The response of manuka (*Leptospermum scoparium*) to homogeneous and heterogeneous distribution of biosolids in soil

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By

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Potentially, biosolids (sewage sludge) could be added to soil to enhance the growth of manuka (Leptospermum scoparium) for the production of honey, essential oils, and ecosystem restoration. Given that manuka is a pioneering species that is adapted to low fertility soils, it was unclear whether there would be a positive growth response to biosolids addition. I aimed to determine the effect of biosolids addition on the biomass, root morphology and elemental composition of manuka. Pots (2.5 L) and Rhizoboxes (15 x 30 x 2.5 cm) were filled with low-fertility soils from Eyrewell Forest (Lismore brown soil) and Kaikoura (sand). Biosolids from Kaikoura (10% of the total weight by mass containing 22g N/kg) were applied either homogeneously or heterogeneously to the surface of the pots and in a 5 cm vertical strip on one side of the rhizoboxes. There was also a control (no biosolids). Each treatment was replicated thrice. Manuka seedlings were grown for 12 weeks and then the biomass, root distribution and chemical composition was determined. The addition of biosolids increased the biomass in both soils. The increases in biomass were not significantly affected by the distribution of the biosolids. However, the distribution of the biomass affected root distribution, with roots proliferating in the biosolids patches in the heterogeneous treatments. In the Kaikoura sand, the addition of biosolids increased the plant concentrations of N, C, P, S, Zn, and Cd, whereas in the Eyrewell soil the biosolids increased N, Zn, Cd and Ni. In Kaikoura there were differences between homogeneous and heterogeneous treatments in plant Zn, Cu and Ni and in Eyrewell differences occurred in Zn and Cd. None of the trace element concentrations in manuka were likely to pose a risk to herbivores or ecosystems. My experiment demonstrated that manuka responds positively to the addition of biosolids and that the positive growth response was not affected by the distribution of biosolids on two soil types. Furthermore, the addition of biosolids did not cause manuka to take up unacceptable concentrations of trace elements. Future research should investigate the performance of manuka over a longer timescale and include treatments where biosolids are applied to the soil

surface of existing manuka stands. Root morphology should also be investigated for deeper understanding of foraging behaviour.

Keywords: Biosolids, manuka, *Leptospermum scoparium*, root distribution, foraging behaviour, biomass distribution, elemental composition, biosolids contaminants.

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Chapter 1

Introduction

The disposal of urban and industrial waste produced by modern society is no longer a problem of the future. It has become strongly established amongst the priority issues in many countries around the world. The final destination of these residues (in rivers, lakes, landfills or ocean) can pose many contamination risks leading to subsequent environmental and public health problems, and financial costs. Therefore, a strong commitment for the development of new techniques is required for the use of biowaste. Biosolids (treated sewage sludge) produced in sewage treatment plants has a strong fertilizer value due to its high levels of organic matter and nutrients (Tian, et.al, 2009).

The land application of biosolids has been a frequent option adopted internationally for sludge management, with many studies being done to assess the risk of using it as fertilizer and soil conditioner in agricultural lands and park areas since it contributes to enhancing soil physical and chemical properties, water retention and fertility (Fahy and Richard, 1999; Speir, et.al. 2004; Tian, et.al, 2009; HIPSITEC, 2010; Salazar et al., 2012;). Reducing the reliance on inorganic fertilizers should be aimed: cutting back on its use could be required since some of its components come from non-renewable sources; phosphorus for instance, is predicted to peak in global production by 2033 (Cordell, et.al. 2009).

However, depending on the source, biosolids may have large quantities of heavy metals, pathogens and organic micropollutants so its application could result in a potential dispersal of these elements to agricultural soils (HIPSITEC, 2010; Spinosa, 2011). These contaminants could be absorbed by plants used for feed production or grazing purposes and result in animal and human exposure to the contaminants through the food chain. (Whatmuff, 1996b; Eriksen et al., 2009; HIPSITEC, 2010; Spinosa, 2011;).

Despite advances in wastewater management (e.g., anaerobic, thermophilic, and mesophilic digestion treatments), many compounds and their metabolites still remain intact following treatment (Kinney, 2006; Roig et.al., 2012). In order to minimize negative consequences for those problems, many countries developed their guidelines for the safe application of biosolids to land. In 2003, a joint initiative in New Zealand of the wastewater industry, central and local government and other key stakeholders aimed to encourage the adoption of the best practice for the application of biosolids to land (NZWWA, 2003).

New Zealand produces approximately 77,000 tonnes of dry solids annually. The combined throughput of these schemes comprises less than 15% of the total potential biosolids with approximately half of it discharged to production forests and half to agricultural land. A small quantity is also sold through garden centres and other retail outlets but all the rest is dumped in Landfills. In countries like United States and the European community the use of biosolids is of 40% and 46%, respectively (NZWWA, 2003).

These examples demonstrate how this resource is underutilized in New Zealand. This is a waste of valuable macronutrients such as nitrogen and phosphorus (two essential elements for crop growth) and micronutrients such as copper, zinc and iron (ANZBP, 2009). The probable cause for the resistance in using biosolids as a soil conditioner is due to concerns about pathogens, heavy metals and cultural concerns so more studies addressing these topics will increase the knowledge and potentially the subsequent usage of biosolids.

Most of the research existing in this field aims to investigate the positive and negative effect of land application (Metzger and Yaron, 1987; Krogmann et al., 1999; Tian et al., 2009; Tian et al., 2013; Roig et al., 2012). Little research has been done in how to minimise its detrimental effect (Prosser, 2014). In some of the studies done in New Zealand, manuka (*Leptospermum scoparium*) a native shrub known to be hardy and tolerant to varying soil and climatic conditions (including elevated heavy metals), responded positively to surface-applied biosolids on a low-fertility soil (Esperschuetz, pers.comm.)

Manuka has been described as probably the most widely distributed, abundant and environmentally-tolerant member of the New Zealand woody flora (Ronghua, 1984). It is being used in land restoration projects of mine sites and degraded areas (Burrows et.al., 1999; Craw et al., 2007) as it shows: (i) improvement of soil quality (ii) promotes high invertebrate numbers and species richness beneath its shrubs (Rufaut and Craw, 2010) (iii) promotes soil ecosystem recovery, (iv) encourages the development of a self-sustaining plant community (Burrows et al., 1999). The use of native species for restoration projects has a better chance of success as the plants needs match the environmental conditions at the restoration site and the populations that subsequently grow can self-organize into functional and resilient communities that adapt to changing conditions: Thus promoting sustainable restoration (Thomas et al., 2014). Therefore, more studies should be conducted in New Zealand's native flora for this purpose.

The study of root development can be positive in understanding some of the plants behaviour and ecology. However, some technical difficulties in observing the roots without disturbance can be a barrier to effective root observations. The use of transparent rhizoboxes to grow the plants can be a

tool for daily roots observations and data collection as regular root scanning can be taken bringing beneficial visual results (Dinkelaker et al., 1993; Moradi et al., 2009).

Chapter 2

Literature review:

2.1 Manuka:

Figure 1: Manuka plant with flowers

Manuka (*Leptospermum scoparium)* also known as tea tree, ranges from a semi-prostrate shrub to a tree up to 4 m tall. It is a member of *Myrtaceae* family and probably the most widely distributed, abundant, and environmentally-tolerant member of the New Zealand indigenous woody flora (Ronghua, et.al. 1984; Stephens, et.al. 2005). It is an indigenous, however not endemic to New Zealand as it also occurs naturally in mainland Australia from the southern coast of New South Wales to western Victoria and is widespread in Tasmania (Thompson, 1989).

It has a dominant role in infertile and poorly drained environments and exhibit two main ecological roles in the vegetation: permanent dominance of extreme environments (in sites that are unfavorable for the development of climax forest as they are too wet, dry, cold, exposed, infertile, or unstable) or as a seral species (in successions to forest where it may be regarded as a woody weed of pasture or a useful species for erosion control, carbon sequestration, and vegetation restoration) (Stephens et al., 2005).

2.1.1 Uses:

Of all the native species in New Zealand, manuka is the one that shows more economic benefits with honey production being highly profitable. The New Zealand honey industry has been growing every year and in 2012/13 exports reached $120 million worth with manuka honey estimated to comprise 80 to 90%. Manuka honey is very valuable commanding a high price compared with other honeys (Ministry for Primary Industries, 2013). In addition, the red-colored wood of manuka tree is hard and durable being used for fencing construction, tool handle manufacture and as firewood, burning with a fierce heat. It is a medicinal plant and has traditional uses in the Maori culture (Salmon, 1980). Manuka can also produce valuable essential oils for the perfume and pharmaceutical industry showing good antimicrobial efficacy against various bacterias; specially gram-positive (Lis-Balchin et al., 2000) as it contain some antibacterial agents (particularly leptospermone) that may end up in the soil via a number of pathways like rhizodeposition from roots or through leaf fall degradation. This characteristic can promote a positive effect as it increases the rate of pathogen die-off in soil (Prosser, 2014) and therefore could minimize some drawbacks of biosolids application.

Manuka has also being shown to be arsenic tolerant being able to grow in high-arsenic substrate in old mining site excluding arsenic from their shoots. This data suggest that revegetation with manuka could be used as phytostabilisation agents on high-arsenic sites (Craw, et.al. 2007) crediting the species tolerance to stressful habitats. Manuka quickly colonizes disturbed land surfaces and steep, erosion-prone pastoral hill country, being efficient for erosion mitigation and soil conservation (Stephens et al., 2005).

2.2 Biosolids

Biosolids are the nutrient rich sludge that remains after sewage is being treated in the municipal wastewater treatment plants. Biosolids can be very different, varying its characteristics according to their origin (animal, human, industry), the treatment process they have gone through, (physicochemical or biological, aerobic or anaerobic digestion, lime stabilization, etc.) and time of the year (Forcier, 2002; Cameron et al., 1997). To be named biosolids, sludge has to be treated and/or stabilized to the extent that it is able to be safely and beneficially applied to land (NZWWA, 2003). Application of unstable or immature types of compost could promote slow plant growth and damage crops by competing for oxygen or causing phytotoxicity to plants due to insufficient biodegradation of organic matter (Brodie et al., 1994). Typically, the most common input in the wastewater treatments come from urban, commercial and industrial sources, therefore their toxic contents will end up in the biosolids. Amongst its components is possible to observe macro and micro-nutrients,

organic compounds, heavy metals, endocrine disrupting compounds, pesticides, herbicides, surfactants, pathogenic helminths, bacteria, viruses and fungi (Cameron et al., 1997; Singh and Agrawal, 2008; Krogmann et al., 1999).

The disposal of sewage effluents into oceans and waterways is a practice that still happens in many countries resulting in depletion of dissolved oxygen, eutrophication, chemical toxicity, and salinity of the waterways (Cameron et al., 1997). The ways to dispose off today include landfilling, incineration and land applications but in New Zealand the most common is landfilling due, primarily, to its low cost (NZWWA, 2003; Cameron et al., 1997). However leachates percolating out of landfill sites can result in aquifer contamination, so strict regulations have now been imposed on landfilling considerations such as site selection, installation, and environmental monitoring. (Cameron et al., 1997; USEPA, 1999;).

2.2.1 Production and qualification:

The guidelines for the safe application of biosolids to land in New Zealand works with a grading system that classifies the biosolid produced, aiming at safe use and disposal. The grading is made up of two parts: the first (capital A or B) represents the stabilization grade. The second (lower case a or b) represents the contaminant grade. If a biosolid does not meet the process and product standards for Aa, Ab, Ba, or Bb biosolids, it should be considered a "sludge" rather than a biosolids and be properly handled and disposed (NZWWA, 2003).

Table 1: Biosolids grading system proposed by the New Zealand guidelines for the safe application of biosolids to land (NZWWA, 2003).

