled 53%. Table 1 and Supplemental Table S1 give the properties of the biosolids.

Untreated pine sawdust was obtained from a local sawmill (Shands Road Sawmills Ltd) in New Zealand. After drying at 60°C to a constant weight, the sawdust was sieved to <4 mm. A further portion of the sawdust was kept moist (θ_g = 25%), as collected. The dried sawdust was pyrolyzed at a range of temperatures for varying lengths of time to produce biochars with contrasting properties. A slow pyrolysis method was used to produce

Table 1. Chemical properties of the materials used in the experiments. Values represent the mean (n = 3), except pH (median). Values in parentheses are the standard error. Concentrations of other elements can be found in the supplemental data.

	pH (H_2O)	CEC	Bulk density	C	N	C/N ratio	NH_4^+	NO_3^-
		$cmol_c$ kg^{-1}	g cm^{-3}	——— %	———		——— mg kg^{-1}	———
Lismore stony silt loam	6.3	13.5 (0.2)	1.1	4.3 (0.1)	0.37 (0.01)	11.6	7.9 (2.9)	181 (10.8)
Biosolids	4.5	16.7 (0.7)	0.7	25.3 (0.4)	2.7 (0.0)	9.4	130 (7.3)	1352 (2.5)
***Pinus radiata* (pyrolysis temperature, time) A = steam activation**								
Sawdust (SD, unpyrolyzed)	5.7	10.6	0.2	51 (0.04)	0.06 (0.00)	850	nd†	nd
Char 350°C, 3 h	5.5	2.2	0.2	71 (0.09)	0.03 (0.00)	2367	nd	nd
Char 350°C, 12 h	5.5	1.3	0.2	72.8 (0.1)	0.03 (0.01)	2427	nd	nd
Bulk biochar 350°C	6.9	9.1	0.2	78.1 (0.08)	0.06 (0.20)	1302	nd	nd
Char 400°C A	6.2	5.9	0.1	75.5 (0.07)	0.04 (0.00)	1888	nd	nd
Char 400°C	5.9	5.2	0.2	75.3 (0.07)	0.04 (0.00)	1883	nd	nd
Char 550°C A	8.1	6.7	0.1	88.4 (0.1)	0.03 (0.00)	2947	nd	nd
Char 550°C	7.9	6.7	0.1	86.5 (0.06)	0.03 (0.00)	2883	nd	nd

† nd, not determined.

NO_3^- sorption also dependent on feedstock type. Other more recent studies, also showing low sorption of NO_3^- by biochar, have generally examined biochar manufactured at pyrolysis temperatures <600°C (Gai et al., 2014; Hale et al., 2013; Zhang et al., 2015). Chintala et al. (2013) showed that NO_3^- sorption of a biochar produced at 650°C was only significant in acidic conditions. Thus, ignoring feedstock type as an issue, the lack of NO_3^- sorption in the current experiment is most likely because the low pyrolysis temperatures in our study were insufficient for the formation of N-containing basic functional groups (Kameyama et al., 2012). Shafeeyan et al. (2010) reported that significant numbers of N-containing basic functional groups only form at temperatures >700°C. Sawdust materials can retain cations, but they are not able to bind anions unless they are chemical modified (Ebrahimi and Roberts, 2013; Keränen et al., 2015; Mishra and Patel, 2009; Sousa et al., 2010; Su et al., 2012). For example, Keränen et al. (2015) modified sawdust to sorb NO_3^- using epichlorohydrin, ethylenediamine, and trimethylamine in the presence of N,N-dimethylformamide. It is therefore unlikely that chemical sorption of NO_3^- by the sawdust or biochars will reduce NO_3^- leaching.

Inorganic Nitrogen Leaching

Ammonium N in the leachate accounted for <1% of N applied (Fig. 3). The assumption is made that given the N content of the biochar (Table 1), the source of the NH_4^+–N in the leachate is the biosolids. When biochar materials were mixed with biosolids in the leaching columns, the biochars reduced the amount of NH_4^+–N leached when expressed as a percentage of the total N initially present in the biosolids (Fig. 3). The effect of increasing biochar rate observed with the bulk biochar treatment was to further reduce NH_4^+ leaching. This is most likely a consequence of the increasing CEC, since the amount of NO_3^-–N leached did not vary with the bulk biochar rate applied (Fig. 4). This also indicates that increasing the rate of biochar addition did not significantly accelerate nitrification via potential liming effects, which could in turn have enhanced subsequent NO_3^-–N leaching (Clough et al., 2013). Incorporating biochar into acidic agricultural soils accelerates nitrification and thus, weakens the liming effects of biochar (Zhao et al., 2014).

The low-temperature biochars (350°C) reduced NH_4^+–N leaching more than the high-temperature chars (400 and 550°C), while steam activation did not have a consistent effect on NH_4^+–N leaching (Fig. 3). Park et al. (2003) and Shafeeyan et al. (2010) reported that although steam activation increased the surface area and micropore volume of biochar, it depleted the surface functional groups, possibly offsetting any increase in sorption capacity.

Another possible mechanism for reducing NH_4^+–N leaching declining with increasing biochar rate is microbial immobilization of NH_4^+. The C to N ratios of most of our biochar–biosolids and sawdust–biosolids mixtures (calculated from Table 1) were above 25, the value required to trigger immobilization (McLaren and Cameron, 1996). We did not measure any microbiological parameters; however, if there were significant microbial immobilization, then there would be a negative correlation between WSC (Supplemental Table S3) and the mass of NH_4^+–N leached (Fig. 3, 5). However, the hot and cold WSC concentrations did not correlate with the reduction in NH_4^+–N leaching observed ($r = -0.35$, $P > 0.05$ NS).

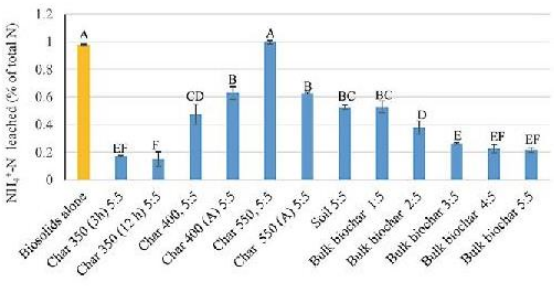

Fig. 3. Ammonium-N leached (as a percentage of total N in the columns) from columns with soil or biochars mixed with biosolids. Number ratios indicate the ration of mass of material (g) to mass of biosolids (g). Bars represent the standard error of the mean (n = 3).

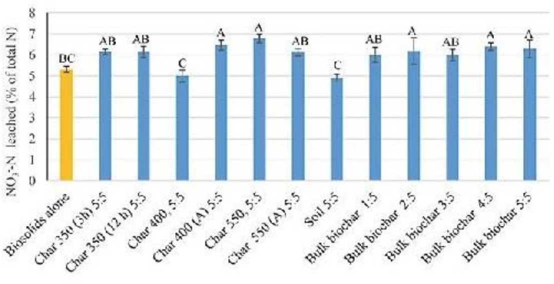

Fig. 4. Ammonia-N (as a percentage of total N in the columns) from columns with soil or biochars mixed with biosolids. Number ratios indicate the ratio of mass of material (g) to mass of biosolids (g). Bars represent the standard error of the mean (n = 3).

While NH_3 adsorption onto biochar can occur (Taghizadeh-Toosi et al., 2011) the likelihood of NH_3 adsorption occurring in the biochar material, as a mechanism for reducing NH_4^+ in solution, is unlikely a result of the pH being too low (<7.0). The pH values of the solutions in our batch sorption experiments ranged from 4.2 to 5.8.

Nitrate leaching from the column experiment accounted for <7% of the N applied (Fig. 4) and showed few differences as a consequences of biochar–biosolids treatment. Most of the N

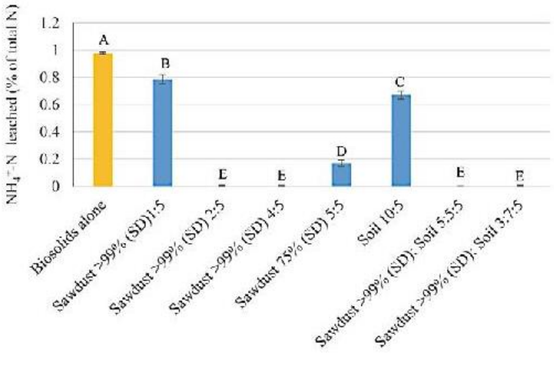

Fig. 5. Ammonium-N leached (as a percentage of total N in the columns) from columns with soil or sawdust mixed with biosolids. Number ratios indicate the ratio of mass of material (g) to mass of biosolids (g). Bars represent the standard error of the mean (n = 3).

Results and Discussion

Inorganic Nitrogen Sorption

All of the materials tested, with the exception of sawdust, sorbed significant amounts of NH_4^+ ranging from 14 to 335 mg NH_4^+ kg^{-1} material (Fig. 1). However, only the biochar produced at 350°C for 12 h and the bulk biochar sorbed more NH_4^+ than the soil (Fig. 1; $p < 0.05$). The amounts of NH_4^+ sorbed by the biochars in the current study were relatively small compared with previous reports. For example, Sarkhot et al. (2013) reported biochar produced from hardwood shavings pyrolyzed at 300°C sorbed up to 5300 mg NH_4^+ kg^{-1}. Differences in biochar sorptive capacity for NH_4^+ have been shown to result from feedstock type, for example, *Thalia* spp. and *Schinus* spp. have been shown to sorb NH_4^+ up to 785 and 3700 mg kg^{-1} by Yao et al. (2012) and Zeng et al. (2013), respectively.

Biochar retention of NH_4^+ is a function of the materials' CEC, which besides being a function of feedstock type, is also the result of the biochar production method (Libra et al., 2011). Specifically, the CEC of a biochar is a function of both the pH and porosity (Mukherjee et al., 2011), which varies with pyrolysis temperature. This was demonstrated by Lehmann (2007), using *Robinia pseudoacacia* L. as a feedstock, who showed a strong correlation between increasing biochar pH and increasing CEC as the pyrolysis temperature was increased, with an optimal CEC of 20 $cmol_c$ kg^{-1} at a temperature of 450°C and pH ~ 9. Similar results were observed by Zhang et al. (2015) for *Quercus* spp. Xiao and Pignatello (2015) demonstrated that maple (*Acer* spp.) wood biochars pyrolyzed at 300 to 700°C all had negative zeta potentials above pH 3 with a large increase in negative charge between pH 3 and pH 5.5. In the current study, increasing the pH during the batch sorption experiments also increased the sorption of NH_4^+ (Fig. 2), and this is consistent with the surface charge varying with pH and directly influencing the biochar's CEC (Lehmann, 2007).

Other studies specifically measuring CEC following the pyrolysis of *Pinus* spp. at 400 and 600°C have shown CEC to range from 10 to 38 $cmol_c$ kg^{-1} at near neutral biochar pH (Mukherjee et al., 2011). The lower CEC values in this range are consistent with the lower CEC values for the materials in the current study (Table 1). For non-*Pinus* spp., CEC is reported to range from 0.2 to 25 $cmol_c$ kg^{-1} and varies with different feedstock and pyrolysis conditions (Cheng et al., 2006; Gundale and DeLuca, 2007; Lehmann, 2007; Nguyen and Lehmann, 2009; Sarkhot et al., 2013). In the current study, there was no significant correlation ($r = 0.19$, $p > 0.05$) between the CEC of the materials tested and their ability to sorb NH_4^+. Sterilizing the solutions using phenol addition during the batch sorption experiments showed no significant differences occurred in terms of NH_4^+ sorption. This observation, and the lack of any increase in the NO_3^- concentration (results not shown), indicates that microbial activity did not affect the results of our batch-sorption experiments.

The lack of any significant sorption of NH_4^+ by the sawdust may be due to several reasons. Sawdust cell walls are active ion exchange sites resulting from the presence of cellulose, lignin, and hydroxyl groups (Shukla et al., 2002). However, cation adsorption onto sawdust is pH dependent, in the case of heavy metals, and thus in the current study, lack of NH_4^+ sorption may be due to nonoptimum pH conditions for maximum CEC expression. Another factor that prevents cation exchange on sawdust includes competition for cation sorption sites. In the current study, the Ca^{2+} ions in the assay matrix may have competed with NH_4^+ and been selectively adsorbed on the sawdust (Shukla et al., 2002). The molar ratio of Ca^{2+} to NH_4^+ in our study was 18:1. Furthermore, Harmayani and Anwar (2012) found the initial cation concentrations and extraction time also affected sorption onto pine sawdust. Thus these factors may not have been optimal in the current study for sorption of NH_4^+ by sawdust. Based on these results, the chemical sorption of NH_4^+ is not a mechanism that will reduce the potential leaching of NO_3^- when mixing biosolids with biochars or sawdust and soil.

None of the materials tested sorbed NO_3^- (data not shown). Using sugarcane (*Saccharum officinarum* L.) bagasse as a biochar feedstock, Kameyama et al. (2012) reasoned that the increased sorption of NO_3^- with increasing temperature was the result of N-containing basic functional groups on the biochar surface increasing in number with increasing pyrolysis temperature. Wang et al. (2015) also found NO_3^- sorption increased with increasing biochar manufacturing temperature. Clough et al. (2013) reviewed the studies examining NO_3^- sorption on biochar and concluded that sorption of NO_3^- onto a biochar surface was unlikely to occur unless the pyrolysis temperature during biochar manufacture was >600°C, with the degree of

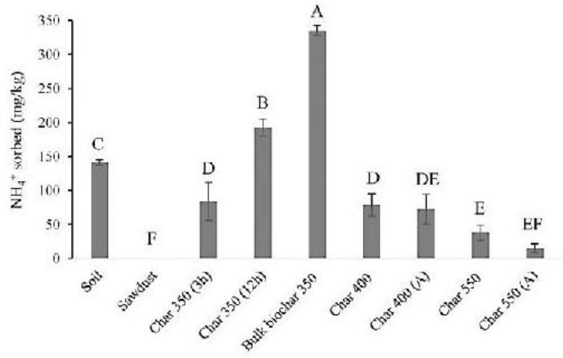

Fig. 1. Ammonium (NH_4^+) sorbed (mg kg^{-1} dry wt.) by soil, sawdust, and biochar from a 100 mg L^{-1} NH_4^+ solution after 6 h of agitation. Material/solution ratio = 1:10. Bars represent the standard error of the mean (n = 3). Bars with the same letter are not significantly different.

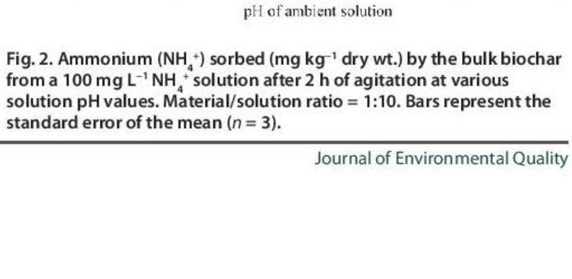

Fig. 2. Ammonium (NH_4^+) sorbed (mg kg^{-1} dry wt.) by the bulk biochar from a 100 mg L^{-1} NH_4^+ solution after 2 h of agitation at various solution pH values. Material/solution ratio = 1:10. Bars represent the standard error of the mean (n = 3).

in the biosolids remained as organic N. None of the biochar treatments caused a significant decrease in NO_3^-–N leaching (Fig. 4), in fact, the high temperature biochars and the high rates of the bulk biochar caused an increase (p < 0.05) in NO_3^-–N leaching. Reasons for the greater NO_3^-–N leaching could include greater aeration of the biosolids material, resulting in higher rates of mineralization and subsequent nitrification causing more NO_3^-–N leaching. Our result deviates from the findings of Knowles et al. (2011), who reported that the bulk biochar significantly reduced NO_3^-–N leaching from biosolids-amended soil. However, the experimental conditions described in Knowles et al. (2011) were significantly different. Their experiment was performed in the field with large lysimeters containing intact soil cores with pasture present (*Lolium perenne* L.) and thus, plant N uptake occurred.

