Biowastes to Establish Plants for Essential Oil Production on Low Fertility Soils

A Dissertation

submitted in partial fulfilment

of the requirements for the Degree of

Doctor of Philosophy

at

Lincoln University

by

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Lincoln University

2019

Abstract of a Dissertation submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy

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Biowastes are unwanted materials of biological origin. They include biosolids (from sewage works), Dairy Shed Effluent (DSE), sawdust and Treated Municipal Wastewater (TMW). When applied to soil, biowastes can provide plant nutrients, but also introduce heavy metals, pathogens, or xenobiotics. Biowastes could improve degraded or low-fertility soils and generate revenue through the production of non-food products such as essential oils (EOs). I grew NZ native plants, mānuka (*Leptospermum scoparium* J.R. Forst & G. Forst) and kānuka (*Kunzea robusta* de Lange & Toelken) as well as Lavender (*Lavandula angustifolia* Mill.), Rosemary (*Rosmarinus officinalis* L.) and Thyme (*Thymus vulgaris* L.) in series of greenhouse experiments in medium-to-low fertility soils. Soils used in the experiments were Bideford clay loam (BCL), Lismore stony silt loam (LSL), Pawson silt loam (PSL) and Craigieburn silt loam (CSL), ordered from highest to lowest fertility, that were amended with either biosolids (up to 13500 kg N ha^{-1} equiv.), biosolids + sawdust (1:0.5, 1250 kg N ha^{-1} equiv.) and DSE (200 kg N ha^{-1} equiv.). Two types of biosolids from Kaikoura (KB) and Christchurch City Council (CB) were used in the experiments. Moreover, biosolids were applied in different methods including incorporation to the soil, surface application and using patches of the biosolids in soil. I had a field experiment to evaluate the effect of TMW (30 kg N ha^{-1} equiv.) on the EO yield and compositions of *L. scoparium* and *K. robusta*. Field surveys were used to evaluate the EO concentration and composition in natural populations of *L. scoparium* and *K. robusta*.

In the greenhouse experiments the maximum biomass increase was related to CB application (3000 kg N ha^{-1} equiv.) that enhanced the biomass of *L. scoparium* by up to 29-fold in the lowest fertile soil (CSL). Generally, the optimum biosolids application rate was 1500 kg N ha^{-1} equiv. in the greenhouse experiments that increased the biomass of *L. scoparium, K. robusta, L. angustifolia* and *R. officinalis* by up to 120%, 170%, 86% and 70 % in PSL and LSL (low fertile soils), while DSE only increased the biomass of *L. scoparium* and *R. officinalis* and not the other plant species. Adding sawdust to KB increased the biomass of *L. scoparium* and *K. robusta* although it offset the *L. scoparium* growth increase by the KB-only treatment. Applying TMW increased the canopy volume of *L. scoparium* and *K. robusta* in the field experiment. Biowastes increased foliar concentrations of some macronutrients (e.g. N, P and S).

Generally, the concentration of TEs including Zn was increased by biosolids and no other biowastes application. Maximum CB application rate increased the Zn concentration of *L. scoparium* and *L. angustifolia* leaves by 4 and 3 times (from 24 to 102 and 38 to 115 mg kg^{-1} dry matter), respectively. Cadmium concentration increased by up to 11-fold and 31-fold (from 0.03 to 0.22 and 0.01 to 0.31 mg kg^{-1} dry matter) in the *L. scoparium* and *K. robusta* leaves when biosolids were applied in high rates. Concentration of the TEs in all treatments and all experiments stayed in the safe range of the food safety standards. Generally, the treatments had a negligible effect on oil concentration except for DSE (200 kg N ha^{-1} equiv.) and biosolids application at rates higher than 1500 kg N ha^{-1} equiv. that decreased the *R. officinalis and L. angustifolia* EO concentrations, respectively. This would offset the effect of biomass increase in terms of oil production. Contrasting methods of biosolids application had similar effects on the EO concentration of *L. scoparium.* Most of the essential oils' evaluated components were unaffected or slightly affected by biowaste addition. The incorporation of biosolids with soil had a greater effect on the EO composition compared to surface application. The field survey showed that the EO concentration of *L. scoparium* and *K. robusta* was significantly higher than the plants grown in the greenhouse. This could be the result of environmental conditions that would induce the EO production of the plants.

This study indicates that biowastes that are disposed into landfills or waterways may be beneficially used to restore native ecosystems on low-fertility or degraded soils to produce essential oils. Applying biosolids (up to 1500 kg N ha^{-1} equiv.) could establish native ecosystems dominated by *L. scoparium* and *K. robusta* that annually would produce up to 103 kg ha^{-1} and 98 kg ha^{-1} of essential oils worth NZ$38400 and NZ$34300, respectively. Further field trials are warranted to elucidate critical ecological variables and production economics in biowaste management. In particular, the effect of adding biowastes to established stands of *L. scoparium* and *K. robusta* should be determined.

Key words: biosolids; dairy shed effluent; degraded soil; essential oil; kānuka; mānuka

Note on the thesis structure and my contribution

This thesis comprises six greenhouse experiments, a field survey and a field trial. Some of these experiments were my sole responsibility, while other were conducted with fellow students and postdocs. In all cases, I analysed the essential oils, which required the adaptation of published analytical procedures for greenhouse and field sampled plants. Below I list my contribution to the individual experiments

Exp. 1 (2013-2014). I conducted the analyses of the essential oils from plant tissue from an experiment that was conducted before I arrived at Lincoln University.

Exp. 2 (2014-2015). I conducted the experiment with two fellow students. I was solely responsible for the analyses of the essential oils.

Exp. 3 (2015-2016). I was solely responsible for the whole experiment.

Exp. 4 (2015-2016). I was solely responsible for the whole experiment.

Exp. 5 (2015-2016). I conducted the experiment with a postdoctoral fellow. I was solely responsible for the analyses of the essential oils.

Exp. 6 (2015-2016). I conducted the experiment with a postdoctoral fellow. I was solely responsible for the analyses of the essential oils.

Exp. 7 (2015 – ongoing as of 2018). The field trial was set up by a team from Lincoln University and the Christchurch City Council. I collected soil samples, and plant samples from the field trial. I helped with the chemical analyses of the soils and plants. I was solely responsible for the analyses of the essential oils.

Exp. 8. Field survey (2014-2015). I was solely responsible for the whole experiment.

Exp. 1 - 7 investigated the effect of biowastes on the biomass, elemental composition, as well as the concentration and composition of essential oils. *Leptospermum scoparium* was grown in all experiments with other species (*Kunzea robusta*, *Lavandula angustifolia*, *Rosmarinus officinalis,* and *Thymus vulgaris*) occurring in some experiments. Exp. 8 investigated the elemental composition, essential oil concentration and composition of natural populations of *L. scoparium* and *K. robusta*. Given the commonalities between the experiments, it was useful to consider the results together, so that the effect of biowastes added under contrasting conditions could be compared. This structure also avoids redundancy that would occur if each experiment had its own introduction and methods section.

Acknowledgements

First, I would like to thank my main supervisor Professor Brett Robinson, who conducted the whole thesis and encouraged me continuously with patience and high understanding of studying while being a mother and supporting me in the difficulties.

I would like to thank my supervisory team: Professor Nicholas Dickinson, associate professor Rainer Hofmann, Dr. Juergen Esperschuetz, Dr. Jacqui Horswell and Jason Breitmeyer. Together, these supervisors gave me a great support and motivation with their experience, knowledge and helping me in the mountains to take soil and plant samples.

As a team, we would like to thank Centre for Integrated Biowaste Research (CIBR) for sponsoring this project, which will affect the native restoration and essential oil export of NZ. Particularly we would like to acknowledge the support from Dr. Jacqui Horswell.

I would like to thank Dr. Juergen Esperschuetz and Dr. Maria Gutierrez-Gines for giving me the opportunity of working beside their experiments for essential oil extraction.

A special thanks to Christine Roberts for supporting me by offering NZ International Doctoral Scholarship (NZIDRS) during my PhD journey and her high understanding of difficult situations.

I would like to thank Sue Bowie, Tania McBeth, Eirian Jones and Robyn Wilson for their great support to receive NZIDRS, Heaton Rhodes and LU writing Scholarships and for their nice and friendly behaviour (always with a smile).

I would like to thank Myra Duthie, Lyn Barclay and Bernadette Mani for their great help and kind support during my studies at Lincoln University.

I thank Janet Bertram, Roger Cresswell, Lynne Clucas, Roger McLenaghen, Leanne Hassall, Dr. Maria Gutierrez-Gines, Dharini Paramashiva, Qian Liang, Vicky Zhang, Joy Jiao and Neil Smith for their guidance, information, help in the laboratory and maintaining the experiment and helping me kindly.

A special thanks to Dr. Jackie Blunt, Fiona Watson, Doreen Lassen and Robbyn Bateman from the LU Health Centre for all their great support (and kind face) during the periods of being unwell.

I would like to thank Brent Richards and Leona Meachin who made the glasshouse work possible with their experience, knowledge and patience.

There has been a group of students who helped me behind the scenes either emotionally or by their knowledge or else helping at the greenhouse. Thanks to Dharini Paramshivam, Obed Lense, Minakshi Mishra, Ardalan Ashrafzadeh, Roshean Woods, Anna Carlton, Shamim Mamun, Dharshika Welikala, Camilla Gardiner, Michael Yi and Carmen Medinacarmona.

Thanks are extended to Nicky Robb at the Motukarara DOC nursery who helped me regarding the NZ Native plants, *L. scoparium* and *K. robusta* with her amazing knowledge and experience.

I would like to have a very special thanks to Christine Robinson, a super elite mum, because of sharing her similar experiences of being a PhD student and a new mum besides taking care of my little boy that made the hard days easier for me to tolerate and giving the courage to continue.

I would like to thank Leanne Hassal, Amal Turkey and Eirian Jones because of their nice and soft cuddles and listening to my happy and sad stories that made the feeling of being far from my mother easier to stand.

I also would like to thank Anu Sharma, Angelica Hofmann, Carol Smith and Victoria Flynn, the group of ladies (mothers), who listened to my motherly concerns, sharing their similar experiences, calm me down and giving me positive energy to continue.

Thanks to the kind manager and teachers at the Lincoln University Early Childhood Centre (LUECC) for a high-quality care of my little boy and updating me with his photos regularly.

Finally, thanks to my great friends and family who kept me up during the PhD process. A special thanks to my lovely husband, Reza, and my little boy, Nikrye (my reason to be) for putting up my absence and having less time to pass with besides packing the lunchbox for the day-care when I was not able to! Thanks to my wonderful family my mum, dad, my lovely sisters plus my cute niece who talking to them has always been a great support even from the long distance. And thanks to my lovely neighbours Deanne and Rachel Hewitt who supported me a lot during pregnancy and offering me babysitting that was a great help and support and gave me some free time to spend for myself. I look forward to playing more with my little one and spending more fun times I have missed.

Table of Contents

List of Tables

List of Figures

Acronyms and Abbreviations

EO= Essential Oil

TE = trace element

NZ = NZ

CEC = Cation exchange capacity

SM = Secondary metabolites

NO_3^--N= Nitrate nitrogen

NH_4^+-N = Ammonium nitrogen

Biowastes

TMW = treated municipal wastewater

CB = Christchurch City Council Biosolids

KB = Kaikoura Biosolids

Soils

LSL = Lismore stony silt loam

PSL = Pawson silt loam

BCL = Bideford clay loam

CSL = Craigiburn silt loam

Elements

N= Nitrogen

P= Phosphorous

K= Potassium

S= Sulphur

Ca= Calcium

Mg= Magnesium

Mn= Manganese

Zn= Zinc

Cu= Copper

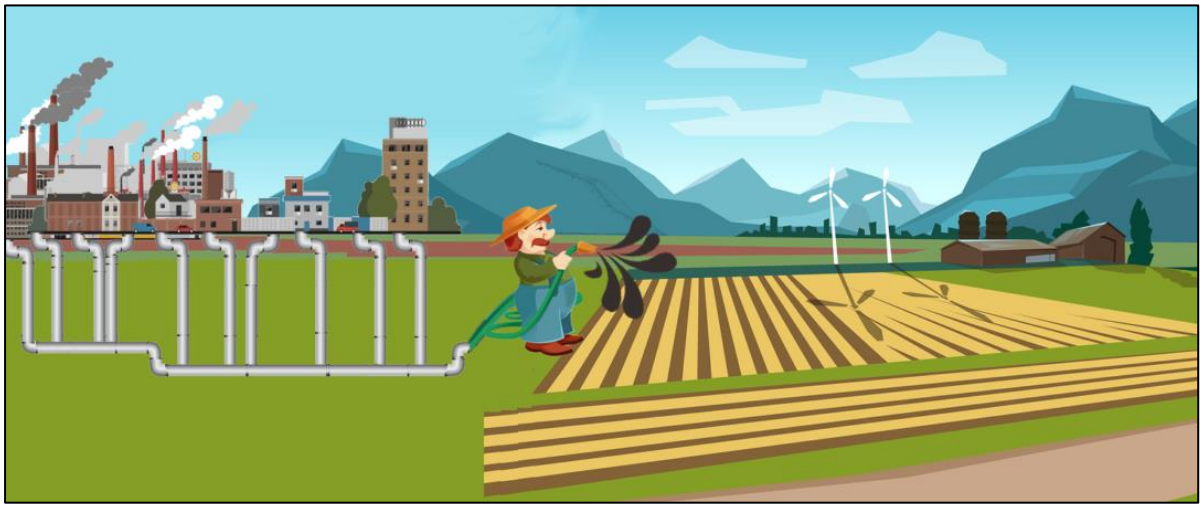
Cd= Cadmium

SECTION I:

Introduction and Background

Chapter 1

Introduction



1.1 General introduction

Biowastes comprise unwanted material of biological origin. Common biowastes include the products of sewage treatment (Sanchez et al., 2009, Guo et al., 2014), animal effluents (Colleran, 2000) as well as crop and silvicultural residues (Kim et al., 2015). Disposal of biowastes can be expensive, such as disposal to landfill (Güereca et al., 2006), or environmentally damaging e.g., disposal in waterways or incineration (McLaughlin and Filmer, 2008). Potentially, biowastes could be beneficially applied to land to improve soil fertility (Esperschuetz et al., 2016b). However, injudicious application can result in environmental degradation and increased risks to human health (Pritchard et al., 2010).

Countries with sewage treatment facilities produce, on average, 52 kg yr^{-1} of biosolids per person, resulting in the global output of >10 Mt yr^{-1} (Bradley, 2008). NZ's production of biosolids is ca. 342,000 t (fresh weight) yr^{-1}, of which ca. 68% goes to landfill (CIBR, 2014). Biosolids contain high concentrations of essential plant nutrients and organic matter, both of which can improve soil fertility (Obi and Ebo, 1995). However, biosolids can also contain pathogens and contaminants (Krogmann et al., 1999, Singh and Agrawal, 2008), which is why they are not typically applied to NZ's high-value soils. Nonetheless, improved treatment technology has resulted in a reduced concentration of TEs and organic contaminants (Chaney, 1990). In 2008 the cost of disposing of biosolids in landfills (excluding transport costs) in NZ, was ca. $33 M yr^{-1} (WCC, 2008). Such costs may be avoided by using biosolids to rebuild soils that have become degraded due to forestry, mining and intensive cropping (Daniels et al., 2003, Novak et al., 2009). Additionally, biosolids can be applied to soils contaminated with Trace Elements

(TEs) to reduce their bioavailability to plants and soil biota (Black et al., 2010). In NZ, biosolids applied to the Stockton mine site resulted in improved foliage growth and reduced erosion and drainage (Christchurch City Council, 2014). Some 60% of the annual biosolids produced in the United States is land-applied (Suppan, 2013). Similarly, other countries including Australia, Canada, China, Czech Republic, England, Hong Kong, Japan, Italy, Korea and Spain apply biosolids to restore degraded lands (Navas et al., 1999, Lu et al., 2012, He, 2015, Kim and Owens, 2010, LeBlanc et al., 2009).

In agricultural countries, animal effluents can cause environmental harm (BPDNZ, 2011) such as the degradation of water quality and increasing greenhouse gas emissions (Baskaran et al., 2009). For example, Dairy Shed Effluent (DSE) which comprises bovine urine and faeces collected during the milking of dairy cattle (Zaman et al., 2002), can contaminate ground and surface waters (Houlbrooke et al., 2004). Between 1994 and 2017, the number of dairy cattle in NZ increased by 69%, from 3.84 to 6.47 million (StatsNZ, 2018a). DSE contains elevated concentrations of N, K, P, and S, as well as pathogens and xenobiotics (Roach et al., 2001). Land-application of DSE reduces the need for chemical fertilizers (Cameron et al., 1995, Bolan et al., 2004). DSE is rich in organic matter and can improve the water holding capacity, aeration, and drainage of soil as well as make the soils less vulnerable to compaction and loss through erosion. (Rahmani and Tabaei-Aghdaei, 2014). However, DSE-borne pathogens can cause human illness (Jiang, 2008). As with any N-containing fertilizer, over-application of DSE results in excessive leaching of nitrates into receiving waters.

NZ produces ca. 900,000 t yr^{-1} of silvicultural residues, including sawdust (FFNZ, 2007), which is often disposed of in piles (Robinson et al., 2007). These residues often occur on or near harvested pine forests, where the soil has become degraded due to the logging (timber harvesting) operation (Paramashivam et al., 2017). These soils are low in both organic matter and concentrations of plant nutrients (Chirino et al., 2010). In 2001, the disposal of logging residues costs ca.NZ$42 t^{-1}, including storage, transportation, and processing (Hall et al., 2001). Using sawdust on-site could significantly reduce the delivery costs.

Potentially, sawdust could be mixed with biosolids as they reduce plant uptake of contaminants from biosolids-amended soils (Esperschuetz et al., 2017). Sawdust has been demonstrated to reduce nitrate leaching from biosolids (Paramashivam et al., 2016). Therefore, mixing biowastes such as biosolids and sawdust may lead to improved environmental and economic outcomes (Paramashivam et al., 2017) when they are applied to degraded lands.

Treated Municipal Wastewater (TMW) comes from households, small companies and occasionally storm water run-off (UNEP, 2015), which would be discharged into the waterways (Kolpin et al., 2002). TMW contains high concentrations of plant essential nutrients (Gupta et al., 1998), although even advanced treatment systems are not able to eliminate all pollutants and chemicals from this material (EC, 2007). Continuous soil irrigation with TMW improves soils nutrient levels (Ramirez-Fuentes et al., 2002, Rattan et al., 2005). TMW could be used in industry, agriculture, artificial recharge of aquifers, rehabilitation of natural ecosystems and to irrigate forests (Palaniappan et al., 2010) or degraded lands.

Gibbs and Salmon (2015) estimated that there are some six million ha of degraded lands worldwide. These lands could be improved physically, chemically and biologically (Rahmani and Tabaei-Aghdaei, 2014, Zebarth et al., 1999, Singh and Agrawal, 2008) by using the advantages of biowastes for cultivation. Currently, some 5% of NZ is covered by *Pinus radiata* (1.3 million ha) (NZGEO, 2018) that would be degraded after harvest (Turner and Lambert, 1988). There is less incentive to replant areas in pine due to reduced prices for timber (MPI, 2018).

Potentially, biowastes could be used on low-fertility lands to produce non-food crops such as essential oils (EOs). This limits human exposure to pathogens and other biowaste-borne contaminants (McLaughlin et al., 2007). EO production may enable profit generation from degraded land and divert biowastes from expensive or environmental-damaging disposal. There is international interest in NZ mānuka (*Leptospermum scoparium* J.R. Forst & G. Forst) EO due to its high β-triketone levels (Douglas et al., 2004). Some 80% of NZ-grown *L. scoparium* EO is exported (G Porter, 2003b). The EO's β-triketone significantly contributes to antimicrobial properties in *L. scoparium* products (Maddocks-Jennings et al., 2005a, Lis-Balchin et al., 2000), and is mainly found in *L. scoparium* from the East Cape region (G Porter, 2003a). *L. scoparium* EO currently sells for NZ$930 kg^{-1} (LO, 2018a). Likewise, kānuka (*Kunzea robusta* de Lange & Toelken) EO contains high levels of α-pinene (>50%) (Porter and Wilkins, 1999). This component has an inhibitory effect on the growth of potential infectious endocarditis causing gram-positive bacteria (Leite et al., 2007, Lis-Balchin et al., 2000) and is sold for NZ$876 kg^{-1} (LO, 2018b). The increasing number of studies on the health benefits and medical uses of the EOs may result in the development of a *L. scoparium* and *K. robusta* EO industry in a similar way to the *L. scoparium* honey industry (Sunders, 2017).

L. scoparium and *K.robusta* form pioneer to mid-successional plant communities prior to the longer-term establishment of podocarps and other large indigenous tree-dominated forests. *L. scoparium*- and *K.robusta*- dominated vegetation support a large assemblage of native floral and faunal biodiversity.

They are resilient to fire that is a significant disturbance to vegetation, particularly since Polynesian settlement of New Zealand. They also have an important role in landscape recovery following harvest of exotic forests (Sunders, 2017, Reid et al., 2009).

Lavender (*Lavandula angustifolia* Mill.) EO is widely used because of its antibacterial and anti-inflammatory properties (Hajiali et al., 2016). This EO is sold for NZ$187 kg^{-1} (EOD, 2018). It is believed that rosemary (*Rosmarinus officinalis* L.) EO improves memory (Moss et al., 2003). The EO of this plant is sold for NZ$226 kg^{-1} (IM, 2018). Thyme (*Thymus vulgaris* L.) EO also has antibacterial activity (Dorman and Deans, 2000) that is widely used.

In 2014, producing natural products such as EOs was highlighted a 'key sector' by NZ Trade and Enterprise (NZTE, 2014). Besides EOs medicinal advantages they could also be used in the perfume industry. The global perfume sales revenue in 2017 was US$37.4 billion, which had a compound annual growth rate increase of 3.7% from 2010 (R&M, 2018). Perfume is made from 2 to 30% of EOs (Pybus and Sell, 2007).