Grade 'Aa`	Have substantially reduced pathogen and vector-attracting compounds, such as volatile solids, such that the product is deemed safe to be handled by the public with minimal risk.
Grade 'Bb`	Can have a lower level of treatment and will contain pathogens. Use is restricted and subject to management to protect the soil and waterways.

In most sewage treatment plants wastewater undergoes preliminary, primary, secondary, and, in some cases a tertiary treatment. Preliminary and primary treatments consist of screening and grit removal being considered a mechanical treatment. The sludge created contains 3%-7% solids. The secondary treatment generally relies on biological treatment using microorganisms to reduce biochemical oxygen demand and remove suspended solids so the remaining water is cleaner and can go into waterways. This step produces less solids contents (0.5%-2%). Tertiary treatment is more common in modern treatment plants and includes biological and chemical precipitation processes to remove nitrogen and phosphorus from the wastewater. The additions of lime, polymers, iron or

aluminum salts done in this process will affect the amount of solids content and the characteristic of the biosolids. Additional treatments occur to meet regulatory requirements that protect public health and environment, facilitate handling, and reduce costs (USEPA, 1999).

The type of treatment that the wastewater went through affects the characteristics of biosolids, which in turn can affect the types of biosolids treatment chosen. The most common types of treatment processes are stabilization (to reduce pathogen levels, odor, and volatile solids content) and dewatering (remove excess water) (USEPA, 1999). Some key features of stabilization processes are summarized in Table 2.

Table 2: Summary of stabilization processes that produce biosolids (Epstein, 2003)

Alkaline Stabilization	
Use of lime or other alkaline materials, such as cement kiln dust, lime kiln dust, Portland cement and fly ash. Increase of pH to reduce pathogens and achieve vector attraction reduction.	The process can produce a Class A or Class B. Major uses are in agriculture, reclamation, slope stabilization, structural fill, and municipal solid waste (MSW) landfills.
Anaerobic Digestion	
Anaerobic digestion involves biologically stabilizing biosolids in a closed vessel to reduce the organic content, mass, odor, and pathogens. During this process methane is generated and can be used as an energy source. Both mesophilic temperatures (35°C, 95°F) and thermophilic temperatures (55°C, 131°F) are used.	The process produces either a Class B at mesophilic temperatures or Class A at thermophilic temperatures. The production of Class B is more common. Principal uses are in agriculture and forestry.
Aerobic Digestion	
Aerobic digestion utilizes oxygen or air to biologically stabilize biosolids in an open or closed vessel or lagoon. The organic matter is converted to carbon dioxide, water, and nitrogen. Pathogens and odors are reduced. High thermophilic temperatures are recently being used.	Generally in most cases a Class B biosolids is produced. High temperature systems can produce a Class A product. The principal uses are in agriculture.
Composting	
Composting is the biological decomposition of the organic matter. Generally biosolids composting is done at thermophilic temperatures (>55°C, 131°F) in order to destroy pathogens. During composting the odorous compounds are reduced.	Composting produces a Class A product. Its principal uses are horticultural, including landscaping, nursery operations, turf, lawn and sod production, agriculture, public works department projects, highway beautification and reclamation.
Heat Drying and Pelletizing	
Heat drying involves using active or passive dryers to remove water from biosolids. It is used to destroy pathogens and remove water, which reduces the volume of material. In some cases heat-dried products are formed into pellets.	Heat drying produces a Class A biosolids product. It is primarily used in agriculture. Other uses are in turf and sod production and golf courses. Heat dried pellets are also blended with other nitrogen, phosphorus, and potassium chemicals to produce fertilizers.

Biosolids have to be analyzed and monitored as the application rate on agricultural land will be limited by the level of contamination from heavy metals, toxic organic chemicals, and pathogens.

Odors can also be an important problem. Odour avoidance requires immediate incorporation into the soil and the sites for application must be selected with respect to population density, air drainage, and the prevailing wind direction. (Parr et al., 1978; Pepper et al., 2006). Figure 2 illustrate the sewage sludge processes and its beneficial uses.

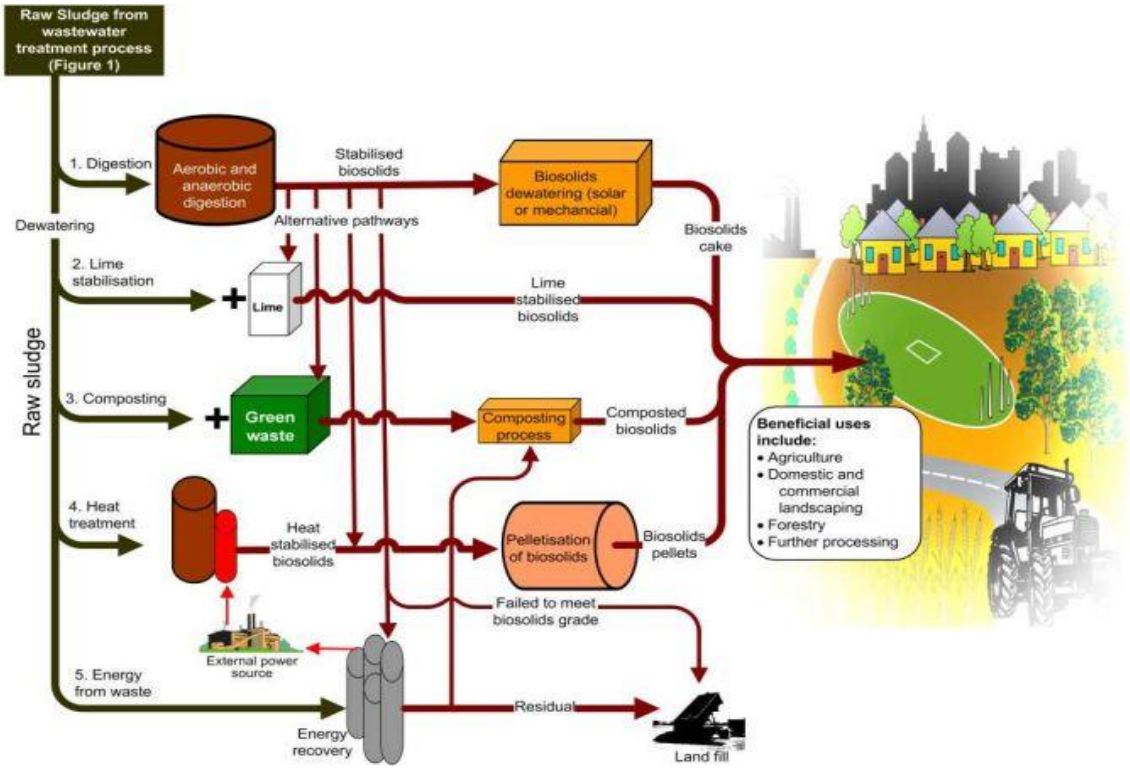


Figure 2: Wastewater treatment processes and biosolids uses. Source: (http://www.biosolids.com.au/what-are-biosolids.php)

As long as a soil is being productive many elements are being consumed so additions of those elements have to be done where anthropogenic interventions occurred in order to keep the soil fertility in good levels. Therefore, the application of biosolids could be done to replace these elements and thus maintain the soil fertility. Within all the chemical characteristics of a biosolid, plant nutrients are amongst the most important ones with farmers valuing the biosolids based on their nitrogen and phosphorus content as they are extremely important for plant growth (Pepper et al., 2006).

2.2.2 Contaminants:

The weathering of parent material is a major contribution to soil function. Therefore, natural soil will contain a wide range of total and available concentrations of most elements depending on the geochemical composition of that parental materials and variations in the intensity of soil-forming processes (Alloway, 2010).

Deficiencies of essential heavy metal such as zinc (Zn), copper (Cu) and manganese (Mn) and metalloids such as selenium (Se) in agricultural soils can affect agricultural productivity and human health since they have important roles as constituents or activators of enzymes in physiological pathways. Although, large quantities of those elements pose potential toxicity problem in living organisms (Epstein, 2003; Pepper et al., 2006; Alloway, 2010) primarily because of their protein-binding capacity and thus their ability to inhibit enzimes activity (Speir and Ross, 2002). Excessive amounts of heavy metals and metalloids in agricultural soils are of great concern as they cannot be biologically or chemically degraded once they enter the soil system. Those elements are amongst the most intractable pollutants to remediate (Dungan and Frankenberger, 2002). Biosolids can contain those and others heavy metals, like lead (Pb), mercury (Hg), Nickel (Ni) and cadmium (Cd) (Knowles et al., 2011) that may cause harm.

To determine whether toxicities or deficiencies will occur, the total amount of these elements in the soil is not the most important factor. The balance between the fraction adsorbed by the plant and the fractions of heavy metals and metalloids in the soil solution depends on the total content of the element, the adsorptive capacity of the soil and physico-chemical factors, such as pH and redox potential (Alloway, 2010). Some plants can also exert significant effects on the availability of these components through the release of exudates from the roots (Bao et al., 2011) so a closer interaction with biosolids (homogeneous distribution in the bulk soil) could affect the uptake by the plant.

The concentrations of heavy metals have been one of the principal driving forces for the regulations governing the land application of biosolids because of their potential toxicity and persistence in the soil. The soil limits recommended in New Zealand is based on a European-type LOAEC approach (lowest observed adverse effects concentrations) and are similar to those adopted in Australia (NZWWA, 2003). The main metals of concern for the human health are cadmium, lead and mercury (Smith, 1996).

For example, table 3 illustrates the levels of some selected heavy metals compared to the NZWWA (2003) guidelines.

Table 3: Concentration of Heavy Metals found in Kaikoura biosolids and the grades A and B benchmarks used in the New Zealand. Concentration in mg/kg. (Knowles et al., 2011; CIBR, 2013).

	Cadmium (Cd)	Chromium (Cr)	Copper (Cu)	Lead (Pb)	Zinc (Zn)	Mercury (Hg)
Kaikora Biosolids	2.8	32	561	96	878	2.3
NZ guidelines Grade A	1	600	100	300	300	1
NZ guidelines Grade B	10	1500	1250	300	1500	7.5

Thousands of organisms are also found in biosolids and some are pathogenic for humans and animal. Amongst them are bacterias (e.g: Salmonella sp, Escherichia coli, Shigella sp, Vibrio cholerae) viruses (e.g: Hepatitis A and B virus, Adenovirus, Norovirus, Sapporovirus, Rotavirus, Enteroviruses) and many helminths and protozoa (e.g: Entamoeba histolytica, Giardia lamblia, Balantidium coli, Toxoplasma gondii, Ascaris sp, Trichuris trichirua, Toxocara canis, Taenia sp) (Pepper et al., 2006). Wastewater treatments, primarily temperature treatment (composting, heat drying, alkaline stabilization and thermal digestion) can significantly reduce certain pathogenic producing biosolids that do not represent health hazard. However the efficiency of removal depends on the organisms and their physical and biological properties (Epstein, 2003; USEPA, 2003).

2.3 Roots Behavior to Heterogeneous Nutrient Distribution:

Soils around the globe exhibit a heterogeneous (patchy) distribution of nutrients. It happens as a result of organic inputs derived from different material (e.g. Leaf litter, dead roots, animal remains and dead microorganisms) and the subsequent microbial decomposition of simple and complex organic materials releasing inorganic nutrients for plant capture (Hodge, 2004). Therefore, each soil has its own unique “patchiness” so the plants should be able to respond to them effectively (Fitter, 1997).

The plant behaviour in response to this heterogeneous or “patchy” nutrient environment is the use of root foraging mechanisms defined as the tropisms and growth activities that vary quantitatively over time (Tian and Doerner, 2013). These include changes to the growth rate (of each meristem), changes in direction (angle, tortuosity), and root density (rate of lateral root formation and emergence, and demography of lateral root meristems per unit cell number of lower order root). These parameters that affect the behaviour of lateral organs are of extreme importance as they define the capacity of the plant to exploit locally enriched resources (Tian and Doerner, 2013).