Volatile organic compounds (VOCs) may be present in biochars and sawdust (Spokas et al., 2011) and can potentially reduce nitrification (Clough et al., 2010) and mineralization. Borchard et al. (2014) demonstrated that VOCs from biochar influence N cycle and can reduce greenhouse gas emissions from soil. The fact that the NO_3^-–N leached as a percentage of N applied was higher (p < 0.05) under the biochar treatments than in the biosolids alone (Fig. 4) indicates that if biochar-borne VOCs were inhibiting nitrification, then the effect was small.

Sawdust caused a significant reduction (p < 0.05) in both NH_4^+–N and NO_3^-–N leaching from both biosolids and biosolids-amended soil treatments (Fig. 5, 6). Rates of more than two parts of sawdust to five parts of biosolids eliminated NH_4^+–N leaching and reduced NO_3^-–N leaching by >40% (Fig. 6). These results cannot be explained by chemical sorption mechanisms because the batch experiments revealed that the sawdust sorbed neither NH_4^+–N nor NO_3^-–N. Adding sawdust increased the C to N ratio (Table 1) of the mixtures, which may have resulted in microbial immobilization of biosolids derived N. The sawdust's C to N ratio of 850 is well in excess of the value required to trigger immobilization (C/N of >25:1 McLaren and Cameron (1996). The WSC extracts (Supplemental Table S3) also indicate that in the unmixed materials, C was readily available for microbial immobilization to occur. Consistent with this theory are the results of Daniels et al. (2001), who showed that adding sawdust to biosolids at a rate of 3:2 reduced NO_3^-–N in soil pore water by >50%. In contrast, Schmidt (2001) showed that a 1:1 biosolids to sawdust mixture was ineffective in reducing NO_3^-–N leaching in the first growing season. The high WSC availability also raises the possibility of other heterotrophic activity, such as denitrification, also consuming NO_3^-–N and contributing to the decrease in NO_3^-–N leaching observed. Schipper and Vojvodic-Vukovic (1998) showed that soil amended with sawdust will remove NO_3^-–N from the groundwater via denitrification. Sawdust with a moisture content of 25% had a significantly smaller effect on NH_4^+–N and NO_3^-–N leaching than dry sawdust (Fig. 5, 6). This indicates that the sawdust may have irreversibly sorbed some of the N-rich pore water from the fresh biosolids and that physical sorption may be an important mechanism for the retention of N in these experiments. Our experiments did not provide any information on the mechanisms of such physical sorption. Biochar containing some partially pyrolyzed or unpyrolyzed material may therefore also mitigate N leaching. In this case, partial pyrolysis may be a low-cost means of drying the material. As the material weathers in the soil, the CEC of the biochar may increase (Glaser et al., 2002; Liang et al., 2006), further retaining NH_4^+–N in the root-zone where plant uptake can occur.

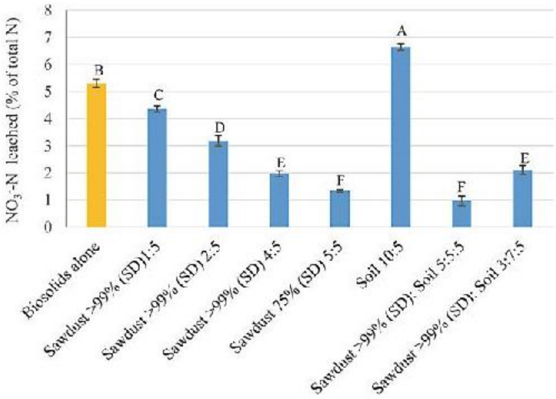

Fig. 6. Ammonia-N leached (as a percentage of total N in the columns) from columns with soil or sawdust mixed with biosolids. Number ratios indicate the ratio of mass of material (g) to mass of biosolids (g). Bars represent the standard error of the mean (*n* = 3).

Conclusions

The potential for unweathered biochars derived from sawdust feedstock to mitigate NO_3^-–N leaching from biosolids-amended soils is low and the biochars may even accelerate NO_3^-–N leaching. However, pine waste and pine biochars significantly reduced NH_4^+–N mobility. Conversely, including raw, dried sawdust when amending soils with biosolids shows significant promise to limit N mobility in biosolids and potentially reduce NO_3^-–N leaching. Future work should look to better understand the reasons for this while optimizing rates and methods to achieve NO_3^-–N leaching mitigation.

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WASTE MANAGEMENT

Biowaste Mixtures Affecting the Growth and Elemental Composition of Italian Ryegrass (*Lolium multiflorum*)

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Abstract

Biosolids (sewage sludge) can be beneficially applied to degraded lands to improve soil quality. Plants grown on biosolids-amended soils have distinct concentrations of macronutrients and trace elements, which can be beneficial or present a risk to humans and ecosystems. Potentially, biosolids could be blended with other biowastes, such as sawdust, to reduce the risks posed by rebuilding soils using biosolids alone. We sought to determine the effect of mixing biosolids and sawdust on the macronutrient and trace element concentration of ryegrass over a 5-mo period. *Lolium multiflorum* was grown in a low fertility soil, typical for marginal farm areas, that was amended with biosolids (1250 kg N ha^{-1}), biosolids + sawdust (0.5:1) and urea (200 kg N ha^{-1}), as well as a control. Biosolids increased the growth of *L. multiflorum* from 2.93 to 4.14 t ha^{-1}. This increase was offset by blending the biosolids with sawdust (3.00 t ha^{-1}). Urea application increased growth to 4.93 t ha^{-1}. The biowaste treatments increased N, P, Cu, Mn, and Zn relative to the control, which may be beneficial for grazing animals. Although biowaste application caused elevated Cd concentrations (0.15–0.24 mg kg^{-1}) five- to eightfold higher than control and urea treatments, these were below levels that are likely to result in unacceptable concentrations in animal tissues. Mixing biosolids with sawdust reduced Cd uptake while still resulting in increased micronutrient concentrations (P, S, Mn, Zn, Cu) in plants. There were significant changes in the elemental uptake during the experiment, which was attributed to the decomposition of the sawdust.

Core Ideas

- Biowastes (biosolids + sawdust) were effective in improving a low-fertility soil.
- The biowaste mixture improved growth and quality of Lolium multiflorum over 5 months.
- The biowaste treatments increased N, P, Cu, Mn, and Zn.
- Mixing biosolids with sawdust reduced Cd uptake.
- Biowaste induced changes in elemental composition increased over the 5-month period.

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Biosolids are a product of municipal wastewater treatment. They are primarily derived from domestic sources, which are combinations of human feces, urine and gray water, as well as small inputs from industry and occasionally stormwater (Lu et al., 2012). Biosolids are produced at an annual rate of 27 kg per person, and their disposal can be expensive (e.g., incineration or in landfills) or environmentally damaging via legal or illegal disposal into waterways (LeBlanc et al., 2009).

Biosolids provide nutrients and organic matter, which can improve soil structure (Antolín et al., 2005; Singh and Agrawal, 2008), enhance plant growth (Miaomiao et al., 2009; Mok et al., 2013), and increase soil microbial activity (Cytryn et al., 2011). The efficacy of biosolids in improving soil quality depends on their provenance and treatment. Biosolids application to soil can also cause negative effects because they can introduce pathogens (Vasseur et al., 1996; Zaleski et al., 2005) and contaminants, including heavy metals (Oliver et al., 1994; Miaomiao et al., 2009; Lomonte et al., 2010; Lopes et al., 2011) that may be hazardous to soil biological processes and to human health. Therefore, the rate of biosolids addition to land is regulated with respect to the levels of heavy metals, organic compounds and pathogens (EEC, 1986; USEPA, 1993; NZWWA, 2003). Excessive biosolids applications to land can result in excessive runoff or leaching of plant nutrients such as nitrate (NO_3^-) and phosphate (PO_4^{3-}) into receiving waters (Agopsowicz et al., 2008; Knowles et al., 2011). Therefore, biosolids are more suitable for rebuilding eroded land, low-fertility with poor soil structure, such as marginal farm areas (Fresquez et al., 1990; Shahid and Al-Shankiti, 2013). Such sites are not directly linked to the human food chain, and the high organic matter contents of biosolids may be more effective than mineral fertilizers for restoring degraded soils.

Some of the negative effects of biosolids addition to soil, namely environmental impacts of heavy metals, can be mitigated

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Abbreviations: PAHs, polycyclic aromatic hydrocarbons; DOM, dissolved organic matter; DOC, dissolved organic carbon; SOM, solid organic matter.

by blending the biosolids with other biowastes including biochar (Knowles et al., 2011), lignite (Simmler et al., 2013) and wood waste (Paramashivam et al., 2015). Bugbee (1999) reported that blending biosolids with sawdust could improve plant growth, while reducing NO_3^- leaching by increasing the C/N ratio. In addition to reducing NO_3^- leaching, wood waste, which can be expensive and environmentally damaging to dispose of, can effectively sorb heavy 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). Bulut and Tez (2007) demonstrated that there was variation in the sorption of individual metals (Pb $\approx$ Cd > Ni), which was attributed to the affinity of each element to the proteins, carbohydrates, and phenolic compounds in the sawdust.

Sawdust undergoes decomposition when mixed with N-rich material. During decomposition, the cation exchange capacity of the sawdust increases, as more functional groups form on the surface of the sawdust particles (Jokova et al., 1997). Therefore, it is likely that the sorption of metals by sawdust will increase, at least temporarily, as it decomposes. Sawdust may be beneficial in countries with forestry operations that produce large quantities of wood waste, which can be expensive and environmentally damaging to dispose of (Robinson et al., 2007).

Previous studies have shown that biosolids increases the growth of ryegrass (Crush et al., 2006; Santibanez et al., 2008). Biosolids also increase the uptake of Cd, Cu and Zn into the plant biomass (Ahumada et al., 2009; Bai et al., 2013; Mugica-Alvarez et al., 2015). 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). In contrast, 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 (Anderson et al., 2012).

We hypothesize that mixing sawdust with biosolids will reduce the solubility and hence the plant uptake of heavy metals from biosolids-amended soil. We aimed to determine whether mixtures of biowastes (biosolids + sawdust) could be used to rebuild a low-fertility soil without resulting in excessive metal concentrations in the aerial portions of ryegrass. Specifically, we sought to elucidate the effect of biosolids, either alone or mixed with sawdust, and urea on the growth and concentration of macronutrients and trace elements in *L. multiflorum*.

Materials and Methods

Experimental Setup

The experiment was conducted at Lincoln University greenhouse facility (43°38′42″ S, 172°27′41″ E). Low-fertility soil, with now history of fertilizer addition, was collected from a marginal farm area near Bideford, New Zealand (40°45′56″ S, 175°54′42″ E). The soil has been classified as an orthic brown soil, and has been chosen as a representative of marginal soils commonly found around farm systems. Biosolids and sawdust were collected from the Kaikoura Wastewater Treatment Plant, New Zealand (42°21′37.40″ S, 173°41′27.35″ E). Biosolids were homogenized thoroughly after sieving (≤10 mm). Tables 1 and 2 show the properties of the soil, biosolids, and sawdust.

Twenty-four 10-L pots (25 cm diam., 29 cm height) were filled with 10 kg of soil to a soil bulk density of 1.3 g cm^{-3}. Pots were incubated at ambient conditions in the greenhouse for 14 wk before treatment application. Three treatments (urea, biosolids, and biosolids + sawdust) and a control were setup randomly with six replicates each within the experimental setup. The treatments comprised urea (2.11 g dry weight), biosolids (245 g dry weight), and the same amount of biosolids mixed with sawdust (123 g dry weight). The applications rates for urea and biosolids were equivalent to 200 and 1250 kg N ha^{-1}, respectively, with the

Table 1. Properties of soil, biosolids and sawdust used in the experiment. Values in parentheses represent standard error of n = 5 replicates.

	Soil	Sawdust	Biosolids
pH	6.1	5.7 (0.1)	4.5 (0.0)
Moisture content, % w/w	25.5	230.4 (2.7)	106.2 (4.2)
Dry matter, % w/w	79.7	30.3 (0.2)	48.6 (1.0)
C/N ratio	14.3	908.4 (154.0)	10.6 (0.1)
Total available N, mg kg^{-1}	43.1	n.d. (n.d.)†	403.8 (7.1)
CEC‡, $cmol_c$ kg^{-1}	21.0	8.0 (0.2)	17.1 (0.6)
total base saturation, %BS	55.0	76.2 (0.8)	86.3 (3.0)
C, % w/w	6.5	47.7 (0.1)	27.1 (0.7)
N, % w/w	0.5	0.1 (0.0)	2.5 (0.6)
P, % w/w	0.05 (0.00)	n.d. (n.d.)	0.59 (0.00)
K, % w/w	0.19 (0.00)	0.05 (0.00)	0.37 (0.00)
S, % w/w	0.04 (0.00)	0.01 (0.00)	0.87 (0.01)
Ca, % w/w	0.41 (0.01)	0.08 (0.00)	0.63 (0.01)
Mg, % w/w	0.20 (0.00)	0.02 (0.00)	0.30 (0.00)
B, mg kg^{-1}	29.0 (0.3)	1.9 (0.2)	26.7 (0.1)
Cu, mg kg^{-1}	4.2 (0.0)	0.8 (0.0)	891.0 (18.9)
Zn, mg kg^{-1}	29 (0)	8 (0)	1073 (27)
Mn, mg kg^{-1}	133.5 (2.9)	47.2 (0.8)	184.9 (4.5)
Fe, mg kg^{-1}	15,461 (108)	116 (6)	14,534 (92)
Cd, mg kg^{-1}	0.05 (0.00)	n.d. (n.d.)	3.97 (0.07)

† n.d. = not detected.

‡ CEC = cation exchange capacity.

Table 2. Plant available [$Ca(NO_3)_2$] nutrient and trace element concentrations in biosolids and sawdust at start of the experiment. Values in parentheses represent standard error of $n = 5$ replicates.

	Sawdust	Biosolids
	mg kg^{-1}	
P	13 (1)	49 (1)
K	295 (6)	170 (5)
S	5 (2)	1193 (64)
Mg	185 (2)	349 (14)
B	n.d. (n.d.)†	n.d. (n.d.)
Cu	0.06 (0.02)	8.90 (0.32)
Zn	6.1 (1.0)	530.7 (12.0)
Mn	33.2 (1.3)	74.0 (2.9)
Fe	0.5 (0.1)	77.6 (1.7)
Cd	0.01 (0.00)	1.32 (0.02)

† n.d. = not detected.

application rate of biosolids equivalent to 50 t ha^{-1} dry weight. The biosolids and biosolids + sawdust mixtures were applied to the surface of the pots before sowing. Urea (50 kg ha^{-1} equivalent) was applied four times during the experimental period. Pots were arranged in a randomized block design.

In September 2013, 2 g of *L. multiflorum* ('Feast II' tetraploid Italian ryegrass) seeds were sown in all pots immediately after treatment application. An automated irrigation system applied a total of 1060 mm of water to each pot over the experimental period of 18 wk 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. The plant biomass was repeatedly cut back to 2 cm above the soil to simulate grazing. Harvesting occurred fortnightly over the summer (southern-hemisphere) starting from 16 Oct. 2013 to 29 Jan. 2014.

Analyses and Statistical Evaluation

At the end of the experiment, soil and plant samples were dried at 70°C until constant weight was obtained, then ground using a Retch ZM200 grinder. Soil samples were collected and passed through a 5-mm stainless steel sieve prior to chemical analyses. Soil and plant C and N concentrations were measured using an Elementar Vario MAX CN analyzer. Soil pH was determined with pH meter (Mettler Toledo Seven Easy) 24 h after shaking 10 g of soil in 25 mL deionized water. Plant-available elements was estimated with a 0.05-M $Ca(NO_3)_2$ extraction following Black et al. (2012), who reported that this extraction was the most effective procedure for determining the plant-availability of metals in biosolids-amended soil. In brief, 5 g soil was weighed into 50-mL centrifuge tubes and extracted with 30 mL of 0.05 M $Ca(NO_3)_2$ after 2 h of end-over-end shaking and centrifuging at 3200 rpm for 15 min (Whatman 52 filter paper). Extracts were stored in sealed containers until chemical analyses.