Using biowastes to re-establish plants on low-fertility lands would improve the aesthetic and ecological value of the NZ landscape, which is consistent with the 'clean green' image that NZ wishes to portray in overseas markets (Ateljevic and Doorne, 2002, Bell, 1996). NZ is introduced as "100% Pure NZ" in the country's Tourism website (TourismNZ, 2018). This shows the importance of beautiful image of clean water and plants. Both native vegetation that are new for the tourists and the familiar plants, which are grown worldwide could help illustrating this image. Tourism plays an important role in the NZ economy by creating goods and services also increasing the chance of employment. In 2017, tourism expenditure increased by 1.9% compared to the previous year, resulted in NZ$36 billion. In this year, domestic tourism outcome increased by 4% (NZ$820 million) to NZ$21.4 billion. In 2017, visitors from overseas to NZ increased by 8.9% and tourists made NZ$3.3 billion in goods and services tax (GST). Moreover, around 8.4% of the country's jobs is from tourism (StatsNZ, 2018d).

1.2 Current knowledge on the effect of biowastes on essential oil production and quality

EOs increase plant survival by attracting pollinators or inhibiting herbivores and plant pathogens (Abdelmajeed et al., 2013). The concentrations of EO depend on both the environment and the plant genotype. Plant EO yields are affected by salinity, soil-fertility, soil moisture and contaminants such as TEs (Abdelmajeed et al., 2013). EOs improve plant survival in adverse conditions and some plants react

to environmental stress by increasing production of EOs (Stevović et al., 2011). The application of nutrient-rich biowaste may, therefore, detrimentally affect EO production because they generally increase soil fertility, diminish the stress on and increase the growth of plants. However, the elevated concentrations of TEs in biosolids may increase environmental stress and correspondingly stimulate the production of EOs.

There are fewer than ten studies investigating the use of biowastes for EO production. Some members of the Lamiaceae family (*Rosmarinus sp.* and *Mentha piperita*) have been investigated by using biowaste applications onto agricultural soils. There are no studies investigating the use of biowastes to rebuild degraded soils for EO production. Nor is there any information on using biowastes to augment EO production of *L. scoparium* or *K. robusta*. Hypotheses on the likely effects of biowastes on EO production can be developed based on studies of the effects of nutrients and contaminants on the yield and quality of EO-producing plants.

The development and EO production of aromatic plants may be affected both positively and deleteriously by the ratio and amount of nutrients in biowastes (Chrysargyris et al., 2016). Munnu (2013) showed that organic mulch and N fertilization did not affect the major EO components of *R. officinalis* (alpha-pinene, 1:8 cineole, camphor and verbenone) and all the components stayed in acceptable international trade standard levels. In a greenhouse experiment on *Mentha piperita* L. (*Lamiaceae*) grown in red Latosol (containing relatively high iron and aluminium oxides), the effect of different levels (unreported N content) of treated biosolids (0, 28, 56, and 112 t ha^{-1}) on the EO quantity and chemical composition was evaluated. Results of hydro-distilled EO assessment by CG-MS showed that biosolids do not change the EO quality but increase the yield (Scavroni et al., 2005). These authors suggested using 28 t ha^{-1} of biosolids and harvesting at 90 days after planting to have the best result with no decrease of menthol (one of the two main EO components of *M. piperita* that are menthol and menthone). Rahmani and Tabaei-Aghdaei (2014) reported that *Rosa damascena* Mill. EO production was increased by cow manure (unreported N concentration) application at the rate of 15 t ha^{-1}. Other research showed that EO production of *Ocimum basilicum* significantly increased by applying 10 t ha^{-1} farmyard manure (1.28% N, 2.14% P, and 0.95% K) (Anwar et al., 2005).

There are some studies on the effect of stress on EO production. Lima et al. (2017) reported that long-pepper (*Piper hispidinervum*) produces more EO under the full sun compared to 50% and 30% shade. Several studies reported the EO production of the plants (e.g. *Salvia officinalis* and *Cymbopogon nardus*) increases by water stress (Bettaieb et al., 2009, Petropoulos et al., 2008, Singh-Sangwan et al., 1994).

Even though this stress would cause changes in the Secondary metabolites (SM) composition (Gershenzon, 1984). Porter and Lammerink (1994) reported that high temperatures may decrease the EO concentrations.

Biosolids, DSE and TMW can change the microbial biomass (Zaman et al., 2002, Zaman et al., 1999b, Sanchez-Monedero et al., 2004, Garcıa-Gil et al., 2000), which in turn affect the EO quality and yield (Wicaksono et al., 2017). High levels of nutrients and C sources from biosolids are beneficial for microbial respiration (Kao et al., 2006). Some microbes present in the rhizosphere appear to increase EO production (Banchio et al., 2008). There is a significant correlation between EO composition and bacterial and fungal community in the soil (Wicaksono, 2016). Increased concentrations of TEs in biowastes, can change the microbial population from bacteria to fungi, which is more tolerant to TEs (Kao et al., 2006) and affect the EO production. It is unclear what role individual species of bacteria and fungi have on EO production.

The concentration of EOs in field-grown plants would likely be different from greenhouse plants. This can be a consequence of more extreme environmental conditions in the field that induces plants to produce more EOs, which have adaptive benefits under stressful conditions (Abdelmajeed et al., 2013). Field grown plants in different locales are likely to have distinct genotypes. Genetic variation plays an important role in the quality and chemistry of EOs in different plants including *L. scoparium*, *K. robusta*, *T. vulgaris* and other plants e.g. beach vitex (*Vitex rotundifolia*) (Perry et al., 1997a, McGimpsey et al., 1994, Perry et al., 1997b, Hu et al., 2007).

1.3 Hypotheses and aims

The overarching aim of this thesis was to determine whether biowastes could be beneficially used to establish EO producing plants on low-fertility soils. While there is a paucity of information on the interactions of biowastes with EO-bearing plants, particularly the NZ native plants *L. scoparium* and *K. robusta*, hypotheses can be developed based on the aforementioned literature. The hypotheses in this thesis are:

1) The addition of biowastes will increase the biomass production of EO-producing plants and that these increases will be greatest on low-fertility soils.

2) The addition of biowastes will increase the macronutrient uptake by the plants and the addition of biosolids will increase the uptake of some TEs, especially Cd, Cu and Zn, to levels that reduce the value of the EOs.

3) The elevated N in many biowastes will reduce the EO concentration in the plants, thereby offsetting any increase in biomass.

4) The biowastes will change the composition of EOs, which may affect their value either positively or deleteriously.

The thesis sought to test these hypotheses through the following specific objectives:

a) To evaluate the growth response of *L. scoparium*, *K. robusta*, *L. angustifolia*, *R. officinalis* and *T. vulgaris* growing in four contrasting medium-to low-fertility soil to addition of two types of biosolids and sawdust + biosolids mixtures, dairy shed effluent, and treated municipal wastewater [Chapter 4].

b) To evaluate how the biowastes affected the accumulation of the nutrients and contaminants by the plants [Chapter 5].

c) To determine how the biowastes affected the concentration and therefore the yield of EOs in the plants [Chapter 6].

d) To determine the extent to which biowastes affected the quality of the EOs [Chapter 7].

e) To evaluate quality of EOs in natural populations of *L. scoparium* and *K. robusta* and compare this to the greenhouse trials [Chapter 7].

Appendices are provided to support information of method development in Chapter 3 (Appendix A), expanded data of the nutrient uptake by the plants in Chapter 5 (Appendix B), the plant EO components in Chapter 7 (Appendix C) and the EO composition.

Chapter 2

Background

Figure 2.1 Biosolids (anaerobically digested and high-heat sterilized) in a farmer's hands (GLP, 2017).

2.1 Biosolids

Biosolids are organic-rich material that result from sewage treatment. Biosolids differ from sewage sludge in that they have been treated to meet the regulatory necessities for land application (Figure 2.1) (NRC, 2002). Biosolids contain high concentrations of essential plant nutrients such as nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S) and organic matter (50-70%) (Banegas et al., 2007, NAWWA, 2003). They also can comprise high concentrations of TEs, specifically zinc (Zn), lead (Pb), cadmium (Cd) copper (Cu), iron (Fe), nickel (Ni), boron (B), molybdenum (Mo) and manganese (Mn) (Esteller et al., 2009) .These TEs can be either beneficial or harmful (at high concentrations) to the plants and soil. Biosolids also may contain unwanted compounds and pathogens (Garrec et al., 2003, Jones-Lepp and Stevens, 2007, Bibby et al., 2010). There are many physiochemical differences between fresh and aged biosolids. During aging, the labile fraction of organic matter is progressively broken down (Samaras et al., 2008). Concentrations of organic matter, N, are normally higher in freshly digested biosolids. However, other elements (Table 2-1) are usually higher in aged

biosolids because of the degradation of the surrounding organic matrix (Cogger et al., 1998). One of the main differences is N speciation: fresh biosolids contain high concentrations of ammonium-N (NH_4^+-N), whereas mature biosolids contain a higher proportion of nitrate-N (NO_3^--N) (Bernal et al., 1998).

Table 2-1 Physiochemical properties of fresh and aged biosolids. Units are mg kg^{-1} unless otherwise indicated. (Bernal et al., 1998, Esteller et al., 2009, Knowles et al., 2011, Rigby and Smith, 2013).

	Fresh biosolids	Aged Biosolids
Moisture content (%)	83	53
EC (mS cm^{-1})	5.2	8.3
pH	6.4	4.1
Total N (%)	4.3	2.7
Total C (%)	45	28
Organic matter (%)	62	60
NH_4^+-N	3400	182
NO_3^--N	5.1	4192
C:N ratio	8.4	6.9
CEC (cmol kg^{-1})	39	41
Olsen- P	5192	4683
Na^+	460	713
K^+	5183	4984
Ca^{2+}	4268	9818
Mg^{2+}	3949	2204
Cu^{2+}	352	561
Cd^{2+}	32	2.8
Zn^{2+}	809	878
Pb^{2+}	79	112

The presence of elevated TEs in biosolids is cited as a factor limiting their reuse. Table 2-2 shows the range of TE concentrations typically found in biosolids.

Table 2-2 Trace Element concentrations (mg kg^{-1}) in biosolids (Haynes et al., 2009)

TEs	Concentration in dry weight
Arsenic (As)	1-20
Cadmium (Cd)	1-70
Chromium (Cr)	50-500
Cobalt (Co)	5-20
Copper (Cu)	100-800
Lead (Pb)	100-600
Mercury (Hg)	1-10
Nickel (Ni)	10-200
Selenium (Se)	5-10
Zinc (Zn)	1000-3000

2.2 Treated Municipal Wastewater (TMW)

Figure 2.2 Municipal waste water treatment plant (left) (GWT, 2016) and waste water (right) (AII, 2018).

TMW (Figure 2.2) is the liquid fraction following sewage treatment, and advanced treatment systems can reduce high levels of pathogens and TEs present in the raw sewage (Cai et al., 2013).

The properties of TMW vary depending on the source (Shraddha, 2018). Generally, TMW can contain high levels of organic material (Karunanithi et al., 2016) plant nutrients including N, P, TEs such as Fe and Mn, salts (e.g. NaCl), bicarbonates (HCO_3^-) in some samples (Niazi et al., 2016) as well as Cu and Zn (Nicholson et al., 2003). It also can contain pathogens (Shannon et al., 2007). TMW has less N and P compared to animal wastewater: the concentration ranges of the total N and P in TMW are approximately (15-90) x 10^{-3} g L^{-1} and (5-20) x 10^{-3} g L^{-1} while in the dairy waste water are (185-2636) x 10^{-3} g L^{-1} and (30-727) x 10^{-3} g L^{-1}, respectively (Cai et al., 2013).

Approximately 70% of the water used by humanity is used for agriculture (Pedrero et al., 2010). Some of this water may be supplemented by TMW which 99.9% water with plant nutrients are comprising most of the remaining 0.01%. The use of TMW for agricultural activities reduces the need for consuming both fresh water and fertilisers as well as reducing the discharge of TMW into surface waters (Pedrero et al., 2010). Nevertheless, excessive application of TMW can cause eutrophication in surface waters (Cai et al., 2013) besides surface water pollution (Pescod, 1992), if it is not applied based on the guidelines.

2.3 **Dairy Shed Effluent (DSE)**

Figure 2.3 Dairy farm effluent pond (Tikkisetty, 2016).

DSE refers to wastes from milking sheds and associated yards (Figure 2.3). It includes the manure and urine as well as the water used to wash-down the waste. Therefore, it comprises dissolved and suspended nutrients, salts and organic matter as well as traces of spilt milk (DAF, 2018). DSE contains plant essential elements including N, P, K, S, Mg and TEs that can be used to increase crop production (BPDNZ, 2011) and decrease irrigation water requirements (DAF, 2018). The concentrations of these components vary depending on the diet and age of the animal, besides the season and period of the storage (Environment-Canterbury, 2007, Dairy-NZ, 2015). For example, the N content varies from 0.03% to 1.8% (Di et al., 1998, Zaman et al., 1999a, Zaman et al., 2002) and P concentration ranges (21-125) x 10^{-3} (g kg^{-1}) (Di et al., 1998, Longhurst et al., 2000). The detergents and other chemicals used for cleaning the milking equipment can be found in the DSE.

If the land application of DSE is not managed appropriately, it may be damaging to the quality of underground and surface water resources (due to run-off). The nutrients in DSE can exacerbate the growth of aquatic weeds and algae resulting in water quality degradation, including a reduction in dissolved oxygen (BPDNZ, 2011). The proposed land application rate of dairy effluent is 150-200 kg N ha^{-1} yr^{1} (Roach et al., 2001). Applying the DSE in the proposed limit has economic benefits due to a reduced need for chemical fertilizers while maintaining the quality of the streams and ground water (VICGOV, 2009).

2.4 Sawdust (wood-waste)

Figure 2.4 Sawdust from wood after cutting trees in the forest (Kobzev, 2018).

Sawdust is a by-product of the timber industry (Figure 2.4). Timber processing includes removing the bark and branches resulting in considerable amounts of on-site waste as sawdust (e.g. 0.69 million cubic meter of *Pinus radiata*) accumulating over time (Paramashivam, 2015, Yao et al., 2013). Sawdust has been widely used in mushroom cultivation and bedding in horse stables, cattle pens, and chicken barns (Poppe, 2000, VC, 2018) . Sawdust can improve structure and aeration of the heavy soils (Overholser, 1955). Sawdust also can help to enrich soil, reduce dehydration (keeps the moisture) and improve overall growth of the plants.

Sawdust is clean to handle, easy to apply and long lasting. It contains total carbon in the range of 45%-51%, N (0.06-0.1) x 10^{-3} g kg^{-1} and CEC of ca. 10.6 cmol kg^{-1} (Paramashivam et al., 2016, Bugbee, 1999). To balance the high concentration of carbonaceous matter a nitrogenous fertilizer is needed to add for meeting the requirements of microorganisms that decompose the woody material (Overholser, 1955). It can be mixed with biosolids to reduce NO_3^- leaching. Paramashivam et al. (2016) and Daniels et al. (2001) showed that adding wood-waste to biosolids-amended soil reduces NO_3^- leaching.

Sawdust can adsorb some inorganic and organic contaminants from soil pore water (Paramashivam et al., 2017). Dried *P. radiata* (pine) sawdust can adsorb NH_4^+-N, NO_3^--N and NO_2^--N from storm water (Harmayani, 2012). Combining sawdust with biosolids is reported as a good strategy to increase plant growth and soil aggregate stability (Bugbee, 1999, Schmidt et al., 2001, Sandoval et al., 2012). Sawdust reduces TE uptake by plants in biowaste-amended soil (Daniels et al., 2001, Fiset et al., 2000).

2.5 Biowaste application rates

Nitrogen is the nutrient that is highly likely to be lost through surface or ground water if it is applied at the high rates. Moreover, N is the element that is needed for the plants in higher quantity than others. Therefore, if the agronomic N rate is applied and the N need of the plant is met, most of other nutrients would be in adequate concentration for the plants (Evanylo, 2009). The author also pointed out that aging biowastes can change both the total and mineral N content, which can potentially lead to increased N-losses. It is therefore prudent to minimise the time between analysis of the biowastes and their application to land.

2.5.1 Effect of biowastes on soil fertility

Most biowastes contain significant quantities of organic matter that can improve soil structure, water holding capacity, aeration, and drainage as well as reducing soil compaction and erosion (Obi and Ebo, 1995, Rahmani and Tabaei-Aghdaei, 2014). Biowastes have also been shown to improve cation exchange capacity (Kabirinejad and Hoodaji, 2012, Hina, 2013). Many biowastes including biosolids, Dairy Shed Effluent (DSE) and Treated Municipal Wastewater (TWW) contain high concentrations of essential plant nutrients including N, P, K, S and TEs (Antoniadis, 2008, Bai et al., 2013, Sarkar et al., 2005, Gottschall et al., 2007, Wang et al., 2010).

Soil C and N, which are the two most important factors affecting soil fertility (Schmidt et al., 2001, Evanylo, 2009, Haynes et al., 2009). Biowastes can be a source of both available and slow-release N, which can provide both short and long-term profits for the soil (Robinson and Polglase, 2000, Zaman et al., 1999a, Sedlak, 1991). Biowastes also alter the soil microbiota, which can result in increased plant growth (Hossain et al., 2017, Oliveira and Ferreira, 2014).

The land-application of biosolids, DSE and TMW onto *Pinus radiata*, *Rosemarinus officinalis*, *Zea mays* and *Hordeum vulgare*, generally results in increased biomass production without excessive levels of contaminant uptake (Zaman et al., 2002, Singh and Agrawal, 2008, Wang and Jia, 2010, Kimberley et al., 2004, Cala et al., 2005a, Krey et al., 2011, Rusan et al., 2007). Biosolids have been used for restoration of TE-contaminated mine sites (Brown et al., 2003b) as this material have the potential to immobilize the Pb, Zn and Cd (Basta et al., 2001). Biosolids have been used as fertilizer to either improve or maintain the soil fertility for sustainable plantation (Fresquez et al., 1990). Biosolids application to low organic matter and clay content soils increases the soil organic carbon and nutrients retention (Antoniadis, 2008). Blending sawdust with N-rich biosolids can reduce nitrate leaching (Paramashivam et al., 2016).

Hawke and Summers (2006) reported that the application of DSE increased the total N, P and plant available nutrients and improved long-term fertility of the soil that can increase the plants biomass.

Biowastes can change the bioavailability of nutrients and contaminants in soil (Singh and Agrawal, 2008, Antolín et al., 2005, Brown et al., 2003a). Bioavailability could be altered through:

- Change in total concentration (Houba et al., 1996).
- Change in pH. An increased pH reduces the bioavailability of cations and generally increases the availability of anions. However, in highly alkaline soil, P becomes less available (Horiba, 2015).
- Change in CEC and also specific adsorption sites (related to CEC). Increased CEC reduces cation solubility (McLaren and Cameron, 1996).
- Change in dissolved organic carbon – binds dissolved metals and renders them unavailable for plant uptake (Robinson et al., 2009).
- Change in salinity – salts can increase the solubility of some elements. It can change (increase or decrease) their uptake by plants. Salts affect soil structure (e.g. clay dispersion) (McLaren and Cameron, 1996).
- Other competing ions, for example, Ca^{2+} competes for binding sites with Cd^{2+} although Ca^{2+} can also increase pH (Plette et al., 1996, Ikebuchi et al., 1991)
- Changes in microbial activity, which thence alter the bioavailability of elements (Rashid et al., 2016).

The initial soil pH and fertility play important roles in the nutrient availability and efficiency of the biowastes addition. For example, at low pH Al and Mn can become more available and more toxic to the most plants whereas Ca and Mg are less available acidic soil (Horiba, 2015). To increase nutrient uptake in acidic soils, lime-amended biosolids are used to neutralise the pH (Pritchard et al., 2007). Lense (2018) reported a 6-10% increase of the pH following the application of treated TMW (500 mm) for 18 months. This shows that this treatment would not be suitable for the high pH soils.

2.6 Degraded or low-fertility lands

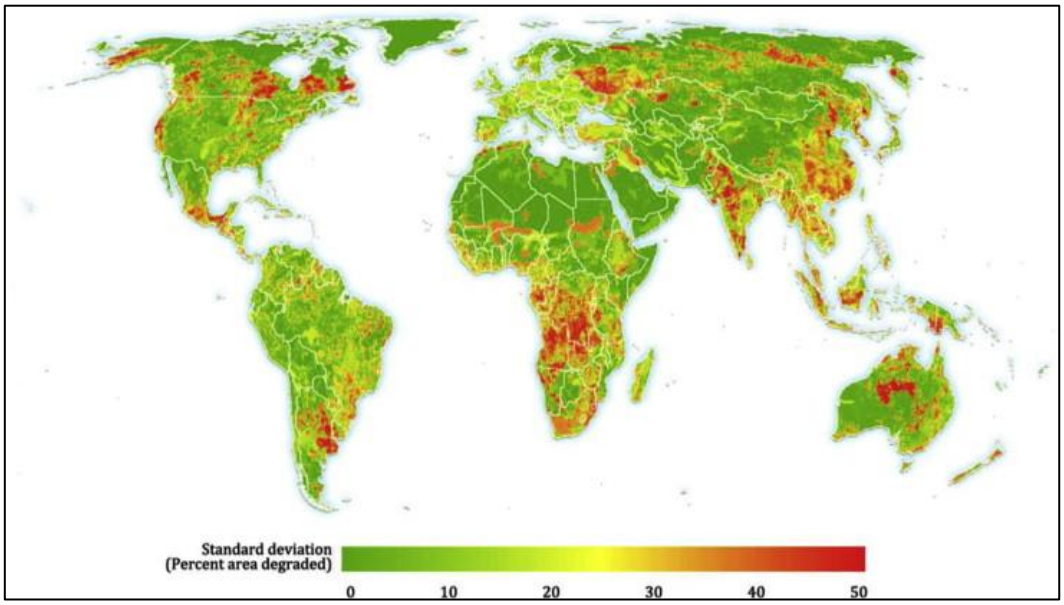


Figure 2.5 World degraded land map (Gibbs and Salmon, 2015).

Gibbs and Salmon (2015) estimated that 75% of the world's land area is degraded (ca. six million ha) (Figure 2.5) and this environmental damage affects the lives of 3.2 billion people (National-Geographic, 2018).

In NZ, soil can become degraded due to forestry. In 2010, NZ had 250,000 ha of land in Radiata pine (*Pinus radiata* D. Don), with soils that are often severely degraded after logging (MAF, 2010), where topsoil is scraped off or eroded. Soils under *P. radiata* forests can be acidic and depleted in plant nutrients (Brockerhoff et al., 2005). Some 192 million t yr^{-1} of NZ soil are lost from erosion (StatsNZ, 2018b). This soil degradation has negative impact on the land, water and climate (Figure 2.6).