Plants have been exposed to heterogeneous above- and belowground environments for centuries. When this condition was added to some limitations imposed by the sessility of the plants a need of adaptation appeared leading the plants to the evolution of foraging mechanisms.

In root ecology, plasticity is what is shown by a genotype when its expression can be altered by environmental influences (Bradshaw, 1965). In the root system it is possible to observe

morphological and/or physiological plasticity, which enhances the plant acquisition of essential resources (Hutchings and de Kroon, 1994; Valladares et al., 2007).

Morphological plasticity: the ability to actively modify their potential of resource absorption by placing the roots selectively within the habitat increasing local root surface area in patches with high nutrient content (Hutchings and de Kroon, 1994).

Physiological plasticity: the increase in nutrient uptake per unit of root surface in the nutrient rich patch. It has been viewed as a beneficial addition to morphological plasticity for the good acquisition of resources (Hutchings and de Kroon, 1994).

2.3.1 Types of Foraging Behavior

Many ways that plants can interact with a nutrient patch in the soil have been observed and some of them are listed below:

No foraging:

Some species of plant do not show root morphological responses to patches of nutrients. There is no increase in the root growth or root biomass in the nutrient rich zone. Be unaffected by the nutrient status of the patches in the soil suggest that the soil is continuously searched (Hutchings and de Kroon, 1994). This foraging behavior seems to be more profitable in environments with low fertility soils where resources become available in the form of transient nutrient pulses giving the plant the ability to absorb some of the nutrient but keep in the search for other patches. This is more frequently observed in slow-growing plants which have higher diameter roots (Campbell, et al, 1991).

Fig. 3: Root showing no response to a nutrient patch

Proliferation:

In this foraging strategy, fine roots grow in and around the patch, absorbing its nutrients but no changes in the main root architecture happens. It is more observed on fast-growing plants as their fine roots are effective exploiters of small and short lived patches. Species presenting this characteristic have the ability to create a dense network of ephemeral rootlet rapidly, consuming all the nutrients in the patch (Fitter, 1994). The root aggregation is the result of an increase in the formation and growth of lateral roots in response to local enrichment by some species (Hutchings & de Kroon, 1994). Some plants use this foraging behavior to compete for soil nutrients depleting the nutrients in some areas making it inaccessible for other species (Tilman, 1988).

Fig 4: Root showing proliferation in the nutrient patch

Signaling:

Foraging strategy where the nutrient patch produce a strong response in the whole root system. It has a signaling process involved that creates a change in the root architecture so the whole roots start to move towards the patch increasing the number of roots in contact with the nutrient rich area and thus increasing their uptake system. This local signaling involves an intricate relationship between nutrients, hormones, and growth. It is enhanced when the internal nutrient availability is limited, becoming a systemic signaling (Ruffel et al., 2011). For example, for nitrate (NO_3^-), one of the most growth-limiting nutrients for plants, root proliferation in NO_3^--rich zones relies on the dual NO_3^-/hormone transport activity of the NO_3^- transceptor. Apart from that, other key regulatory

components of the NO_3^- perception and signaling pathway have to work together to regulate root activity according to its nutrient status (Ruffel et al., 2011). Despite some progress in the area, the signaling mechanisms remain largely unknown.

Fig 5: Root responding to signaling mechanism towards nutrient patch

Avoidance

This is when the root avoids the nutrient rich path, growing in another direction. It can happen when an element of the patch is highly concentrated or toxic for the plant. This behavior was observed on the roots of *Arabidopsis* seedlings that changed their downward course when encountering high levels of salinity. The researchers showed that salinity induced endocytosis of the cell membrane of a regulator of auxin transport, on the side of the root encountering the salt. Consequently, concentrations of auxin, a hormone that helps determine the direction of root growth, were redistributed, and the direction of root growth changed course (Galvan-Ampudia et al., 2013).

Fig 6: Roots avoiding nutrient patch

Growth Inhibition

Like avoidance, this mechanism occurs when the root finds a really concentrated or toxic patch in the soil. However, in this case the growth of the root ceases in the part that encountered the patch. An inhibition in the root growth of *Arabidopsis thaliana* was observed when an increased in the levels of metals (cadmium and copper) occurred in the environment in comparison with a nutrient-rich environment (Universiteit hasselt, 2014). Some plants have adaptive mechanisms for accumulate or tolerate high contaminant concentrations in their rhizospheres, it is present only in tolerant phenotypes (Khan et al., 2000) so, it is probable that species without these phenotypes will show root growth inhibition for some contaminants if encounter it.

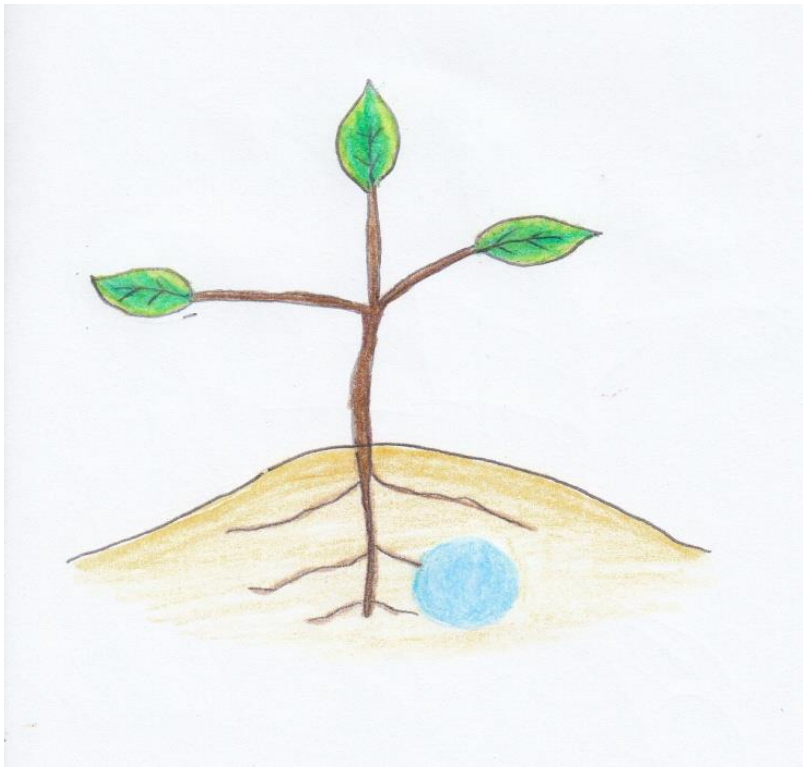


Fig 7: Roots showing no growth in the nutrient patch

Foraging with diffusion gradient

This behavior occurs when the concentration of the nutrient in the patch is very high, however when in normal concentrations it is not toxic for the plant. To be able to absorb it without any negative effect, the plant takes the nutrients slowly by growing many fine roots only around the area with high nutrient concentration without getting inside of it. This mechanism was observed in the root system of wheat plants whose roots were provided with a concentrated band of ammonium sulphate

fertilizer. To get the nutrients in a level that would not be toxic for the plant, the roots proliferated in its vicinity and eventually formed a dense cylindrical cluster as they progressively took up the fertilizer. This may be a positive response when it comes to competition with other species or in preventing nutrient leaching in the soil (Atwell et al., 1999).

Fig 8: Roots showing diffusion gradient growth

Analysis have demonstrated that some crop species proliferate more roots on areas of high nutrient concentration (Robinson, 1994; Fransen et al., 1998) and that heterogeneously nutrient distribution in soil could promote higher yield and plant nutrient concentration than homogeneous soil (Kembel and Cahill, 2005).

Therefore, knowing how manuka reacts to different biosolid applications both in terms of root behaviour, root and shoot elemental concentration and biomass will help in the development of better techniques for land management and also in the general understanding of the plant and the biosolids.

Chapter 3

Research Questions and Aim

The lack of fertile soils in many areas of New Zealand is a fact. Only 5.5% of the country's soils are considered to be of high value for food production (Hewitt, 2012) Excess use of fertilizers, avoidance or exodus from some areas are the options many farmers may have to choose from and sometimes none seems positive. In order to minimize those problems in some infertile areas, planting manuka could be a good choice together with applications of biosolids to further improve the soil quality. In this way marginal lands could be productively used for honey and essential oils production or ecosystem restoration and excess of biosolids could be recycled, creating living and profitable conditions for many families as well as giving a beneficial destiny for some of the human waste.

However, as a pioneering species that is adapted to low fertility soils, it is unclear if manuka will show a positive growth response to biosolids addition.

My research aim is to investigate if manuka will show a positive growth response to the addition of biosolids.

To do this, my hypotheses are:

1) The above and below ground biomass of manuka will increase with the addition of biosolids.

2) Manuka will demonstrate root foraging behavior in the addition of biosolids.

3) Manuka foliage will sequester major elements (N, P, K, S, Mg, Ca) and heavy metals (Zn, Cu, Cd, Ni) with the addition of biosolids.

To test these hypotheses, I will investigate the growth response of manuka seedlings in two different soil substrates amended with biosolids and a control treatment. Specifically I will investigate:

1) The root distribution of manuka seedlings.

2) The above and below ground biomasss of manuka seedlings.

3) The elemental composition of the manuka seedlings foliage.

Chapter 4

Material and methods

4.1 Collection and preparation of materials:

These experiments (pot trial and rhizobox trial) used 2 naturally low nutrient soil materials (a sand and Lismore brown soil) as substrate to contrast with the rich nutrient biosolids in the treatments.

50 Kg of sand was sourced from the top 40 cm (approximately) from a beach at Kaikoura Flat, 5 km north of Kaikoura, Canterbury (42°21'37.7"S 173°41'28.1"E).To prepare the sand the salt content was reduced by washing it with tap water. A thin fabric mesh was individually placed inside 20 pots of 2.5kg capacity and 2 kg of sand was placed in each pot. The mesh held all the sand inside the pots but allowed the water to flow out. The pots were washed with tap water for 3 minutes once a day for 5 days. The sand was then placed in 2 big trays (100x60 cm) and left to dry for three days. The sand was sub-sampled for soil analysis (See table 4).

160 kg of Lismore soil (Orthic Brown Soil, Hewitt, 2010) was sourced from the top 40 cm of a pine plantation in Eyrewell forest, 26km south east of Oxford, Canterbury (-43°43’87.11", 172°45’30.79") and sieved on site using a 2cm metal sieve. The material (<2cm) was then transported to Lincoln University and homogenized manually by mixing the whole amount with shovels. This homogenized sample was then sub-sampled to give 25kg of soil which was then used for the subsequent experiments and for soil analysis (see table 4). This Lismore soil is referred to from now on as Eyrewell soil.

20 kg of partially treated biosolids were collected from a stockpile at the Kaikoura Regional treatment works, at Kaikoura, Canterbury. Before being stored in the stockpile, the biosolids went through initial treatment of sedimentation and anaerobic digestion in settlement ponds. This reduces pathogen and odors and makes it usable for agricultural purposes. 15 kg of biosolids were collected from eight different locations across the pile and bulked. This bulked sample was analyzed for pH, total carbon (C) and total nitrogen (N) and elemental composition before its use in the experiment (Table 4).

The biosolids used contain 2.2% of N. It represents 22g N/kg of biosolids. The same amount of N was applied in the homogeneous and heterogeneous treatments of each trial. Treatments in the pot trial received 4.4g N/pot and in the rhizobox trial they received 2.4g N/rhizobox.

Table 4: Chemical properties of biosolids and soils used in trial. Values are means and in brackets are standard errors, n=5.