Pseudo-total elemental analysis was performed using microwave digestion in 8 mL of nitric acid (Aristar; ±69%), filtered through Whatman 52 filter paper, and diluted with filtered (MilliQ) water to a volume of 25 mL. Concentrations of B, Ca, Cd, Cu, Fe, K, Mg, Mn, P, S, and Zn were determined using inductively coupled plasma optical emission spectrometry (ICP–OES Varian 720 ES). For quality assurance, reference soil and plant material from Wageningen University, the Netherlands (International Soil analytical Exchange 921 and International Plant analytical Exchange 100) was analyzed with the samples. Recoverable concentrations were 81–112% of the certified values.

Significant differences ($\alpha = 0.05$) between control soil, urea, biosolids, and biosolids + sawdust treatments were determined by analysis of variance (Trillas et al., 2006), followed by Duncan post-hoc tests at $p \leq 0.05$. The analyses were performed using SPSS v.22 (IBM, 2013). Correlation analyses between dry biomass production and element concentrations were performed in Microsoft Excel 2013 (Microsoft Office, 2013).

Results

Figure 1 shows the cumulative pasture biomass production over the 18 wk experimental period. Control treatments showed a total average of 10.56 g biomass dry weight per pot, equivalent to 2.15 t ha^{-1}. Urea fertilization increased the cumulative biomass to 24.19 g, equivalent to 4.93 t ha^{-1}. Biosolids application also resulted in a significant biomass response (20.32 g, equivalent to 4.14 t ha^{-1}), while mixing sawdust with biosolids lowered the biomass growth of *L. multiflorum* compared to biosolids alone (14.72 g, equivalent to 3.00 t ha^{-1}). Six weeks after sowing, significant differences were detected in the growth response of *L. multiflorum* as a result of different treatments ranking in order of urea > biosolids > biosolids + sawdust > control, which remained unchanged throughout the duration of the experiment.

Table 3 shows that there were significant differences macronutrient uptake between untreated control and the treatments. Biosolids addition significantly increased the concentrations of P and S, but surprisingly not N, relative to the control. Biosolids decreased plant K concentration. Biosolids + sawdust increased both N and P. Urea application only caused a significant increase in N and caused significant decreases in P, K and S.

Within treatments, there were significant differences in the uptake of K, P, and S over the experimental period (Fig. 2). Whereas K concentration in the plant biomass showed a decreasing trend in all treatments (Fig. 2b), the highest concentrations of P and S were detected in plant biomass harvested 10 and 12 wk after sowing, as well as at the end of the experiment (Fig. 2c and 2d). For the control, biosolids, and biosolids + sawdust treatments, the N concentration varied between 2 and 3% throughout the experimental period. Concentrations of N were between 3 and 5% during the experiment in urea treatments, with distinct peaks in the initial harvest and at the 10-wk harvest, whereas N concentrations in control, biosolids and biosolids + sawdust treatments ranged between 2 and 3% (Fig. 2a). Plant P at individual harvest time points was negatively correlated with the corresponding biomass in the biosolids and biosolids + sawdust treatments ($r = -0.97, p \leq 0.001$; $r = -0.89, p \leq 0.01$), as well as plant S ($r = -0.88, p \leq 0.01$; $r = -0.78, p \leq 0.05$, data not shown).

The application of biosolids and biosolids + sawdust increased the average plant Zn concentrations up to nine- and sixfold, respectively. Foliar Cu concentrations were increased by up to 50% in the biosolids, and 70% in the biosolids + sawdust treatments (Table 4). Average concentrations of Mn were increased by approximately 50% after biosolids application compared to control treatments. Plant Cd concentrations in the biosolids treatment were approximately eightfold higher than the control

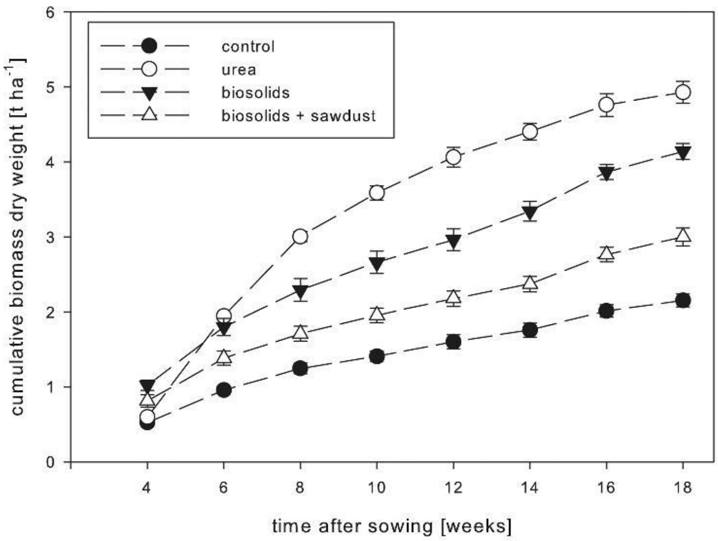

Fig. 1. Cumulative biomass (dry weight) in t ha^{-1} equivalent during the 18-wk experimental period. Each point is the average of six replicates with bars representing the standard error of the mean. Non-overlapping bars indicate significant differences ($p \leq 0.05$).

and urea treatments. This increase was only a fivefold increase in the biosolids + sawdust treatment. Urea application did not cause significant differences in average foliar concentration of any trace element.

As with the macronutrients, there was significant variation in the uptake of trace elements over the 18-wk experimental period (Fig. 3). In the biosolids and biosolids + sawdust treatments, the concentrations of Zn, Cd and Cu increased throughout the experimental period (Fig. 3b, 3c, and 3e). In contrast, there was little difference in the Cu concentration between biosolids and biosolids + sawdust treatments (Fig. 3e). For Cd and Zn, the difference between the biosolids and the biosolids + sawdust treatment increased over time (Fig. 3b and 3c). Between the 6- and 8-wk harvests, the results showed a pronounced increase in the elemental concentrations of Fe, Cd, Mn and Cu, especially in biosolids treatments.

Discussion

The total biomass production (2–5 t ha^{-1} equivalent) of *L. multiflorum* over 18 spring and summer weeks under biosolids and biosolids + sawdust treatments is comparable to the average

Table 3. 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 ($n = 8$).

	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

† Different lowercase letters indicate significant differences between treatments at $p \leq 0.05$.

biomass production of 2.2 and 8.7 t ha^{-1}, depending on the growth period, reported for 'Feast II' (Hanson et al., 2006; Moir et al., 2013). Smith and Tibbett (2004) reported annual biomass production of 1.7, 2.0, and 2.4 t ha^{-1} in pastures receiving 4, 8, and 16 t ha^{-1} of dried biosolids, which is somewhat lower than our study equating to approximately 50 t ha^{-1} of dried biosolids. Biosolids and biosolids + sawdust hence were effective in increasing plant growth on a low-fertility soil. The results indicate that mixing sawdust with biosolids significantly reduced the growth increase compared to biosolids alone and that neither biosolids nor biosolids + sawdust was as effective as urea in increasing biomass. The lower biomass production of the biosolids + sawdust treatment compared to the biosolids-alone treatment is consistent with sawdust immobilizing N. While the average N concentration in the biosolids + sawdust was not significantly lower than the biosolids-alone treatment (Table 3), the mass that was extracted (biomass × N concentration) was significantly higher for the biosolids alone treatment.

In the control treatment, the N concentration in our study was in a similar range of the value reported for annual ryegrass (*L. multiflorum*) in a study comparing different grass species under different rates of N loading (Moir et al., 2013). That the urea treatment (N) resulted in a greater increase in biomass than either of the biosolids treatments (N plus a suite of other plant nutrients) indicates that other components in the biosolids, such as heavy metals, 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. Most of the N in biosolids is locked up in organic compounds which need to undergo (microbial) transformation processes to become available (Sommers, 1977).

With the exception of N, the concentrations of macronutrients in our study were similar to those reported for perennial ryegrass (Harrington et al., 2006). Even though urea significantly increased the biomass, the concentrations of other essential

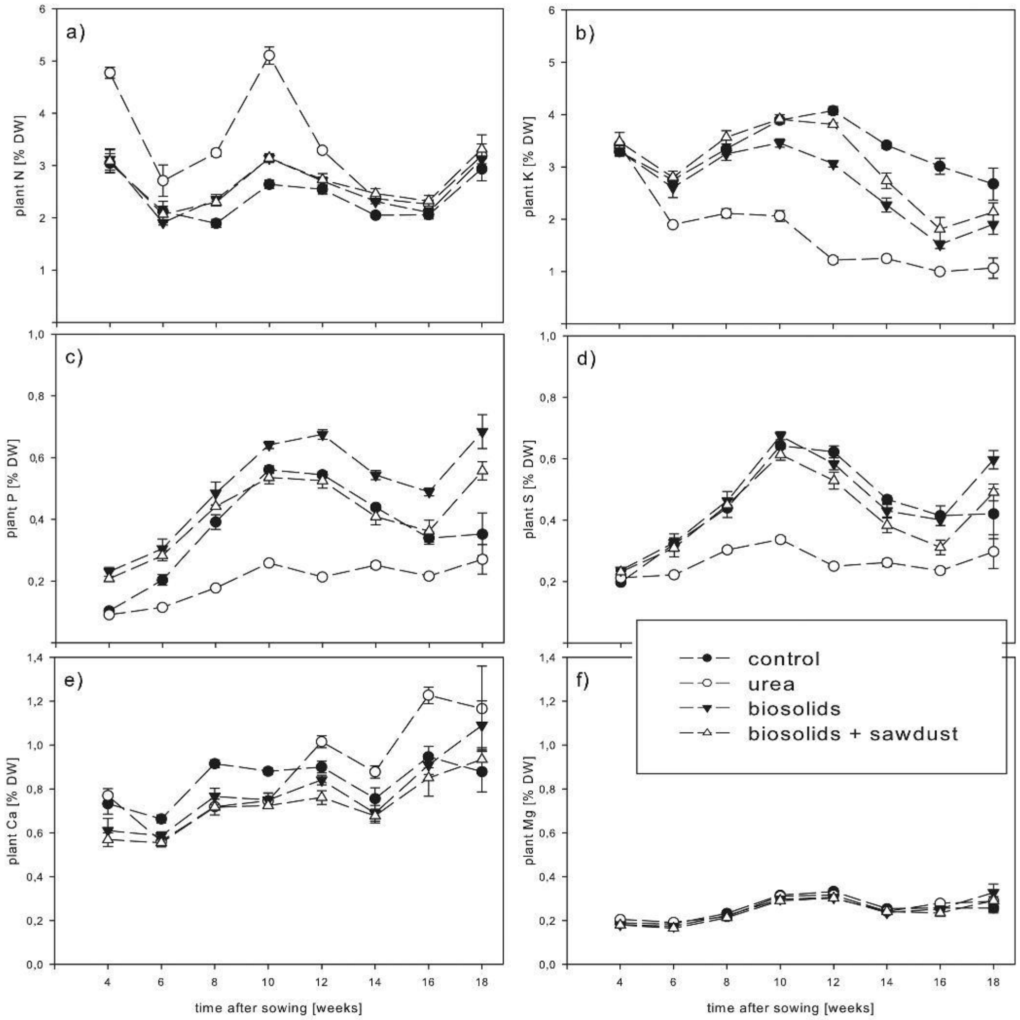

Fig. 2. Average concentrations of macronutrients over the experimental period ($n = 6$). Error bars represent the standard error of the mean. Non-overlapping error bars indicate significant difference between means ($p \leq 0.05$).

Table 4. 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 ($n = 8$).

	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

† Different lowercase letters indicate significant differences between treatments at $p \leq 0.05$.

macronutrients, namely P, S and K were significantly lower in the urea treatment, indicating that these elements were not limiting in the control soil. These elements dropped to near-deficient concentrations (McNaught, 1970) in the urea treatment, possible due to a dilution-by-growth effect. The biosolids + sawdust treatment (Table 3) showed that the concentrations of K, P, and S were higher than the critical deficiency threshold concentrations (28, 2.1, and 1.8 g kg^{-1}, respectively) reported for perennial ryegrass (*L. perenne*) (McNaught, 1970; Smith et al., 1985). This is consistent with using biosolids and biosolids + sawdust not only to improve plant growth, but also to enhance plant nutrient uptake in a low fertility environment. The concentration of plant K decreased throughout the experimental period, which could

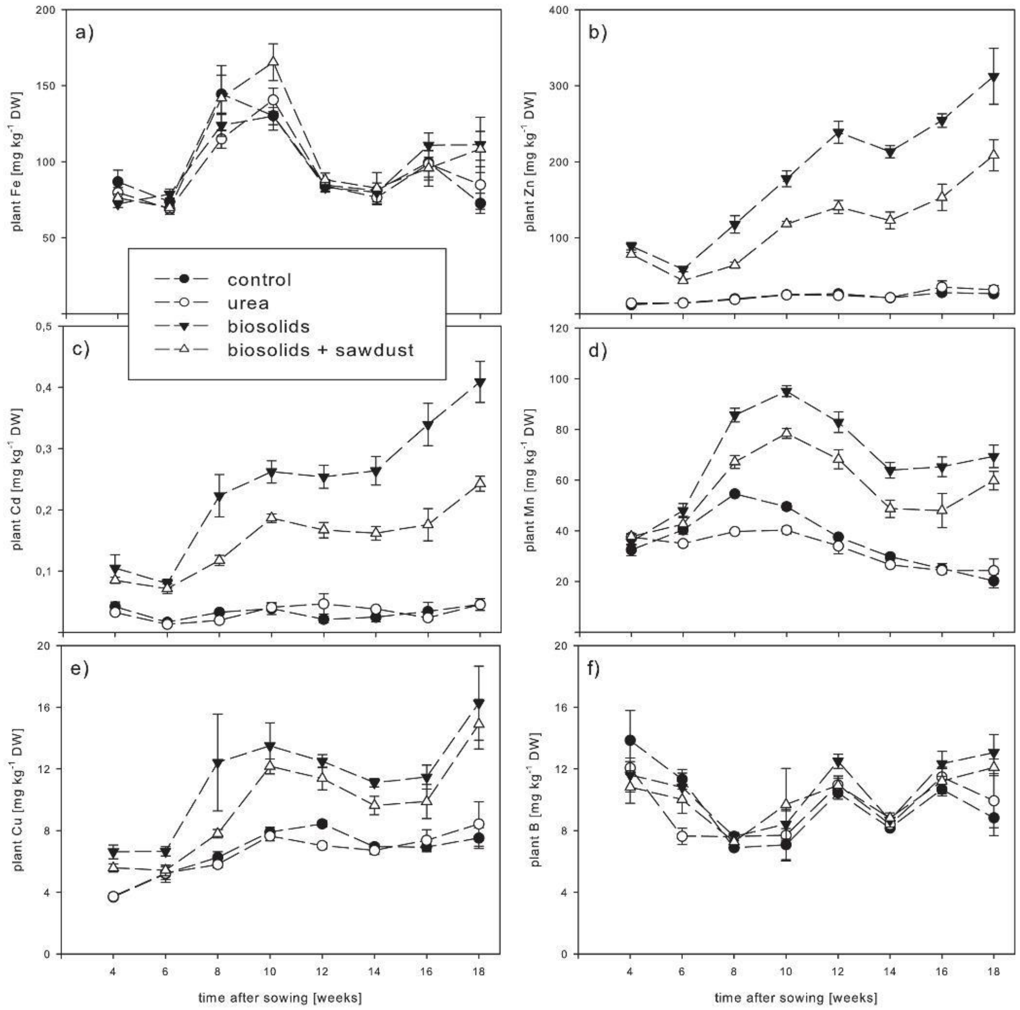

Fig. 3. Average (n = 6) concentrations of trace elements over the experimental period. Error bars represent the standard error of the mean. Non-overlapping error bars indicate significant difference between means ($p \leq 0.05$).

be attributed to a limited supply of K from the initial soil, as well as biosolids and biosolids + sawdust treatments (Table 2).