Land	Fresh water	Marine	Atmosphere & climate	Air
Soil quality changes can put added pressure on indigenous plant species and raise opportunities for invasive species.	Water quality and the ecological health of rivers, streams, and lakes can be degraded when eroded sediment, contaminants, and nutrients enter waterways.	A reduction in survival rates of many species and the loss of important marine habitats occurs when eroded sediment reaches estuarine and coastal areas.	Soil is a major carbon sink. Land use change can cause the soil carbon stock to increase or decrease, especially if there is a disturbance of the topsoil as in agriculture and production forestry. When a land use change decreases the soil carbon stock, carbon dioxide, a greenhouse gas, is emitted to the atmosphere. When a land use change increases the soil carbon stock, carbon is removed from the atmosphere.	Dry, bare soil can be eroded by wind, suspending particles in the air and causing respiratory issues, dust nuisance, and loss of fertile soil.
Erosion damages infrastructure such as fencing and roads on farms, and when it occurs, can cause significant impacts to roads, housing, and infrastructure (such as water pipelines).	Compacted soils are often slow draining, which can lead to more sediment and nutrients moving off the land and affecting waterways.		Soil carbon is an indicator of soil organic matter, which is important in supplying and retaining nutrients for plants and farm production and reducing the amount of nutrients leached to water.	
Erosion reduces the amount and quality of soil, leading to reductions in plant and animal productivity.	Sediment that enters waterways can build up in river channels leading to an increased flooding hazard and risk to infrastructure such as bridges.		We have limited understanding of the relationship between erosion and the storage/release of carbon.	
Reductions in soil quality can limit plant growth leading to reductions in animal productivity.			Soils that are wet or compacted are likely to have increased emissions of nitrous oxide, a greenhouse gas.	

Figure 2.6 Environmental impact of the soil degradation (StatsNZ, 2018c).

2.7 Essential oils[1]

2.7.1 What are essential oils?

Essential oils are concentrated hydrophobic liquid comprising volatile aroma compounds from plants (EBI, 2017). EOs are combination of active volatile molecules (Peters and Ebrary, 2016). Some EO components attract pollinators, deter herbivory or inhibit pathogens (Abdelmajeed et al., 2013, Peters and Ebrary, 2016). EOs differ from other oils in that they have a significant volatile fraction and often contain a high proportion of aromatic as opposed to aliphatic compounds (Peters and Ebrary, 2016). The aromatic EOs in the Myrtaceae and other families' leaves is released if they are crushed by fingers (ANPSA, 2018).

EOs are Secondary Metabolites (SMs) (Dhifi et al., 2016). SMs are products that are biosynthesized from one or more primary metabolites through a broader range of pathways, which are available for primary metabolism and are not essential to growth and life of the producing organism (Verpoorte, 2000). The purpose of many SMs production and their profits to the plants are unknown, while some are known for specific reasons. For example, toxins protect plants against the predators (Pohnert et al., 2007).

Macro and micronutrients affect the SMs production that is controlled by the environmental condition and plants species (Hassan, 2012). Nitrogen can affect EO production, both positively and deleteriously (Hamisi et al., 2012, Biesiada et al., 2008, Scavroni et al., 2005). Plants allocate the C and N to produce SMs, only after primary needs and growth requirements are met. Therefore, if the N fertilisation increases to excess levels of growth requirements, production of N-based SMs (e.g. alkaloids) may be increased. In the same way, increasing the carbohydrates rises the C-based SMs (e.g. phenolics) production (Hassan, 2012).

The main constituents of the plants EOs are terpenes and oxygenated compounds (Bakkali et al., 2008). These are normally extracted from the plant using steam distillation, solvent extraction as well as carbon dioxide extraction and expression, which based on the EO properties and the final product are used (Chemat et al., 2006, Toma et al., 2001, Guan et al., 2007)

[1] A natural oil typically obtained by distillation and having the characteristic fragrance of the plant or other source from which it is extracted OXFORD 2018. essential oil. *Oxford Dictionaries.*

2.7.2 Distribution of essential oils in plant tissues

Plants store EOs in either external or internal secondary structures that are on the surface or inside the plant. If the EO is stored in the external structure, the aroma can be noticed by just a touch, while to reach the EO stored in the internal secretory structures, we need to break the plant EO producing organ. External structures are called glandular trichomes (Figure 2.7). *Ocimum basilicum*, *Lavandula angustifolia*, *Origanum majorana*, *Origanum vulgare*, *Mentha piperita* and *Rosmarinus officinalis* are keeping the EO by this structure.

Internal structure consists of secretory cavities and ducts (Figure 2.7) that occur as spherical spaces and are most commonly found in the Myrtaceae and Rutaceae families (Shutes, 2015).

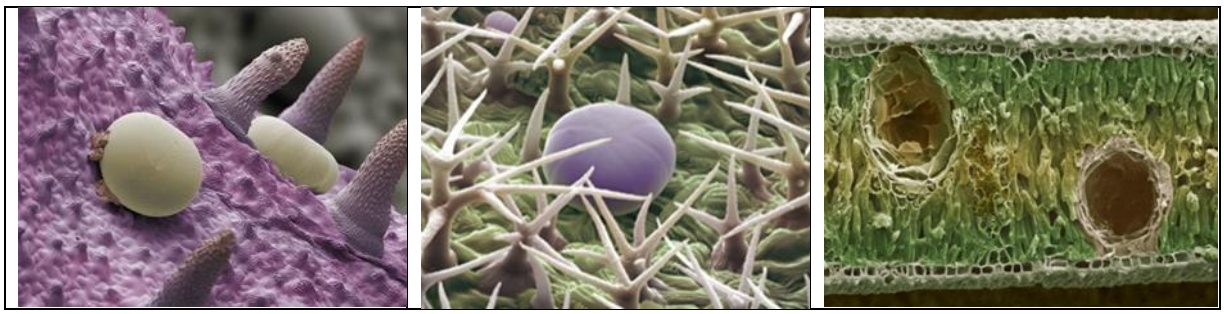


Figure 2.7 External (two left) and internal (right) structures of keeping essential oils (glandular trichomes and secretory cavities and ducts) (Shutes, 2015).

2.7.3 The global and NZ essential oil market

The supply of EOs was estimated between US$8 billion and $10 billion in 2013 (Ngai-Tahu, 2014). Other than the use of the EOs in food and beverage industry, the increased use of EOs in medications affected the development of the overall market. Due to the knowledge about therapeutic properties of EOs, using them in medicines has been increased. Moreover the use of EOs in spa & relaxation and aromatherapy industries is increasing (EOM, 2018).

Perfume types are defined by the amount of EOs included; the higher the percentage of EOs the costlier the fragrance that increases the revenue. The global sales profits of the perfume business was US$29 billion in 2013 (Ngai-Tahu, 2014). Population growth and urbanization has increased demand for perfumes (R&M, 2018). Figure 2.8 shows the economic significance of fragrance industry in the world.

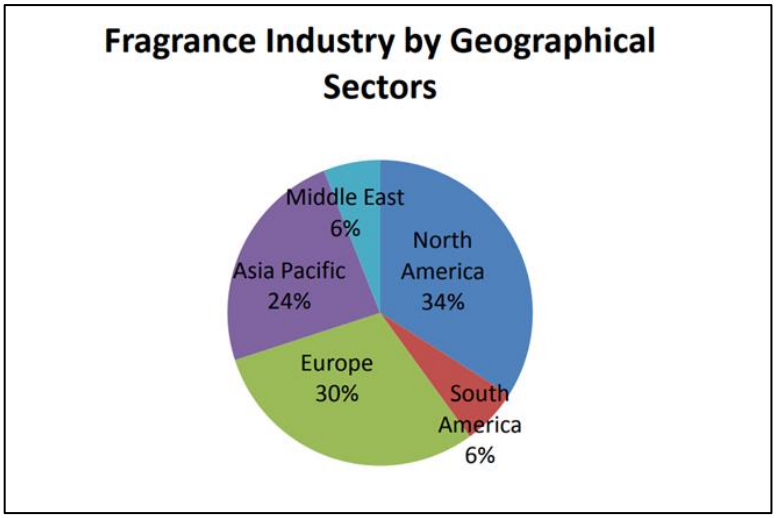


Figure 2.8 The fragrance industry in terms of economic significance by geographical sectors (Ngai-Tahu, 2014).

NZ's latitude (34 ° -47 ° S) is comparable to the North Africa to mid-France one. As NZ is in the dynamic boundaries of the South Pacific and Australian plates, it has various land forms, topography and contrasting climates between regions. Because of the wide range of soil-climate combination, the condition is appropriate for growing sub-tropical to cool temperature species (Porter, 2003).

There are some NZ native EOs, e.g. *L. scoparium* that are internationally recognised (Douglas et al., 2004). The NZ cosmetic industry is restricted mainly to blending and packing, while there is a possibility of having a wide range of products manufactured in the country (Ngai-Tahu, 2014).

2.8 Plants used this research and their essential oil characterisation

In the experiments of this study five EO-producing plant species are used. Table 2-3 shows their classification and growth habit.

Table 2-3 List of the plants used in the study (Akhondzadeh et al., 2003, PFAF, 2012, Munné-Bosch and Alegre, 2000, Stahl-Biskup and Venskutonis, 2012, Sunders, 2017, Lis-Balchin et al., 2000).

Plant species	Binomial name	Family	Details
Mānuka	*Leptospermum scoparium* J.R. Forst & G. Forst	Myrtaceae	perennial woody shrub 1 – 3m high when mature, native to NZ and Australia
Kānuka	*Kunzea robusta* de Lange & Toelken.	Myrtaceae	perennial woody shrub 3 – 15m high when mature, native to NZ
Lavender	*Lavandula angustifolia* Mill.	Lamiaceae	evergreen shrub growing to 1.2 m by 1 m, native to Mediterranean region
Rosemary	*Rosmarinus officinalis* L.	Lamiaceae	evergreen shrub growing to 1.5 m by 1.5 m, native to Mediterranean region
Thyme	*Thymus vulgaris* L.	Lamiaceae	evergreen shrub growing to 0.2 m by 0.3 m, native to southern Europe

2.8.1 Mānuka (*Leptospermum scoparium* J.R. Forst & G. Forst) (Myrtaceae)

Traditional Māori lore states that *L. scoparium* plant is a female (Maddocks-Jennings et al., 2005b). *L. scoparium* has different varieties that exist in NZ or Australia (Lis-Balchin et al., 2000, Killeen et al., 2016). *L. scoparium* EO has several benefits including inhibition of bacterial and fungal infections, diminishing depression, anxiety, anger and stress, curing allergic reactions, fading skin scars, providing relief from cough and cold and helping to treat body inflammation (Patil, 2018). *L. scoparium* EO is stored in oil glands in the leaves (Figure 2.9).

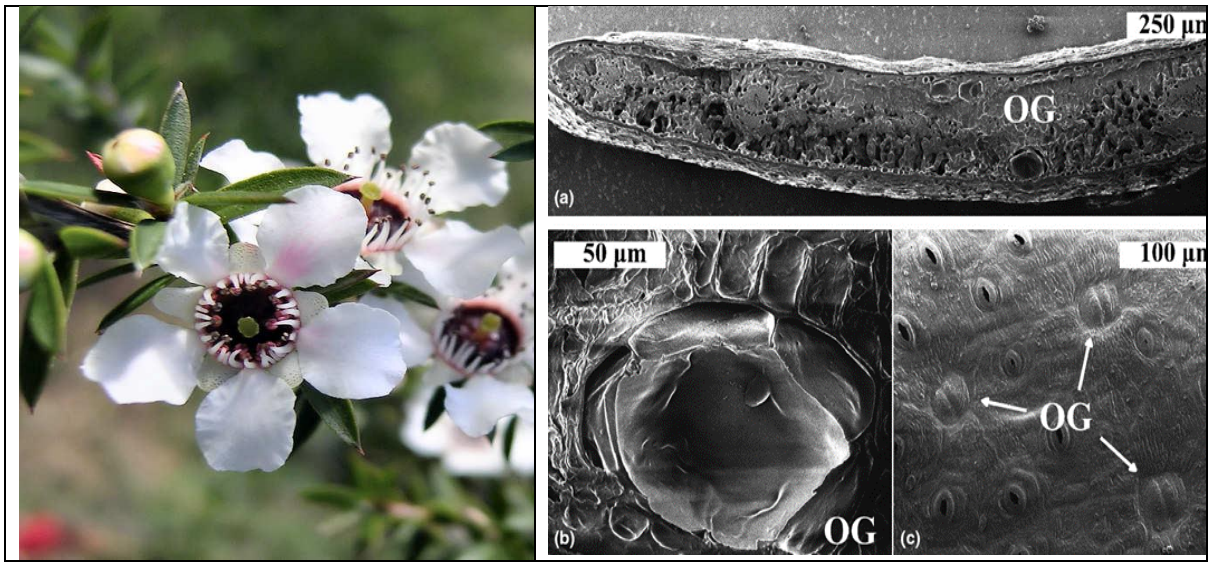


Figure 2.2 *L. scoparium* plant (left) (Inetgardens, 2018) and electron microscopy of the leaf (right): (a) cross-section showing oil gland (OG); (b) oil gland; (c) ventral surface with oil glands (Killeen et al., 2015).

L. scoparium EO is out of interest because of its high β-trikenone (Figure 2.10) levels (Douglas et al., 2004) that enhances the antimicrobial properties (Maddocks-Jennings et al., 2005b). The chemical composition of *L. scoparium* is affected by both the genotype of the plant and the environment (Maddocks-Jennings et al., 2005b, Perry et al., 1997a, Sunders, 2017). EOs from different locations have unique aromas, colour, clarity, and texture. Three dominant *L. scoparium* EO chemotypes have been defined throughout NZ (C&F-Research, 2000):

a) High pinene content (in the far north).
b) High triketone (in the East Cape and Marlborough Sounds regions).
c) Complex of sesquiterpenes (over the rest of NZ) (Perry et al., 1997a).

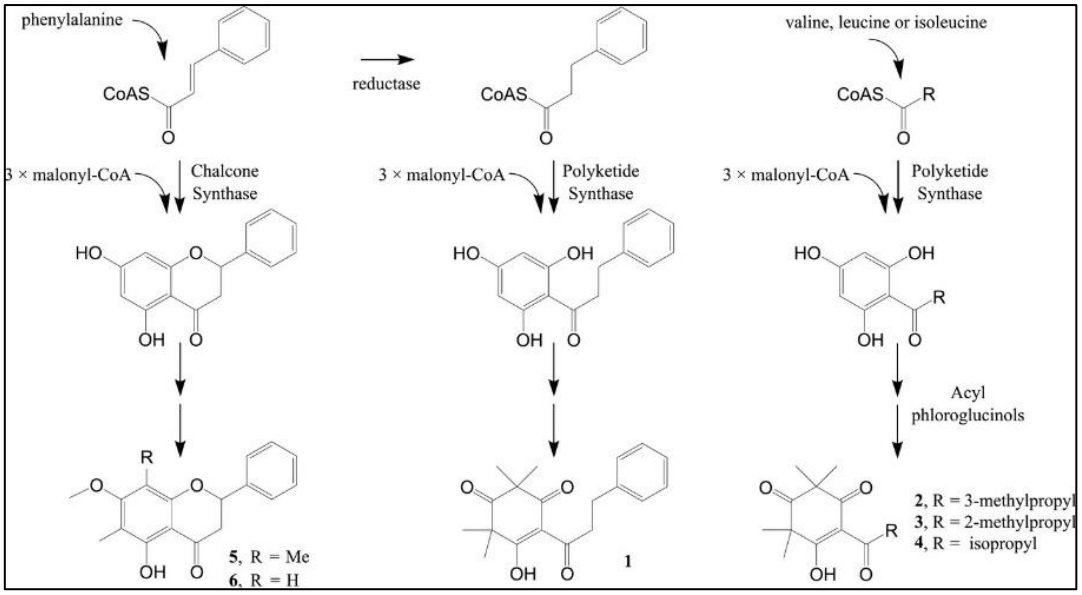
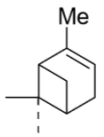
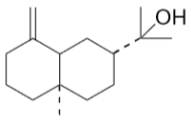
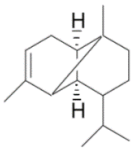
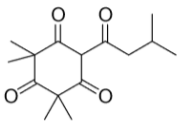
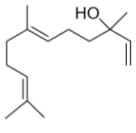
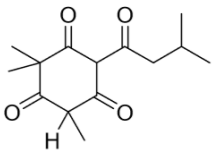


Figure 2.10: Biosynthesis of β-triketones and flavonoids in *Leptospermum* (Killeen et al., 2015).

There are six major groups of volatile (steam distillable) compounds are in *L. scoparium* EO that are illustrated in Table 2-4 and Figure 2.11 (Senanayake, 2006a).

Table 2-4 The steam distillable compounds of *L. scoparium* essential oil.

Group	Name	Example compound		Name	Example compound
A	monoterpenes	 α-pinene	**D**	eudesmols	 β-eudesmol
B	sesquiterpenes	 α-ylangene	**E**	triketones	 Leptospermone
C	oxygenated sesquiterpenes excluding eudesmols	 β-nerolidol	**F**	nor-triketones	 Nor-leptospermone

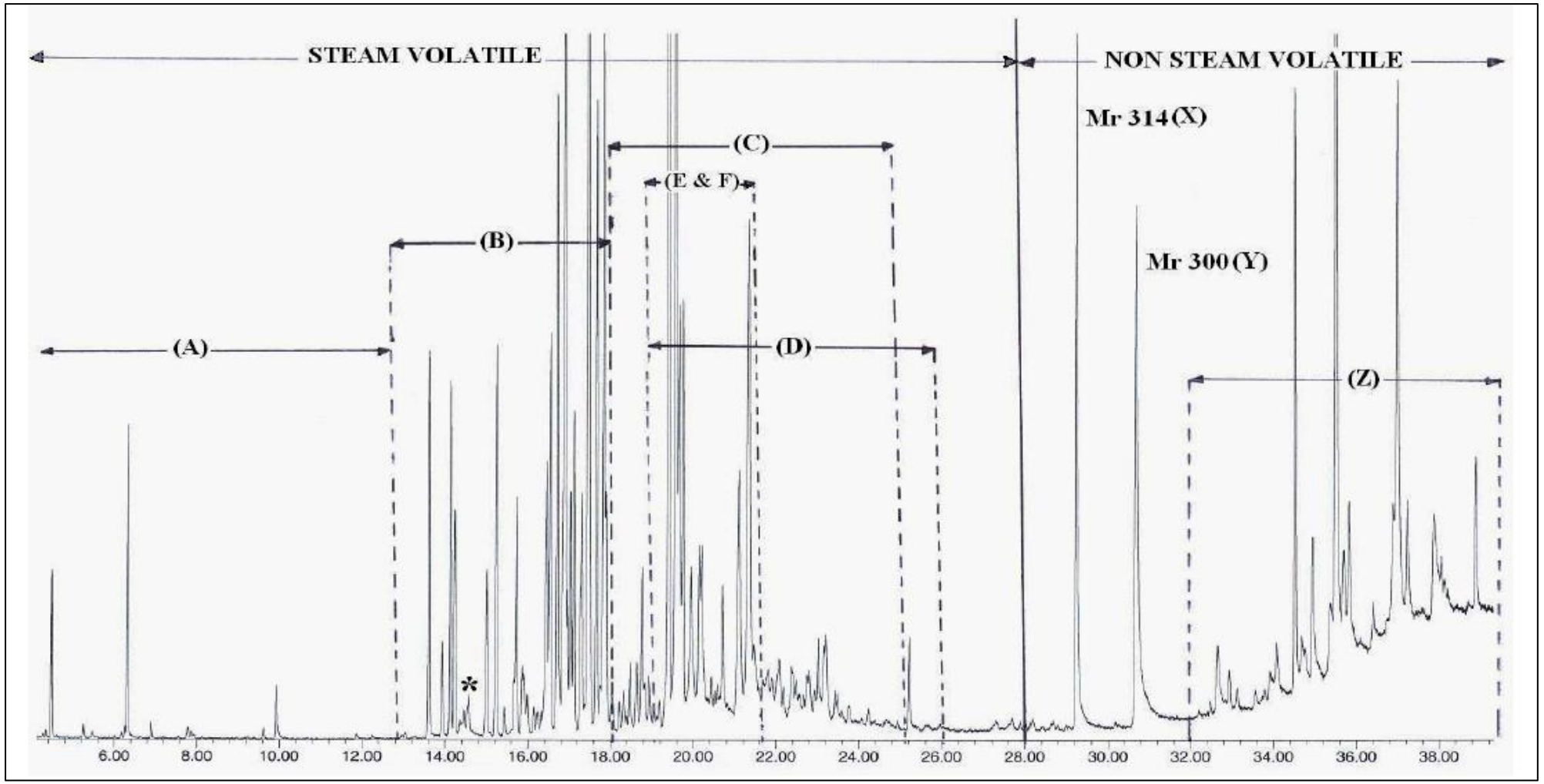


Figure 2.11 Groups of volatile (steam distillable) compounds in *L. scoparium* oil (Senanayake, 2006a)

2.8.2 Kānuka (Kunzea robusta de Lange & Toelken) (Myrtaceae)

Figure 2.12 *K. robusta* plant (ALCW, 2016).

Māori knowledge introduces the *K. robusta* as a male (Figure 2.12) (Maddocks-Jennings et al., 2005b). *K. robusta* EO has lower antimicrobial properties compared to *L. scoparium* EO (Harkenthal et al., 1999). Of the *K. robusta* EO major properties is elevated levels of monoterpene hydrocarbons, mainly α-pinene, and the presence of moderate levels of some sesquiterpenes e.g. viridiflorene, calamenene (an aromatic sesquiterpene), viridiflorol and ledol (Porter and Wilkins, 1999). The storage and physiology of EOs in *K. robusta* are presumably similar to *L. scoparium* although this has not been investigated as *L. scoparium*. The chemotype of *K. robusta* EO is almost the same throughout NZ. The EO is usually high in α-pinene (higher (77%) in northern NZ and lower (52%) in south). Nevertheless, two major chemotypes have been recognised:

a) Low in the p-cymene (most common).
b) High in p-cymene levels (>5%) (C&F-Research, 2000).