	Biosolids	Eyrewell	Kaikoura
pH	4.3(0.01)	4.9(0.004)	8.3(0.01)
Cond.(µS/cm)	2637.5(22.9)	98.1(1.4)	19.4(1.2)
C (%)	23	3.9	0.1
N (%)	2.2	0.2	< 0.1
P (mg/kg)	5658(230)	372(69)	476(36)
S (mg/kg)	9006(199)	210(3.7)	112(12.5)
Ca (mg/kg)	11012(332)	2732(84)	8855(522)
Mg (mg/kg)	3872(91)	4072(54)	6426(169)
K (mg/kg)	3777(50)	4728(60)	3893(225)
Na (mg/kg)	397(14)	224(4)	242(12)
Cd (mg/kg)	2.2(0.1)	< 0.1	< 0.1
Mn (mg/kg)	254(7.2)	338(15)	429(11)
Cu (mg/kg)	611(16)	3 (0.2)	11(1.5)
Ni (mg/kg)	23.3(2.4)	8.2(0.3)	11.2(0.5)
Zn (mg/kg)	1239(45)	68.8(1.6)	50(1.1)
Pb (mg/kg)	120(4.3)	15(0.8)	14(0.4)
Cr (mg/kg)	49(8.1)	23(0.3)	17(0.6)

Approximately 10kg of this bulked sample was passed through a 2cm metal sieve prior to use in the pot trial. A sub-sample (1kg) was ground using a ceramic mortar and pestle and sieved using a 2mm Nylon sieve (reducing metal contamination). This 2mm screened biosolids sub-sample was used in the rhizoboxes.

Aproximatelly 100 manuka (*Leptospermum scoparium*) seedlings were obtained from commercial plant nursery (Wai-Ora, Christchurch, New Zealand) in sprouting trays to be used in this experiment. The seedlings used in both trials ranged in above ground size from 4cm to 6.5cm when planted. Roots were also small having a main root with similar size of the above ground plant and a few laterals. The small size was important for a better observation of the effect of biosolids in the plant growth.

4.2 Greenhouse Experiment:

Pots and rhizoboxes were randomly distributed in a greenhouse at Lincoln University's Nursery, Lincoln, New Zealand, during the summer of 2014. A fan ventilates the greenhouse cooling the air if it exceeds 24 °C. The mean temperature during the experiment period was 20.2 °C with maximum temperature of 32 °C and minimum of 9.8 °C.

4.3 Pot trial:

The pot trial consisted of four replicates of three treatments for each soil material (Eyrewell soil and Kaikoura sand) giving a total of 24 pots. The treatments were control, homogeneous, heterogeneous and their details are explained below.

The control treatment consisted of 2kg of soil in a 2.5L pot. Manuka seedlings were then transplanted from the sprouting tray to the pots after all the potting mix had being carefully removed from its roots by gently brushing off. The same process was done for planting into Eyrewell soil and Kaikoura sand.

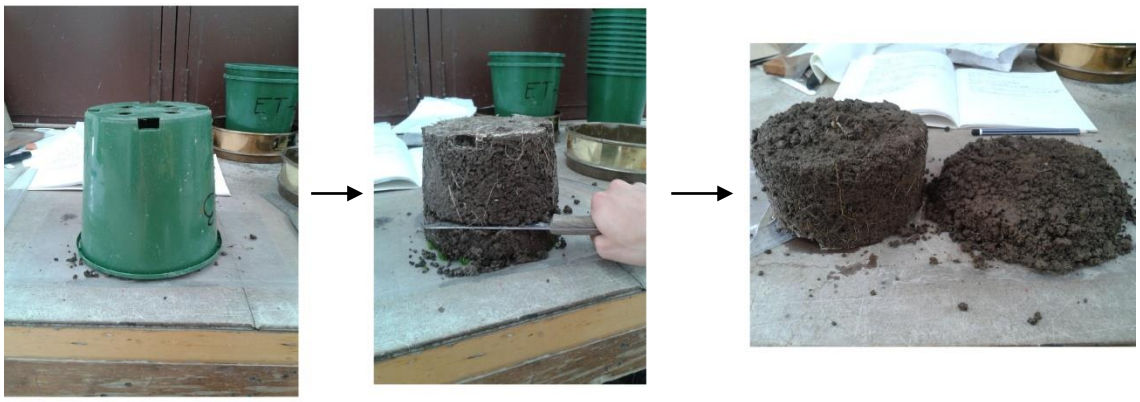
The homogeneous treatment consisted of an evenly distributed mixture of biosolids and soil substrate. 800 grams of biosolids (10% of the total weight) were added to 7.2Kg of soil and continuously mixed for 3 minutes using a plastic scoop to avoid metal contamination. 2kg of the mixture were placed in 4 pots with each pot containing 4.4g N/pot (200 g of biosolids per pot). Manuka seedlings were then transplanted from the sprouting tray to the pots, after having all the potting mix being carefully removed from its roots. The same process was done for planting into Eyrewell soil and Kaikoura sand.

The heterogeneous treatment consisted of 1.8kg of soil placed inside each pot. In this treatment the manuka seedlings were transplanted before the addition of 200g of biosolids that was carefully placed on the surface of the pots. Each pot thus contained 4.4g N/pot. The same process was repeated for Eyrewell soil and Kaikoura sand.

Plants grew from late November 2014 until February 2015. Irrigation was done manually, everyday, using a hand-held sprinkling hose with approximately 100mL of water added per application, maintaining the pots at field capacity.

Plants were harvested after 12 weeks of growth. The shoots were cut 0.5mm above the first roots, rinsed with tap water and dried in a 65-70^{o}C oven until constant weight. After dried, the leaves were separated from the stems by hand. Both were weighed and sampled individually.

After the removal of the shoot, the soil and roots in the pot were cut separating the top 3 cm from what was left in the base (approximately 11 cm). In that way differences in the root distribution could be observed for the different treatments. Figure 9 shows the cutting process.



(a) (b) (c)

Figure 9: (a) + (b) illustrate the cutting process to divide the roots in top and base layers. (c) shows the end product with the top 3 cm separated from the rest of the pot (approximately 11 cm).

The roots had all the soil gently brushed off and meticulously washed in tap water before being individually sampled in top and base roots. After being dried in a 65-70^{o}C oven until constant weight, the weight was measured for biomass analyses.

Biomass was analyzed for root and shoot (stem+leaves); elemental composition, carbon and nitrogen were analyzed from the leaves.

4.4 Rhizobox trial:

For a better observation of the growth and root architecture of manuka plants in the different treatments 12 rhizoboxes (4 for each treatment: control, homogeneous, heterogeneous) were also used.

Rhizoboxes are a perspex box where the side and end plates are glued to each other and the back plate forming an air-tight seal on three sides and a 15 x 30 x 2.5 cm cavity on the inside. A removable rigid and highly transparent front plate completes the box. That enables a good vision of the root system allowing the observation of the root behavior and growth in the different treatments. There are 10 holes through the back plate for water irrigation if needed, tapped to accommodate a screw adapter (see figure 11 for more details).

The rhizoboxes were randomly arranged in the greenhouse from December 2014 until February 2015. Watering was done 6 days per week using a spray bottle for top applications and a syringe for hole application. The watering maintained rhizoboxes at field capacity with amounts applied varying according to the evaporation to the air and absorbance by the plant, ranging between 5mL to 15mL per day.

To ensure root growth along the transparent lid the rhizoboxes were arranged in a slope position (at a 45^{o} angle). After 40 days of growth in the rhizobox the lid was temporarily removed so the roots could be scanned for posterior image analysis. This process was done once a week for 4 weeks, using a Canon scanner (CanoScan LIDE 210). In the rhizobox trial only sand was used as it is a very low fertility substrate, allowing more contrast between treatments with and without biosolids. Figure 10 shows their arrangement in the greenhouse:

Figure 10: Rhizoboxes were arranged randomly in a 45◦ angle. The black plastic was placed around the soil line to prevent light and algae growth.

The treatments were as follows:
In the control treatment 1.05kg of sand was placed in four layers of approximately 262g to maintain similar bulk density along the whole rhizobox. A distance of 5 cm was left empty on the top so the rhizobox could be placed at an angle without losing any substrate from the top opening.

The homogeneous treatment consisted of an evenly distributed mixture of biosolids and sand substrate. Each rhizobox had 1.110kg of the mixture sand+biosolids, maintaining the biosolids at 10% concentration. To achieve that, 3.996kg of soil was mixed with 444g of biosolids in a container and

then the homogeneous mixture was divided within the 4 replicates, each one containing 2.4g N/rhizobox (111g of biosolids per rhizobox). Manuka seedlings were carefully transplanted from the sprouting tray to the rhizobox after having all the potting mixture removed from the roots by gently brushing it off.

The heterogeneous treatment the rhizobox was divided into two sections (one third, two thirds). A thin plastic stick was positioned in the division point to help with the substrate allocation (as shown by the blue line in the figure 11) leaving 10cm on one side and 5 cm on the other. In the bigger section 1 kg of sand was carefully added while the smaller section received the mixture sand+biosolids at 10% of the total box weight. The plastic stick was them removed.

Biosolids and sand have different densities so, to make accurate stripes, their volume was calculated so the mixture would have the same amount of biosolids (and thus nutrients) used in the homogeneous treatment and constant volume between all the rhizoboxes. To do that the total volume of the four strips was marked in a 5L box by using water. Afterwards, 444g of biosolids (the same amount as in the homogeneous treatment) was placed in the 5L box and sand was added until it reached the volume mark of 4 strips. In the end 560g of sand was added resulting in strips with approximately 44% of biosolids. Each rhizobox received 111g of biosolids and 2.4g N/rhizobox.

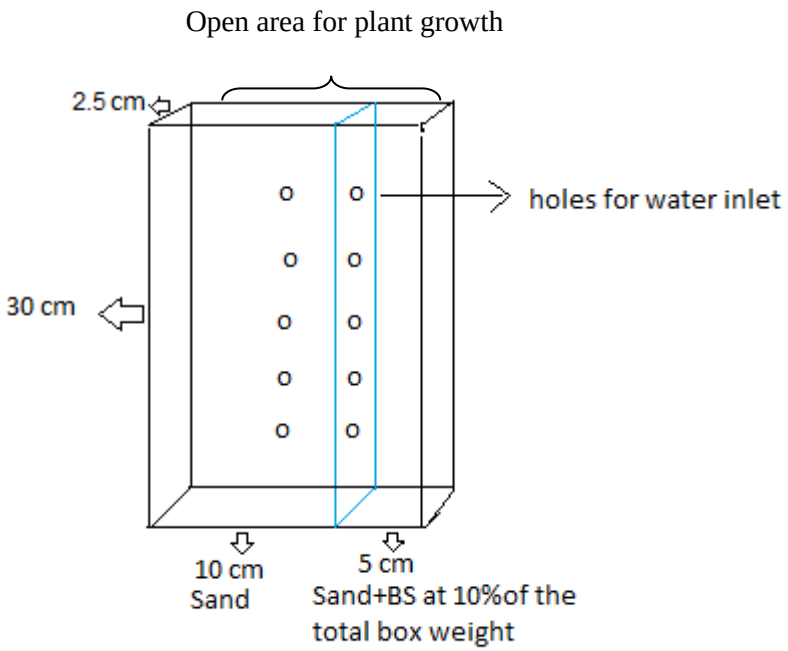
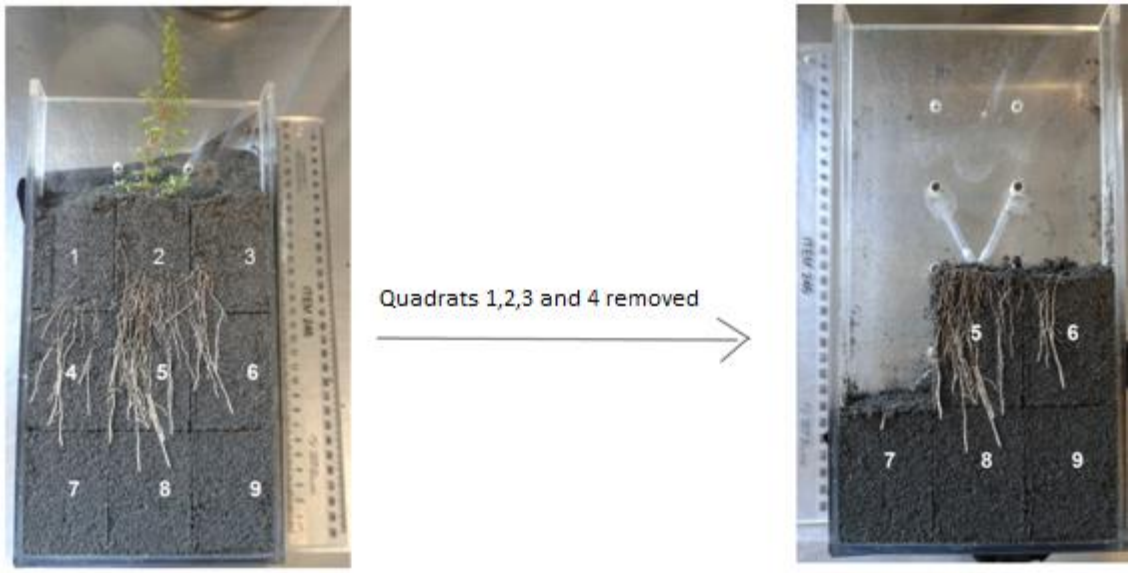