Lolium multiflorum growing in the biosolids and biosolids + sawdust treatments had significantly higher concentrations of Cd, Cu and Zn compared to control and urea treatments. Mixing sawdust with biosolids significantly decreased the Cd and Zn concentrations compared to the biosolids treatment. Clearly, this is beneficial in the case of Cd; sawdust addition can reduce the entry of this toxic element into fodder and food plants. In our study, Cd concentrations 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 our study (Table 3) were lower compared to others studies where biosolids had been used as a soil conditioner at similar rates (Antoniadis and Alloway, 2001; Black et al., 2012).

Some of the negative effects of elevated Cd may be offset by the elevated Zn concentrations (Oliver et al., 1994; Khoshgoftar et al., 2004; Reiser et al., 2014). Since Cd is absorbed by the root Zn transporter, a low supply of plant available Zn promotes Cd accumulation by plants (Khoshgoftar et al., 2004). Applying Zn fertilizer inhibits Cd uptake and translocation, especially in soils with low plant available Zn (Oliver et al., 1994). Khoshgoftar et al. (2004) reported that when Zn fertilizer was applied in a greenhouse experiment, Zn concentration in wheat shoot increased from 26 to 56 mg kg^{-1}, and Cd concentration was reduced from

0.90 to 0.09 mg kg^{-1}. In our study, foliar Zn concentrations were similar to the 129 to 390 mg kg^{-1} range reported by Santibanez et al. (2008) and Torri and Lavado (2009), who used higher rates of biosolids addition (150–400 t ha^{-1}) for perennial ryegrass. Biomass Zn concentrations in *L. multiflorum* in this study were higher than those reported for similar or lower rates of biosolids addition in combination with *L. perenne* (Antoniadis and Alloway, 2001; Ahumada et al., 2009; Black et al., 2012), hence they could have offset Cd uptake into plant biomass. The high Zn concentrations in our study can be explained by the relatively high Zn content in the used biosolids, as well as the mildly to moderately acidic nature of the soil and biosolids respectively (Table 1). The Zn concentrations in *L. multiflorum* in the biosolids treatment were within the range that Anderson et al. (2012) reported to cause a beneficial increase in blood Zn concentrations in sheep.

Although Cd and Zn were significantly higher in biosolids compared to biosolids + sawdust, plant Cu concentrations in plant biomass increased after mixing biosolids with sawdust compared to pure biosolids application. Copper deficiency is a widespread problem in all agricultural systems (Sinclair and Edwards, 2008; White and Broadley, 2009); thus increasing Cu uptake by plants by mixing biosolids with sawdust can provide agricultural benefits. However, the Cu concentration in our study were generally lower than those reported for *L. perenne* (Antoniadis and Alloway, 2001; Ahumada et al., 2009; Black et al., 2012). Urea application caused significant differences in uptake of B, Cu, and Zn, however, the differences were small and unlikely to be of agricultural significance.

During the experimental period, an accumulation of Zn, Cd and Cu was observed in *L. multiflorum* biomass. An increase of these elements at the end of the growing season may be related to decreased metabolic processes and smaller changes in the plant biomass, as suggested in studies investigating seasonal variations in trace metal uptake by *Phragmites australis* (Kastratović et al., 2013; Eid and Shaltout, 2014). This is consistent with the results obtained from total biomass harvests (Fig. 1), which show only a small growth increase toward end of the experiment. In the biosolids + sawdust treatment, Cd and Zn concentrations increased at a lower rate compared to the biosolids treatments, indicating that sawdust reduced the mobility of these elements. It was likely that the sawdust started to decompose during the experiment, resulting in increased metal sorption. Kostov et al. (1991) showed that the C/N ratio of *Picea excelsa* sawdust decreased from 251 to 62 only 6 mo after treatment with nutrient solution. Sawdust decomposition could explain the greater difference between the biosolids + sawdust treatment and biosolids treatments at the end of the experiment compared to the harvests before 6 wk, where difference were minimal.

The application of biosolids and biosolids mixed with sawdust improved the growth of *L. multiflorum* on a low-fertility soil, while the biowaste mixture (biosolids + sawdust) was less effective in restoring fertility compared to biosolids alone. Although less growth promoting, the advantage of using sawdust was seen in a reduction of the Cd uptake by the plants. There were significant changes in the elemental composition of the pasture over time, with the differences between the biosolids and biosolids + sawdust treatments increasing over time to favorable agronomic levels. Our results indicate that a single harvest of pasture can be insufficient to determine the effect of a soil treatment on element uptake, since results highly vary with environmental conditions, plant growth, and metabolism. Future work could involve a field study to reveal the effect of sawdust decomposition on the long-term fertility of soils amended with a mixture of biowastes.

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TECHNICAL REPORTS

WASTE MANAGEMENT

Production of Biomass Crops Using Biowastes on Low-Fertility Soil: 1. Influence of Biowastes on Plant and Soil Quality

J. Esperschuetz, C. Anderson, S. Bulman, O. Lense, J. Horswell, N. Dickinson, R. Hofmann, and B. H. Robinson*

Abstract

Land application of biosolids to low-fertility soil can improve soil quality by increasing concentrations of macronutrients and trace elements. Mixing biosolids with sawdust could reduce the risks of contaminant accumulation posed by rebuilding soils using biosolids alone. We aimed to determine the effects of biosolids and biosolids-sawdust on the plant quality and chemical composition of sorghum, rapeseed, and ryegrass. Plants were grown in a greenhouse over a 5-mo period in a low-fertility soil amended with biosolids (1250 kg N ha^{-1}), biosolids-sawdust (0.5:1), or urea (200 kg N ha^{-1}). Biosolids application increased the biomass of sorghum, rapeseed, and ryegrass up to 14.0, 11.9, and 4.1 t ha^{-1} eq, respectively. Mixing sawdust with biosolids resulted in a growth response similar to biosolids treatments in rapeseed but nullified the effect of biosolids in sorghum. Urea fertilization provided insufficient nutrients to promote rapeseed growth and seed production, whereas seed yields after biosolids application were 2.5 t ha^{-1}. Biosolids and biosolids-sawdust application enhanced plant quality by increasing element concentrations, especially Zn, and potentially toxic elements (Cd, Cr, Ni) did not exceed food safety standards. An application of 50 t ha^{-1} of biosolids, equivalent to 1250 kg N ha^{-1}, did not exceed current soil limits of Cu, Zn, and Cd and hence was effective in rebuilding soil without accumulating contaminants. The effect of mixing sawdust with biosolids varies with plant species but can further enhance plant nutrient quality in biomass and seeds, especially P, Cu, Zn, Mn, Fe, S, and Na.

Core Ideas

- Biosolids application showed potential for Zn enrichment in all plant species.
- Biosolids and sawdust applied to soil enables growth of rapeseed in low-fertility soil.
- Toxic elements like Cd were not increased to levels dangerous for human health.
- Biosolids and sawdust increased seed quality and hence potentially plant products.

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Global increases in population and wealth have resulted in increased production of biowastes, which require sustainable management strategies for their disposal and recycling (Panagos et al., 2013; Río et al., 2011). Biosolids (sewage sludge) are a product of human and industrial effluent. Application of biosolids to agricultural land is widely practiced and can reduce the requirement for mineral fertilizers. Biosolids have also proven suitable to rebuild degraded land (Dere et al., 2012; Oladeji et al., 2013; Speir et al., 2003; Stehouwer et al., 2006), where they can indirectly increase soil C stocks when CO_2–C is fixed by plants whose growth has been promoted by biosolids and their effect on soil quality in terms of physical, chemical, and biological fertility (Torri et al., 2014). Furthermore, in this context biosolids can increase C sequestration by introducing recalcitrant C (Tian et al., 2009), and the formation of metal–organic complexes can limit microbial and enzymatic access, thereby protecting C from rapid mineralization (Keiluweit et al., 2015).

Biosolids can contain elevated concentrations of heavy metals, organic contaminants, and pathogens. This poses potential risks for quality assurance in the human food chain and may negatively affect soil health and function as well as plant growth (Alloway, 2013; Bolan et al., 2014). In addition, organic contaminants and pathogens may pose risks to human health and the environment due to their persistence and potential bioaccumulation in food webs (Clarke and Smith, 2011; Horswell et al., 2010; Sidhu and Toze, 2009). However, treatment technology and processing of biosolids have improved over recent years, and land application as a waste management strategy has become increasingly popular (Park et al., 2011).

Plants grown in biosolids-amended soils can add value to the land through the use of plant parts for industry (e.g., cosmetics and medicine) and bioenergy (e.g., bioethanol, biogas, and biomass burning) purposes. In plants, some of the metals applied with biosolids serve as macro- and micronutrients and

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Abbreviations: DW, dry weight.

are essential for growth at low concentrations. Over the last century, there has been a reduction in crop quality due to insufficient contents of macro- and micronutrients in soil (Fan et al., 2008; Thomas, 2003); hence, application of micronutrients via biosolids could benefit plant and seed quality (Alloway, 2013). Although in many cases risks of metal accumulation or translocation to grains is limited, plants grown in biosolids-amended soils must be carefully monitored to ensure they do no accumulate metal concentrations in plant tissues above toxicity thresholds (Vamerali et al., 2010).

Some of the negative effects of biosolids addition to soil can also be mitigated by blending the biosolids with wood waste (Ammari et al., 2012). Sawdust can immobilize contaminants (Fiset et al., 2000) and thereby reduce plant uptake. Immobilization and removal of heavy metals, such as Cu, Cr, Ni, Zn, and Cd, by sawdust and other wood waste has been shown in wastewater (Ajmal et al., 1998; Bouziane et al., 2012; Bryant et al., 1992; Bulut and Tez, 2007), where the exchange capacity and general sorption characteristics depend on the materials' contents of cellulose, hemicellulose, pectin, lignin, proteins, and phenolic groups (Bulut and Tez, 2007; Kumar, 2006; Randall et al., 1974).

Plants grown for bioenergy purposes may have a small but significant role in future energy policies (Dickinson et al., 2009). Marginal and degraded land may provide an excellent alternative for growing plants for bioenergy (Field et al., 2008). Combining soil remediation with energy crop production has become increasingly popular because the management of soil contaminants can be combined with waste recycling and profitable output (Dickinson et al., 2009; Evangelou et al., 2012; Gadepalle et al., 2007). Therefore, we chose rapeseed, sorghum, and ryegrass as plant species on the basis that they could add value to the land through fiber or pulp production, through providing materials for food, or through the production of bioenergy through bioethanol, biogas, and biomass burning.

We aimed to determine the plant growth and element concentrations in a low-fertility soil amended with biosolids and a mixture of biosolids-sawdust. Specifically, we sought to identify the effect of nutrients, trace elements, and heavy metals on plant and seed quality. The effects of organic contaminants and pathogens were beyond the scope of the study. We hypothesize that biosolids increase plant quality and that contaminants do not accumulate in plant tissues at concentrations above toxicity thresholds. Because sawdust can be used to immobilize elements, a sawdust application in combination with biosolids can prevent plant uptake of elements that are not essential for plant growth and development. The focus in this manuscript is on plant and soil nutrients and trace elements; N transformation processes are discussed in Part 2 of this study (Esperschütz et al., 2016a).

Materials and Methods

Experimental Setup

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 of New Zealand near Bideford, mainly classified as orthic brown soil with a clay-loam texture (40°45′56″ S, 175°54′42″ E). Soil analyses showed the soil at a medium pH range (pH 6.1), with medium carbon (6.5%) and nitrogen (0.46%) levels and a C/N ratio of 14.3. Cation exchange capacity was determined at 21 me 100 g^{-1}. Potassium, Mg, and Na occurred at concentrations of 0.30, 0.63, and 0.14 me 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 wk before treatment application.

The experiment was set up in four soil treatments (control, biosolids, biosolids-sawdust, 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 radiate* 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 ($\leq$10 mm). Table 1 provides a detailed description of sawdust and biosolids. 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 biosolids-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. Concentrations of total and $Ca(NO_3)_2$–extractable, plant-available elements in the initial sawdust and biosolids material are shown in Table 1.

Seeds of ryegrass (*Lolium multiflorum* LAM. Feast II tetraploid Italian ryegrass; 2 g), sorghum [*Sorghum bicolor* (L.) Moench 'Sudanese'], and rapeseed (*Brassica napus* L. 'MAKRO') were sown directly into the lysimeters after treatment application. After germination, sorghum and rapeseed were thinned to three and five plants per lysimeter, respectively. The experiment was maintained for 18 wk in the greenhouse with temperatures ranging between 9 and 20°C during the nighttime (10 PM until 6 AM) and between 14 and 28°C during the daytime. The lysimeters were weeded fortnightly. 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. Soil moisture was kept above field capacity to allow drainage. The total irrigation for rapeseed, sorghum, and ryegrass was 2160, 1190, and 1060 mm, respectively. The amount of leachate was sampled and recorded weekly throughout the experimental period. Aliquots were stored at −20°C until further analyses (see Esperschütz et al., 2016a). Ryegrass was repeatedly cut back to 2 cm above the soil to simulate grazing. Individual harvests were analyzed separately as reported in Esperschütz et al. (2016b).

Table 1. Initial conditions of the sawdust and biosolids used for the lysimeter experiment at the Lincoln University Plant Growth Unit (n = 5; SE <10% if not indicated otherwise).

	Sawdust		Biosolids	
	Total	Extractable†	Total	Extractable†
pH	5.7 ± 0.1	n.d.‡	4.5 ± 0.0	n.d.
Dry matter, %	30.3 ± 0.2	n.d.	48.6 ± 1.0	n.d.
Total C, %	47.7 ± 0.1	n.d.	27.1 ± 0.7	n.d.
C/N ratio	908 ± 154	n.d.	10.6 ± 0.1	n.d.
CEC,§ me 100 g^{-1}	8.0 ± 0.2	n.d.	17.1 ± 0.6	n.d.
Total base saturation, %BS	76.2 ± 0.8	n.d.	86.3 ± 3.0	n.d.
N, %	0.1 ± 0.0	b.d. ± b.d.¶	2.5 ± 0.6	403.8 ± 7.1
P, mg kg^{-1}	42 ± 1	13 ± 1	5941 ± 42	49 ± 1
K, mg kg^{-1}	455 ± 6	295 ± 6	3653 ± 34	170 ± 5
S, mg kg^{-1}	70 ± 1	5 ± 2	8681 ± 140	1193 ± 64
Ca, mg kg^{-1}	838 ± 11	n.d. ± n.d.	6331 ± 91	n.d. ± n.d.
Mg, mg kg^{-1}	212 ± 3	185 ± 2	3005 ± 34	349 ± 14
Na, mg kg^{-1}	40 ± 2	26 ± 1	202 ± 1	54 ± 2
B, mg kg^{-1}	1.9 ± 0.2	n.d. ± n.d.	26.7 ± 0.1	n.d. ± n.d.
Cu, mg kg^{-1}	0.8 ± 0.0	0.1 ± 0.0	891.0 ± 18.9	8.9 ± 0.3
Fe, mg kg^{-1}	116 ± 6	0.5 ± 0.1	14,534 ± 92	77.6 ± 1.7
Mn, mg kg^{-1}	47 ± 1	33 ± 1	185 ± 5	74 ± 3
Zn, mg kg^{-1}	8.4 ± 0.4	6.1 ± 1.0	1073.1 ± 26.8	530.7 ± 12.0
Cd, mg kg^{-1}	n.d. ± n.d.	0.01 ± 0.00	3.97 ± 0.07	1.32 ± 0.02
Ni, mg kg^{-1}	0.6 ± 0.5	0.03 ± 0.01	20.7 ± 0.4	3.97 ± 0.06
Cr, mg kg^{-1}	0.2 ± 0.0	n.d. ± n.d.	47.6 ± 0.8	0.03 ± 0.00
Pb, mg kg^{-1}	n.d. ± n.d.	n.d. ± n.d.	151.3 ± 3.2	n.d. ± n.d.