2.8.3 Lavender (*Lavandula angustifolia* Mill.) (Lamiaceae)

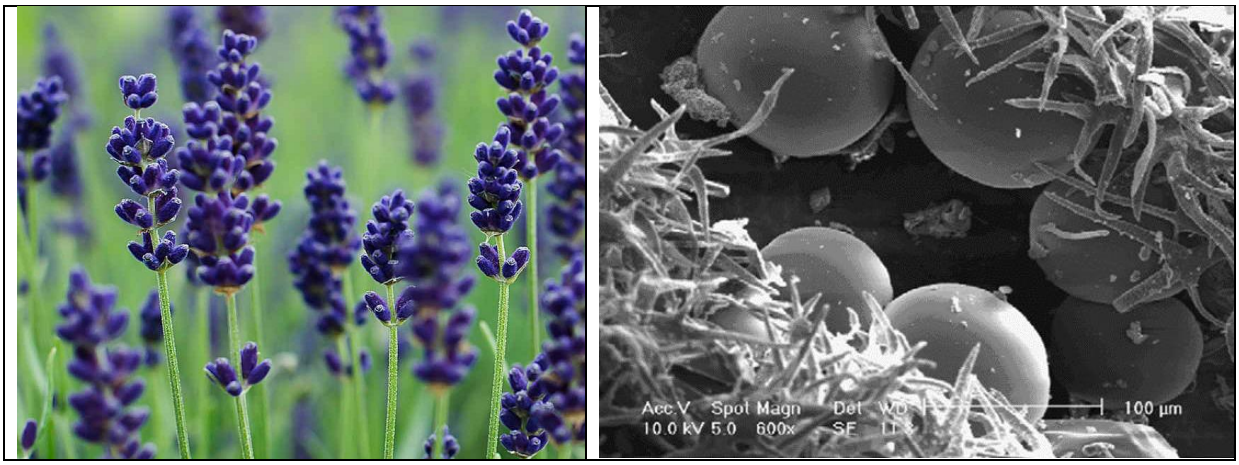


Figure 2.13 *L. angustifolia* plant (left) (HedgesDirect, n.d.) and the oil glands (right) (Sahraoui et al., 2008).

L. angustifolia EO is kept in the flowers oil glands (Figure 2.13) and has antibacterial and anti-inflammatory properties (Giovannini et al., 2016). *L. angustifolia* EO can make people more content by decreasing the performance of working memory and reaction times for both memory and attention-based tasks (Moss et al., 2003).

The most important components of *L. angustifolia* EO are α-thujene, α-pinene, camphene, β-pinene, 1.8 cineole, trans-β-ocimene, γ-terpinene, terpinolene, linalool, camphor, borneol, lavandulol, α-terpineol, linalyl acetate, bornyl acetate, β-caryophyllene (Hajhashemi et al., 2003, Ghelardini et al., 1999).

2.8.4 Rosemary (*Rosmarinus officinalis* L.) (Lamiaceae)

Figure 2.14: *R. officinalis* plant (left) (Jardinitis, n.d.) and electron micrographs of the leaves capitate trichomes (right) (Bousbia et al., 2009).

R. officinalis EO is stored in trichomes in the leaves (Figure 2.14) (Bousbia et al., 2009). *R. officinalis* EO improves memory, also can make people more alert and content (Moss et al., 2003). The major components of *R. officinalis* EO are 1,8-cineole, α-pinene, camphor, camphene and β-pinene (Wang et al., 2008).

2.8.5 Thyme (*Thymus vulgaris* L.) (Lamiaceae)

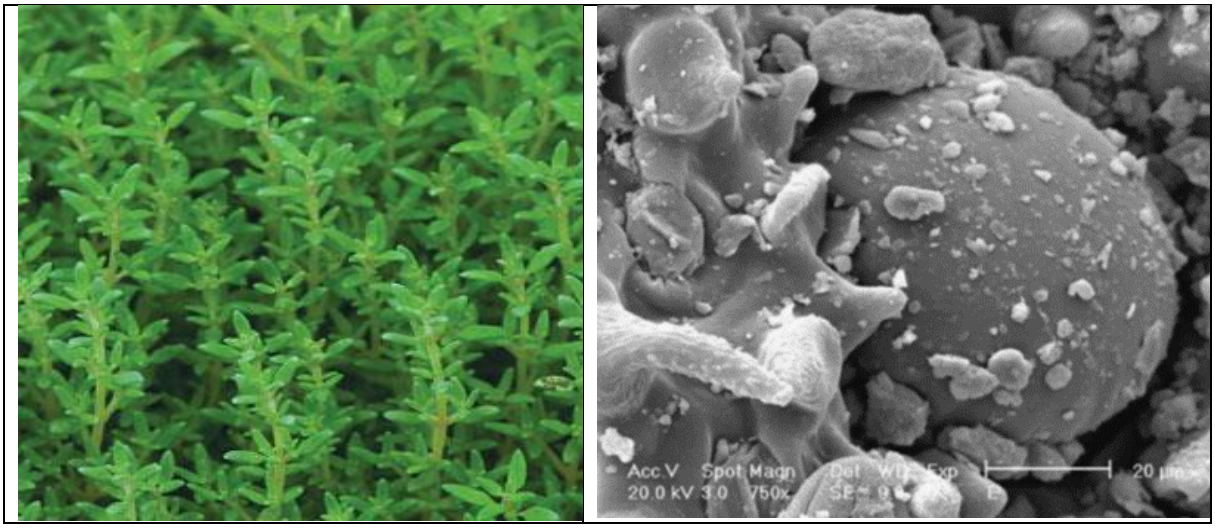


Figure 2.15 *T. vulgaris* plant (left) (Ballseed, n.d.) and scanning electron micrographs of the leaves (right) (Golmakani and Rezaei, 2008).

T. vulgaris EO is kept in the leaves oil glands (Figure 2.15) (Golmakani and Rezaei, 2008). The major components of *T. vulgaris* EO are thymol, carvacrol, linalool, α-terpineol, and 1,8-cineole (Lee et al., 2005). *T. vulgaris* EO can help in reducing pain, skin problems, fatigue, sleeping problems, nervousness and anxiety. Moreover, it can work against some bacteria such as *Salmonella* spp., *Enterococcus* spp., *Escherichia coli* spp. and *Pseudomonas* spp. (Mercola, 2016).

2.9 Essential oil producing plants and the environment

Esperschuetz et al. (2017) showed that there is little nitrate leaching (<2 kg ha^{-1} equiv.) from a low-fertility soil amended with 1250 kg N ha^{-1} equiv. biosolids and planted with *L. scoparium* and *K. robusta* compared to *Lolium perenne* (>30 kg ha^{-1} equiv.). Downward (2013) hypothesised that the SMs produced by these myrtaceous species were inhibiting nitrifying microorganisms. Prosser (2011) demonstrated that *L. scoparium* enhanced the die off of *E. coli* in biosolids-amended soil. Therefore, these species may mitigate some of the environmental risks associated with applying biosolids to land.

Section II

Materials and Methods

Chapter 3

Greenhouse experiments

The thesis is comprised of six separate greenhouse experiments (Exp. 1 – Exp. 6). All greenhouse experiments were performed at the Lincoln University Plant Growth Unit (43o38'42"S 172o27'41"E) from 2013 to 2016. The pots in all experiments were placed in a randomized block design and regular irrigation as well as weeding were done for all the experiments.

3.1 **Soil**

Soils were collected from central NZ (Figure 3.1). A clay loam soil was taken from Bideford, NZ (40° 45' 56" S, 175° 54' 42" E). The NZ soil classification is a Brown soil (Hewitt, 2010b). A Lismore Stoney Silt Loam (LSL) classified as a Pallic Firm Brown Soil in the NZ Soil Classification (Hewitt, 2010b) was collected from Eyrewell Forest (43°43'87"S, 172°45'31"E), which formerly was under *Pinus radiata* cultivation. The soil has a stone content of about 5% and a particle size distribution (<2 mm fraction) in the A horizon (0-180 mm) of 25% clay, 30% silt, 45% sand and in the B horizon (180-400 mm) of 17% clay, 35% silt and 48% sand (Francis and Knight, 1993). A Pawson Silt Loam (PSL) was taken from Banks peninsula (43°47'31.18"S, 172°58'18.05"E). The PSL is a Pallic Firm Brown soil in NZ soil classification (Hewitt, 2010b). Soil was collected from Coleridge-Lyndon Rd, Canterbury, NZ (S 43° 20' 35'', E 171° 36' 59''). The Craigieburn Silt Loam (CSL) is an allophanic Brown soil (Hewitt, 2010a). For the latter experiments, approximately 100 kg of the soil was collected from different horizons (Ah: 0 - 15 cm, Bw; 20 – 40cm, and BC: 40 - 70 cm), and packed separately. A 4:1 proportion mixture of Ah and Bw horizon samples was used for the Exp. 5 and separate horizons were used in Exp. 6. All soils were taken after removing the surface vegetation. Soil samples were homogenised and passed from a 10 mm sieve to separate the stones while maintaining soil aggregates and structure. Subsamples were taken for chemical analyses (Table 2-1 and Table 3-2).

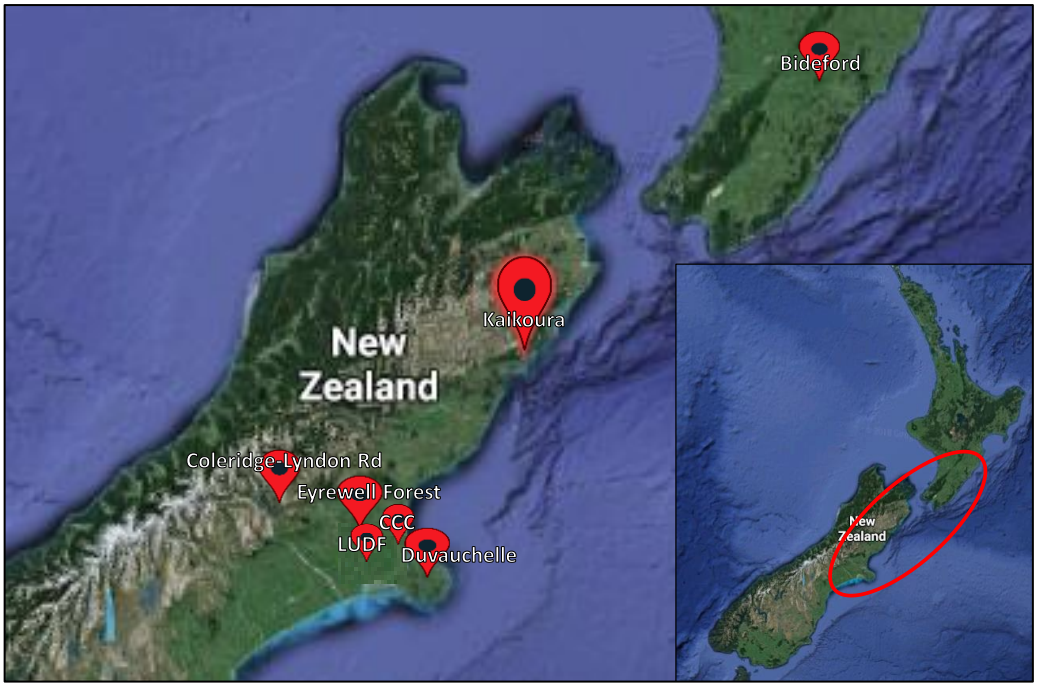


Figure 3.1 Map of the four soil and two biowastes sampling sites. Insert is a map showing the sites locations within NZ. CCC and LUDF represent Christchurch City Council and Lincoln University Dairy Farm, respectively (Google maps).

Table 3-1 Parameters of the soils used in the Exp. 1- Exp. 5. Concentrations are in mg kg^{-1} dry matter unless otherwise indicated. Standard errors are given in parenthesis where available. (T) and FW represent the total element concentration and fresh weight, respectively.

	Exp. 1	**Exp. 2 & 3**	**Exp. 4**	**Exp. 5**
Soil type	Bideford Clay Loam (BCL)	Lismore Stoney Silt Loam (LSL)	Pawson Silt Loam (PSL)	Craigieburn Silt Loam (CSL)
N.Z. Soil Classification	Brown Soil	Pallic Firm Brown soil	Pallic Firm Brown soil	Allophanic Brown Soil
pH	6.1	5.2 (0.01)	4.9 (0.01)	5.5 (0.01)
CEC (me 100 g^{-1})	21	13	12.8	36
C (T) (%)	6.5	4.5 (0.2)	3.1 (0.07)	1.6
N (T) (%)	0.50	0.23 (0.01)	0.38 (0.05)	0.22 (0.01)
C/N	14	20 (0.4)	10 (0.1)	7
NH_4^+- N (mg kg^{-1} FW)	2	3.5 (0.11)	6.8 (0.2)	< 0.01
NO_3^-- N (mg kg^{-1} FW)	0.6	28 (1.6)	25 (1.26)	< 0.01
Olsen - P	11	13	15	18 (1.1)
P (T)	544 (5)	383 (7.3)	812 (14.7)	717 (5.2)
K (T)	1886 (46)	4468 (37)	2929 (39)	3510 (51)
S (T)	405 (2)	210 (5.5)	375 (8.2)	380 (2.4)
Ca (T)	4063 (67)	2472 (41)	4448 (45)	4840 (58)
Mg (T)	1962 (22)	3768 (33)	2580 (11)	5620 (30)
Fe (T)	15461 (108)	22293 (270)	18876 (52)	27800 (50)
Mn (T)	133 (3)	288 (2.8)	577 (4)	510 (1.9)
Cu (T)	4.2 (0.0)	3.4 (0.3)	8.1 (0.43)	8.4 (0.1)
Na (T)	207 (5)	268 (4.1)	182 (1.4)	210 (8.4)
Ni (T)	4.1 (0.0)	7.3 (0.1)	7.9 (0.1)	10 (0.07)
Zn (T)	29 (0.0)	75 (2.6)	51 (1)	94 (1.4)
Pb (T)	8.3 (0.1)	14 (0.1)	11.7 (0.15)	26 (0.6)
Cd (T)	0.05 (0.00)	0.43 (0.01)	≤ 3*10-4	0.32 (0.03)
Cr (T)	14.0 (0.2)	22 (0.3)	14.2 (0.16)	35 (0.3)

Table 3-2 Parameters of the Craigieburn Silt Loam (CSL) soil used in the Exp. 6. Concentrations are in mg kg^{-1} dry matter unless otherwise indicated. (T), (E) and FW represent the total, exchangeable elements and the fresh weight, respectively.

Parameter	Horizon Ah	Horizon Bw	Horizon BC
pH	5.57 (0)	5.77 (0.04)	5.89 (0.06)
EC (µS cm^{-1})	36 (1.0)	17 (0.7)	7.9 (0.3)
C (T) (%)	1.46 (0.18)	1.03 (0.07)	0.70 (0.05)
N (T) (%)	0.24 (0)	0.12 (0)	0.07 (0.00)
NH_4^+ -N (mg kg^{-1} FW)	0 (0)	0 (0)	0 (0)
NO_3^- -N (mg kg^{-1} FW)	0.04 (0.02)	0.29 (0.04)	0.17 (0.03)
Olsen P	15 (0.37)	15 (0.37)	6.2 (0.23)
P(T)	755 (13)	654 (15)	449 (3.5)
S (T)	380 (8.5)	270 (3.5)	230 (3.3)
S (E)	0 (0)	0 (0)	0 (0)
K (T)	3430 (88)	3750 (112)	4200 (85)
K (E)	87 (12)	36 (9)	23 (6)
Ca (T)	4980 (96)	3730 (98)	4220 (36)
Mg (T)	5570 (60)	5650 (109)	6040 (52)
Mg (E)	134 (14)	57 (12)	9.1 (1.8)
Na (T)	210 (7)	220 (8.4)	210 (3.7)
Na (E)	13 (2)	18 (4.2)	8.2 (1.9)
Mn (T)	520 (2)	440 (6.3)	340 (6.2)
Mn (E)	4.8 (0.6)	1.2 (0.5)	0.4 (0)
Cu (T)	8.5 (0.22)	8.5 (0.26)	10.7 (0.14)
Cu (E)	0 (0)	0 (0)	0 (0)
Zn (T)	90 (1.3)	104 (2.6)	79 (0.7)
Zn (E)	0.13 (0.05)	0.02 (0.01)	0.06 (0.04)
Cd (T)	0.3 (0.03)	0.29 (0.03)	0.15 (0.04)
Pb (T)	26 (0.6)	25 (0.6)	20 (0.6)

3.2 Biowastes

This thesis used contrasting biowastes including biosolids, DSE and sawdust collected from central NZ. Biosolids were collected from Kaikoura (KB) Regional Treatment Works, Kaikoura, Canterbury, NZ (42°21'37.40"S, 173°41'27.35"E). These KB had minimal industrial input and were stockpiled in the oxidation pond and weathered. Similarly, biosolids were collected from the Christchurch City Council (CCC) Wastewater Treatment Plant (CB). These CB had moderate industrial input and had undergone anaerobic digestion. The moisture content of the CB was <5% w/w (CCC, 2018), whereas the KB had a water content of 53% w/w. *Pinus radiata* sawdust was collected from Kaikoura Wastewater Treatment Plant, NZ (42°21'37.40"S, 173°41'27.35"E). DSE was collected from Lincoln University Dairy Farm (LUDF) (43° 38' 38.07" S, 172° 26' 1.96" E). Figure 3.1 shows the biowastes sampling sites locations. Biosolids and sawdust were sieved (≤10 mm) and biowastes (including DSE) were mixed and

homogenized before application. Subsamples of all material have been taken to analyse the chemical properties that is shown in Table 3-3.

Table 3-3 Parameters of the biowastes used in the experiments. Concentrations are mg kg^{-1} dry matter for biosolids and sawdust and mg kg^{-1} fresh material for DSE unless otherwise indicated. Standard errors are given in parenthesis where available. n.a. = not applicable. KB, CCC, LUDF and FW represent Kaikoura biosolids, Christchurch City Council, Lincoln University Dairy Farm and fresh weight, respectively.

	KB	CCC Biosolids	Kaikoura sawdust	LUDF dairy effluent
pH	4.5 (0.06)	6.8 (0.02)	5.7	7.5 (0.01)
CEC [me $100g^{-1}$]	17.1	36.5 (0.32)	8.0	n.a.
Total C [%]	27 (0. 7)	30 (0.03)	48	0.11 (0.00)
Total N [%]	2.6 (0.1)	4 (0.0)	0.1	0.02 (0.00)
C/N	11 (0.1)	8 (0.01)	908	5.9 (0.2)
NH_4^+- N (mg kg^{-1} FW)	101 (6)	2375 (14)	≤0.1	82 (2)
NO_3^-- N (mg kg^{-1} FW)	305 (9)	3.56 (0.23)	≤0.1	0.05 (0.01)
P	5941 (42)	16247 (10)	42 (1)	17 (0.4)
K	3653 (34)	2164 (20)	455 (6)	143 (2)
S	8681 (140)	14029 (90)	70 (1)	19 (0.3)
Ca	6331 (91)	30493 (220)	838 (11)	65 (2)
Mg	3005 (34)	5022 (24)	212 (3)	15 (0.4)
Fe	14534 (92)	22356 (122)	116 (6)	3.2 (0.1)
Mn	185 (5)	411 (2.2)	47 (1)	0.59 (0.01)
Cu	891 (19)	291 (2.4)	0.8 (0.0)	0.12 (0.003)
Na	269 (6.5)	648 (5.9)	40	27 (0.5)
Ni	20.7 (0.4)	27.5 (0.08)	0.6 (0.5)	0.01 (0.004)
Zn	1073 (27)	993 (1.8)	8.4 (0.4)	0.28 (0.01)
Pb	151 (3)	54 (0.6)	$\leq 3*10^{-3}$	$\leq 3*10^{-3}$
Cd	3.97 (0.07)	1.6 (0.01)	$\leq 3*10^{-4}$	$\leq 3*10^{-4}$
Cr	47.6 (0.8)	127 (0.8)	0.2 (0.0)	$\leq 4*10^{-4}$

3.3 Plants

L. scoparium and K. robusta seedlings were obtained from Waiora Nursery Ltd, Christchurch, NZ (http://www.waioralandscapes.co.nz/pages/nursery/) or seedlings were purchased from Motukarara Native Plant Nursery, Department of Conservation, Canterbury, NZ (http://www.doc.govt.nz/our-work/motukarara-conservation-nursery/). All seedlings are sourced from Canterbury province in NZ. *L. angustifolia* (Lavender Grosso- English lavender hybrid) and *R. officinalis* plants were purchased from Oderings Nursery, Christchurch, NZ (https://www.oderings.co.nz/). *T. vulgaris* seeds were purchased (McGregor's Brand), planted at Lincoln University Nursery and the seedlings were transferred to the pots after four weeks of growth. Before planting all the seedlings, roots were fully washed with tap water to remove the potting mix.

3.4 **Experiment 1:**

Figure 3.2 The pots (small lysimeters) arrangement and automatic irrigation system in Exp. 1.

Pots (25 cm in diameter x 29 cm high) were filled with 2 kg of pea gravel at the bottom (and a fleece sheet on top) to facilitate drainage. Soil (BCL) was filled into pots and packed to an average bulk density of 1.3 g cm^{-3}. The treatments comprised 245 g dry weight KB (1250 kg N ha^{-1} equiv.) and the same rate of biosolids combined with sawdust at a ratio of 1:0.5. The controls received no biosolids or sawdust. *L. scoparium* and *K. robusta* were planted in the pots. Each treatment was replicated four times. Planting occurred in September 2013 and allowed plants to establish for six weeks before applying the treatments on the soil surface. Exp. 1 continued for 18 weeks in the greenhouse. Average night and day temperature during the Exp. 1 in the greenhouse were 14.5 °C and 21°C (minimum 9°C and maximum 28°C). An automatic irrigation system was installed (Figure 3-2), and supplementary manual irrigation occurred as required.

3.5 **Experiment 2:**

Figure 3.3 The small lysimeters (top two) and pots (two below) arrangement and automatic irrigation system in Exp. 2.

Leptospermum scoparium* and *Kunzea robusta

Pots of the same dimensions as Exp. 1 were filled with 2 kg of gravel at the bottom overlain with Eyrewell LSL soil mixed with 1 kg of KB (2800 kg N ha^{-1} equiv.) (Figure 3-3). The DSE treatment comprised the soil and a total of 200 kg N ha^{-1} equiv. The DSE was slowly poured on to the soil surface (3 hours after irrigating the pots) in ten weeks (500 mL per week and 5000 mL in total) from three months after the plants were established. The plan for applying DSE was as below:

1) The first two weeks (from January 12th to 26th) weekdays DSE application of 100 mL to each pot.