Figure 11: Description of the rhizobox in the heterogeneous treatment. The blue line was the division point between sand and sand+BS

After 9 weeks of growth the plants from the rhizoboxes were harvested. The shoots were cut 0.5mm above the first roots and rinsed with tap water. Shoot were dried in a 65-70^{o}C oven until constant weight to measure biomass. For the root analysis, the soil material from each rhizobox was divided in 9 quadrats of the same size (approximately 5×7.4×2.5 cm) respecting the 5cm width of the biosolid layer in the heterogeneous treatment. The roots were then collected from each quadrat, washed with tap water and sampled individually. They were dried in a 65-70^{o}C oven until constant weight and measured. Figure 12 illustrates the division used.



(a) (b)

Figure 12: (a) is a rhizobox without the front plate showing the division of the 9 quadrates in a homogeneous treatment. (b) is the same rhizobox after the removal of 4 quadrats.

4.5 Sample preparation and chemical analysis

Soil samples were left to dry for one week at room temperature. After that, their pH was measured using a Toledo Metler pH meter and for the conductivity, a Toledo Metler conductivity meter. Both methods were done according to Blakemore, et.al. (1987).

For the leaves elemental composition, 0.3 g of dried sample was individually placed into a microwave vessel. 2.0mls trace element grade Nitric acid and 2.0ml of 30% hydrogen peroxide was added. The vessel was then sealed and vortexed to ensure the acids and sample was well mixed. Vessels were loaded into the turntable and place into the microwave cavity. Two ramps were set up to ensure that the whole sample was completely digested. The first ramp reached 90^{o}C over 15mins, holding for 5 minutes, and the second ramp 185^{o}C over 10 minutes, holding for 15 minutes. The cooled samples were uncapped and made up to 15ml using MilliQ water.

For the soil analysis, 0.5 g of dried sample was individually placed into a microwave vessel. 2.0mls trace element grade Nitric acid and 2.0ml of 30% hydrogen peroxide was added. Vessels were then sealed and vortexed. The ramps were 90^{o}C over 15mins, holding for 5 minutes and the second reaching 200^{o}C over 15 minutes, holding for 20 minutes. The cooled samples are uncapped and made up to 25ml using MilliQ water. Soil samples were filtered using Whatman 52 filter paper.

The internal temperatures of the vessels were continually monitored by 2 Infrared sensors in the bottom of the microwave cavity.

Samples were then analysed using Varian 720 ICP-OES Australia.

Total Carbon and Nitrogen were analyzed in plant and soil material using an Elementar Vario-Max CN Elemental Analyzer (Cresswell and Hassall, 2015).

Due to the small amount of leaves in the control treatments the minimum weight required for analysis was not reached in all the cases. Therefore, the rhizobox trial did not have the elemental composition analyzed in the control treatment and discussion was only done with data for the pot trial.

4.6 Data Analysis:

Microsoft Excel was used for the calculations of the averages, standard errors and standard deviations for all of the biomass data. Before being statistically analyzed, the results of the leaf elemental composition went through calculations to correct the fraction of trace elements in the leaves originating from surface-deposited dust that may incorporate particles into the waxy layers of the leaves. The mass fraction of the soil on the leaf sample, Msoil (mg/kg) was calculated as:

$$Msoil = \frac{Tplant - Rplant}{Tsoil} \qquad (1)$$

where Tplant is the measured indicator element concentration in the plant tissue (mg/kg), Rplant is the baseline concentration of the indicator element that the plant has accumulated through the roots and translocated to the shoots (in this case Fe [mg/kg]), and Tsoil is the concentration of the indicator element in the soil (mg/kg). (Robinson et.al , 2008)

Therefore, the corrected plant concentration of the target element, Cplant* (mg/kg) was calculated by:

$$Cplant^{*} = Cplant - Msoil \,.Csoil \qquad (2)$$

where Cplant and Csoil are the measured concentrations (mg/kg) of the target element in the plant and soil. (Robinson et.al , 2008).

Plant and soils data were analyzed using Minitab 17 One-Way ANOVA to determine any treatment effect. Grouping information using Fisher LSD Method was carried out for all the elements.

Chapter 5

Results and Discussion

5.1 Root Distribution:

The distribution of the roots in the pots and rhizoboxes was analyzed to determine whether or not root foraging behaviour was occurring as by comparing the areas of root allocation, patterns can be found. More complex investigations about root morphology including number and type of lateral roots are outside the scoop of this dissertation.

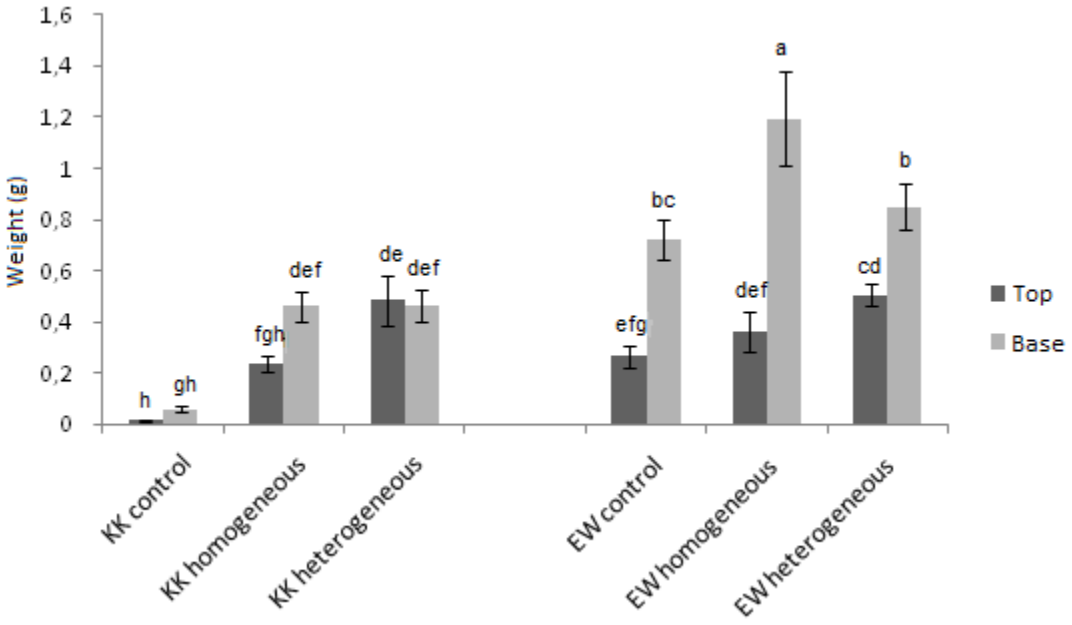


Figure 13: Biomass of the root level (top and base) in the pots for all the treatments in Kaikoura sand (KK) and Eyrewell soil (EW). Bars represent the standard error of the mean. Values with the same letter are not significantly different.

A dimensional comparison between the areas of root growth showed that the root distribution differed significantly between treatments. For the EW control treatment of the pot trial (where no biosolids were applied) there were relatively more roots in the base than in the top 3 cm. This was expected because the base represents most of the volume of the pot. In the homogeneous treatment, where the biosolids were applied evenly throughout the pot, the biomass was increased for both the KK and EW treatments. However, there were significant differences between the KK and EW treatments.

In the heterogeneous treatment, KK and EW had different responses to the biosolids application. In KK, top and base levels did not show differences and EW followed the same pattern as control but showed a smaller ratio base:top when compared to homogeneous and control indicating that more

roots grew in the amended patch (top). Similar response was observed in the rhizobox treatment growing in KK (see figure 15). Here the biosolids were applied in the right hand side of the heterogeneous treatments. Note that the centre line always has the higher biomass as it is the location of the main root. In this trial, the homogeneous treatment also showed a general increase in the biomass when compared to the control, with no differences between sides within the treatment. The heterogeneous treatment showed significant differences in the centre and the right hand side (where biosolids were applied) when compared to the homogeneous and control.

This result indicates that a foraging behavior is happening in the plant as more roots are occurring in areas where biosolids were applied. Similar results were found in other studies where higher root density was found in patches of soil supplied with nutrients than in control patches that did not receive them (Drew, 1975; Caldwell et.al., 1991b; Hutchings and de Kroon, 1994; Fitter, 1994). This proliferation response can be seen in Figure 14, which shows the root architecture in a heterogeneous replicate of the rhizobox, highlighting the difference in the root structure between the left hand side (un-amended soil) and the right hand side (amended soil).