† Estimation of plant-available elements using 0.05 mol L^{-1} $Ca(NO_3)_2$ extraction.

‡ Not determined.

§ Cation exchange capacity.

¶ Below detection.

Analyses and Measurements

A final destructive harvest of all lysimeters was performed after 18 wk. 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. Soil attached to roots ≤2 mm was considered as rhizosphere soil. Rhizosphere soil was harvested, subsampled, and stored for further analyses after sieving (≤5 mm). Plant and soil samples were ground using a Retch ZM200 grinder. Plant C and N concentrations were measured using a Vario MAX CN analyzer (Elementar). Pseudo-total elemental analysis was performed using microwave digestion in 8 mL of Aristar nitric acid (±69%), filtered using Whatman 52 filter paper, and diluted with milliQ water to a volume of 25 mL. Sample digestion and analyses was performed using an inductively coupled plasma optical emission spectrometer (720-ES ICP–OES, Varian) as described by Esperschütz et al. (2016b). Concentrations of Al, Co, Sr, and Pb were below concentrations toxic to plants and were unlikely to be significant for human health or ecosystem functioning (Aral and Vecchio-Sadus, 2008; Wuana and Okieimen, 2011) and hence are not further discussed in the present study.

An estimation of the plant-available elements was made using a 0.05 mol L^{-1} $Ca(NO_3)_2$ extraction following Black et al. (2012), who reported that this extraction was the most effective procedure for determining the plant availability of metals in biosolids-amended soil. In brief, 5 g soil were weighed into 50-mL centrifuge tubes and extracted with 30 mL of 0.05 mol L^{-1} $Ca(NO_3)_2$ after 2 h of end-over-end shaking and centrifuging at 3200 rpm for 15 min (Whatman 52 filter paper).

Statistical analyses were based on four individual replicates for rapeseed and sorghum and six replicates for ryegrass, respectively. Using SPSS 22 (IBM SPSS statistics), ANOVAs were performed followed by Duncan's post hoc tests to identify homogenous subsets for α = 0.05. Graphs were prepared using SigmaPlot 11.0 (Systat Software Inc.).

Results

Biomass and Seed Yield

Biosolids application resulted in a positive growth response in all plant species compared with control treatments during the 18-wk experimental period (Fig. 1a). Mixing sawdust with biosolids resulted in a growth response similar to biosolids treatments in rapeseed but nullified the biosolids effect in sorghum. Urea application increased sorghum and ryegrass biomass but showed no effect on rapeseed biomass compared with control treatments.

When grown on unamended soil, rapeseed produced negligible biomass and did not respond to urea application; hence, no seeds were obtained from control and urea treatments at the end of the experiment. No significant differences were observed between rapeseed seed biomass in biosolids and biosolids-sawdust treatments (Fig. 1b). Soil amendments significantly increased the seed biomass of sorghum, with highest yields in biosolids and urea treatments.

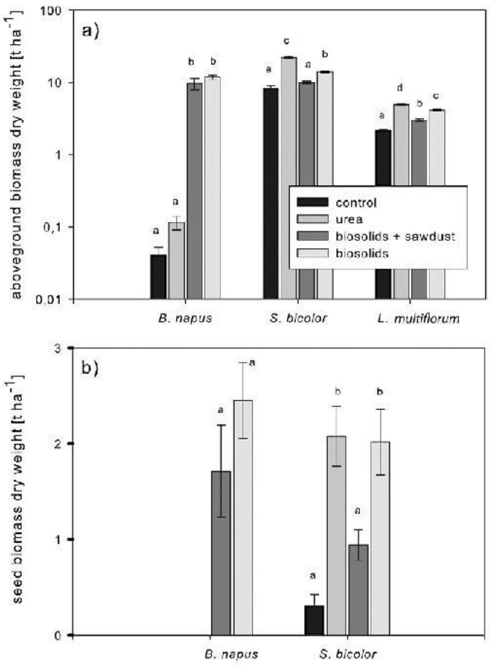

Fig. 1. Total aboveground plant biomass (a) and total seed biomass (b) of sorghum (*S. bicolor*), rapeseed (*B. napus*), and ryegrass (*L. multiflorum*) at the end of the experiment. Significant differences ($p \leq 0.05$) are represented by lowercase letters.

Nutrient and Trace Element Concentration in Plants and Seeds

Biosolids and biosolids-sawdust application to rapeseed showed lower N, S, Ca, and Mg concentrations compared with controls. There was no effect on P and K accumulation (Table 2). An increased accumulation of S and Mg was detected in sorghum biosolids and biosolids-sawdust treatments. In combination with sorghum, urea application showed increased N concentration, whereas decreased concentrations of P and K were detected compared with control treatments. In ryegrass treatments, biosolids and biosolids-sawdust caused an increase in plant P but resulted in lower concentrations of K and Ca compared with controls. Compared with rapeseed and sorghum, the highest concentrations of P and K were detected in ryegrass, irrespective of soil treatments. A detailed discussion of N uptake and leaching was performed by (Esperschütz et al., 2016a).

In rapeseed, biosolids and biosolids-sawdust treatments resulted in lower B and Fe but showed up to 10-fold higher concentrations of Zn compared with control treatments (Table 3). Biosolids and biosolids-sawdust caused fivefold and eightfold higher Zn concentrations in sorghum, respectively. Biosolids-sawdust application resulted in Cu and Mn accumulations that were up to 30 and 40% higher than biosolids treatments. In ryegrass, biosolids treatments showed increased Cu, Mn, and Zn concentrations up to 65, 85, and 540%, respectively, and levels in biosolids-sawdust treatments were increased by 65, 110, and 400%, respectively, compared with controls. Whereas no differences between treatments were detected in Mo concentration in rapeseed, higher Mo concentrations were measured in sorghum and ryegrass biosolids and biosolids-sawdust treatments compared with controls. In ryegrass and in sorghum, a decrease in Mo concentration was detected after urea application.

Macronutrient concentration (Supplemental Table S1) in rapeseed seeds was not significantly different between biosolids and biosolids-sawdust. Concentrations of N, P, and S in sorghum treatments were up to 60, 108, and 57% higher after biosolids application compared with controls. Among micronutrients (Supplemental Table S2), Cu, Fe, and Zn were increased in sorghum seeds after biosolids-sawdust application up to 25, 305,

Table 2. Total macronutrients in plant biomass in response to different soil amendments.†

	Control	Urea	Biosolids-sawdust	Biosolids
	%			
Rapeseed				
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
Sorghum				
N	0.21 ± 0.05a	1.51 ± 0.16b	0.29 ± 0.01a	0.19 ± 0.03a
P	0.10 ± 0.01bc	0.04 ± 0.00a	0.17 ± 0.00c	0.10 ± 0.01b
K	0.81 ± 0.03bc	0.31 ± 0.00a	0.92 ± 0.07c	0.61 ± 0.11b
S	0.07 ± 0.00a	0.06 ± 0.00a	0.09 ± 0.00b	0.09 ± 0.01b
Ca	0.40 ± 0.02a	0.49 ± 0.03b	0.50 ± 0.02b	0.52 ± 0.03b
Mg	0.11 ± 0.00a	0.16 ± 0.01c	0.12 ± 0.01ab	0.14 ± 0.01b
Ryegrass				
N	2.39 ± 0.04a	3.35 ± 0.09c	2.63 ± 0.12b	2.56 ± 0.05ab
P	0.30 ± 0.01b	0.17 ± 0.00a	0.35 ± 0.02c	0.43 ± 0.02d
K	3.21 ± 0.03d	1.93 ± 0.02a	3.00 ± 0.12c	2.73 ± 0.06b
S	0.38 ± 0.01bc	0.26 ± 0.00a	0.35 ± 0.02b	0.40 ± 0.01c
Ca	0.80 ± 0.01c	0.77 ± 0.02bc	0.66 ± 0.02a	0.73 ± 0.01b
Mg	0.23 ± 0.00b	0.24 ± 0.01bc	0.21 ± 0.01a	0.23 ± 0.00b

† A detailed investigation of macronutrients in individual ryegrass biomass throughout the experiment is reported in Esperschütz et al. (2016b).

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

Table 3. Total micronutrients in plant biomass in response to different soil amendments.†

	Control	Urea	Biosolids-sawdust	Biosolids
	mg kg^{-1}			
Rapeseed				
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
Sorghum				
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
Ryegrass				
B	11.4 ± 1.0b	8.9 ± 0.3a	9.9 ± 0.8ab	10.5 ± 0.3ab
Cu	5.9 ± 0.1a	6.0 ± 0.2a	8.7 ± 0.4b	10.3 ± 0.6c
Fe	96.0 ± 3.9a	105.8 ± 13.6a	105.5 ± 7.1a	118.7 ± 14.4a
Mn	37.4 ± 1.0a	35.2 ± 0.8a	51.0 ± 2.4b	60.2 ± 1.7c
Zn	21.6 ± 2.3a	19.8 ± 0.7a	97.1 ± 3.7b	150.4 ± 8.3c
Mo	1.11 ± 0.08b	0.68 ± 0.02a	2.33 ± 0.48c	4.24 ± 0.29d

† A detailed investigation of macronutrients in individual ryegrass biomass throughout the experiment is reported in Esperschütz et al. (2016b).

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

and 51%, respectively. Concentrations of Cu, Fe, Zn, and Mn were higher compared with biosolids treatments. Rapeseed seeds showed higher concentrations of Mn in biosolids-sawdust treatments compared with biosolids.

Contaminant Uptake into Plants

Compared with controls, a higher concentration of Ni was detected in rapeseed and ryegrass after biosolids and biosolids-sawdust addition (Table 4). Ryegrass showed Ni concentrations between 2.5-fold and 5-fold higher compared with sorghum and rapeseed, irrespective of soil treatment. The accumulation of Cd in rapeseed was not affected by any treatment. Biosolids and biosolids-sawdust treatments showed increased Cd concentrations in ryegrass, to a similar level as in rapeseed. In sorghum biomass, Cd concentrations were low ($\leq$0.1 mg kg^{-1}) and close to detection limit (0.01 mg kg^{-1}). Cadmium concentrations in rapeseed seeds were not affected by mixing sawdust with biosolids compared with pure biosolids (Supplemental Table S3). In sorghum, similar to rapeseed, no differences were observed regarding Cd accumulation. Biosolids and biosolids-sawdust resulted in an increase in Li and As uptake in ryegrass and an increased Li accumulation in rapeseed, respectively (Table 4).

Extractable Elements in Rhizosphere Soil

Limited amounts of rhizosphere soil was harvested from rapeseed; hence, analyses of macronutrients and trace elements in rhizosphere soil were only performed for sorghum and ryegrass treatments. A plant-available fraction of macro- and micronutrients was estimated after $Ca(NO_3)_2$ extraction. Available N (NO_3^-, NO_2^-, and NH_4^+) was determined after KCl extraction and has been discussed in detail by (Esperschütz et al., 2016a). Sorghum rhizosphere soil showed increased plant-available P in response to biosolids application and an increase in S after biosolids and biosolids-sawdust application (Table 5). In ryegrass treatments, Mg and Zn increased in response to biosolids application, whereas P, S, and Mn showed higher concentrations in biosolids- and biosolids-sawdust–amended soil. The potential contaminants Cd, Cr, Ni, and Pb were below detection limit in all treatments (<0.01, <0.02, <0.08, and <0.13 mg kg^{-1}, respectively; data not shown).

Plant species had a significant effect on extractable elements in the rhizospheres of plants in control, urea, and biosolids treatments but not in the biosolids-sawdust treatment. Control, urea, and biosolids treatments with sorghum reduced available K compared with ryegrass (Table 5). In urea treatments, sorghum growth lowered concentrations of K, Mg, Mn, and Zn compared with ryegrass. After biosolids application, available K, S, Mg, Cu, and Zn concentrations were reduced compared with ryegrass. Mixing biosolids with sawdust negated any plant species effect on the availability of nutrients and trace elements in soil.

Discussion

Plant and Seed Yield in Response to Biowaste Application

According to the characterization of the initial soil used for the experiment, all plants grown in the control treatment encountered a low-fertility environment, particularly N and P. Urea application increased sorghum and ryegrass biomass significantly compared with controls, biosolids, and biosolids-sawdust treatments. In urea treatments, 200 kg ha^{-1} of plant-available N was applied. Because urea rapidly hydrolyses to form NH_4^+ (Robertson and Groffman, 2015), the urea treatments may have provided more available N than the biowaste treatments, which contain >95% organic N (Gilmour et al., 2003). The lack of rapeseed growth in the urea treatment indicates that nutrients other than N were limiting growth. Rapeseed has shown higher requirements for S and P compared with other species, such as wheat or maize (Abdallah et al., 2010; Ahmad et al., 2007; Chen et al., 2015; Jackson, 2000). High N levels in rapeseed control and urea treatments were most likely the effect of a N concentration effect due to scarce biomass growth in these treatments compared with biosolids and biosolids-sawdust. In the control soil, Olsen P was only 11 mg L^{-1}, and the soil total S content in the initial soil was measured at 405 mg kg^{-1}, which was characterized as S-deficient according to Rajendram et al. (2008). The application of the equivalent of 375 kg ha^{-1} of total S and 250 kg ha^{-1} of total P with biosolids and biosolids-sawdust, of which 41.5 and 2.5 kg ha^{-1}, respectively, were available to plants, provided sufficient S and P for rapeseed growth. By applying nutrients and trace elements with biosolids and biosolids-sawdust,

Table 4. Contaminant accumulation in plant biomass in response to different soil amendments.†

	Control	Urea	Biosolids-sawdust	Biosolids
	mg kg^{-1}			
Rapeseed				
Cd	0.24 ± 0.03a‡	0.27 ± 0.04a	0.30 ± 0.02a	0.26 ± 0.03a
Ni	0.43 ± 0.26a	0.48 ± 0.24a	1.41 ± 0.12b	1.25 ± 0.15b
Cr	0.87 ± 0.71a	0.48 ± 0.16a	1.00 ± 0.26a	0.77 ± 0.23a
As	<0.50	0.22 ± 0.02	<0.18	<0.02
Li	<0.07	0.07 ± 0.00a	0.28 ± 0.02b	0.23 ± 0.03b
Sorghum				
Cd	n.d. ± n.d.§	0.01 ± 0.01a	0.11 ± 0.02b	0.09 ± 0.02b
Ni	1.02 ± 0.22bc	0.52 ± 0.09a	0.73 ± 0.02ab	0.97 ± 0.08b
Cr	2.21 ± 0.53c	0.97 ± 0.10a	1.50 ± 0.08ab	1.71 ± 0.07bc
As	<0.38	0.35 ± 0.11a	<0.18	0.50 ± 0.23a
Li	<0.01	0.02 ± 0.02a	0.01 ± 0.00a	<0.03a
Ryegrass				
Cd	0.03 ± 0.01ab	0.02 ± 0.00a	0.13 ± 0.00b	0.26 ± 0.06c
Ni	2.57 ± 0.22a	2.52 ± 0.32a	3.79 ± 0.14b	4.47 ± 0.21b
Cr	0.02 ± 0.00a	0.03 ± 0.00a	0.03 ± 0.00a	0.03 ± 0.00a
As	0.36 ± 0.07a	0.15 ± 0.04a	1.03 ± 0.08b	0.71 ± 0.04b
Li	0.17 ± 0.03a	0.09 ± 0.01a	0.92 ± 0.08b	0.58 ± 0.02b

† A detailed investigation of macronutrients in individual ryegrass biomass throughout the experiment is reported in Esperschütz et al. (2016b).

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

§ Not determined.

Table 5. Extractable ($CaNO_3$) nutrient and trace element concentrations in soil detected in combination with different plant species and soil amendments.