2) The next three weeks (from Jan 26th to Feb 16th) the DSE was applied three days a week (Monday, Wednesday and Friday) at rates of 150 mL for two days, and 200 mL for one day.

3) The next month (from Feb 2nd to March 3rd), 250 mL of DSE was applied two days a week (Monday and Friday).

4) In the last week 500 mL of DSE was applied at once.

Control treatments did not receive biowastes. There were four replicates of each treatment. Exp. 2 was conducted for 24 weeks from September 2014. Average night and day temperature during Exp. 2 in the greenhouse were 17°C and 21°C (minimum 9.6°C and maximum 33°C). An automatic irrigation system was used supplemented by manual watering when required.

Lavandula angustifolia, *Rosmarinus officinalis* and *Thymus vulgaris*

Two litre pots of 15 cm diameter and 15 cm height were used for *L. angustifolia*, *R. officinalis* and *T. vulgaris* in Exp.2. Pots were filled with Eyrewell LSL soil (1.35 kg) mixed with 0.15 kg of KB (2800 kg N ha^{-1} equiv.). For DSE and control treatments 1.5 kg of soil was used per pot. The equivalent of 1 % of the soil weight lime was added to the pots (0.015 kg for control and DSE treatments and 0.0135 kg for the biosolids treatments). Pots were irrigated manually once a day to field capacity. DSE application started in January 2015. A total of 2 litres of DSE were applied to the pots in portions of 50 mL (four days a week) for ten weeks. Note that the average temperatures in the greenhouse for Exp. 2 using *L. angustifolia, R. officinalis,* and *T. vulgaris* are different because the experimental periods were different. Details are given below.

L. angustifolia plants were grown for 4 months from November 2014 to February 2015. The data used in the analysis is related to clusters of four weeks (started 10 days after application of DSE). Average night and day temperatures during this experiment in the greenhouse were 17.2°C and 22°C (minimum 9.6°C and maximum 33°C).

R. officinalis plants were grown for 30 weeks from September 2014 to April 2015 and have been cut one time before final harvest. DSE was applied 17 weeks after plantation. The average night and day temperature during this experiment in the greenhouse were 17.2°C and 21.5°C (minimum 9.6°C and maximum 33°C).

T. vulgaris plants were grown for 27 weeks from October 2014 to April 2015. Plants were trimmed one time before final harvest. DSE were applied 14 weeks after plants were cultivated. Average night and day temperature during this experiment in the greenhouse were 17.2°C and 21.5°C (minimum 9.6°C and maximum 33°C).

There were four replications of each treatment for all the plants.

3.6 **Experiment 3:**

Figure 3.4 Pots arrangement and plants used in Exp. 3.

Exp. 3 (Figure 3-4) comprised four plants: *L. scoparium*, *K. robusta*, *L. angustifolia* and *R. officinalis* and five biosolids treatments. Pots (22.5 cm diameter x 17 cm high) were filled with Eyrewell LSL soil. CB were mixed in with the soil at rates of (0, 50, 150, 450 and 1350)*10^{-3} kg pot^{-1}, equivalent to 0, 500, 1500, 4500 and 13500 kg N ha^{-1}. There were five replicates of each treatment. Lime was added to the pots related to *L. angustifolia* and *R. officinalis* at the rate equivalent of 1 % of the soil weight for increasing the soil pH and make the optimum element uptake by the plants.

For *L. scoparium* and *K. robusta,* Exp. 3 was conducted for 16 weeks from October 2015 to February 2016. Average night and day temperature during the experiment in the greenhouse were 17°C and 22.3°C (minimum 9.5°C and maximum 32°C).

For *L. angustifolia* and *R. officinalis,* Exp. 3 was continued from November 2015 to February 2016 for 11 weeks and 15 weeks, respectively. Average night and day temperature during the experiment in the greenhouse for *L. angustifolia* were 17.3°C and 21.7°C (minimum 8.7°C and maximum 43°C) and for *R. officinalis* were 17.4°C and 22.1°C (minimum 8.7°C and maximum 43°C). Pots were irrigated once per day to field capacity.

3.7 **Experiment 4:**

Figure 3.5 Pots arrangement and plants used in Exp. 4.

Pots with the same dimensions as Exp. 3 were filled with PSL soil and the CB were mixed in at rates of 0 and 0.15 kg, equivalent to 0 and 1500 kg N ha^{-1}. *L. scoparium* and *K. robusta* were planted in the pots. There were five replicates of each treatment. Exp. 4 was conducted from November 2015 to February 2016 (12 weeks). Average night and day temperature during Exp. 4 in the greenhouse were 17°C and 22°C (minimum 8.7°C and maximum 43°C). Pots were irrigated once per day to the field capacity.

3.8 **Experiment 5:**

Figure 3.6 Pots arrangement and plants used in Exp. 5.

Exp. 5 (Figure 3-6) used 35, four litre pots (19.5 cm diameter * 19.5 cm height) were used. Exp. 5 had seven treatments stated below replicated five times. Treatments comprised the control, three quantities of CB (ca. 16, 48 and 145 t ha^{-1}) that were applied either on top or mixed with the soil.

1- Control (no biosolids added) ≈ 3.2 kg — 0 kg N ha^{-1} biosolids — of soil only

2- 1.4% biosolids mixed with the soil

3- 1.4% biosolids applied on soil top

630 kg N ha^{-1} biosolids — ≈ 0.045 kg biosolids+ 3.155 kg soil

4- 4.3% biosolids mixed with the soil

5- 4.3% biosolids applied on soil top

1900 kg N ha^{-1} biosolids — ≈ 0.138 kg biosolids+ 3.062 kg soil

6- 12.8% biosolids mixed with the soil

7- 12.8% biosolids applied on soil top

5700 kg N ha^{-1} biosolids — ≈ 0.41 kg biosolids+ 2.79 kg soil

In Exp. 5 only, *L. scoparium* seedlings were planted in the pots. Exp. 5 was conducted for four months and was irrigated daily to field capacity. The average temperature during Exp. 5 was 20.2 °C (maximum 32 °C and minimum 9.8 °C).

3.9 Experiment 6:

Figure 3.1 Rhizoboxes and *L. scoparium* root growth in Exp. 6.

Rhizoboxes were made with a wooden frame and two glass sides (Figure 3-7). The inside dimensions were 80 x 80 x 2.5 cm. These dimensions were selected to have a soil profile with different horizons, which let the plants grow for 4 months (December 2015 to March 2016) period. Three 25 cm horizons were considered on top of a 2 cm gravel (to facilitate the drainage) to fill the rhizoboxes. The rhizoboxes were filled in a horizontal position to maintain a similar bulk density in the whole profile (approximately 0.88 g cm^{-3} for Ah horizon, 1.12 g cm^{-3} for Bw horizon and 1.30 g cm^{-3} for BC horizon). To force the plant roots for growing along one of the transparent sides, rhizoboxes were placed on a 30° angle.

Exp. 6 comprised four treatments stated below replicated three times. In all the biosolids treatments 0.15 kg of CB (4% of the Ah horizon weight) were used.

1- Control- no biosolids added (C)

2- Biosolids applied on top (T)

3- Biosolids mixed homogeneously mixed with the Ah horizon (M)

4- Patch of biosolids concentrated in one third section of the Ah horizon (P)

Table 3-4 shows a summary of the all greenhouse experiments and the treatments applied.

Table 3-4 Summary table of the greenhouse experiments and treatments. KB, DSE and CB represent the Kaikoura Biosolids, Dairy Shed Effluent and Christchurch City Council Biosolids, respectively.

Experiment	Soil	Treatments (kg N ha^{-1})
Exp. 1	Bideford Clay Loam	KB (1250), KB+Sawdust (1250)
Exp. 2	Lismore Stony Silt Loam	KB (2800), DSE (200)
Exp. 3	Lismore Stony Silt Loam	CB (0, 500, 1500, 4500 and 13500)
Exp. 4	Pawson Silt Loam	CB (0 and 1500)
Exp. 5	Craigieburn Silt Loam	CB (0, 630, 1900 and 5700)
Exp. 6	Craigieburn Silt Loam	CB (3000)

3.10 Plant harvest

Fresh samples of EO producing organs (leaves or flowers) from each plant was taken randomly over the plant from young and old parts. Samples were placed in plastic vials and plunged in liquid nitrogen immediately after harvesting and kept at -80°C until solvent extraction of EO.

For all experiments, the aerial parts of the plants were harvested and analysed for biomass and EO quality and quantity at the end of the experiments (periods of the Exp. 1 - Exp. 6 are explained in sections 3.4-3.9). The shoot biomass of the plants was weighted immediately after harvest and washed with deionized water and air dried. Aboveground portions of the plants were oven dried (at 70 °C until a constant weight was obtained) to calculate the moisture content and oven dried equivalent of the plants. Oven dried leaves were used to study the nutrient and TE status.

Field experiment and field survey

3.11 Duvauchelle field study

The field experiment (Exp. 7) was installed on ca. 1000 m^2 of land in July (2015) at Pipers Valley Road, Duvauchelle, NZ (Figure 3.8 and Figure 3.10). The number of 1350 native plants in 27 blocks (5 x 5 m) of three different vegetation types were planted in nine rows (Table 3-5 and Figure 3.9).

Figure 3.8 The field trial in Piper's valley road (1.5 years after planting).

Table 3-5 Three different vegetation types used in the field study at Pipers Valley Road (Exp. 7).

Vegetation type 1		**Vegetation type 2**		**Vegetation type 3**	
mānuka	*Leptospermum scoparium*	Akiraho	*Olearia paniculata*	Kapuka	*Griselinia littoralis*
		Puahou	*Pseudopanax arboreus*	Tarata	*Pittosporum eugenioides*
kānuka	*Kunzea robusta*	Karamu	*Coprosma robusta*	Tī kōuka	*Cordyline australis*
		Hall's tōtara	*Podocarpus cunninghamii*	Harakeke	*Phormium tenax*
				Whaririki	*Phormium colensoi*

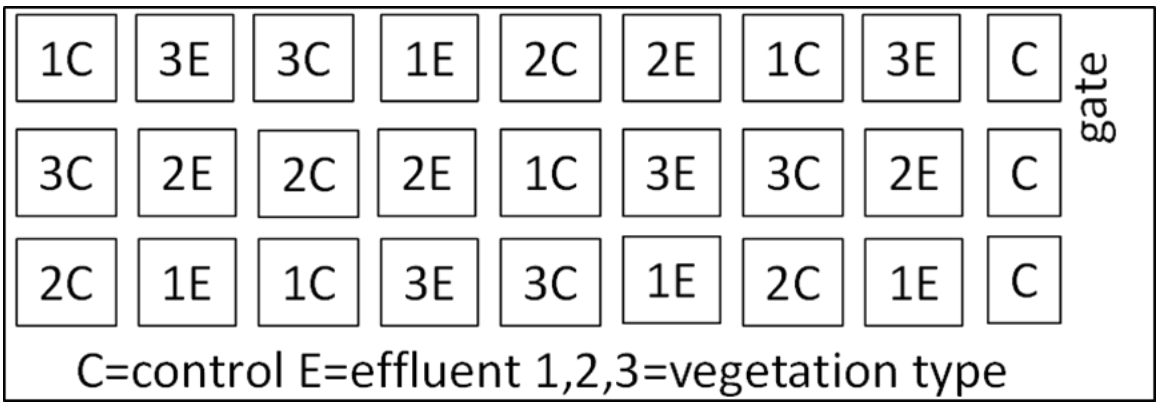


Figure 3.2 The field trial setup in Piper's valley road (Exp. 7).

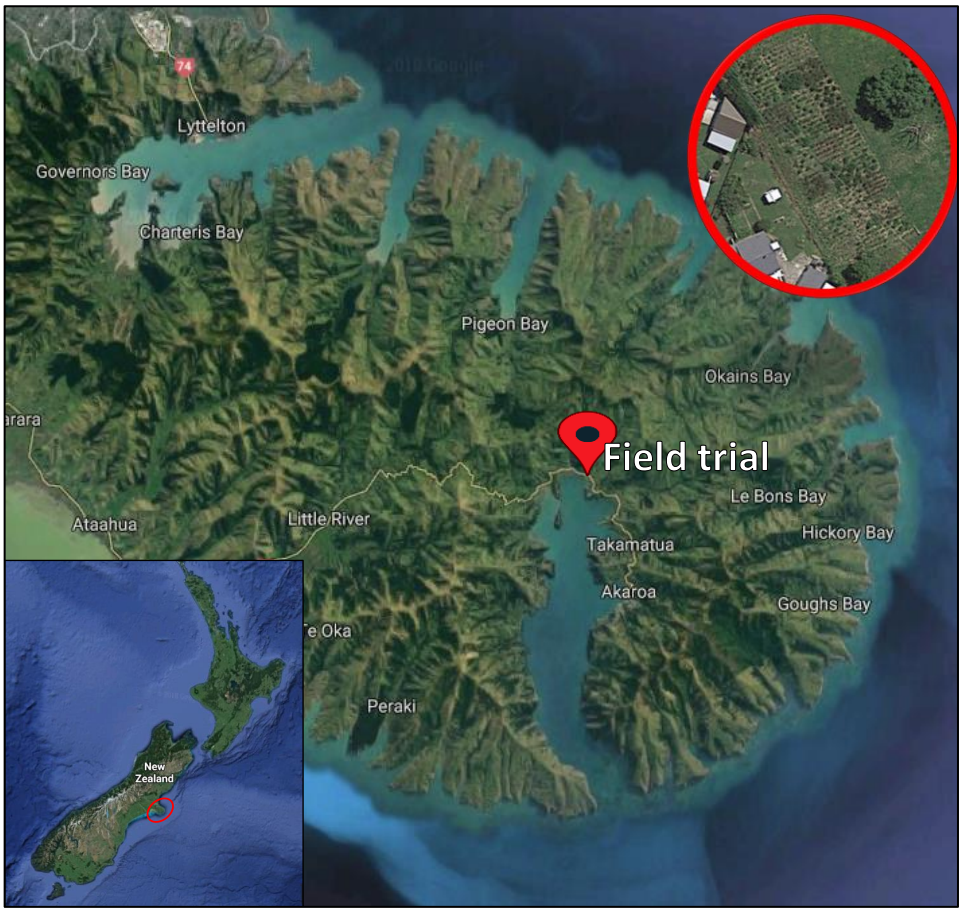


Figure 3.10 Map of the field trial location. Insert is a map showing the site situation within NZ (Google maps).

Note that this thesis only considers the oil producing plants *L. scoparium* and *K. robusta*. Growth and chemical results from the other species can be found in the report of Appendix D (A lysimeter experiment and field trial to determine options for the beneficial reuse of wastewater from Duvauchelle and Akaroa, Banks Peninsula, 2017).

During the growing season (October – April), four rows (12 blocks) received TMW at a rate of 500 mm (30 kg N ha^{-1} equiv.), a similar amount to what is used on an irrigated dairy farm in Canterbury (Robinson et al., 2017). TMW was obtained from the Akaroa Wastewater Treatment Plant, sited about 500 m 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. TMW and the Barry's soil properties are in Table 3-6. The TMW irrigation started in January 2016. The site was keeping tidy using a lawnmower during the experiment.

Soil and plant samples was taken for chemical analysis. In June 2017, all areas within the plot that were not under native vegetation were planted with silver tussock (*Poa cita*) to prevent weeds growth. The plants were monitored and recorded for existence in May 2017.

Table 3-6 Characteristics of the Treated Municipal Wastewater and soil used in Exp. 7. Values in brackets represent the standard deviation of the mean (*geometric mean and standard deviation range). n=54 except trace elements n=9.

	Treated Municipal Wastewater	Barry's soil (Duvauchelle)
pH	7.5	5.2
EC (uS cm^{-1})	423 (40)	-
Total suspended solids (g $m3^{-1}$)	32	-
NH_4^+-N (mg L^{-1})	0.49 (0.15 – 0.80)*	10.1 (7.5)
NO_3^--N (mg L^{-1})	18 (7.5)	17.1 (13.2)
NO_2^--N (mg L^{-1})	0.86 (0.09)	-
Total C (%)	-	4.4 (0.6)
Total N (%)	<25	0.38 (0.05)
Al (mg L^{-1})	0.43 (0.11 – 1.7)*	32731 (1418)
B (mg L^{-1})	0.10 (0.04)	-
Ca (mg L^{-1})	59 (12)	6770 (393)
Cd (mg L^{-1})	<0.001	-
Cu (mg L^{-1})	0.04 (0.03)	7.7 (0.2)
Fe (mg L^{-1})	0.96 (0.25 – 3.6)*	20155 (2852)
K (mg L^{-1})	22 (5.0)	4491 (346)
Mg (mg L^{-1})	19 (5.5)	4251 (76)
Mn (mg L^{-1})	0.06 (0.03)	624 (9)
Na (mg L^{-1})	95 (21)	290 (10)
P (mg L^{-1})	11 (5.0)	1046 (30)
S (mg L^{-1})	25 (11)	490 (21)
Zn (mg L^{-1})	0.17 (0.11)	68 (3)
Sodium Accumulation Ratio (SAR)	15 (2.6)	-

In February 2017, *L. scoparium* and kānuka plant samples were taken from the plants of the "vegetation type 1" blocks. Plants were labelled and kept in paper envelopes and carried to the laboratory. Subsamples were oven-dried for at 70°C until a constant weight was obtained (c.a. six days). The remainder of the samples were saved in the sealed polythene sacks to prevent absorption of moisture from the air and transferred to -80 °C freezer until the solvent extraction of the EO.

3.12 Field survey

For the field survey, naturally occurring *L. scoparium* and *K. robusta* plants and underlying soil samples were taken from contrasting natural habitats during the summer time (growing season) (Figure 3.11). Plant samples (leaves on the stems) were collected from the young and old parts of the plants and stored in the paper bags for transport to the laboratory. Soil samples were taken from the top 15 cm under the plants, after removing the surface foliage. Upon returning to the laboratory (a maximum 6 hours after plants were sampled), they were washed with DI water, then kept frozen in plastic bags in a -80°C freezer until the solvent extraction of the EO. Subsamples were oven dried for the elemental analysis. Subsamples of the soils were kept frozen to evaluate the plant available elements. Table 3-7 shows the results of chemical analysis of soils from the sampling sites.

Figure 3.11 Sampling natural populations of *L. scoparium* and *K. robusta*. Nikau Palm Gully (the top two) and Quail Island (the two below).

The sampling sites (Figure 3.12) were as below:

- Nikau Palm Gully (43°51’30”S 172°57’00”E),
- Quail Island (43°37'48.00" S 172°41'24.00" E),
- Bridle Path at Lyttleton (43° 36' 11.7216'' S, 172° 43' 9.5880'' E)
- Yarrs Flat, off Goodrick’s road (43°41'1.95"S 172°27'4.34"E)

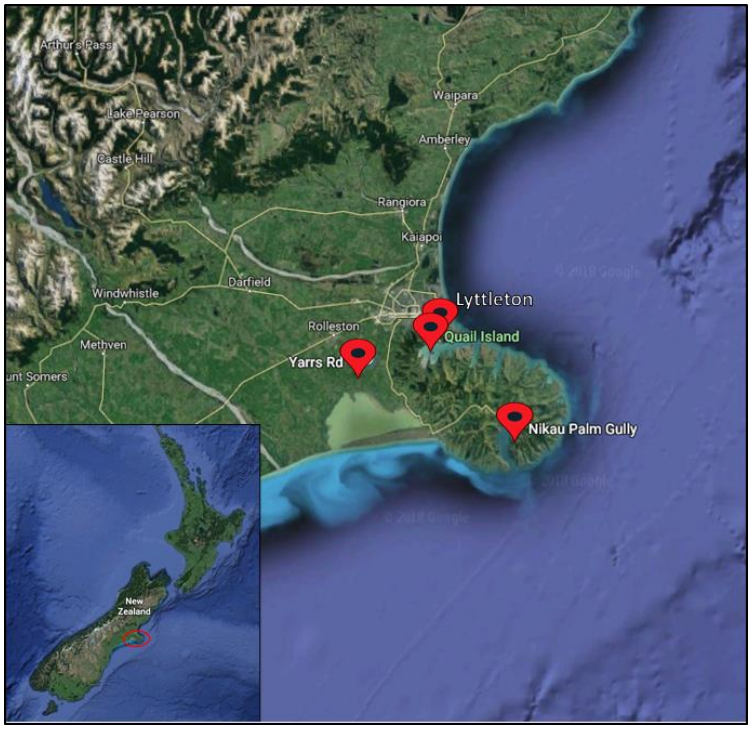


Figure 3.3 Map of the sampling sites of *L. scoparium* and *K. robusta* natural populations in South Island. Insert is a map showing the site location within NZ (google map).

Table 3-7 Soil properties of field sampled *L. scoparium* and *K. robusta*. Units are mg kg^{-1} unless otherwise indicated. Values in brackets represent the standard error of the mean (5>n<7). FW represents the Fresh Weight.