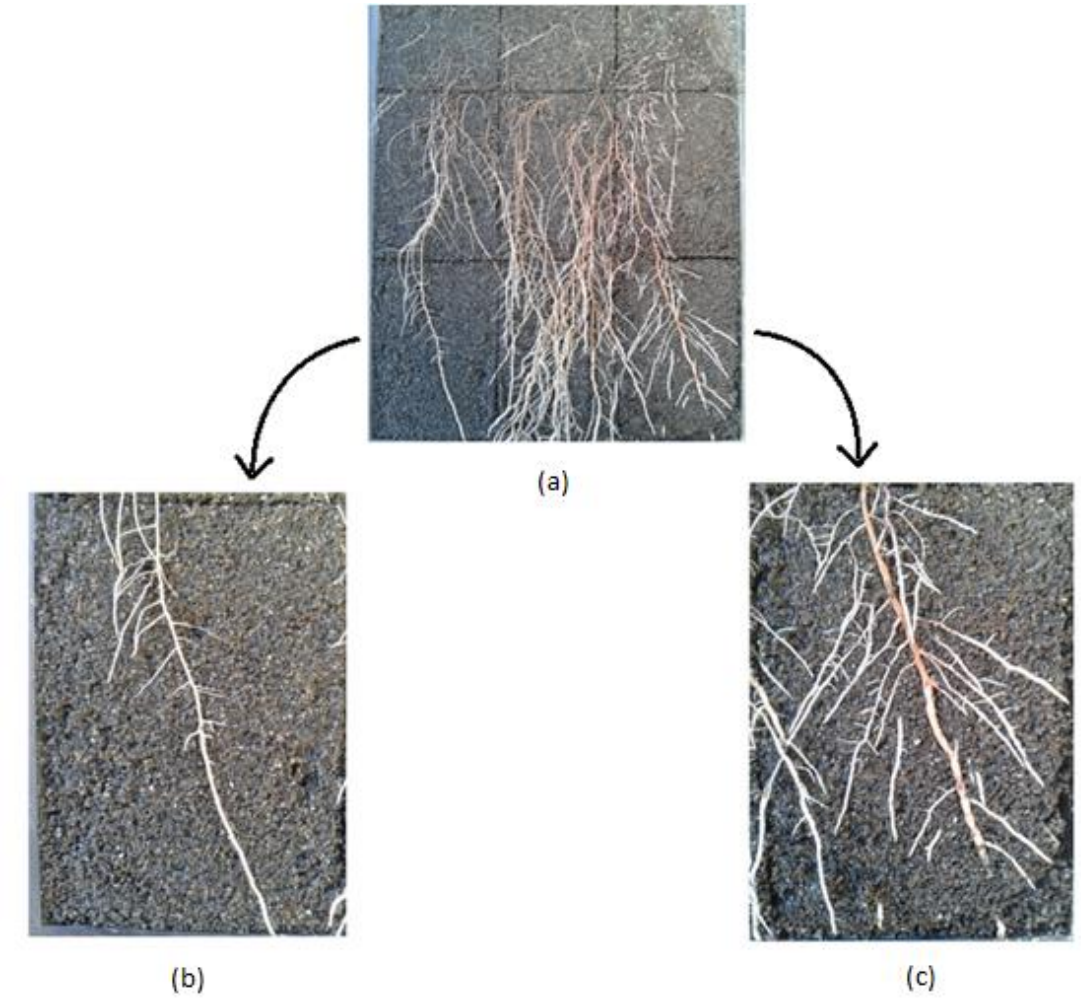


Figure 14: (a) shows the root architecture of a heterogeneous replicate of the rhizobox trial, highlighting the (b) bottom left and (c) bottom right quadrats. (c) is in the biosolids amended line showing an increase in the number of roots in comparison to the roots in the un-amended side (b).

The high concentration of nutrients in the biosolids patch was sensed by the plant which subsequently responded by producing more fine roots in that area, and being able to uptake more nutrients in the rich nutrient patch (Drew, 1975; Jackson and Caldwell, 1989; Hutchings and De Kroon, 1994; Fitter, 1994). Whereas in the un-amended soil, roots were less developed (lower number of lateral roots) indicating that the plant continues to search the soil for nutrients (Drew, 1975; Hutchings and de Kroon, 1994).

By observing the roots in the un-amended patch, it may be possible to say that in the heterogeneous treatment the supply of nutrients could be also causing a systemic response in the whole plant because in this area, the root growth is much higher when compared to the control treatment that did not received nutrient addition. Further images of the different treatments of the rhizoboxes can be seen in appendix 2.

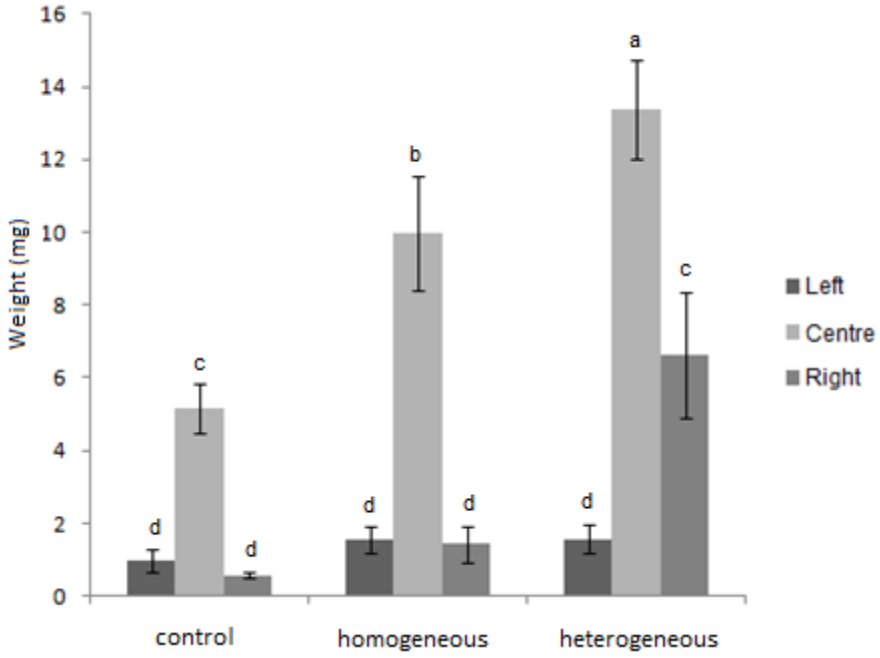


Figure 15: Root biomass per vertical line in the different treatments of the rhizobox trial. Bars represent the standard error of the mean. Values with the same letter are not significantly different.

5.2 Biomass

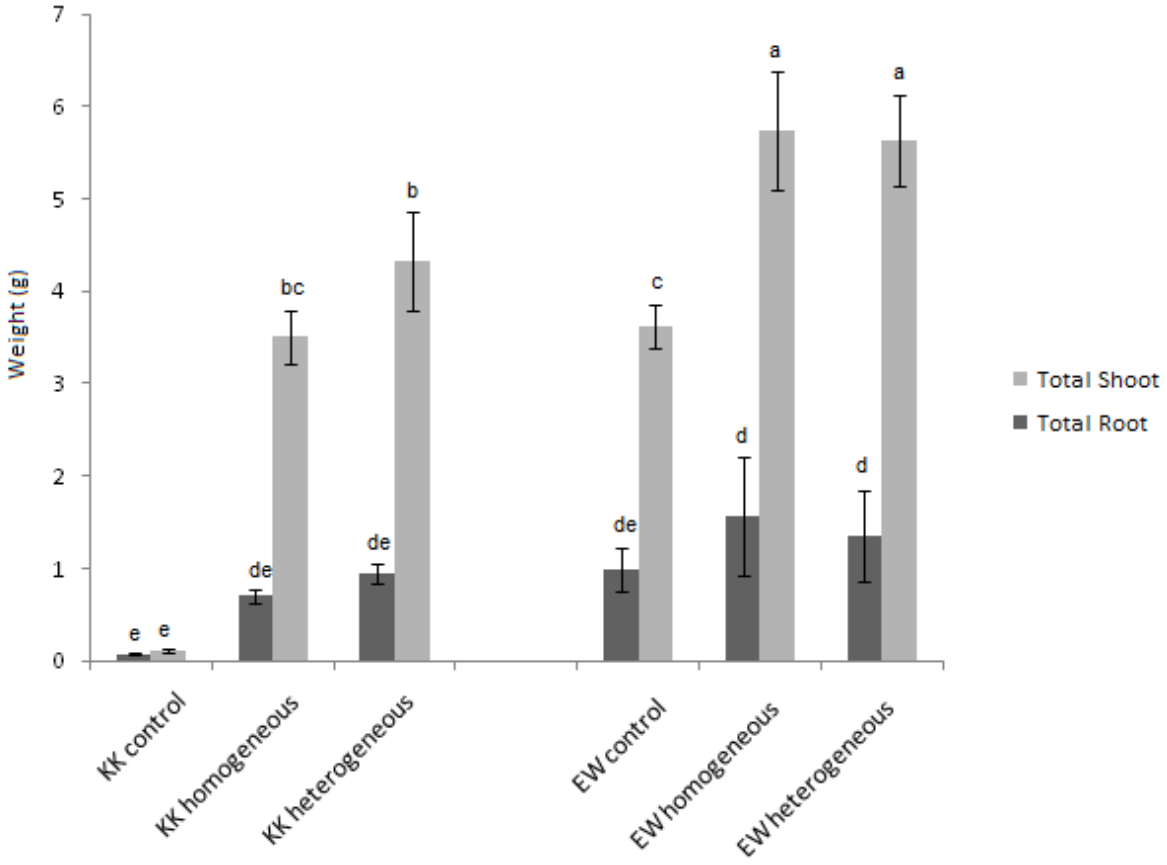


Figure 16: Total root and total shoot biomass for all the treatments in the pot trial. Bars represent the standard error of the mean. Values with the same letter are not significantly different.

Figure 16 shows that total shoot biomass increased with biosolids addition in both KK and EW substrates. Significant differences occurred in KK between control and homogeneous and control and heterogeneous treatments but this difference did not occur between homogeneous and heterogeneous. The same happened for EW showing that biosolids did improve the plant growth but that this growth was not affected by the type of treatment. This data is consistent with Longsdon (1993) that reported a 35% yield increase for both barley and wheat when biosolids were ploughed into the fields at a rate of 4.5 dry t/ha/yr prior to planting.

The increase in biomass was more intense in KK than EW when compared to their respective control. The reasons may be due to the low levels of organic matter (0.1% Total C), low levels of N (<0.1%), and high pH of KK. With mean value of pH 8.3 in the pure sand a reduced biomass production was expected (Lauchli and Grattan, 2012). However, with the addition of biosolids (pH of 4.3) the mean value of the mixture decreased allowing for a bigger growth in the homogeneous and heterogeneous treatment as nutrients become more available for plant uptake (Lauchli and Grattan, 2012). This new

value is much closer to what is found under naturally occurring manuka trees. In this previous study the average pH (pH moist) in the A and upper B horizons was 5.5 in a Kaingaroa silty sand (McIntosh, 1980). Soils with pH >8 are considered highly alkaline causing the plants to be strongly affected in its nutrient availability, nutrient uptake and ion toxicity (Lauchli and Grattan, 2012). The addition of biosolids also increased the levels of organic matter. According to Epstein (2003) this is most important in sandy soils as it increases the water holding capacity, soil aggregation and cation exchange capacity that is a very important property for supplying plant nutrient. Therefore, the use of biosolids was extremely important for plant growth in both substrates but specially for KK .

In KK, total root biomass was different between control and homogeneous and between control and heterogeneous but similar between homogeneous and heterogeneous treatments. In EW the results were different, with the total root biomass similar between control, homogeneous and heterogeneous applications. By having similar total biomass in the roots but an increase in the total biomass in the shoots the hypothesis that root foraging is happening is validated. This indicates that the same root biomass could promote a bigger shoot growth. An increase in nutrient uptake occurred in both treatments as a result of an optimal biomass allocation increasing the plant nutrition and growth.

The rhizobox trial, considering they contained the same substrate as KK pots, presented different results to the pot trial. Differences in shoot biomass were observed between all the treatments with heterogeneous treatment having the highest values (see figure 17). In the KK pot trial, homogeneous and heterogeneous treatments had similar results for both total root and total shoot. This shows that in this experiment manuka did not consistently show a statistically significant preference between top or incorporated applications. Studies have shown that different species respond differently to biosolids application. Castillo et.al (2011) found that incorporation of biosolids increased elephantgrass dry matter yield and nutrient removal compared to surface application and allowed biosolids to replace a greater proportion of inorganic N fertilizer. However, another study said that surface applied biosolids increased shoot biomass of two perennial grasses which was partly a result of the increased soil nitrate-N concentrations that followed biosolids application (Mata-González et.al, 2001).

However, all the results point for a positive effect of biosolids addition by bringing pH to a plant growth range and increasing nutrient availability leading the plants to develop root foraging behavior. In a pattern where roots do not forage, a bigger increase for homogeneous in comparison to heterogeneous would occur because most of the roots in the heterogeneous treatment would be in the unamended soil.