	Control	Urea	Biosolids-sawdust	Biosolids
	mg kg^{-1}			
Sorghum				
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 ± 0.32b	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↓
Ryegrass				
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	5.19 ± 0.35a	3.34 ± 0.15a	11.85 ± 1.59b	14.54 ± 0.93c↑
Mg	94.1 ± 3.20a	88.8 ± 3.41a↑	98.7 ± 4.09ab	106.8 ± 4.65b↑
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↑

† Lowercase letters indicate significant differences between treatments at $p \leq 0.05$. Arrows indicate significantly higher (↑) or lower (↓) concentrations ($p \leq 0.05$) between plant species grown under the same treatment.

rapeseed biomass was recorded in a similar range compared with mineral fertilization with 150 kg N ha^{-1} (Muchecheti et al., 2011). Although an equivalent of only 20 kg ha^{-1} available N was applied with biosolids, biosolids application improved soil fertility and performance of rapeseed in the low-fertility soil compared with urea-only treatment.

In sorghum treatments, a high biomass response was observed with mineral N (urea) fertilization, which was in accordance with Fellet and Marchiol (2011) and Marchiol et al. (2007), who found greater biomass production in mineral-fertilized sites (22.1 t ha^{-1}) compared with organically fertilized sites (16.9 t ha^{-1}). A higher biomass yield due to mineral fertilization has been explained by mineral fertilization causing a longer vegetative period, thereby delaying the senescence of the canopy (Fellet and Marchiol, 2011).

Total seed yields obtained from rapeseed biosolids and biosolids-sawdust treatments were comparable to the study by Ahmadi (2010), where plants received biosolids and biosolids-sawdust fertilization at rates between 50 and 150 kg N ha^{-1}, respectively. Jackson (2000) identified a rapeseed seed yield of 2.65 t ha^{-1} at an optimum of 200 kg N ha^{-1}. Seed yields in our study were 2.5 t ha^{-1} in biosolids treatments, achieved with an application of only 20 kg ha^{-1} of plant-available N applied with biosolids. Biosolids application to degraded soil would therefore provide a viable alternative to urea fertilization to grow rapeseed while simultaneously achieving an optimum seed yield.

Sorghum produced seeds in all treatments. Both urea and biosolids resulted in a seed biomass range of 2 t ha^{-1}, which is in the lower range of recorded grain yield of sweet sorghum in North China (Zhao et al., 2009). Biosolids application increased nutrient use efficiency, offering a low-cost strategy to achieve similar grain yield with only 20 kg ha^{-1} equivalent available N, compared with 200 kg ha^{-1} available N for urea. An increased seed yield in biosolids treatments can be further linked to Zn because increasing levels of Zn have been shown to improve conditions for pod formation and the number of seeds per pod, thereby enhancing seed yield (Olama et al., 2014). Biosolids-sawdust did not increase biomass or seed yield when compared with control treatments. This could be related to C increase as a result of the sawdust component, stimulating heterotrophic bacteria that immobilize N while they degrade the C substrate (Schmidt et al., 2001).

Nutrient and Trace Elements in Vegetative Plant Biomass

In sorghum and ryegrass plants, we generally detected higher or similar element concentrations in biosolids treatments compared with urea. Higher concentrations of several macro- and micronutrients were found in rapeseed urea compared with biosolids treatments. This was most likely a concentration effect due to scarce biomass growth in the urea treatment. Urea application caused a decrease of P and K in sorghum biomass, presumably a dilution effect, related to a high biomass increase that diluted plant P and K, in combination with a lack of P and K uptake, because only small amounts of P and K were available in soil (Jarrell and Beverly, 1981). These results are in accordance with Riedell (2010), who describes lower shoot P and K concentrations in maize after high-N application. Biosolids application increased Ca, Mg, S, and Cu concentrations in vegetative plant parts of sorghum compared with controls. Although these elements were increased, the levels in all treatments were in the reported range of 1000 to 50,000, 1500 to 3500, 1000 to 5000, and 1 to 10 mg kg^{-1}, which are levels typically found in food crops (Alloway, 2013).

Irrespective of plant species, biosolids and biosolids-sawdust treatments increased plant Zn in the order of rapeseed > ryegrass > sorghum, above the typical range found in crop species (Alloway, 2013). Zinc concentrations in sorghum were below 15 to 20 mg kg^{-1} in control and urea treatments, a level that has been reported as a typical range for adequate growth in most crop species (Marschner, 1995). In rapeseed, Zn concentrations in biosolids and biosolids-sawdust treatments were 150% higher compared with sorghum and ryegrass. Generally, Zn is important in many biological functions, but recent studies increasingly show free ionic Zn (Zn^{2+}) as more biologically toxic than traditionally presumed (Plum et al., 2010). Visible Zn toxicity in plants has been described above 300 mg kg^{-1} (Broadley et al., 2007). Our results indicate that biosolids as well as biosolids mixed with sawdust could potentially overcome Zn deficiency in crop species without reaching toxic thresholds.

Contaminant Uptake into Plants

Nickel concentrations increased with biosolids and biosolids-sawdust application in all plant species but were detected in a range typical for food crops (Alloway, 2013). Concentrations of Cd in rapeseed were measured at 0.1 to 0.3 mg kg^{-1}, which has been reported as a normal range in plants (Alloway, 2013; Chaney, 1989). In sorghum and ryegrass biomass, Cd was significantly increased due to biosolids and biosolids-sawdust applications compared with controls but in a range not toxic to human or animal health (Alloway, 2013; Chaney, 1989). This indicates that biosolids and biosolids-sawdust 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. Plant species like rapeseed did not accumulate contaminants such as Cd from biosolids and biosolids-sawdust treatments; hence, potentially higher rates of biosolids could be applied without reaching threshold levels for food products. Although there were higher Li concentrations in ryegrass and rapeseed biosolids and biosolids-sawdust treatments, as well as higher As concentrations in biosolids and biosolids-sawdust ryegrass treatments, these levels were below concentrations shown to be toxic to plants and were unlikely to be significant for human health or ecosystem functioning (Aral and Vecchio-Sadus, 2008; Wuana and Okieimen, 2011).

Influence of Biosolids and Sawdust on Seed Quality

In rapeseed treatments, an increase in seed quality compared with control treatments could not be verified because no seed production was obtained in control and urea treatments. Macro- and micronutrients in seeds in biosolids and biosolids-sawdust treatments (Supplemental Tables S1 and S2) were in a range as expected under normal P fertilization or higher in cases of Ca, Zn, Cu, or Fe (Ding et al., 2010). Copper concentrations were higher than the critical content of 2.2 mg kg^{-1} for Cu deficiency as stated by Khurana et al. (2006); hence, seed Cu concentrations are not correlated to the Cu deficiency of vegetative parts as discussed above. This indicates that biosolids application can increase the quality of rapeseed seeds and therefore potentially plant oil. Increased mineral concentrations in seeds, especially P and Zn, could be beneficial for seedlings and result in a faster seedling establishment and higher yields (Broadley et al., 2007; Zhu and Smith, 2001).

Sorghum seeds showed higher N, P, and S concentrations in biosolids treatments compared with controls, and biosolids-sawdust further increased Cu, Fe, and Zn compared with control treatments. Using biosolids and sawdust as soil amendments could potentially overcome nutrient deficiency in plant foods, which is becoming an increasingly important global problem (Musa et al., 2012). Concentrations of Cd and Ni in seeds in biosolids and biosolids-sawdust treatments remained low (0.05 and 1.5 mg kg^{-1}, respectively) and did not endanger food safety according to current thresholds (Codex, Alimentarius Commission 2001).

Nutrient Availability in Soil

Biosolids and biosolids-sawdust application influenced P and S concentrations in the rhizosphere; however, different effects were observed in biosolids-sawdust depending on the plant species. This could be related to different nutrient mobilization via different compounds exuded by plant species (Carvalhais et al., 2011; Nardi et al., 2000; Ström et al., 2002). However, plants might have influenced nutrient availability via uptake, evapotranspiration, or preferential flow down though root channels (Allaire et al., 2011; Mitchell et al., 1995; Vasquez, 2008). In addition, sawdust could have altered nutrient availability by exerting influence on microbial communities through compounds leached into the soil from sawdust. It has been previously reported that organic compounds such as phenols, tannins, lignin, and terpenes can be leached from sawdust, which could potentially alter microbial processes in toxic concentrations. (Hedmark and Scholz, 2008; Keeling and Bohlmann, 2006; Rupar and Sanati, 2005; Tao et al., 2005).

An increase of Mg, Mn, and Zn was seen in ryegrass rhizosphere in response to biosolids. This could be due to a lower demand for plant nutrients due to lower biomass production compared with sorghum and hence higher concentrations of available elements remaining in the soil. Cadmium, Cr, and Ni were not detected in plant-available concentrations, which is in accordance with the biomass results, suggesting these contaminants do not accumulate in plants after a biosolids application rate of 50 t ha^{-1}.

Sorghum and ryegrass exerted a diverse influence on the availability of nutrients and trace elements in soil, presumably due to root growth and exudation (Nascimento and Xing, 2006). Potassium, S, Mg, Mn, Cu, and Zn had lower availability in sorghum treatments compared with ryegrass. Root exudates, including organic anions, are responsible for metal complexation and uptake into plants or immobilization in soil (Bais et al., 2006). Our results suggest a higher influence of sorghum exudates on the nutrient availability in soil compared with ryegrass. However, biosolids-sawdust application was the only treatment where no differences occurred between sorghum and ryegrass treatments with regard to nutrient availability. Sawdust may well provide higher amounts of available C, which likely attracted heterotrophic bacteria, consuming root exudates and available nutrient sources in soil (Cébron et al., 2015). Therefore, sawdust could have overcome the difference in plant exudation (in volume as well as composition) between sorghum and ryegrass by stimulating the rhizosphere microbial biomass.

The leaching of heavy metals from biosolids-amended soils has been discussed in several studies with different types of biosolids and soil types (Brown et al., 1983; Esteller et al., 2009; Gove et al., 2001). Heavy metal leaching in these studies was reported in very low or negligible concentrations and hence was not focus of our experiment. However, because an increasing amount and repeated biosolids application to land in the near future could potentially pose risks in terms of heavy metal leaching into groundwater, the leaching composition of heavy metals is an interesting topic for further research.

Conclusions

High rates of biosolids application to low-fertility land in combination with rapeseed, sorghum, and ryegrass provided a complete fertilization with macro- and micronutrients without significantly increasing contaminants such as Cr, Ni, or Cd in the plant biomass. Biosolids application increased plant and seed biomass as well as seed and crop quality by enhancing the concentration of important elements in the vegetative biomass and seeds, especially Zn and P. Results from blending biosolids with fresh sawdust varied strongly depending on plant species, and therefore the use of sawdust in these scenarios must be decided on a case-by-case basis depending on the required outcome. The use of sawdust can immobilize and reduce available N for plant growth; however, sawdust can also enhance plant quality with respect to individual nutrients in biomass and seeds, including P, Cu, Zn, Mn, Fe, S, or Na. Further investigation of biosolids application rates in combination with sawdust and different plant species could maximize the plant quality benefits derived from sawdust, thereby improving its value for recycling in combination with biosolids on land instead of increasing landfill deposition. In addition to metal contaminants and N, analyses of organic contaminants and pathogens have to be included into future studies.

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Production of Biomass Crops Using Biowastes on Low-Fertility Soil: 2. Effect of Biowastes on Nitrogen Transformation Processes

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Abstract

Increasing production of biowastes, particularly biosolids (sewage sludge), requires sustainable management strategies for their disposal. Biosolids can contain high concentrations of nutrients; hence, land application can have positive effects on plant growth and soil fertility, especially when applied to degraded soils. However, high rates of biosolids application may result in excessive nitrogen (N) leaching, which can be mitigated by blending biosolids with other biowastes, such as sawdust. We aimed to determine the effects of biosolids and sawdust on growth and N uptake by sorghum, rapeseed, and ryegrass as well as N losses via leaching. Plants were grown in a greenhouse over a 5-mo period in a low-fertility soil amended with biosolids (1250 kg N ha^{-1}), biosolids-sawdust (0.5:1), or urea (200 kg N ha^{-1}). Urea application increased biomass production of sorghum and ryegrass but proved insufficient for rapeseed on low-fertility soil. Biosolids application increased plant N concentrations in ryegrass and rapeseed and increased N uptake into the seeds of sorghum, increasing seed quality. Biosolids application did result in lower N leaching compared with urea, irrespective of plant species, and N leaching was unaffected by mixing the biosolids with sawdust. There was an indication of biological nitrification inhibition in the rhizosphere of sorghum. Rapeseed had similar growth and N uptake into biomass in biosolids and biosolids-sawdust treatments and hence was the most promising species with regard to recycling fresh sawdust in combination with high rates of biosolids on low-fertility soil.

Core Ideas

- Mixing sawdust with biosolids did not reduce NO_3^- leaching irrespective of plant species.
- Compared with urea, biosolids application did not result in higher N loss via leaching.
- Results indicated biological nitrification inhibition of sorghum.

Worldwide, biowaste production is increasing because of a rising population, agricultural intensification, and the need for improved food production (Río et al., 2011). Biowastes include crop residues, wood wastes, animal manures, food processing waste, and waste from municipal sewage treatment plants. Sustainable management strategies for disposal and recycling are required to reduce costs and negative environmental outcomes of landfill deposition (Amajirionwu et al., 2008). For some of these wastes, especially animal manure and biosolids (treated or stabilized sewage sludge), application to agricultural land is widely practiced and has shown positive effects on soil fertility and plant biomass (Miaomiao et al., 2009; Mok et al., 2013), along with improvements in soil chemical, physical, biological, and microbial properties (Cytryn et al., 2011; Rogers and Smith, 2007; Singh and Agrawal, 2008).

Biosolids are rich in organic matter and can contain essential plant nutrients such as nitrogen (N), phosphorus, sulfur, and potassium (Al-Dhumri et al., 2013). Therefore, land application of biosolids can have positive effects on soil fertility and plant growth (Corrêa, 2004; Petersen et al., 2003; Smith and Durham, 2002; Westerman and Bicudo, 2005), but there are drawbacks that need to be considered because biosolids may contain elevated concentrations of heavy metals, organic contaminants, and pathogens (Bolan et al., 2014). Because of the potential risks in using biosolids for agriculture, the notion of using biosolids to rebuild degraded land has become increasingly popular (Dere et al., 2012; Mbakwe et al., 2013; Meyer et al., 2001; Oladeji et al., 2013; Speir et al., 2003; Stehouwer et al., 2006). Biosolids application may promote topsoil development and enhance the reestablishment of vegetation, especially in degraded environments (Hearing et al., 2000; Lu et al., 2012).

Most N in biosolids is contained within the organic matter and thus 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

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Abbreviations: BNI, biological nitrification inhibition; DW, dry weight.

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. However, high loads of potentially available N applied with biosolids can lead to excessive NO_3^- leaching, which can negatively affect waterways (Smith, 2003). Therefore, best management practices have to be followed to ensure protection of soil, food, feed, and waterways. Total and mineral N concentrations in biosolids vary with type and treatment of biosolids, which has been extensively reviewed recently by Rigby et al. (2016). Several factors have been identified influencing N transformations in biosolids-amended soil, such as biosolids type, C/N ratio, application rate, soil texture and organic matter content, soil temperature, soil moisture, and soil pH (Rigby et al., 2016).

The negative effects of biosolids addition to soil can be mitigated by mixing biosolids with other biowastes. Positive results have been reported by adding biochar and wood wastes (Ammari et al., 2012; Knowles et al., 2011). Biosolids-sawdust mixtures have a beneficial effect on plant growth and soil aggregate stability while reducing NO_3^- leaching (Bugbee, 1999; Sandoval et al., 2012). In combination with other organic wastes, sawdust has the potential to improve the physical, chemical, and nutritional properties of soils (Paramashivam et al., 2016; Sandoval et al., 2012). Therefore, blending biowastes can enable recycling strategies that result in decreased landfill deposits. The optimal ratios of mixing other biowastes with biosolids have to be determined to avoid negative effects on plant growth (Schmidt et al., 2001).