Specie	***L. scoparium***			***K. robusta***		
	Nikau Gully	Quail Island	Yarrs Flat	Nikau Gully	Quail Island	Bridle Path
N (%)	0.25 (0.03)	0.5 (0.03)	2.1 (0.1)	0.25 (0.03)	0.37 (0.01)	0.28 (0.04)
C (%)	4.1 (0.7)	6.6 (0.4)	40 (1.0)	4.1 (0.7)	4.4(0.16)	3.6 (0.54)
NH^{+}_{4}-N (mg kg^{-1} FW)	1.35 (0.24)	1.53 (0.13)	1.2 (0.11)	1.3 (0.2)	1.2 (0.1)	1.0 (0.2)
NO^{-}_{3}-N (mg kg^{-1} FW)	0.05 (0.00)	0.36 (0.1)	0.08 (0.02)	0.1 (0.0)	0.4 (0.1)	0.3 (0.1)
Olsen P	6.9 (2.5)	21.7 (1.6)	50.2 (12)	6.9 (2.5)	10.5 (0.5)	26.7 (6.4)
P	481 (41)	873 (66)	873 (66)	481 (41)	741 (23)	907 (131)
Al	22087 (719)	22302 (1583)	2577 (461)	22087 (719)	20359 (246)	18476 (1765)
As	2.0 (0.16)	1.8 (0.18)	1.8 (0.14)	2.0 (0.2)	2.1 (0.1)	2.7 (0.4)
B	2.5 (1.6)	5.0 (0.7)	14.3 (2.4)	1.1 (0.8)	5.9 (0.2)	9.0 (0.9)
Ca	4830 (354)	5187 (460)	19401 (1187)	4830 (354)	5311 (159)	7726 (129)
Cd	<0.01	<0.01	<0.01	<0.01	0.01 (0.0)	0.04 (0.0)
Cr	14.0 (0.6)	34.2 (3.3)	7.0 (1.4)	14.0 (0.6)	22.5 (0.1)	13.5 (1.1)
Cu	4.6 (0.21)	15.8 (1.07)	3.2 (0.25)	4.6 (0.2)	16.6 (0.9)	14.0 (1.2)
Fe	17015 (1148)	24436 (1933)	8503 (1120)	17015 (1148)	20155 (564)	21410 (2149)
K	2672 (76)	2670 (140)	889 (77)	2672 (76)	2556 (54)	3230 (328)
Mg	3235 (146)	2621 (93)	1721 (171)	3235 (146)	2838 (44)	3335 (65)
Mn	486 (59)	852 (75)	285 (131)	486 (59)	710 (37)	497 (95)
Na	319 (44)	258 (18)	780 (123)	319 (44)	301(10)	561 (84)
Ni	13.6 (5.5)	23 (1.3)	2.6 (0.94)	13.6 (5.5)	19.4 (1.1)	8.5 (0.5)
Pb	8.5 (0.4)	14.0 (0.67)	15.1 (4.8)	8.5 (0.4)	12.5 (1.0)	14.0 (0.5)
S	344 (52)	606 (29)	4326 (577)	344 (52)	446 (16)	340 (52)
Zn	39.3 (1.8)	99.4 (3.0)	3.69 (0.45)	39.3 (1.8)	92.2 (2.3)	73.7 (7.7)

Chemical analyses

3.13 Essential oil extraction and GC-MS analysis

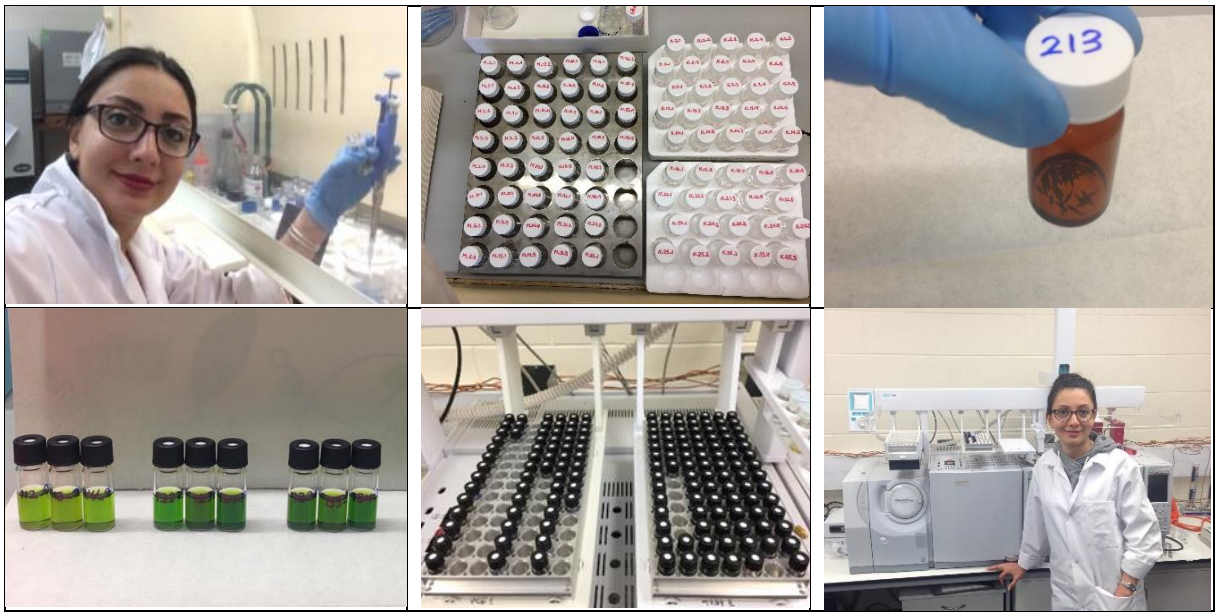


Figure 3.4 The procedure of essential oil solvent extraction and analysis by CG/MS.

Precisely 0.1 g of fresh sample (leaves for *Leptospermum scoparium*, *Kunzea robusta*, *Rosmarinus officinalis* and *Thymus vulgaris*- flowers for *Lavandula angustifolia*) was soaked in 2 mL of the solvent in glass vials at room temperature. The solvent and soaking time used for different plants were as below:

L. scoparium and *K. robusta*: ethanol + dichloromethane (1:1) for 18-20 hours (Senanayake, 2006b).
L. angustifolia: hexane for 19 hours
R. officinalis: hexane+ ethanol (9:1) for three hours
T. vulgaris: hexane+ ethanol (9:1) for 18 hours[1]

After soaking the samples, one mL of the extracts was transferred to GC vials and 100 µL internal standard (eicosane- C20- 125 mg L^{-1}) was added. The solvent extracts were analysed using the GC/MS (Figure 3.13).

L. scoparium and *K. robusta* samples were analysed and interpreted according to commonly found components detected in studies on the plant EOs throughout NZ (Porter and Wilkins, 1999, Maddocks-Jennings et al., 2005b). *L. angustifolia, R.s officinalis* and *T.s vulgaris* main EO components were

[1] Methods development is described in Appendix A.

analysed using previous researches on the plants (Sahraoui et al., 2008, da Silva Bomfim et al., 2015, Baranauskienė et al., 2003).

The analysis of volatile organic compounds (VOCs) from EO plant extracts was performed by GC/MS and followed the method described by (Brophy et al., 1989) for *L. scoparium* and *K. robusta*. A Shimadzu QP2010 Ultra (GC/MS) fitted with a Restek RTX-5ms[1] capillary column (30m x 0.25mm i.d x 0.25 µm film thickness) was used to provide chromatographic separation, with the carrier gas set to a constant linear velocity of 44.3 cm sec^{-1}. A CTC[2]-Combipal autosampler was used to inject 0.5 µL of sample extracts into the injection port operated in splitless high-pressure injection mode (168 kPa) at a temperature of 250 °C for 40 seconds. The GC column oven was set to an initial temperature of 45.0 °C and held for 1.33 minutes before being ramped to 65.0 °C at 10.0 °C min^{-1} with a final ramp to 285.0 °C at 6.0 °C min^{-1} held for 10 minutes to release high boiling point components such as flavonoids and wax hydrocarbons.

The mass spectrometer was operated in electron impact mode (EI) at an ionization energy of 70 eV and a mass scanning range of 33.0 to 500 m/z. The ion source and interface temperatures were set to 200 °C and 280 °C respectively. Compounds were identified by comparing acquired mass spectral data with those held in NIST11 and Wiley10 mass spectral libraries and confirmed using published linear retention indices and the retention times of purchased standards. Mass spectral data for *L. scoparium* and *K. robusta* leaf extracts characterized by Porter and Wilkins (1999), was used to help identify triketones and nor-triketones. Compounds were tentatively quantified by comparing the amount of each compound identified to that of the internal standard added to each sample extract.

Shimadzu software GCMS solution version 2.72 was used to both acquire and process the chromatographic data. The chemotypes of the *L. scoparium* and *K. robusta* plants were identified based on Douglas et al. (2004). The total concentrations of EO components were calculated based on retention times from a study at Waikato University (Senanayake, 2006b), the components that eluted before internal standard (eicosane- C20) were considered as volatile EO constituents.

[1] Rtx-5ms is the code name for the GC column. Restek is the company that make the GC columns and they have two types labelled RTX (standard) and RXI (premium).

[2] CTC Combi Pal auto sampler refers to an auto sampler known as the Combi Pal made by a company in Switzerland called CTC Analytics AG.

For *L. angustifolia*, *R. officinalis* and *T. vulgaris* the chromatography settings were as those used for the *L. scoparium* and *K. robusta* samples. The only difference was the solvent used for extraction (mentioned above) and the different injection volume, namely 1 µL rather than 0.5 µL. Appendix A provides the complete GC/MS setting for *L. angustifolia*, *R. officinalis* and *T. vulgaris*.

To evaluate the EOs of *L. angustifolia*, *R. officinalis* and *T. vulgaris*, standard commercial EOs were analysed by GC/MS. The chromatographs (Figure 3.14) showed that the EO components of these plants elute early from the column. Therefore, same calculation of the *L. scoparium* and *K. robusta* plants has been used (all components that were eluted before internal standard have been considered as the volatile EO components).

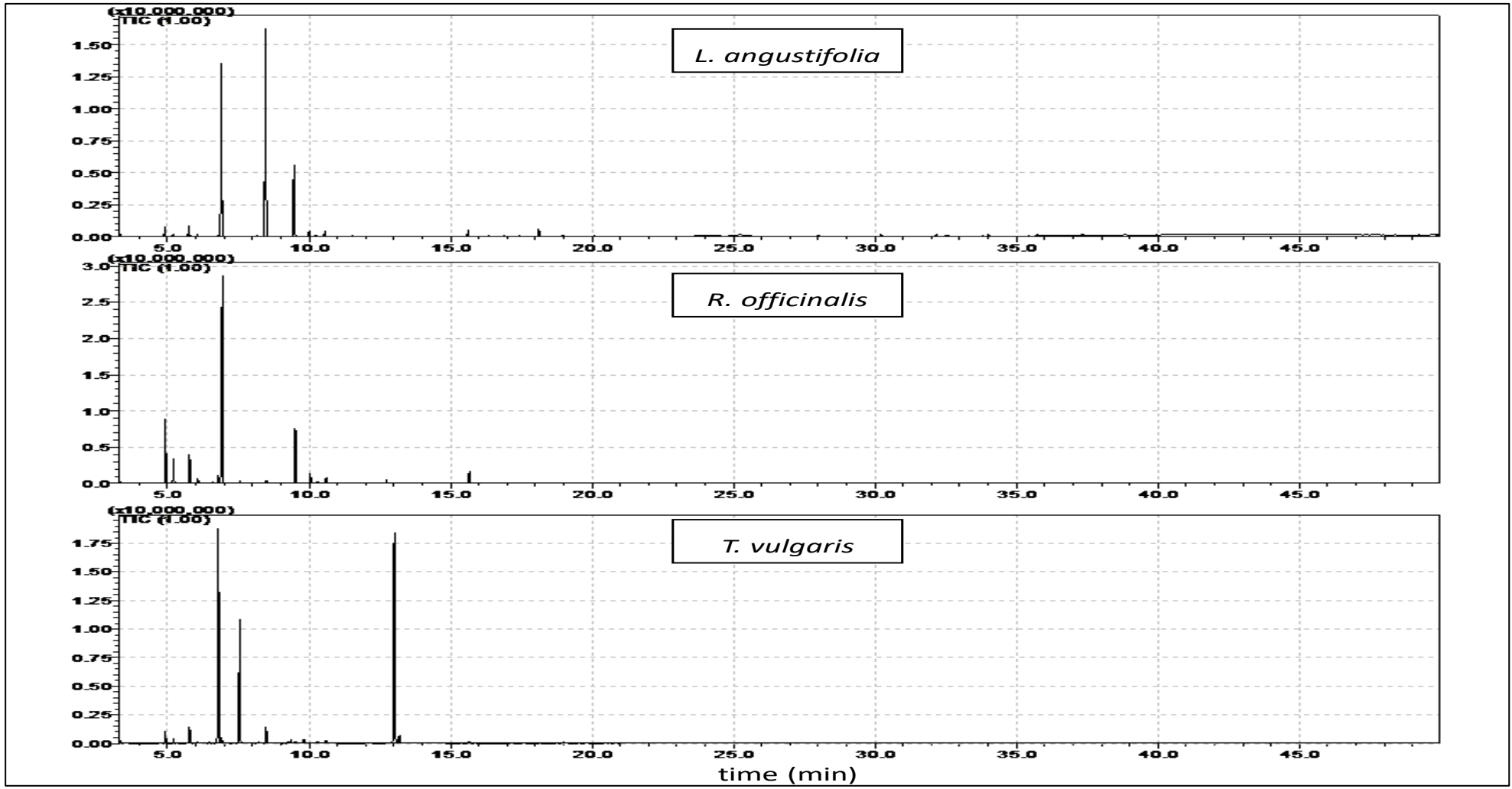


Figure 3.5 Chromatograms of commercial *L. angustifolia*, *R. officinalis* and *T. vulgaris*.

3.14 Essential oil calculations

3.14.1 Essential oil concentrations

The most important and commonly found components of the EOs were obtained from the literature (Maddocks-Jennings et al., 2005b, Douglas et al., 2004, Verma et al., 2010, Wang et al., 2008, Lee et al., 2005). These compounds are termed "evaluated components". In Exp. 1 - 8 not all of these components identified from the literature were above detection limits (ca. 0.03 mg L^{-1} in the solvent extract). For each component, I have only reported values that were above detection limits.

The total concentrations of EOs were calculated and stated on a fresh matter basis because the solvent extraction required fresh material to be used. On average, dry matter content was 30%, with some variations. There were two units used for the EO concentrations:

1- Total concentrations of the EO were calculated based on the weight of EO producing organ (mg g^{-1}). This value can be used to calculate the total oil yield of the plant, but does not indicate the quality of the oil.

2- Concentrations of the individual EO components were calculated based on the concentration in the solvent extract (mg L^{-1} in the solvent extract). This value indicates how the biowastes treatments changed the oil quality.

3- For the EO components that comprised a major fraction of the EO, their concentrations are expressed as a percentage of the EO. For *L. angustifolia, R. officinalis,* and *T. vulgaris*, the quality of the EO is mostly dependent on these major components, rather than compounds present in trace concentrations (Lis-Balchin and Hart, 1999, Jiang et al., 2011, Bagamboula et al., 2004), although the minor components are critical to the synergistic activity of the oil (Burt, 2004). Given the limited knowledge on factors affecting EO quality of *L. scoparium* and *K. robusta*, this assumption may not be valid. Before this information is available, it makes little sense to conduct an exhaustive comparison of components that occur at only trace concentrations. Nevertheless, I have included these concentrations in Appendix C.

3.14.2 Oil production

The EO extraction was performed by using the fresh plant material. Therefore, in EO production calculations fresh weight of the plant material was used:

$$essential\ oil\ production = FW\ (g) \times Concentration\ of\ the\ oil\ (\frac{mg}{g})$$

Where: FW is the fresh weight of the plant EO producing organ.

Evaluation of the *L. scoparium* and *K. robusta* plants samples showed that the EO-bearing leaves comprised ca. 40% of the plant biomass. This figure was used when calculating EO production. By using the pots or rhizoboxes surface area, further calculations have been done that resulted the kg ha^{-1} equiv. of the EO production.

3.15 Soil and plants analyses

Soil samples were crushed by ceramic mortar and pestle and sieved using a 2 mm Nylon sieve. Soils were air-dried for further analysis.

3.15.1 Moisture content

Soils were weighed and oven-dried at 105°C in the metal containers, until reaching a constant weight. After cooling down, the soils were re-weighed. The gravimetric moisture content (g H_2O/g dry material) was calculated for further evaluations.

3.15.2 pH

The soil pH was measured using the method of by Rayment and Lyons (2011) and Blackmore et al. (1987). To evaluate the pH, (10±0.05) x 10^{-3} kg of air-dried samples was mixed with 25 mL DIW, stirred and left to stabilise for 24 hrs. The pH meter (Mettler Toledo, Columbus, OH, USA), was calibrated by buffers of pH 4 and pH 7, then the samples pH was measured.

3.15.3 Electrical Conductivity (EC)

For reading the EC the method of Rayment and Lyons (2011) and Blackmore et al. (1987) was used. The quantity of (10±0.05) x 10^{-3} kg of air-dried soil samples was mixed with 50 mL DIW in the centrifuge tube. The samples were mixed using an end over end shaker for half an hour. Samples were left for half an hour to allow the soil settle. The conductivity meter was calibrated by 1413 µS cm^{-1} standard and EC was recorded using the EC meter.

3.15.4 Cation Exchange Capacity (CEC)

CEC were measured using the method described by Rayment and Lyons (2011) and Blackmore et al. (1987). Silver thiourea (AgTU) 0.01 M reagent was prepared by dissolving 75 x 10^{-3} kg thiourea in 1.5 L of de-ionized water in a 5000 mL volumetric flask. A magnetic stirrer used for mixing. Silver nitrate (8.49 x 10^{-3} kg) was dissolved in 2500 mL of de-ionized water. The silver nitrate solution was then slowly added to the thiourea solution and made up to a final volume of 5000 mL.

Standards were prepared in five 100 mL volumetric flasks as below:

1. 100 mL thiourea,
2. 25 mL AgTU and 75 mL thiourea,
3. 50 mL AgTU and 50 mL thiourea,
4. 75 mL AgTU and 25 mL thiourea,
5. 100 mL AgTU.

These standards corresponded to 0, 0.25 × 10^{-2} M, 0.50 × 10^{-2} M, 0.75 × 10^{-2} M, and 1.0 × 10^{-2} M AgTU. Subsamples of soils were weighted to calculate the moisture content. Air dried soil samples were weighed (0.7 x 10^{-3} kg) into 50 mL centrifuge tubes, 35 mL of 0.01 M AgTU was added and the samples were shaken on an end-over-end shaker for 16 hr. Samples were then centrifuged at 3000 rpm for 10 min and filtered through Whatman 40 filter paper and collected in plastic vials. These samples were analysed for Ag, Mg, Ca, K, Al, Mn and Na on an Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES Varian 720 ES-USA).

Calculating CEC

$$\boldsymbol{CEC_{AgUT}\left(\frac{cmolc}{kg}\right) = \left(1 - ([Ag^{+}] \times 10^{-2})\right) \times 50}$$

Where:

[Ag^{+}] = the Ag^{+} from the ICP-OES expressed in molarity

50= dilution factor

3.15.5 Elemental analysis

After harvesting, the plants were weighted and washed with Deionised Water. Partials of the plants were dried at 70° C until a constant weight was reached. To analyse the chemical elements in the plant, the oven dried above ground parts were ground by a Retch ZM200 grinder.

Plants elements (Cd, B, Ca, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, P, S and Zn) concentrations were determined in the acid digests using ICP-OES. A microwave digestion (MARSXPRESS, CEM Corporation, USA) of 0.5*10^{-3} kg of sample in 8 mL of AristarTM nitric acid (± 69%) were used and filtered by filter paper (Whatman no. 52- pore size 7 µm) after dilution with milliQ water (double de-ionised water) to a volume of 10 mL. 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 detection limit

(Appendix B) of the ICP-OES for all TEs. Wageningen (ISE 921, IPE 100) and NIST (1573a) Certified Reference Materials were analysed in the same sample sets. Recoveries ranged from 91 – 112%.

The C and N content of plant material was determined using an Elementar Vario MAX CN element analyzer (Elementar GmbH, Hanau, Germany). The samples were ignited at 900°C in an oxygen atmosphere. The ignition process transformed any elemental C and N into CO_2, N_2 and NO_x. The NO_x was then reduced to N_2. After that, these gases were passed through a thermal conductivity cell and the concentrations of CO_2 and N_2 were evaluated. The percentage of C and N were calculated from the initial sample weights ignited.

Elemental analysis of the soil was conducted using ICP-OES (Varian 720 ES-USA) and an Elementar Vario MAX CN element analyzer (Elementar GmbH, Hanau, Germany).

3.15.6 Extractable inorganic- N species (NH_4^+-N and NO_3^--N) from the soil and biosolids

Soil nitrate (NO_3^-) and ammonium (NH_4^+), the mineral nitrogen concentrations, were determined using a KCl extraction from frozen soil following the method of (Blackmore et al., 1987). Forty mL of 2 M KCl was added to 4 g of soil and the solution was shaken on an end-over-end shaker for 60 min, centrifuged at 827 g for 10 min and subsequently filtered through pre-leached Whatman 41 filter paper. A flow injection analyser (FIA FS3000 twin channel analyser, Alpkem, USA) was used to determine NO_3^- and NH_4^+. Oven dried samples were milled using a Cyclotech type 1093 cyclone grinder with an aluminium rotor. Plant material (0.5 g) was digested in 5 mL HNO_3. The digests were diluted with Milli Q (Barnstead, EASYpure RF, 18.3 MΩ-cm) to a volume of 25 mL and filtered with a Whatman 52 filter paper (pore size 7 µm).

3.15.7 Bicarbonate (Olsen) extractable P (plant available or soluble P)

The air-dried, ground and sieved (0.2 cm) soil samples were used to analyse the plant available P Olsen P method (Watanabe and Olsen, 1965). The samples (10^{-3} kg) was shaken for 30 min (using an end over end shaker) with 20 mL 0.5 M sodium bicarbonate (NaHCO3). The extractant was centrifuged (10 min at 2000 rpm) and then filtered through Whatman No. 42 filter paper. Five mL of Murphy Riley colour reagent (Murphy and Riley, 1962) was added to the filtrate (10 mL) and the sample was made up to 50 mL with DIW. After half an hour the absorbance was measured and read at 880 nm on a Shimadzu UV mini-1240 spectrophotometer.

Calculating extractable P

$$P\left(\frac{mg}{kg}\right) = \frac{\left(\frac{mg}{mL} - blank\right) \times 5}{Weight\ (g)}$$

3.16 **Statistical analysis**

Excel (©2016) was used to tabulate the data and calculate basic statistics.

Minitab® 16 (Minitab Inc., State College, Pennsylvania, USA) was used for ANOVA analysis and Fisher's Least-Significant-Difference ($p < 0.05$) as a post-hoc test to compare means. One-way ANOVA was used to investigate the effect of treatments on the plants' biomass, EO production and quality, individual EO components as well as the elements concentrations. Data were tested for normality and non-normally distributed data were log-transformed to meet the assumption of normality[1]. Where appropriate, the statistics of log-normal data were reported as geometric means and standard deviation ranges.

Correlation analyses

NS *p>0.05* not significant

S $0.05 > p > 0.01$ significant

S* $0.01 > p > 0.001$ highly significant

S** $p<0.001$ very highly significant

[1] The data for essential oil components concentrations were transformed using Johnson transformation.