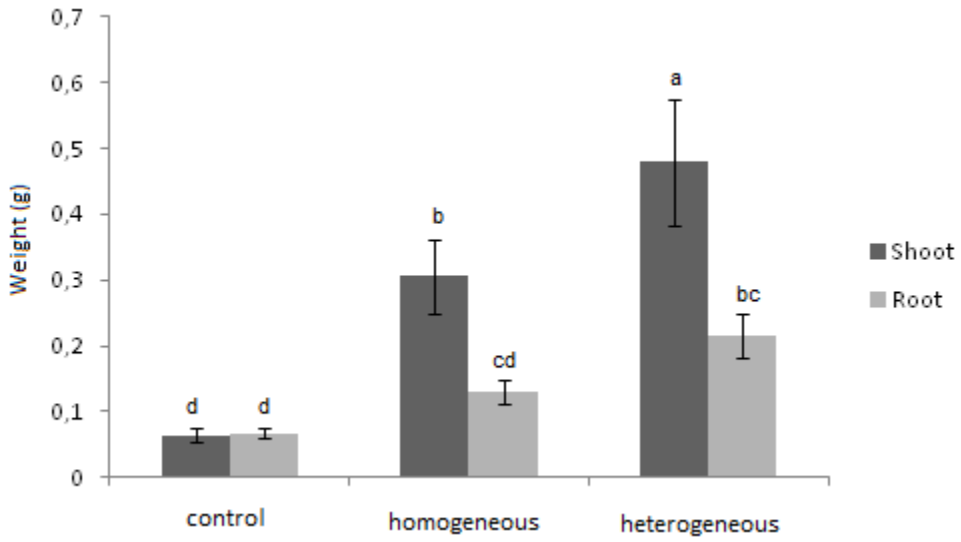


Figure 17: Root and shoot biomass in all the treatments used in the rhizobox trial. Bars represent the standard error of the mean. Values with the same letter are not significantly different.

5.3 Uptake of nutrients and contaminants

Table 5: Concentration of elements in plant leaves in each treatment for the Kaikoura sand (KK) and Eyrewell soil (EW) in the pot trial. Values are means, in brackets are standard errors, n=4, KK control n=3.

	KK control	KK homogeneous	KK heterogeneous	EW control	EW homogeneous	EW heterogeneous
N (%)	1.2(0.2)^{c}	2.6 (0.2)^{a}	2.6 (0.2)^{a}	1.6(0.1)^{c}	2.0 (0.1)^{b}	2 (0.06)^{b}
C (%)	46.3 (0.1)^{b}	47.7(0.4)^{a}	48.5(10.2)^{a}	48.4 (0.2)^{a}	48.3 (0.5)^{a}	48.6 (0.07)^{a}
C:N	39.4 (7.2)^{a}	18.5 (1.1)^{c}	19 (1.4)^{c}	31 (2.0)^{b}	24 (1.4)^{c}	23 (0.8)^{c}
P (mg/Kg)	1459 (40.1)^{c}	1958 (326)^{ab}	2104 (119)^{a}	887 (58)^{d}	1505 (134)^{bc}	945 (122)^{d}
K (mg/Kg)	13385 (447)^{a}	8844 (1032)^{b}	7756 (209)^{b}	8218(216)^{b}	8520 (228)^{b}	7888 (144)^{b}
S (mg/Kg)	2245 (169)^{b}	3934 (854)^{a}	3578 (473)^{a}	1306(162)^{b}	1979(88)^{b}	1884 (93)^{b}
Mg(mg/Kg)	5387(162)^{a}	2142(137)^{b}	2028(45)^{bc}	1852(51)^{bc}	1927(110)^{bc}	1736 (151)^{c}
Ca(mg/Kg)	25727 (1254)^{a}	12324(764)^{b}	13721(731)^{b}	5881(154)^{d}	8431(332)^{c}	7702 (581)^{cd}
Zn (mg/Kg)	41 (0.9)^{d}	133 (12.0)^{b}	208 (8.0)^{a}	20 (1.2)^{d}	153 (13.5)^{b}	105 (11.6)^{c}
Cu (mg/Kg)	10 (0.7)^{a}	10 (1.9)^{a}	5.6(0.5)^{b}	3.8 (0.2)^{c}	6 (0.4)^{bc}	4.4 (0.5)^{bc}
Cd (mg/Kg)	0.01(0)^{c}	0.3(0.1)^{ab}	0.2 (0.04)^{b}	0.06(0.03)^{bc}	0.6 (0.1)^{a}	0.2 (0.04)^{b}
Ni (mg/Kg)	1.8 (0.15)^{a}	0.3 (0.05)^{cd}	0.9 (0.07)^{b}	0.05(0.06)^{d}	0.5 (0.1)^{c}	0.3 (0.1)^{cd}

Table 5 shows that biosolids applications increased leaf N and S concentrations. N increased over 100% in the KK substrate and by 20% in EW. S increased over 60% in KK but in EW it was not

significantly higher. However, the calculation for the extracted masses of N and S increased in the homogeneous and heterogeneous treatments in both trials (table 6).

The C:N ratio had a significant decrease with biosolids applications with no differences between homogeneous and heterogeneous treatments in either trials. The smaller ratio is a consequence of higher levels of available N in the litter (McLaren and Cameron, 1996).

The similarities in N and S mass extracted between the treatments where biosolids were applied could be chemical evidence that the roots are foraging for N and S not only morphologically (as was explained in previous section) but also physiologically by increasing the nutrient uptake per unit of root surface. (Hutchings and de Kroon, 1994). Figure 13 shows that an increase of roots occurred in the biosolids patch in the heterogeneous treatments, however, they contain approximately 50% of the roots in un-amended patch. In contrast, the homogeneous treatments have 100% of roots growing in contact with biosolids, albeit at a lower concentration. Since both treatments in both trials sequestered similar amounts of N and S, there may be increased N and S sorption by roots in the patch of biosolids (physiological foraging). Many species use a combination of morphological and physiological techniques to acquire nutrients with the balance of importance between these techniques depending on the type of nutrient availability in the habitat occupied (Hodge, 2004; Hutchings and de Kroon, 1994).

The biosolids contained elevated P concentrations (5658 mg/kg). This resulted in significantly higher P concentrations in KK treatments. However, EW has control and heterogeneous with similar values. Unlike the N and S concentrations, the P concentrations were significantly higher in the homogeneous treatment of the Eyrewell soil.

K, Mg and Ca followed similar trends in KK, decreasing with biosolids applications. In EW, K and Mg concentrations did not change between treatments and Ca increased in the homogeneous treatment.

Table 6 shows that all the macronutrients tested in this experiment increased their mass extracted by the plant with biosolids applications.

Table 6: Macronutrients extracted by plants (leaf biomass) in the pot trial. Values are means (mg) n=4, KK control n=3.

	KK control	KK homogeneous	KK heterogeneous	EW control	EW homogeneous	EW heterogeneous
N	2(0.1)^{c}	53(2.6)^{a}	64(1)^{a}	32(2.7)^{b}	68(3.4)^{a}	67(1)^{a}
P	0.1(0.003)^{d}	4(0.5)^{ab}	5(0.7)^{a}	2(0.1)^{c}	5(0.3)^{a}	3(0.7)^{bc}
k	1(0.4)^{c}	17(1.5)^{b}	19(1.4)^{b}	17(0.6)^{b}	28(3)^{a}	25(3.5)^{a}
S	0.1(0.01)^{b}	8(1.6)^{a}	9(2.1)^{a}	3(0.7)^{b}	6.5(1)^{a}	6(0.8)^{a}
Mg	0.4(0.01)^{d}	4(0.1)^{bc}	5(0.5)^{abc}	3(0.1)^{c}	6(0.9)^{a}	5(1.2)^{ab}
Ca	2(0.1)^{d}	25(0.7)^{b}	34(2.8)^{a}	12(0.6)^{c}	28(3.3)^{ab}	24(3.5)^{b}

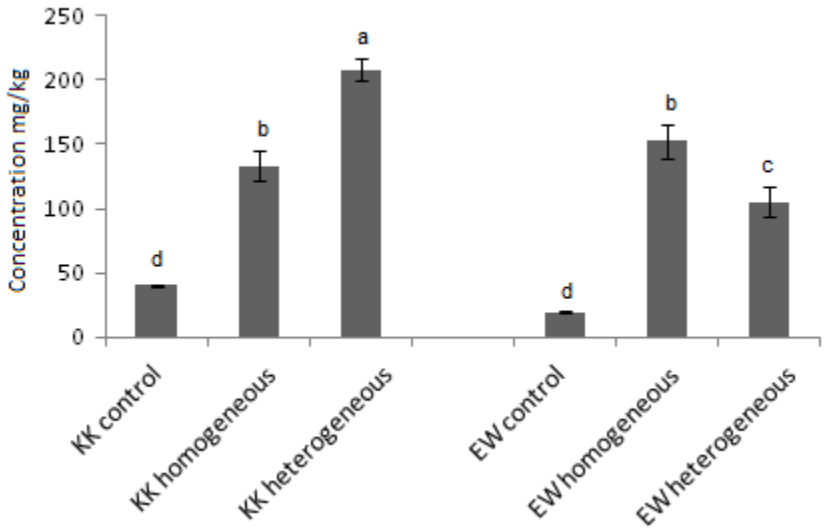


Figure 18: Zn concentration in the leaf biomass in all the treatments for Kaikoura sand (KK) and Eyrewell soil (EW) in the pot trial. Bars represent the standard error of the mean. Values with the same letter are not significantly different.

Figure 18 shows that there were significant higher concentrations of Zn in the biosolid treatments compared to the control. The heterogeneous treatment of the KK took up more Zn than the homogeneous treatment; however, this was reversed in EW. The levels of zinc found in the biosolids used in these experiments (1239 mg/kg) is in accordance with other studies that show that Zn is one of the most abundant heavy metals in sewage sludge (Mosquera-Losada et al., 2010a; Knowles et al., 2011). That is >70% more than the limit for remediation intervention in soils (Provoost et al., 2006). However, the increased Zn by the plant is unlikely to cause toxicity, either to the plant (Domínguez et al., 2008) or to animals (Bester et.al, 2013).

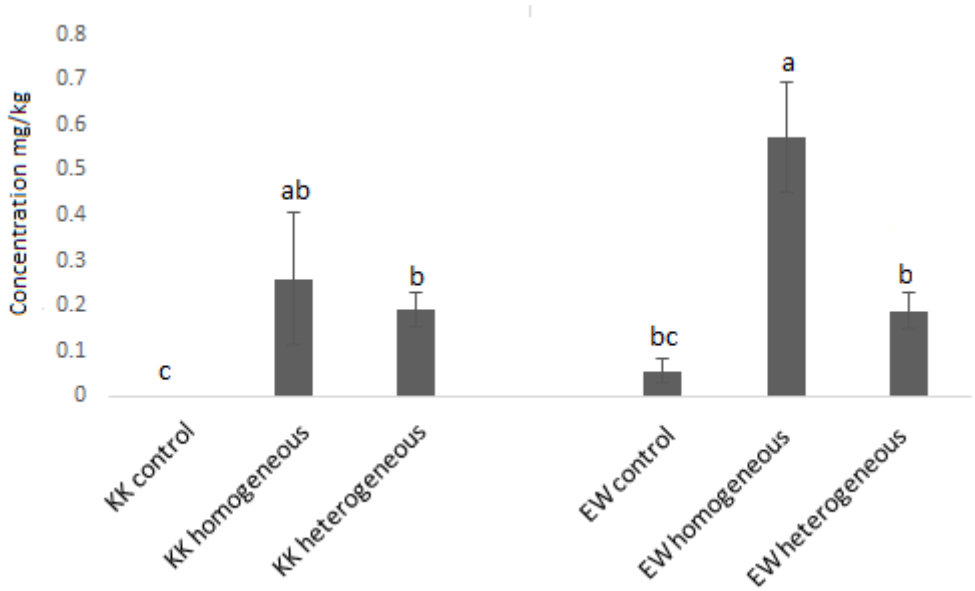


Figure 19: Cd concentration in the leaf biomass in all the treatments for Kaikoura sand (KK) and Eyrewell soil (EW) in the pot trial. Bars represent the standard error of the mean. Values with the same letter are not significantly different.