Rapid nitrification, the conversion of NH_4^+ to NO_3^-, can result in the inefficient use of N due to NO_3^- loss from agricultural systems through leaching or the emission of N_2O after subsequent denitrification (Robertson and Groffman, 2015). By restricting nitrification, N retention in the soil is increased because NH_4^+ is less likely to be lost via leaching and denitrification (Subbarao et al., 2013). Several plant species have shown the potential to inhibit nitrification, which could further mitigate N loss from agricultural systems (Fillery, 2007; Subbarao et al., 2009, 2013). Biological nitrification inhibition (BNI) through exudation of nitrification inhibiting compounds from roots has been shown for tropical pasture plants (Subbarao et al., 2009), but biomass crop species such as sorghum (*Sorghum bicolor* L.) and rapeseed (*Brassica napus* L.) have also shown potential to inhibit nitrification (Brown and Morra, 2009; Zakir et al., 2008). Growing crops with BNI properties on biosolids-amended soils could therefore influence N transformation processes and reduce the risk of N leaching after high rates of biosolids application.

We aimed to determine the effects of biosolids and sawdust on the growth and N uptake by sorghum, rapeseed, and ryegrass as well as N losses via leaching. Following high biosolids application rates on low fertility soil, we hypothesize that potential loss of N can be mitigated by blending biosolids with sawdust and by the selection of plant species with high N requirements or nitrification inhibition properties. The focus of this manuscript is on plant and soil N transformation processes; nutrients and trace elements are discussed in Part 1 of this study (Esperschütz et al., 2016a)

Materials and Methods

Experimental Setup

An experiment was set up at the Lincoln University plant growth facility, as described in detail by Esperschütz et al. (2016a). In brief, low-fertility soil, as defined according to its low Olsen P of 11 mg L^{-1}, was collected from a marginal farm area (40°45′56″ S, 175°54′42″ E) and placed into small lysimeters (25 cm in diameter; of 29 cm). To measure NO_3^- leaching, a leachate-sampling device was installed in the bottom of each lysimeter. Lysimeters were incubated at ambient conditions in a greenhouse for 14 wk before treatment application. Rapeseed (*Brassica napus* L. 'MAKRO'), sorghum (*Sorghum bicolor* L. Moench 'Sudanese'), and ryegrass (*Lolium multiflorum* Lam. Feast II tetraploid Italian ryegrass, 2 g) were grown in four different treatments (control, biosolids, biosolids-sawdust, urea) in individual lysimeters, randomized within the experiment with four replicates for rapeseed and sorghum and six replicates for ryegrass, respectively.

Biosolids were collected from settlement ponds of the Kaikoura Sewage Treatment Plant; sawdust was obtained from an adjacent wood-waste disposal area (Kaikoura, New Zealand, 42°21′37.40″ S, 173°41′27.35″ E). Biosolids (untreated pond sludge, characterized as Grade "Bb" according to NZWWA [2003]) were homogenized thoroughly after sieving (≤10 mm). Fresh *Pinus radiata* sawdust was used to mix with the biosolids. A characterization of soil, sawdust, and biosolids is presented in Esperschütz et al. (2016a). Fresh biosolids (245 g dry weight [DW]) and biosolids mixed with sawdust (245 g DW + 123 g DW) were applied at rates of 1250 kg N ha^{-1}, respectively, with biosolids application equivalent to 50 t ha^{-1} DW. Urea was applied four times over the experimental period (50 kg N ha^{-1} equivalent) up to a total amount of 200 kg N ha^{-1}. Seeds were sown directly into the lysimeters after urea and biosolids application.

The experiment was maintained for 18 wk. The temperature in the greenhouse ranged between 9 and 20°C during nighttime (10 PM until 6 AM) and between 14 and 28°C during the daytime. Using automatic and manual irrigation, soil was maintained at near-field capacity conditions. A total of 2160 mm of irrigation was applied to rapeseed, 1190 mm to sorghum, and 1060 mm to ryegrass.

Analyses and Measurements

The amount of leachate was sampled and recorded weekly throughout the experimental period; aliquots were stored at −20°C until further analyses. Nitrate-N (NO_3^-–N), nitrite-N (NO_2^-–N), and ammonium-N (NH_4^+–N) were determined using a flow injection analyzer (FIA FS3000 twin channel analyzer, Alpkem). Evapotranspiration was calculated as the volume of water irrigated (mL), reduced by the volume recovered as drainage (mL), and subsequently added week by week over the experimental period.

The biomass of ryegrass was repeatedly harvested and analyzed for its macro- and micronutrient speciation throughout the experiment (Esperschütz et al., 2016b). In this study, the cumulative ryegrass biomass was calculated based on eight harvests performed fortnightly with the first harvest 4 wk after sowing. The cumulative ryegrass biomass was compared with the biomass of sorghum and rapeseed, respectively, obtained from a final destructive harvest of all lysimeters after 18 wk. The total plant biomass of sorghum and rapeseed was weighed and oven-dried at 70°C until a constant weight was achieved. Dried plant parts were further separated into roots, leaves, and seeds and ground to a fine powder using a Retch ZM200 grinder for analyses. Soil that has been attached to the plant roots ≤2 mm was considered as rhizosphere soil. Rhizosphere soil was sieved (≤5 mm) before chemical analyses. Based on the different harvesting protocol for ryegrass, no seeds or roots were harvested from *L. multiflorum* plants. Total C and N in plant and soil material were analyzed from ground material using a CNS-2000 Element Analyzer (LECO Australia Pty Ltd).

The soil inorganic N speciation was determined using a KCl extraction from fresh soil (4°C) within 4 d after harvest according to (Blakemore et al., 1987). After adding 40 mL of a 2 mol L^{-1} KCl reagent to 4 g of soil, the solution was shaken on an end-over-end shaker for 1 h, centrifuged at 827 *g* for 10 min, and filtered through Whatman 41 filter paper. Nitrate-N (NO_3–N) and ammonium-N (NH_4–N) were determined using a flow injection analyzer (FIA FS3000 twin channel analyzer, Alpkem).

Statistical analyses were based on four individual replicates for rapeseed and sorghum and six replicates for ryegrass, respectively. Using SPSS 22 (IBM SPSS statistics), ANOVAs were performed followed by Duncan's post hoc tests to identify homogenous subsets for $\alpha = 0.05$. Significance between treatments during the 18-wk period were investigated using a full factorial, multivariate model followed by Duncan's post hoc tests. Results were illustrated in SigmaPlot 11.0 (Systat Software Inc.).

Results

High rates of biosolids application to low-fertility soil resulted in a significant growth response of rapeseed, sorghum, and ryegrass compared with control treatments (Fig. 1a). Rapeseed produced negligible biomass in the control and urea treatments due to a lack of nutrients available to maintain the growth of this species (Esperschütz et al., 2016a). Mixing sawdust with biosolids reduced the growth of sorghum and ryegrass compared with biosolids-only, whereas similar biomass was harvested in rapeseed treatments. Urea application increased biomass production of sorghum and ryegrass but had no effect on rapeseed grown on low-fertility soil.

Biosolids application significantly increased total N uptake into plant biomass in combination with rapeseed and ryegrass (Fig. 1b). Higher N contents in sorghum and ryegrass were seen in urea treatments compared with control, biosolids-sawdust, and biosolids treatments. For rapeseed and sorghum, the N uptake into total plant biomass was similar in biosolids-sawdust and biosolids treatments, whereas ryegrass grown with the biosolids-sawdust mix showed lower N uptake compared with biosolids.

Due to the different harvesting procedure for ryegrass, no seeds or roots were harvested from *L. multiflorum* plants at the end of the experiment. The scarce growth of rapeseed in control and urea treatments has not resulted in seed production in these treatments; hence, seeds could only be harvested from rapeseed biosolids-sawdust and biosolids treatments. No difference was detected in the seed N concentration between biosolids and biosolids-sawdust treatments in rapeseed (2.9 and 3.0%, respectively). The percentage distribution of N between sorghum leaves, seeds, and roots is shown in Fig. 2. Urea application increased leaf-N by up to 74.8% compared with the control, whereas a decrease was observed in seed-N (−4.6%) and root-N (−20.6%) concentration. Biosolids-sawdust application caused an increase in seed-N up to 15.7% relative to controls. Nitrogen was further partitioned into the seeds in the biosolids alone treatment (28.9%).

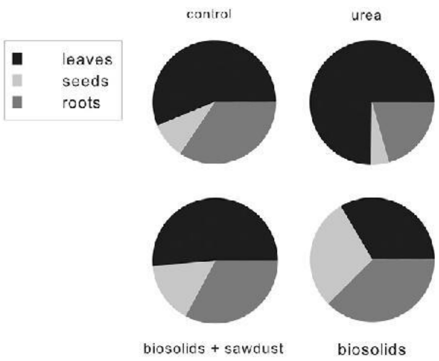

Fig. 2. Distribution of N (%) between sorghum (*S. bicolor*) leaves, seeds, and roots at final harvest after 18 wk.

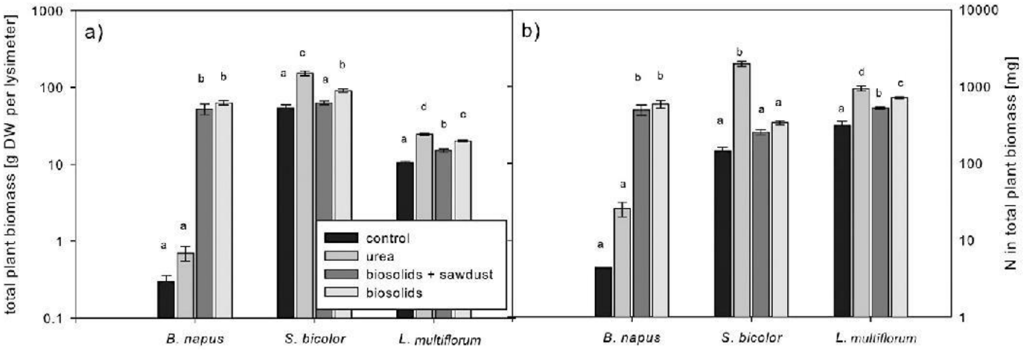

Fig. 1. Total plant biomass (including seed and root biomass) at the end of the experiment (a) and total N recovered from plant biomass (b) from sorghum (*S. bicolor*), rapeseed (*B. napus*), and ryegrass (*L. multiflorum*). Different levels of significance $p \leq 0.05$ are represented by lowercase letters.

At the end of the 18-wk experiment, NH_4^+ concentrations were below detection limit (0.1 mg L^{-1}, equivalent to 5 mg kg^{-1}) in the rhizosphere of all plant species. Nitrate was not significantly different between treatments in rapeseed and ryegrass (12.7–20.5 mg kg^{-1}), whereas NO_3^- concentrations were below detection limit (0.1 mg L^{-1}, equivalent to 5 mg kg^{-1}) in the rhizosphere of sorghum (data not shown).

Drainage and evapotranspiration of different plant species in combination with soil treatments were calculated for each week. Under ryegrass, no significant difference was detected between treatments up to Week 4. Five weeks after the start of the experiment, the cumulative drainage was lowest in biosolids treatments (79 mm) compared with biosolids-sawdust (105 mm) until the end of the experiment (366 and 476 mm, respectively) (Fig. 3a).

In combination with rapeseed, the cumulative drainage at the end of the experiment was significantly higher in urea and control treatments (670 and 676 mm, respectively) compared with biosolids and sawdust-biosolids treatments (450 and 477 mm, respectively) (Fig. 3b), with the differences between these two groups consistent after 8 wk ($p \leq 0.05$).

Drainage from the sorghum treatments ranged from 289 mm in urea treatments to 392 mm in the biosolids-sawdust treatments at the end of the experiment (Fig. 3c). The urea and biosolids treatments had similar drainage compared with the control until Week 15 but separated significantly during the last 3 wk, with negligible drainage recovered from urea treatments. In biosolids-sawdust treatments, drainage was consistently higher compared with other treatments after 5 wk.

At the end of the experiment, the cumulative evapotranspiration of ryegrass was significantly higher in biosolids treatments (627 mm) compared with the control and biosolids-sawdust treatments (532 and 516 mm, respectively). The cumulative evapotranspiration of urea and biosolids was consistently higher after the first 8 wk (302 and 312 mm, respectively) compared with biosolids-sawdust (262 mm) (Fig. 4a), whereas control treatments showed similar evapotranspiration as biosolids-sawdust (276 mm). In the rapeseed treatments, a higher cumulative evapotranspiration was detected in biosolids and biosolids-sawdust treatments, consistently significant after 10 wk (Fig. 4b). Sorghum had a consistently lower evapotranspiration in the biosolids-sawdust treatments compared with all other treatments after Week 6 (585 mm). The urea, control, and biosolids treatments used similar amounts of water during the experiment, with the urea treatments significantly higher (676 mm) at Week 18 compared with the control (627 mm) and biosolids (638 mm) treatments (Fig. 4c). Irrespective of soil treatments, cumulative evapotranspiration was highest in rapeseed (814–1017 mm) compared with sorghum (585–675 mm) and ryegrass (516–627 mm).

Leaching of NO_3^- varied depending on plant species, whereas NO_2^- and NH_4^+ concentrations were always below detection limits (data not shown). No differences were observed between biosolids-sawdust and biosolids treatments for any plant species in the experiment. Rates of NO_3^- detection in leachate were 135 to 148 mg NO_3^- under rapeseed (Fig. 5b), 218 to 220 mg NO_3^- under sorghum (Fig. 5c), and 79 to 115 mg NO_3^- under ryegrass (Fig. 5a).

No differences were detected in the amount of NO_3^- leached from soil treatments with ryegrass during the first 3 wk of the experiment (Fig. 5a), but significantly higher contents

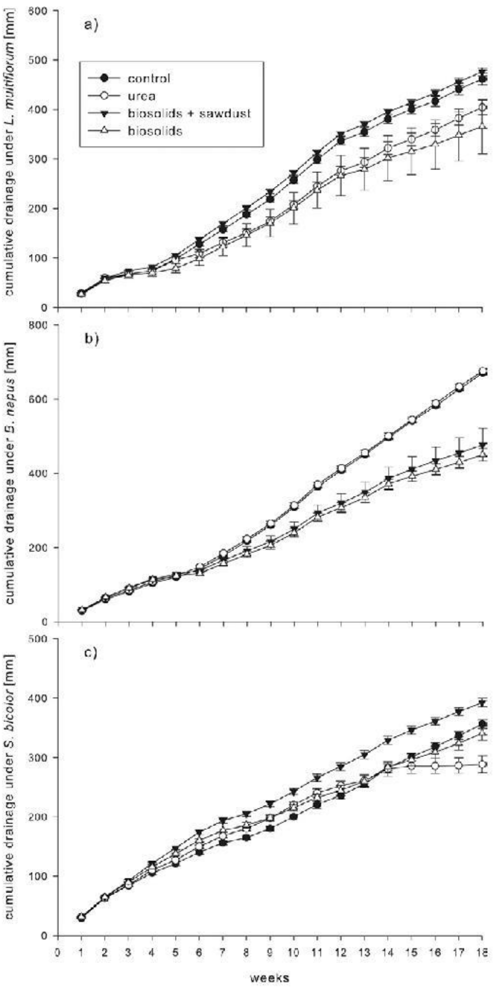

Fig. 3. Cumulative drainage [mm] of ryegrass (*L. multiflorum*) (a), rapeseed (*B. napus*) (b), and sorghum (*S. bicolor*) (c) during the experimental period. Differences $p \leq 0.05$ are represented by nonoverlapping SEM.

of NO_3^- were collected from drainage of biosolids-sawdust and urea treatments compared with the control from Week 5 onward. Leaching of NO_3^- from biosolids treatments was higher compared with the control but lower compared with urea and biosolids-sawdust until the end of the experiment. However, no significant differences were recorded between either of these treatments. After 18 wk, the total NO_3^- leached was 116 and 113 mg from urea and biosolids-sawdust treatments, respectively, whereas 95 mg was leached from biosolids treatments and 79 mg leached from the control.