SECTION III

Results and Discussion

Chapter 4

Effect of the biowastes on biomass of the plants

4.1 Introduction

Biowastes contain organic matter and valuable plant nutrients that potentially could be applied to degraded or low-fertility lands to enhance the physical, chemical and biological conditions of the soil and increase the growth and biomass of the plants (Hue and Sobieszczyk, 1999). Biowastes have been demonstrated to increase the growth of some EO producing species including *Leptospermum scoparium, Kunzea robusta*, *Lavandula angustifolia, Rosemarinus officinalis* and *Thymus vulgaris* (Esperschuetz et al., 2017, Agulló et al., 2011, Yadegari and Mosadeghzad, 2012, Sakr, 2017).

In the greenhouse experiments (Exp. 1– Exp. 6) of this thesis, the aboveground biomass of the plants (whole plant biomass for *L. scoparium, K. robusta*, *R. officinalis*, *T. vulgaris* and clusters of *L. angustifolia*) at harvest ranged from 0.9 – 205 g DM (Figure 4.1- Figure 4.7). In general, the N-containing biowastes (CB, KB, DSE and TMW) significantly increased the growth of *L. scoparium*, *K. robusta*, *L. angustifolia, R. officinalis* and *T. vulgaris*. The growth increase was greatest in the CSL (380-2765% increase of the *L. scoparium* growth), which was the lowest fertility soil. Figure 4.1, Figure 4.2 and Figure 4.6 show representative examples of the plants in Exp. 1– Exp. 6. Note that all biomass data is presented on a dry matter basis.

4.2 Mānuka (*Leptospermum scoparium* J.R. Forst & G. Forst) and kānuka (*Kunzea robusta* de Lange & Toelken.)

Applying biosolids (KB or CB) to the BCL, LSL and PSL significantly increased the dry biomass of *L. scoparium* and *K. robusta* by up to 120% and 170%, respectively (Figure 4.3). In the CSL, which had the lowest C and N content (Table 3-1 and Table 3-2), the application of the CB increased the biomass of *L. scoparium* by 2765% (Figure 4.4 F). The relative biomass increase of each species upon the addition of biowastes was inversely proportional to the soil fertility. *L. scoparium* had a larger biomass increase in the CSL (Exp. 6) *K. robusta* had greater biomass increase in the PSL (Exp. 4). The CSL and PSL had lower fertility (in terms of C and N) than the other soils in these experiments.

Up to a rate of 1500 kg N ha^{-1} equiv., the biomass increase of *L. scoparium* was proportionate to the CB application rate. Higher application rates resulted in progressively lower biomass increases or even decreases compared to the control. The application CB at 13500 kg N ha^{-1} equiv. biosolids killed the plants (Figure 4.1 C).

Figure 4.3 shows the effect of contrasting biowastes and application rates on the biomass of the *L. scoparium* and *K. robusta* plants in the BCL, LSL, and PSL (Exp. 1- Exp. 4). In general, biosolids resulted in a larger biomass increase compared to the other biowastes used. Blending biosolids with sawdust offset some of the biomass increase in *L. scoparium* but not *K.* robusta (Figure 4.3 A). In the LSL (Exp. 2), Dairy Shed Effluent (DSE) application increased the biomass of *L. scoparium* but not *K. robusta* (Figure 4.3 B).

Figure 4.4 shows the effect of different rates and methods of CB application on *L. scoparium* biomass in Exp. 5 and Exp. 6. There was no growth increase difference between *L. scoparium* when same rate of CB was surface-applied, mixed or used as patches in these experiments.

In the field experiments (Exp. 7), the growth of TMW (30 kg N ha^{-1} equiv.) irrigated plants was the same or greater than the unirrigated trees (Figure 4.5). The canopy volume of *L. scoparium* increased from 0.15 m^3 to 0.25 m^3 by TMW application, while *K. robusta* canopy volume did not increase. There were no signs of toxicity. As the field experiment is ongoing at the time of writing, the crown volume was used as a proxy for biomass.

Figure 4.1 Effect of different biowastes and application rates on *L. scoparium* biomass in Exp. 1-Exp. 4 (A-D, respectively). The numbers in the grey boxes show the concentration of N kg ha^{-1} equiv. of the treatments.

Figure 4.2 Effect of different biosolids application rates on *L. scoparium* biomass in Exp. 5 either surface-applied or mixed with the soil. The numbers in the grey boxes show the concentration of N kg ha^{-1} equiv. applied in the treatments.

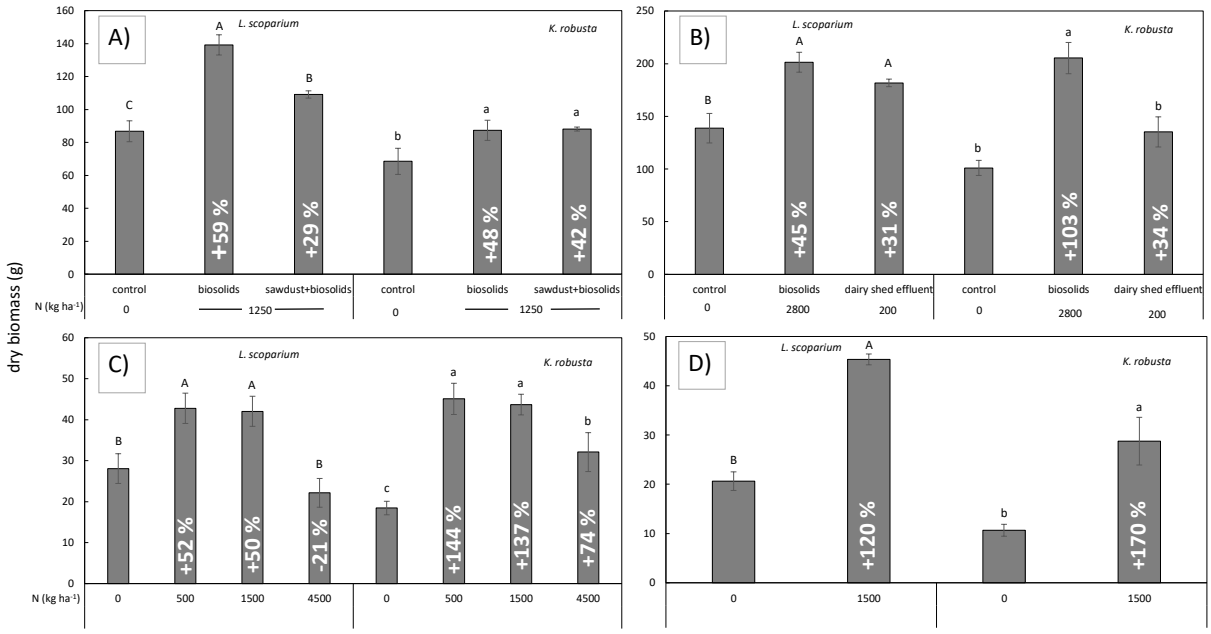


Figure 4.3: Aboveground biomass (g DW) of *L. scoparium* and *K. robusta* in A- Exp. 1 (n=4 ± se), B- Exp. 2 (n=3 ± se), C- Exp. 3 (n=5 ± se) and D- Exp. 4 (n=5 ± se). Significant differences between the treatments at p ≤ 0.05 are indicated by capital letters (A, B, C) for *L. scoparium* and lower-case letters (a, b, c) for *K. robusta* within the plant species. Exp. 1- Exp. 4 continued for 18, 24, 16 and 12 weeks, respectively. Numbers in the bars represent the percentage of changes caused by the treatments compared to the control.

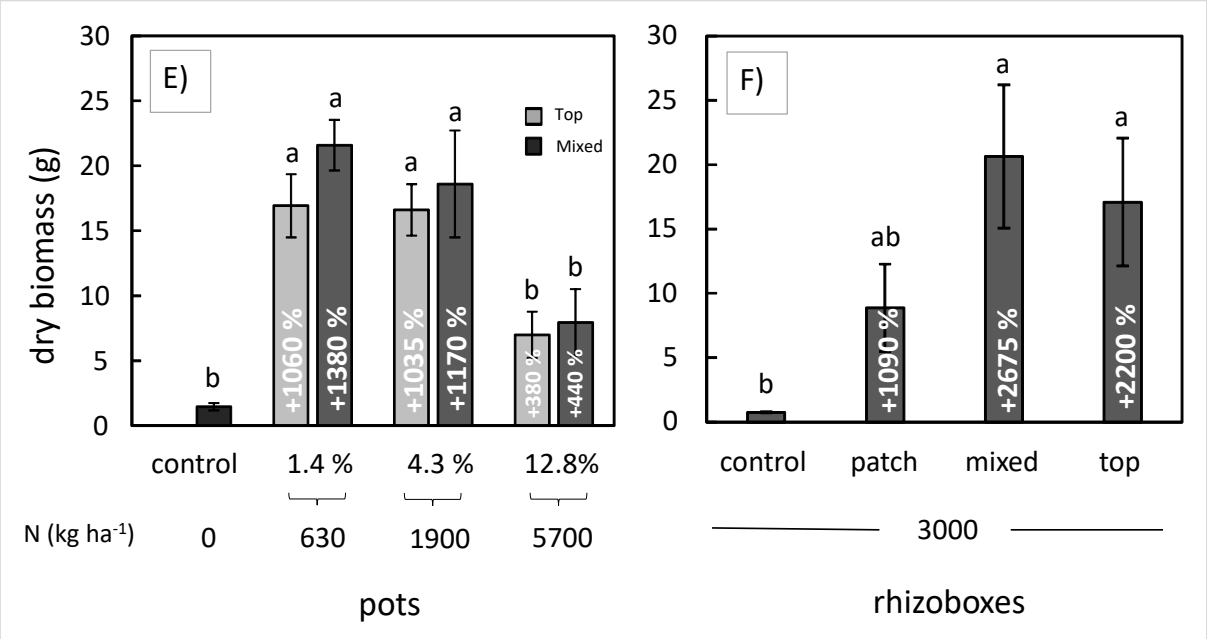


Figure 4.4: Aboveground biomass (g DW) of *L. scoparium* in E- Exp. 5 (pots, n=5 ± se) and F- Exp. 6 (rhizoboxes, n=3 ± se). Different letters (a, b, c) indicate significant differences between the treatments at p ≤ 0.05. Exp. 5 & 6 continued for 16 weeks. Numbers in the bars represent the percentage of changes caused by the treatments compared to the control.

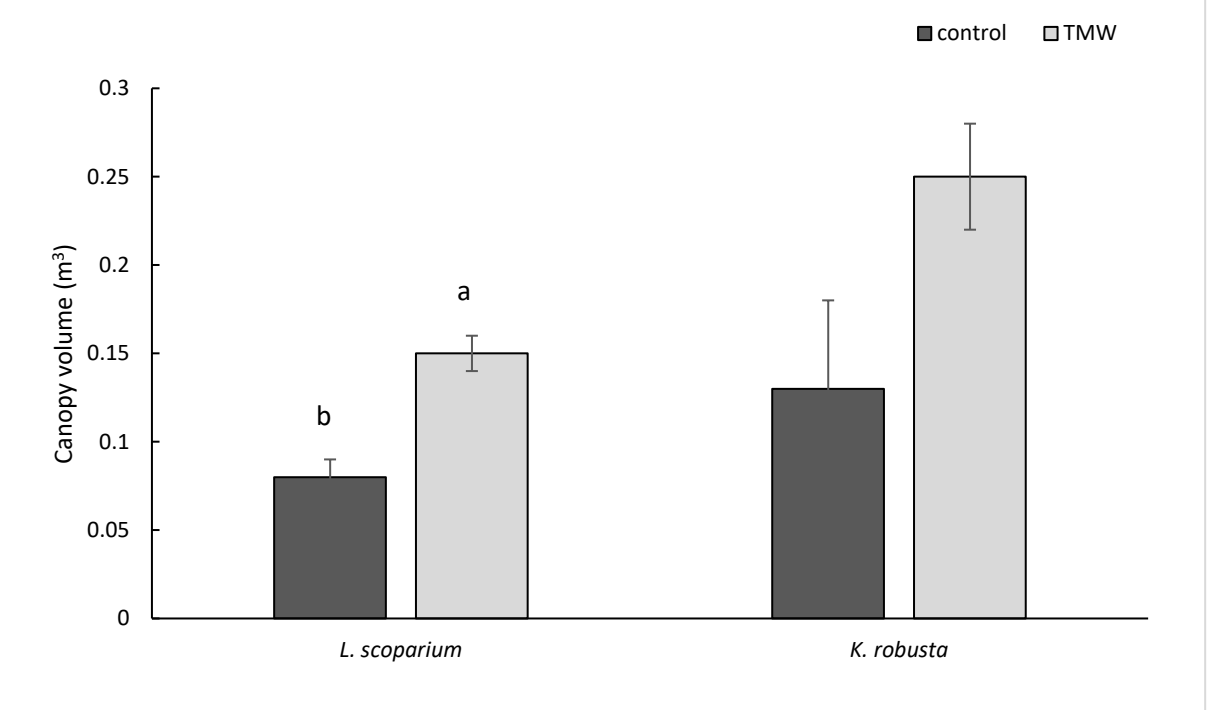


Figure 4.5 Canopy volume of the plants in the field plot on Pipers Valley Road as of May 2017 (Exp. 7). Different letters (a, b, c) indicate significant differences between the treatments at p ≤ 0.05. Error bars represent the standard error of the mean of three plots, with each plot containing 5 – 25 plants. TMW=Treated Municipal Wastewater.

4.3 Lavender (*Lavandula angustifolia* Mill.), rosemary (*Rosmarinus officinalis* L.) and thyme (*Thymus vulgaris* L.)

Biosolids (CB) application of up to rate of 1500 kg N ha^{-1} equiv. significantly increased the dry biomass of *L. angustifolia* and *R. officinalis* by 86% and 70%, respectively and applying KB at the rate of 2800 kg N ha^{-1} equiv. increased the biomass of *T. vulgaris* by 62% in the LSL (Exp. 2 and Exp. 3) (Figure 4.6 and Figure 4.7). The maximum biomass increase for *L. angustifolia* clusters occurred when the CB (1500 kg N ha^{-1} equiv.) were added to the LSL. As with the other species, higher rates of application did not significantly increase growth and the highest rate of application (13500 kg N ha^{-1} equiv.) killed the plants (Figure 4.7). The KB application of 2800 kg N ha^{-1} equiv. to the LSL significantly increased the dry biomass of *L. angustifolia* by 60% (Figure 4.7). The application of 200 kg N ha^{-1} equiv. of DSE to the LSL increased the growth of *R. officinalis* by 60% compared to the control. However, DSE application did not increase the biomass of the other two species.

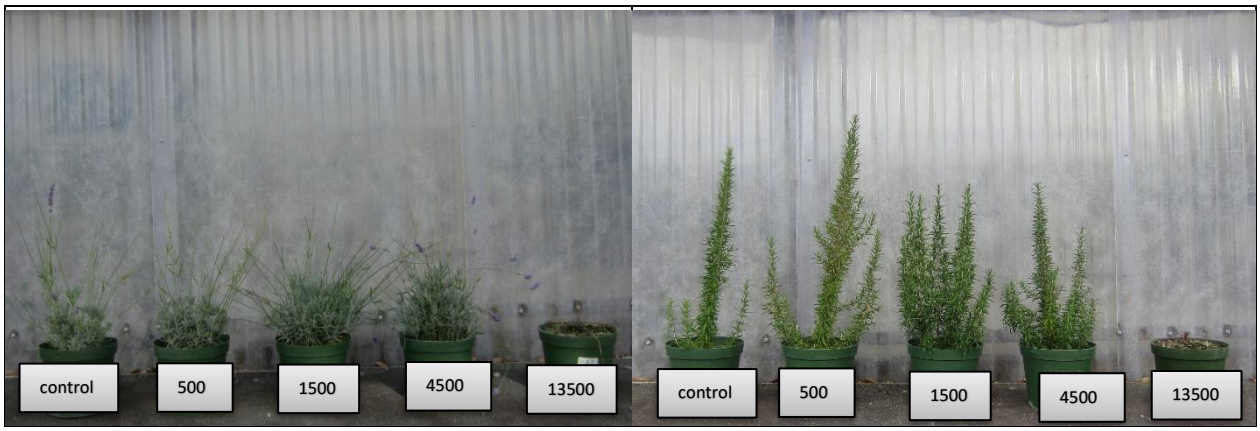


Figure 4.6 Effect of different biosolids application rates on *L. angustifolia* (left) and *R. officinalis* (right) biomass in Exp. 3. The numbers in the grey boxes show the concentration of N kg ha^{-1} equiv. biosolids applied in the treatments.

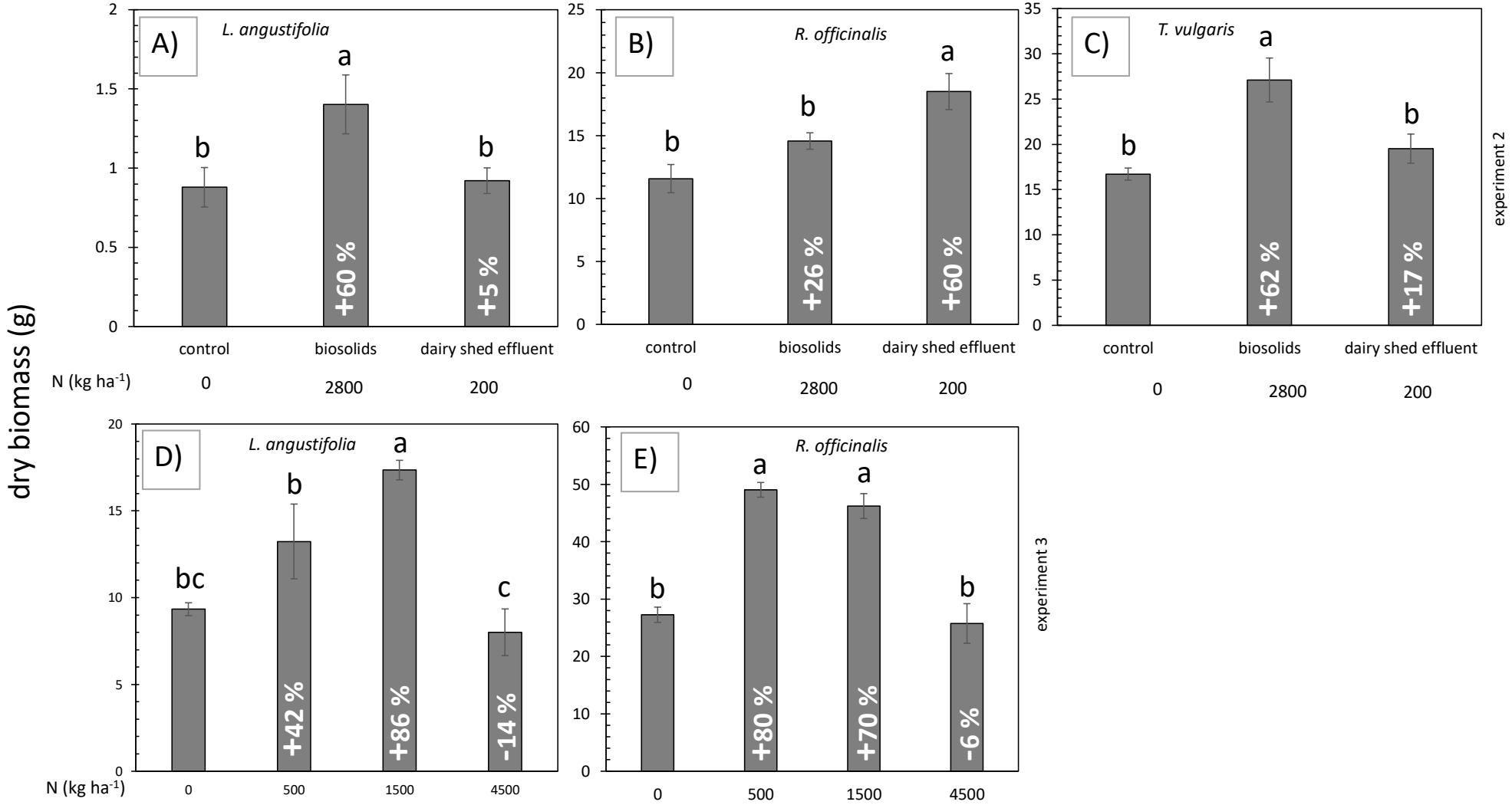


Figure 4.7: Aboveground biomass (g DW) of *L. angustifolia*, *R. officinalis* and *T. vulgaris* in A, B and C- Exp. 2 (n=4 ± se) in addition to D and E- Exp. 3 (n=5 ± se). The biomass of the *L. angustifolia* is related to the plant clusters (oil producing part). Different letters (a, b, c) indicate significant differences between the treatments at p ≤ 0.05 within the plant species. Numbers in the bars represent the percentage of changes caused by the treatments compared to the control.

4.4 Discussion

Biosolids additions of up to 1500 kg N ha^{-1} increased the growth of *L. scoparium, K. robusta*. *L. angustifolia* and *R. officinalis*. There was no benefit to higher rates of addition. While this rate of application exceeds the regulatory threshold for the annual application of N (Gibbs, 2003) most of the biosolids N is present as organic N and is therefore less likely to result in excessive N-leaching due to the slow release of N as the organic matter in the biosolids oxidises (Paramashivam, 2015).

DSE was applied at a lower rate (200 kg N ha^{-1} equiv.) because it commonly contains a higher percentage of plant-available inorganic N. However, DSE only produced a significant biomass increase for *L. scoparium* and *R. officinalis*. While higher rates may have resulted in greater biomass increases, the land application of DSE is limited to 200 kg N ha^{-1} equiv. in most jurisdictions (BPDNZ, 2011).

Milne et al. (2015) and Troeh and Thompson (2005) reported that soil C and N are of overriding importance in soil fertility. The four soils used had C contents ranging from 1.6% - 6.5% (Table 3-1). Soil N ranged from 0.22% - 0.5%. Based on these numbers the fertility of the soils would be BCL > LSL > PSL > CSL. Therefore, the addition of biowastes to the CSL should have result in a greater biomass increase than the other soils. This pattern occurred in the experiments.