In both soil substrates the homogeneous treatments had significantly elevated Cd compared to the control. Heterogeneous treatment was similar to control in EW but not in KK. Cd concentrations in biosolids did not exceed standards warning levels for contaminants (Provoost et al., 2006). In KK, the Cd concentrations were within the range found in different vegetables (Bester et al., 2013) and are unlikely to pose a risk to plant health (Domínguez et al., 2008) or animals (Madejon et al., 2006). However, in EW the homogeneous treatment showed mean plant uptake values of 0.57 mg/kg, going above the tolerate level for animals of 0.5 mg/kg (dry matter) (Madejon et al., 2006). Therefore, biosolids applications where roots have less direct contact with biosolids could result in lower plant Cd-uptake.

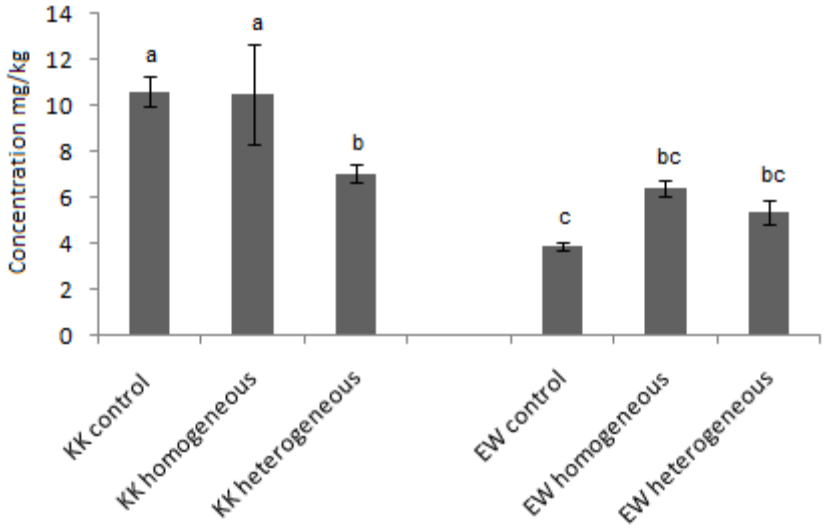


Figure 20: Cu concentration in the leaf biomass in all the treatments for Kaikoura sand (KK) and Eyrewell soil (EW) in the pot trial. Bars represent the standard error of the mean. Values with the same letter are not significantly different.

Cu concentration in the biosolids was high, with values >300% above the soil remediation intervention (Provoost et al., 2006). Surprisingly, Cu leaf concentrations were decreased by the biosolids treatments in KK but was increased in EW. However, the mass of Cu extracted by the plants in both substrates was significantly higher in the biosolids treatments.

Manuka did not show any preference in the type of application, increasing its biomass and nutrient up-take in either homogeneous or heterogeneous biosolids applications. An increase in root allocation in the area of higher nutrient content also occurred, however the analysis of the data does not indicate whether the plant was foraging a specific element or not.

The fact that a plasticity of biomass allocation occurs as a response of nutrient enrichment should be considered when managing biosolids applications, as well as that levels of proliferation varied according to soil type. Surface applications in some areas may cause the plants to collapse if strong winds occur as a high percentage of roots are in the surface instead of properly functioning as plant anchorage (Roychoudhry and Kepinski, 2015). This type of application can also facilitate pathogenic contamination as a consequence of direct contact of biosolids with animals and humans as well as the smell in the area after application (NZWWA, 2003). Therefore, incorporation of biosolids in the soil prior to planting can be a good option.

However, the incorporation process can cause a lot of disturbance and may not be possible where large plants or trees already exist. In manuka forests, surface application of biosolids can be positive in replenishing Zn and Cu that can be deficient in New Zealand soils (Will, 1990).Even though

biosolids contained high levels of these and other metals, manuka did not show to up-take then in in potentialy harmfull levels. These metals are less mobile in the soil (Bolan et al., 2014), however, the N that is also present in biosolids can easily percolate and reach growndwater causing environmental harm (McLaren and Cameron, 1996). Surface applications can be beneficial, as the metals will have less direct contact with the roots whereas the N will percolate through the whole soil profiles creating more chances for plant up-take and thus prevent excessive leaching.

Chapter 6

Conclusion

As a pioneering species adapted to low fertility soils, it was unclear whether manuka (*Leptospermum scoparion*) would positively respond to biosolids addition. This study showed that biosolids application promoted a positive growth result in manuka by increasing total root and total shoot biomass in both pot and rhizobox trials. No difference in treatment was observed, with homogeneous and heterogeneous application showing similar growth, therefore manuka did not demonstrate preference between the two types of application.

The increase in root and shoot biomass in both treatments were higher in the lower fertility soil showing that soil type has an influence in the effect of biosolids in the plant.

A proliferation response was observed in the roots of manuka plants following biosolids application either homogeneously or heterogeneously. However it was not clear if the plants were foraging a specific element.

The nutrient status of the plants also improved after the addition of biosolids. The type of soil had an influence on the uptake of elements with the Kaikoura sand showing an overall higher uptake. However, all macronutrients showed an increase in their masses extracted by the plant after application in both soil substrates. Generally, the uptake of the metals analyzed increased in both soils and both treatments but not to a level of toxicity for animals or plants.

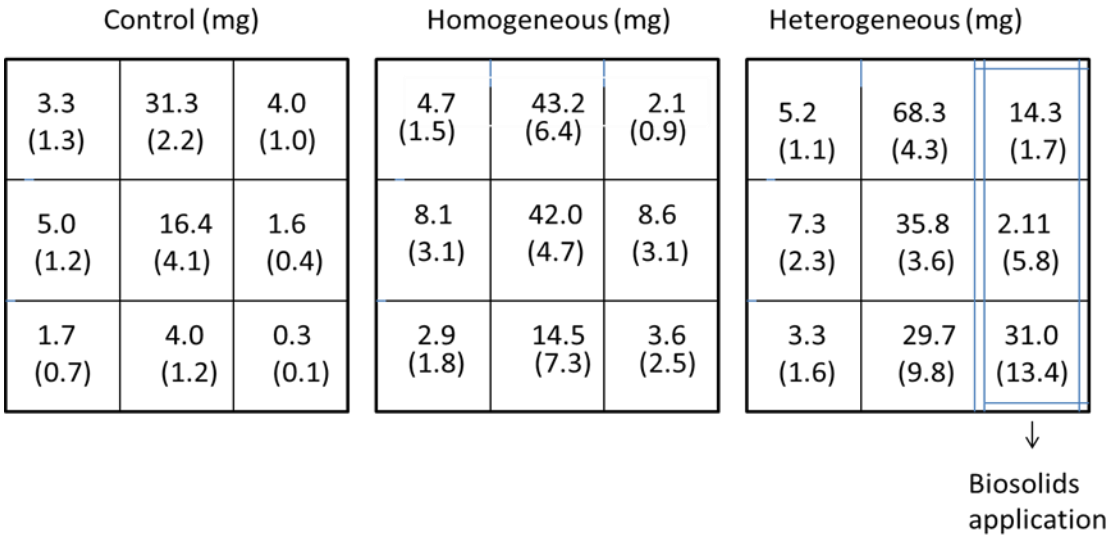
Biosolids application in low fertile areas can be beneficial to improve soil nutrient status for plant growth as long as conditions that represent little risk for soil, living organisms and water contamination are respected. Applications should be managed according to the type of soil as well as climatic conditions for an optimal response.

For a deeper understanding of how the plants interact with biosolids future research should investigate the performance of manuka over a longer timescale and include treatments where biosolids are applied to the soil surface of existing manuka stands. For a deeper understanding of the root behaviour, root morphology should also be investigated.

Appendix A

Root biomass from each quadrat

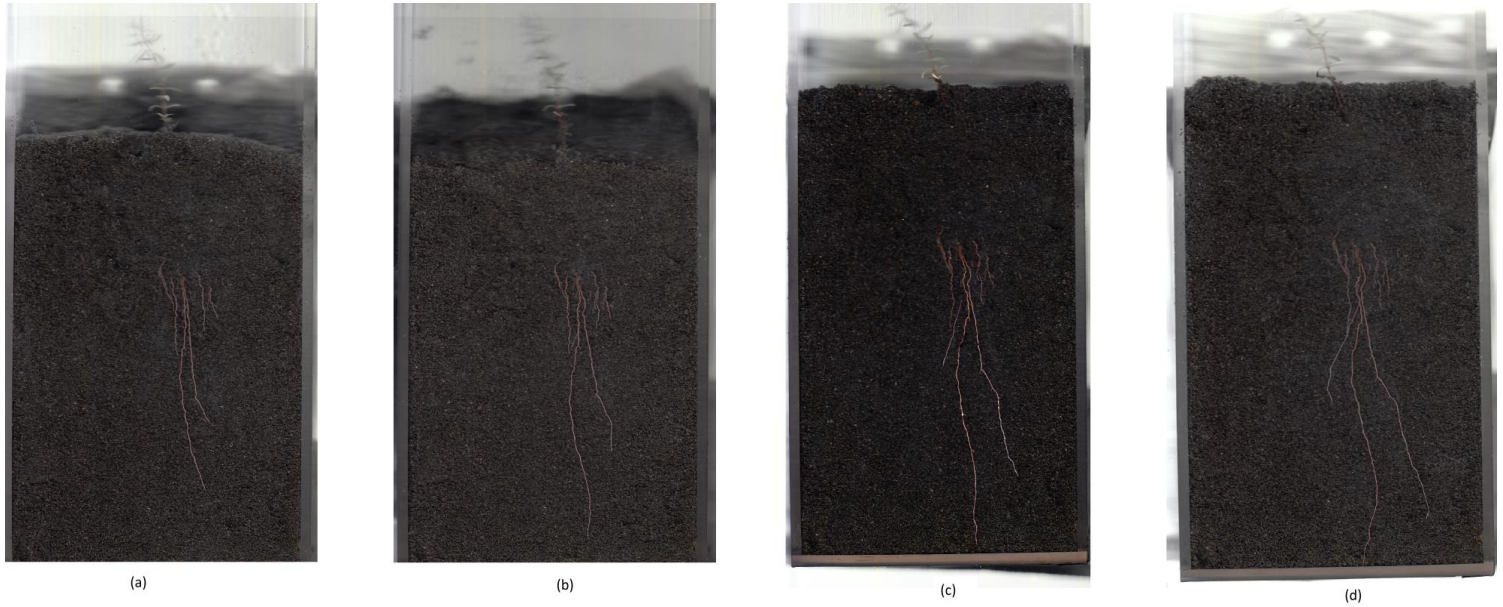
The diagram below shows the mean root biomass in each quadrat of the rhizobox treatments. The blue line in the heterogeneous treatments show where biosolid was applied. Values in brakets are standard errors. n=4.



Appendix B

Images of the root distribution in the rhizobox

B.1 Control treatment



Sequence scan images of the same replicate of the control treatment, rhizobox trial. Images show one month of root growth. Scans were taken once a week starting on image (a).

B.2 Homogeneous treatment

Sequence scan images of the same replicate of the homogeneous treatment, rhizobox trial. Images show one month of root growth. Scans were taken once a week starting on image (a).

B.3 Heterogeneous treatment

Sequence scan images of the same replicate of the heterogeneous treatment, rhizobox trial. Images show one month of root growth. Scans were taken once a week starting on image (a).

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