In combination with rapeseed, the highest amount of cumulative NO_3^- (796 mg) leached in drainage was detected in the urea treatments, followed by control treatments (357 mg) and then biosolids and biosolids-sawdust treatments (162 and 180

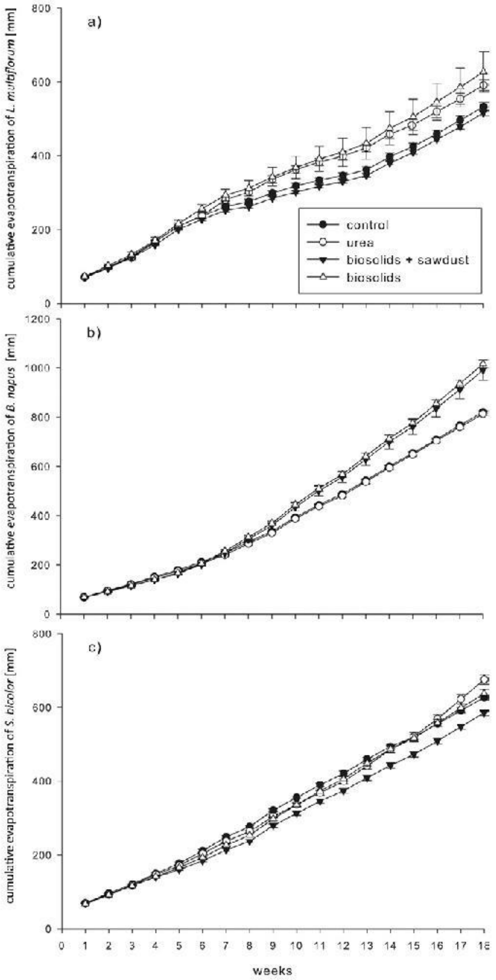

Fig. 4. Cumulative evapotranspiration [mm] of ryegrass (*L. multiflorum*) (a), rapeseed (*B. napus*) (b), and sorghum (*S. bicolor*) (c) during the experimental period. Differences ($p \leq 0.05$) are represented by nonoverlapping SEM.

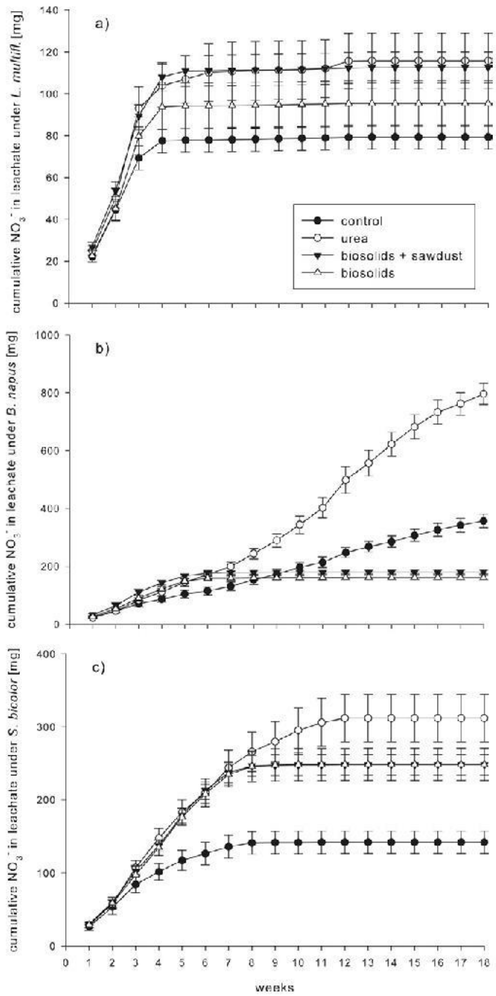

Fig. 5. Cumulative NO_3^- in leachate [mg] under ryegrass (*L. multiflorum*) (a), rapeseed (*B. napus*) (b), and sorghum (*S. bicolor*) (c) vegetation during the experimental period. Differences ($p \leq 0.05$) are represented by nonoverlapping SEM.

mg, respectively) (Fig. 5b). Urea treatments leached significantly higher amounts than the other treatments after only 8 wk, whereas control treatments were consistently higher compared with biosolids and biosolids-sawdust after 12 wk. No differences were found between biosolids and biosolids-sawdust treatments throughout the 18-wk experimental period.

Cumulative NO_3^- leached from sorghum controls (142 mg) was lower than the urea, biosolids, and biosolids-sawdust treatments (Fig. 5c). Higher amounts of NO_3^- were recovered from urea treatments (312 mg) but were not significant ($p = 0.076$) compared with biosolids and biosolids-sawdust treatments.

Discussion

Biosolids and Sawdust Application Increase Growth and Biomass N in Rapeseed

Rapeseed showed a very scarce biomass production in control and urea treatments, which was likely the result of other limiting nutrients than N, mainly S and P (Esperschütz et al., 2016a). Growth of rapeseed increased due to biosolids-sawdust and biosolids. A biomass increase of rapeseed after biosolids application confirms findings from Shaheen and Tsadilas (2013), who reported a biomass increase after different rates of biosolids were applied in a pot experiment using low-fertility soil comparable (to some extent) to the soil used in our study.

Nitrogen accumulation in the total plant biomass reflected results from total plant biomass. Biosolids and biosolids-sawdust application increased the total plant N content and thereby enhanced plant quality. At the end of the growing period, the total N content in rapeseed biomass was equivalent to 110 kg ha^{-1} in the biosolids and biosolids-sawdust treatments. In rapeseed, neither plant growth, plant N content, nor N uptake into seeds was influenced in biosolids-sawdust treatments compared with pure biosolids application. This may indicate that mixing sawdust with biosolids is a suitable way of recycling fresh sawdust in combination with high rates of biosolids on a low-fertility soil in combination with rapeseed.

The total mass of NO_3^- leached in rapeseed was higher in the control and urea treatments compared with biosolids and biosolids-sawdust treatments. A lack of plant establishment in urea and control treatments (<1 g) resulted in NO_3^- leaching comparable to bare soil. Compared with pure biosolids treatments, biosolids-sawdust application did not affect plant growth, drainage, or evapotranspiration during the experimental period, as shown for ryegrass and sorghum. This may indicate that rapeseed is more sensitive to other macronutrients, such as P and S, as discussed elsewhere (Esperschütz et al., 2016a), and that N was not the limiting factor for rapeseed.

Decreased Growth and N Uptake of Ryegrass in Sawdust Treatments

Biosolids as well as urea application resulted in a total biomass production of ryegrass similar to the reported average for a Feast II Italian ryegrass cultivar (Hanson et al., 2006), as discussed in detail by Esperschütz et al. (2016b). Biosolids-sawdust application in combination with ryegrass caused a decrease in plant growth and reduced N accumulation into plants. As discussed in Esperschütz et al. (2016b), our results showed that biosolids and biosolids-sawdust resulted in less growth compared with urea application. Only a limited amount of the total N applied with biosolids (1250 kg ha^{-1}) was immediately plant available, with most of the N in biosolids locked up in organic compounds. Such organic N needs to undergo (microbial) transformation processes to become available (Sommers, 1977). In addition, other properties of the biosolids, such as elevated trace elements or salinity, may have reduced the effectiveness of the added N.

Throughout the 18-wk experimental period, available N from urea and biosolids application was taken up for biomass growth, resulting in a better growth response, lower drainage, and higher evapotranspiration compared with control treatments (Di Paolo and Rinaldi, 2008; Kim et al., 2008). Higher drainage in biosolids-sawdust treatments compared with biosolids or urea application is compatible with reduced plant biomass (due to less available N) and therefore less need of water to support biomass growth. Leaching of NO_3^- occurred mainly in the first weeks of the experiment, immediately after treatment application. This was probably due to leaching through rather bare soil until significant plant biomass was established after 4 wk. After biomass harvests began, drainage was sampled weekly with NO_3^- <0.5 mg, which indicates the utilization of available N by ryegrass plants in all treatments. Biosolids application at a rate of 1250 kg ha^{-1} N hence did not result in higher N loss via leaching compared with 200 kg N ha^{-1} applied with urea.

Using sawdust in combination with biosolids provided no additional benefit over biosolids alone with regard to plant growth and evapotranspiration. This could have been related to an immobilization of N by heterotrophic bacteria that were likely stimulated by the C-rich sawdust (Robertson and Groffman, 2015; Schmidt et al., 2001) or to adsorption reactions of NO_3^- with functional groups on sawdust, as suggested by Harmayani and Anwar (2012). Because there is insufficient N in sawdust to allow microbes to build proteins, they must accumulate N from their environment, in this case from biosolids; hence, biosolids-N might have already been immobilized by sawdust when sawdust was mixed with biosolids before application.

Mixing biosolids with sawdust caused a reduction of plant-available N, which resulted in reduced plant growth compared with biosolids and urea treatments; hence, lower evapotranspiration was calculated on the basis of total plant biomass. This is in contrast to a study growing a hybrid ryegrass (*Lolium* × *hybridum* Hausskn. 'Belinda') in combination with different biosolids and sawdust ratios applications in a degraded soil (Sandoval et al., 2012). Whereas biosolids-sawdust application in our study (using Feast II Italian ryegrass) showed a negative growth response, comparable treatments in Sandoval et al. (2012) indicated a biomass increase. We therefore suggest the influence of soil type and fertility, as well as the *Lolium* species and cultivar type, need to be taken into account when determining biosolids-sawdust ratios to optimize plant growth and soil regeneration.

Response of Sorghum to Mineral and Organic Soil Amendments

Both urea and biosolids enhanced the biomass production of sorghum by providing N sources readily available for plant growth (Esperschütz et al., 2016a). However, higher growth response was obtained in urea treatments, likely due to higher amounts of N readily available for plant uptake compared with biosolids, as discussed for ryegrass above. The high biomass response of sorghum to mineral N (urea) fertilization was in accordance with Fellet and Marchiol (2011), who suggested that higher biomass due to mineral fertilization was caused by a longer vegetative period, delaying the senescence of the canopy. In our study, however, a mineral fertilization rate of 200 kg N ha^{-1} resulted in a growth response similar to control treatments without N addition, carried out by Turgut et al. (2005). This may indicate that in addition to N limitation, the total plant yield was likely affected by the low fertility soil used in our study, specifically the lower availability of macronutrients P and K.

In our study, sorghum had a high response to N (urea) fertilization, with high N uptake into aboveground vegetative plant parts. This result can be of potential interest at sites with high N because plants showed a fast uptake of available N, hence removing N susceptible to leaching. Whereas biosolids and biosolids-sawdust application did not cause a significant N increase in the total sorghum plant, a clear shift could be observed in the plant N distribution toward higher contents of N in seeds, indicating an increase of seed quality and a change in the plant N translocation through organic amendment treatments (Fig. 2).

In sorghum, more drainage in biosolids-sawdust treatments was observed compared with biosolids or urea treatments. This is in accordance with lower evapotranspiration and is likely related to less available N and therefore less need of water for biomass growth. Biosolids application increased evapotranspiration (less drainage) compared with the control treatment, which is consistent with the findings of (Fiasconaro et al., 2013), who showed an increased evapotranspiration of leguminous plants after sewage sludge treatment. Using sawdust in combination with biosolids had no benefit with regard to plant growth and evapotranspiration in sorghum. Directly after biosolids application, an increasing amount of NO_3^- (up to 33 mg) in leachate in the first 4 wk could be explained by the phase of plant development. The amounts of NO_3^- recovered in leachate was likely the result of N flow through the bulk soil before significant plant biomass was established.

Neither NH_4^+ nor NO_3^- was measured in the rhizosphere of sorghum after 18 wk, whereas in both rapeseed and ryegrass, rhizosphere NO_3^- was detected between 15 and 20 mg (data not shown). However, no difference was found in rhizosphere NO_3^- at the end of the experiment in rapeseed and ryegrass treatments, which may indicate a contamination of rhizosphere with bulk soil, making NO_3^- less accessible for roots. Because large amounts of NO_3^- might have been leached from sorghum bulk soil before plant growth and preferential flow, less "bulk soil NO_3^-" was available at the end of the experiment to "contaminate" the rhizosphere sample.

Commonly, NO_3^- and NH_4^+ are the main sources of N for plant growth (Robertson and Groffman, 2015), with uptake mechanisms varying between plant species and growth stages and depending on environmental parameters, along with plant physiological processes (Ruffel et al., 2014). Nutrient availability in the rhizosphere may be influenced by evapotranspiration or differences in the preferential flow due to different root system architecture (Allaire et al., 2011; Mitchell et al., 1995). In our study, sorghum plants may have compensated high N requirements by mobilizing high amounts of soil-available N in addition to utilizing biosolids-available N. In this context, root exudates may have been involved, influencing root–microbe interactions and thereby inhibiting nitrification (Nardi et al., 2000; Subbarao et al., 2013). This would be in accordance with recent findings, where nitrification-inhibiting compounds have been detected in root exudates of sorghum (Zakir et al., 2008). Biological nitrification inhibition (BNI) would have decreased the NO_3^- in rhizosphere with NH_4^+ serving as major N source taken up by the plants. In a nitrification assay, performed from rapeseed, ryegrass, and sorghum control soil, sorghum showed lower NO_3^- and higher NH_4^+ concentrations compared with rapeseed and ryegrass (Supplemental Fig. S1). This may indicate the presence of BNI compounds due to root exudation and hence an inhibition of nitrifying bacteria, oxidizing NH_4^+ to NO_3^-. In this context, methyl 3-(4-hydroxyphenyl) was isolated as an active BNI compound in sorghum root exudates and was found in higher concentrations in the presence of NH_4^+ (Zakir et al., 2008). However, this could not be verified in our study because a nitrification assay was performed only from control soil, but this finding opens new lines of research studying urea and biosolids application in combination with sorghum plants.

Effect of Adding Sawdust to Biosolids on N Leaching

No significant difference between biosolids and biosolids-sawdust treatments were detected regarding the amount of NO_3^- leaching in combination with any plant species. Nitrate was recovered in leachate from both treatments, which is in contrast to other studies (and discussed above with regard to NO_3^- immobilization by sawdust) because this process should result in less NO_3^- leached in biosolids-sawdust treatments. An inhibitory effect of sawdust regarding N and other elements was shown by Kováčik et al. (2013) when fresh sawdust had been applied with dry pig manure to reduce nutrient mobility in soil. However, we suggest that in our study the available inorganic N applied with biosolids has been immobilized, whereas the NO_3^- recovered in leachate was likely to be of soil origin, because throughout the experiment NO_3^- leaching could be observed from more or less bare soil (i.e., rapeseed control treatments).

The total N in soil at the beginning of the experiment was calculated as 7200 kg ha^{-1}, which was increased by 1250 kg ha^{-1} in biosolids and biosolids-sawdust treatments, and 200 kg ha^{-1} in urea treatments, respectively. Although, depending on the plant species, between 20 and 150 kg ha^{-1} of N was removed with plant biomass and between 10 and 80 kg ha^{-1} was lost via leaching, any change in soil N at the end of the experiment will be in the order of 3%. Although we measured the residual N concentration in the rhizosphere soil, we could not resolve this difference, particularly in the case of the biosolids-amended soils where there would have been significant redistribution of N within the soil profile.

Conclusions

Biosolids application to low-fertility soil provided sufficient nutrients to ensure adequate growth of all plant species in this experiment. Biosolids application rates equivalent to 1250 kg N ha^{-1} did not result in an increased N loss via leaching compared with urea treatments. The use of sawdust did not reduce NO_3^- leaching but instead may have immobilized and reduced available N for plant growth in combination with sorghum and ryegrass. Further investigation of different biosolids/sawdust ratios and different plant species could increase the prevalence of sawdust and biosolids recycling on land, instead of increasing landfill deposits. The BNI properties of sorghum affected rhizosphere N but showed no effect on NO_3^- leaching. In this context, mixing biosolids with soil instead of topsoil applications could be of interest for future experiments. To investigate N leaching and BNI, stable isotope experiments could identify different sources of NO_3^- and further identify the effect of plant exudates on N transformation processes in combination with biowastes. Future work should involve field measurements in large plantations, where edge effects are less important than in a lysimeter experiment.

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