An increase in biomass of *L. scoparium* and *K. robusta* following biosolids application has been reported by Reis et al. (2017), Lense (2018), Esperschuetz et al. (2017). The maximum increase of *L. scoparium* biomass in this study (28-fold in 16 weeks by 3000 kg N ha^{-1} equiv. CB application) was less than findings of Reis et al. (2017). Although the plant available-N content of biosolids applied in this study was almost two times higher than the Reis et al. (2017) , they reported a 40-fold increase of *L. scoparium* growth in 12 weeks in a low-fertility sand by applying 2070 kg N ha^{-1} (90 t ha^{-1} equiv.) of fresh biosolids. This difference may be attributed to the substrate. The soil used by Reis et al. (2017) had low organic matter content (0.13% total C) and lower concentrations of plant nutrients (<0.05% total N) compared to this study. This supports the expectation that adding biowastes to higher fertility soil would cause less biomass increase compared to the low-fertility soils. The higher biomass increase in the sandy soil could have resulted from the effect of organic matter in the biosolids that improves soil structure and plant growth.

Other studies have similarly reported biomass increase by biowastes application (Monterumici et al., 2015, Esperschuetz et al., 2016a, Massa et al., 2016, Gutiérrez-Ginés et al., 2017). The

positive effect of biowastes on plant growth could be due to their available nutrients including N, P, K and S. The biowastes, including biosolids, TMW and DSE contain organic material that increases Cation Exchangeable Capacity (CEC) (Antolín et al., 2005, Weber et al., 2007) that results in retaining nutrients and making some of them available for the plants (Weber et al., 2007, Kaur et al., 2008).

The results showed no significant differences between surface applied biosolids and biosolids that were mixed into the soil in terms of biomass increase. Given that N and S are the only major nutrients that are mobile in the soil system (McLaren and Cameron, 1996), these results are consistent with the hypothesis that that these elements were limiting plant growth in our soils. The limited mobility of the other plant nutrients would result in negligible bio-accessibility when the biosolids were surface applied. The greater effect of the CB on plant growth could be related to the higher plant available-N (ca. 6 times) and S (ca. 2 times) compared to the KB. The hypothesis that the plants are N-limited is further supported by the observation that mixing biosolids with sawdust offset the growth benefits of adding biosolids alone (Figure 4.3): Sawdust reduces plant-available N through immobilisation (McLaren and Cameron, 1996).

Even surface applied biosolids will improve the long-term soil fertility as the suite of plant nutrients become incorporated into the soil (Hawke and Summers, 2006). Xue et al. (2018) showed that biosolids affect the soil properties up to 50 cm below the level that they are applied. Biomass increases may also result indirectly from the growth of beneficial rhizobacteria and fungi (Wang et al., 2017) resulting from improved soil nutrients and organic matter (Hawke and Summers, 2006).

The increase in the biomass of the EO producing plants in the current study was similar to other research, which showed that various biowastes (biosolids, municipal solid waste and farmyard manure) increased the above-ground biomass of *R. officinalis*, *Ocimum basilicum, L. scoparium* and *K. robusta* (Esperschuetz et al., 2017, Cala et al., 2005b, Anwar et al., 2005, Piri et al., 2017). Increased biomass in *L. angustifolia* and *R. officinalis* following biosolids application is consistent with the findings of Agulló et al. (2011) and Cala et al. (2005b). Similarly, (Yadegari and Mosadeghzad, 2012) showed that manure application increased the biomass and EO production of *T. vulgaris*. However, adding biowastes to high fertility soils may cause an adverse effect on plant biomass (Tabatabaie and Nazari, 2007) and reduce EO production (Rahmani and Tabaei-Aghdaei, 2014, Petropoulos et al., 2009, Tabatabaie and

Nazari, 2007). The results show that high levels of biosolids addition reduce plant growth, presumably due to toxic agents within the biosolids.

Increasing the dry matter of plant may increase EO yield (Scavroni et al., 2005) providing the concentration and quality of the EOs is not adversely affected. The following Chapters (5, 6 and 7) investigate the effect of the biowastes on levels of contaminating TEs in the plants as well as the concentrations of evaluated components in the oils.

Given that these experiments investigated just a single application of biosolids, long-term repeated applications may have different outcomes due to the increased availability of other nutrients and the accumulation of potentially toxic TEs in the soil (Black, 2010).

4.5 Conclusions

Low-fertility soils could be beneficially rebuilt using a single biosolids application equivalent to 1500 kg N ha^{-1}. This would give the greatest growth response while likely remaining within environmental constraints relating to nitrate leaching. Regulations may need to be adjusted to allow this. Clearly, as the EO crops mature, further nutrients will need to be applied. The results show that in the soils tested, N is the most limiting element. Nitrogen could be applied through mineral fertilisers or alternatively through further applications of biowastes. While the DSE and TMW could be safely applied, repeated applications of biosolids may result in the accumulation of toxic TEs in the soil. Future work should comprise long-term experiments where biowastes are applied to larger plants.

Chapter 5

Effects of the biowastes on the elemental concentrations in the plants

5.1 Introduction

Changes in the soil elemental composition following the application of biowastes alters the elemental concentrations in the plants (Gutiérrez-Ginés et al., 2017). Esperschuetz et al. (2017) showed that *Leptospermum scoparium* and *Kunzea robusta* accumulate more N, P and TEs following the application of 1250 kg N ha^{-1} equiv. biosolids to a degraded soil. Similarly, the application of macronutrients (N, P, S and Ca) can increase the foliar N and P concentrations in Australian *Eucalyptus regnans* (myrtaceae) grown in low-fertility lands (Ringrose and Neilsen, 2005). Another study on *Eucalyptus tereticornis* grown in low P soil showed that the application of 50 kg ha^{-1} yr^{-1} of P, increased the uptake of this essential nutrient by 52% (Crous et al., 2015). *Eucalyptus grandis* and *Eucalyptus camaldulensis* showed higher nutrient uptake when they were grown with different rates of NPK fertilizers (Hunter, 2001). Chrysargyris et al. (2016) showed that the application of N and P (up to 250 and 70 mg L^{-1}, respectively) to *Lavandula angustifolia* growing in hydroponic conditions would increase the concentration of these elements as well as K, Ca, Mg and P in the leaves. The P concentration of *Salvia officinalis* leaves was significantly increased (1.4 fold) following the application of P fertilizer (Nell et al., 2009).

The uptake of TEs by plants growing in biowaste-amended and TE-contaminated soils is well studied (Lake et al., 1984, Sposito et al., 1982, McBride, 1995, Kidd et al., 2007, Zhu et al., 2011). Chaiyarat et al. (2011) showed that *Ocimum gratissimum* grown in the Cd and Zn contaminated soil, would uptake lower Cd (compared to the acceptable level of 0.2 mg kg^{-1}) when cow manure was applied. Zheljazkov and Warman (2003) showed that growing basil in the soil amend with 60% of high-Cu compost (1200 mg kg^{-1}) increased the concentration of this element in the plant tissue by ca. 9-fold. Several studies show that aromatic plants can be safely grown in TE-contaminated soils with organic amendments (Scora and Chang, 1997, Zheljazkov and Nielsen, 1996a). Even while TEs are accumulated in the shoots, there is minimal transfer into the EOs (Scora and Chang, 1997, Zheljazkov et al., 2006).

Lydakis-Simantiris et al. (2016) showed that chamomile (*Matricaria recutita*), sage (*Salvia officinalis*), and thyme (*Thymus vulgaris*) could be cultivated in soils containing elevated TEs

(up to 30 mg kg^{-1} Cd, 1800 mg kg^{-1} Pb and 600 mg kg^{-1} Ni) while still safely producing EOs. They showed that although these plants accumulated high concentrations of TEs in the roots, the concentration of these elements were low in the aboveground biomass and TE concentrations in the EOs were small. *L. scoparium* can tolerate soil concentrations up to 3.6 x 10^{-3}, 3.8 and 1 g kg^{-1} of As, Ni and Cr (Craw et al., 2007, Lee et al., 1983). Cultivation of this plant for EO production could have additional benefits such as producing *L. scoparium* honey that is sold up to NZ$1400 kg^{-1} (Farmers-Weekly, 2018). Moreover, cultivation of aromatic plants has been suggested for phytoremediation of TE-contaminated soils (Zheljazkov and Warman, 2003).

5.2 Effects of the biowastes on the elemental concentrations in mānuka (*Leptospermum scoparium*), kānuka (*Kunzea robusta*), lavender (*Lavandula angustifolia*), rosemary (*Rosmarinus officinalis*) and thyme (*Thymus vulgaris*)

In most of the experiments in this thesis, the application of biosolids increased the concentration of macronutrients in the *L. scoparium*, *K. robusta*, *L. angustifolia*, *R. officinalis* and *T. vulgaris* leaves compared to the control (Table 5-1, Table 5-2, Table 5-5, Table 5-6 and Table 5-7). At or around the rate for optimal biomass increase (1500 kg N ha^{-1} equiv.), the application of CB significantly increased N (by 10 – 100%), P (0 – 40%), and S (0 – 250%). Changes in K, Ca, and Mg were inconsistent and usually not significant. These increases were greatest in the low fertility soil (CSL) and lowest in the high fertility soil (BCL). Interestingly, although the CSL had the lowest fertility in terms of C and N contents, the N concentration in *L. scoparium* grown in this soil was nearly threefold higher than the N concentration in the highest fertility soil (BCL).

Mixing sawdust with the KB (Exp. 1), offset the increase of N compared to adding KB alone for *L. scoparium* and *K. robusta* (Table 5-1 and Table 5-2). Concentrations of P, K and S were either unchanged or further increased in the sawdust+KB treatments (Table 5-1). Surprisingly, there were no significant differences in the macronutrient concentrations in *L. scoparium* receiving surface applied CB compared to plants where the CB had been incorporated into the soil (Table 5-1).

The application of DSE had little effect on N, P and S concentrations but significantly increased K and correspondingly decreased Ca and Mg in some species (Table 5-1, Table 5-2, Table 5-5, Table 5-6 and Table 5-7). In general, the macronutrient increases correlated with the biomass increases for each species (Chapter 4). The application of TMW to the PSL significantly

increased foliar N, P, K and S for *L. scoparium* but only foliar P for *K. robusta* (Table 5-3). It is to be noted that the biomass of *L. scoparium* was significantly increased by the TMW whereas there was no such increase for *K. robusta* (Chapter 4).

The concentrations of Cd and Cu were not significantly different from the control when CB at rates up to 1500 kg N ha^{-1} was applied (Table 5-1, Table 5-2, Table 5-5 and Table 5-6). There were small but significant increases in foliar Zn concentrations. Higher rates of biosolids addition (both CB and KB) significantly increased the concentrations of Cd, Cu and Zn in some, but not all treatments (Table 5-1, Table 5-2, Table 5-5, Table 5-6 and Table 5-7). The concentration of Cd in the plants was compared to food safety standards (ANZFSC, 2015) as a conservative indicator of whether they increased TEs in the leaves may pose a risk to human health. The maximum safe concentration of Cd in the fresh leaves is 0.1 mg kg^{-1}. In this research, dried leaves were used for elements measurement that shows the concentrations higher than the fresh weight. *L. scoparium* and *K. robusta* had the water content of ca. 70%. Therefore, the Cd concentration up to 0.33 mg kg^{-1} of the dry leaves for these plants is accepted as safe level. Cd concentration in other three plants were ≤ $3*10^{-4}$ mg kg^{-1}.

Table 5-4 shows the elemental concentrations of *L. scoparium and K. robusta* from the field survey. There were significant differences in both the macronutrient and TE concentrations at the different sampling locations for both *L. scoparium* and *K. robusta.* The concentrations of N, P, K, S in the field sampled plants were often greater than *L. scoparium* and *K. robusta* in Exp. 1- Exp. 4 grown in unamended BCL, LSL, PSL and similar to plants receiving DSE, CB at 500 kg N ha^{-1} or KB at 2800 kg N ha^{-1} in Exp. 1- Exp. 4. *L. scoparium* growing in the CSL (Exp. 5 and Exp. 6) had significantly higher foliar macronutrient concentrations than the plant sampled in the field (Table 5-1 and Table 5-4). These results indicate that adding biowastes to BCL, LSL and PSL resulted in foliar macronutrient and TE concentrations that were similar to the natural populations that were sampled.

Table 5-8 shows the concentrations of the elements in standard commercial EOs from the plants used these experiments. Comparing Table 5-1- Table 5-7 with Table 5-8 shows that the macronutrient and TE concentrations are significantly lower in the essential oils. This indicates that contaminating TEs are excluded from the oils and therefore even if leaf TE concentrations are at or close to threshold values, the TE concentrations in the EOs are likely to be significantly lower.

Table 5-1: Elemental concentrations of *L. scoparium* leaves in Exp. 1- Exp. 6. Numbers in in the treatments represent the concentration of N equiv. (kg ha^{-1}) of biowastes applied to the soils. Different letters (a, b, c, d) represent significant differences between the treatments of each experiment (based on Fisher's Least-Significant-Difference test at P ≤ 0.05). Standard errors are given in the parentheses. T and M represent surface (top)-application and mixed with the soil, respectively. (n.d.=not determined). KB and CB represent Kaikoura Biosolids and Christchurch City Council biosolids, respectively.

	Treatment (kg N ha^{-1} equiv.)	N	P	K	S	Ca	Mg	Zn	Cu	Cd
		g kg^{-1} dry matter				mg kg^{-1} dry matter				
Exp. 1	**Control (0)**	7.3 (0.1)^{b}	783 (93)^{b}	3458 (92)	1112 (67)	14359 (738)	1761 (185)	13 (0.23)^{b}	2.3 (0.3)	≤ 3*10^{-4}
	KB (1250)	8.6 (0.4)^{a}	915 (7)^{ab}	3248 (42)	1171 (30)	11746 (1190)	1410 (28)	17 (1.2)^{a}	2.5 (0.3)	≤ 3*10^{-4}
	Sawdust+ KB (1250)	8.2 (0.3)^{ab}	1055 (44)^{a}	3528 (140)	1200 (67)	12427 (500)	1459 (57)	14 (1.4)^{ab}	2.3 (0.2)	≤ 3*10^{-4}
Exp. 2	**Control (0)**	11 (0.4)	916 (46)	5875 (579)	1183 (69)	9039 (520)^{b}	2799 (223)	10 (1.2)^{b}	2.3 (0.2)^{b}	0.02 (0.01)
	KB (2800)	12 (0.5)	1323 (125)	5713 (302)	1142 (51)	11636 (951)^{a}	3236 (243)	68 (22)^{a}	3.4 (0.3)^{a}	0.07 (0.04)
	Dairy shed effluent (200)	11 (0.4)	1096 (182)	6557 (414)	1173 (82)	10929 (357)^{ab}	3065 (307)	11 (2.3)^{b}	3.3 (0.2)^{a}	0.02 (0.01)
Exp. 3	**Control (0)**	11 (0.6)^{d}	1003 (186)^{c}	4146 (183)	913 (66)^{d}	11920 (909)^{cd}	3042 (333)^{b}	24 (3.6)^{c}	2.5 (0.5)^{c}	0.03 (0.01)^{a}
	CB (500)	16 (0.5)^{c}	1194 (46)^{bc}	4535 (109)	1638 (129)^{c}	11782 (685)^{d}	3023 (162)^{b}	33 (3.2)^{bc}	3.1 (0.2)^{c}	≤0.01*
	CB (1500)	20 (0.9)^{b}	1476 (87)^{b}	4588 (196)	2306 (106)^{b}	13929 (386)^{bc}	3728 (199)^{ab}	43 (5.2)^{b}	4.0 (0.2)^{b}	0.11 (0.10)^{a}
	CB (4500)	26 (0.8)^{a}	2515 (109)^{a}	4165 (190)	3182 (194)^{a}	15775 (657)^{ab}	3959 (348)^{a}	69 (6.0)^{a}	6.8 (0.6)^{a}	0.22 (0.16)^{a}
	CB (13500)	22*	2310 (84)^{a}	4432 (391)	3200 (481)^{a}	18267 (998)^{a}	4295 (21)^{a}	102 (45)^{a}	4.2 (0.1)^{b}	≤0.08*
Exp. 4	**Control (0)**	11 (0.3)^{b}	1136 (84)^{b}	4836 (179)	1192 (54)^{b}	12687 (794)	3082 (171)	20 (1.4)^{b}	3.0 (0.5)	≤ 3*10^{-4}
	CB (1500)	24 (1.2)^{a}	2898 (403)^{a}	5028 (149)	2995 (272)^{a}	12630 (888)	3515 (253)	68 (9.2)^{a}	5.8 (0.2)	≤ 3*10^{-4}
Exp. 5	**Control (0)**	20 (0.4)^{b}	1360 (41)^{cd}	12400 (530)^{a}	3180 (66)^{abc}	14100 (950)^{ab}	4080 (230)^{a}	61 (16)^{bc}	6.3 (0.8)^{a}	0.02 (0.02)^{bc}
	CB-1T (630)	24 (0.6)^{ab}	1490 (99)^{bcd}	8950 (780)^{b}	3100 (150)^{abc}	8900 (350)^{d}	1900 (120)^{d}	36 (2.3)^{d}	5.3 (0.5)^{ab}	0.01 (0.01)^{c}
	CB-2T (1900)	26 (0. 3)^{a}	2040 (130)^{a}	9040 (900)^{b}	3040 (120)^{bc}	11900 (630)^{bc}	2450 (120)^{bc}	42 (3.7)^{cd}	4.3 (0.4)^{bc}	0.13 (0.01)^{ab}
	CB-3T (5700)	27 (1.7)^{a}	1810 (200)^{abc}	6140 (700)^{c}	2910 (170)^{cd}	10600 (500)^{cd}	2650 (200)^{b}	41 (4.3)^{cd}	3.7 (0.7)^{c}	0.15 (0.01)^{ab}
	CB-1M (630)	22 (1.0)^{b}	1180 (93)^{d}	8520 (410)^{bc}	2540 (180)^{d}	9680 (650)^{cd}	2080 (210)^{cd}	40 (5.7)^{cd}	3.2 (0.4)^{c}	0.01 (0.01)^{c}
	CB-2M (1900)	27 (0.5)^{a}	1890 (180)^{ab}	9130 (1700)^{b}	3440 (140)^{ab}	14200 (670)^{ab}	2610 (50)^{b}	62 (9.4)^{b}	5.5 (0.5)^{ab}	0.12 (0.04)^{ab}
	CB-3M (5700)	28 (2.6)^{a}	2110 (220)^{a}	6050 (450)^{c}	3480 (160)^{a}	17000 (2180)^{a}	3650 (380)^{a}	88.0 (9.3)^{a}	5.6 (0.6)^{ab}	0.22 (0.03)^{a}
Exp. 6	**Control (0)**	n.d.* *	2450 (180)^{a}	8230 (910)	3000 (390)	17800 (1300)^{a}	4690 (390)^{a}	41 (4.8)^{a}	7.1 (0.9)^{a}	0.00 (0.00)
	Mixed (3000)	23 (0.6)	1380 (40)^{b}	8000 (1430)	2730 (110)	15300 (1400)^{a}	2590 (70)^{b}	42 (7.9)^{a}	5.2 (0.2)^{b}	0.03 (0.03)^{a}
	Patch (3000)	23 (0.9)	2260 (150)^{a}	8000 (470)	2920 (110)	10500 (460)^{b}	2470 (200)^{bc}	29 (2)^{ab}	5.6 (0.3)^{ab}	0.04 (0.00)^{b}
	Top (3000)	22 (0.9)	1030 (60)^{b}	6660 (1040)	2360 (60)	10400 (490)^{b}	2020 (140)^{c}	21 (0.7)^{b}	4.4 (0.3)^{b}	0.01 (0.01)^{b}

*Only one sample was evaluated. Calculating the mean and statistical comparison was not possible.

** There was insufficient biomass for the determination.

Table 5-2: Elemental concentrations of *K. robusta* leaves in Exp. 1- Exp. 4. Numbers in in the treatments represent the concentration of N equiv. (kg ha^{-1}) of biowastes applied to the soils. Different letters (a, b, c, d) represent significant differences between the treatments of each experiment (based on Fisher's Least-Significant-Difference test at P ≤ 0.05). Standard errors are given in the parentheses. KB and CB represent Kaikoura Biosolids and Christchurch City Council biosolids, respectively.

	Treatment (kg N ha^{-1} equiv.)	N	P	K	S	Ca	Mg	Zn	Cu	Cd
		g kg^{-1} dry matter			mg kg^{-1} dry matter					
Exp. 1	**Control (0)**	8.7 (0.9)	2106 (348)	3290 (82)	2090 (298)	10465 (573)^{a}	1913 (160)^{a}	23 (2.1)^{b}	2.3 (0.8)	0.02 (0.02)
	KB (1250)	9.6 (0.5)	1563 (539)	2715 (929)	1457 (490)	5678 (1985)^{b}	1030 (349)^{b}	37 (14)^{a}	2.3 (0.8)	0.02 (0.02)
	Sawdust+ KB (1250)	8.4 (1.0)	1123 (378)	2531 (870)	1279 (447)	6997 (2361)^{ab}	1075 (360)^{b}	32 (11)^{a}	1.5 (0.7)	0.06 (0.06)
Exp. 2	**Control (0)**	7.6 (0.5)	1523 (170)	7221 (389)^{a}	995 (65)^{ab}	4789 (369)^{b}	1828 (176)	30 (6.7)^{b}	1.3 (0.3)^{b}	0.01(0.00)^{b}
	KB (2800)	8.6 (0.6)	1747 (101)	5927 (86)^{b}	1310 (117)^{a}	7216 (685)^{a}	1886 (239)	119 (5.8)^{a}	2.3 (0.2)^{a}	0.31 (0.09)^{a}
	Dairy shed effluent (200)	8.8 (1.1)	1672 (371)	5915 (374)^{b}	962 (94)^{b}	5836(154)^{ab}	2226 (168)	41 (8.5)^{b}	1.5 (0.2)^{b}	0.02 (0.01)^{b}
Exp. 3	**Control (0)**	9.7 (0.9)^{d}	1540 (298)^{b}	4944 (216)^{b}	1302 (126)^{d}	6714 (705)^{b}	2979 (338)	76 (11)^{a}	1.7 (0.4)^{b}	≤ 3*10^{-4}
	CB (500)	15 (0.9)^{c}	2056 (240)^{ab}	5087 (216)^{b}	1687 (89)^{c}	7813 (965)^{ab}	3073 (346)	84 (5.2)^{a}	3.0 (0.5)^{ab}	≤ 3*10^{-4}
	CB (1500)	21 (0.7)^{b}	1904 (120)^{ab}	4848 (75)^{b}	2350 (166)^{b}	9212 (819)^{a}	3131 (288)	114 (16)^{a}	2.8 (0.6)^{ab}	≤ 3*10^{-4}
	CB (4500)	25 (2.2)^{a}	2729 (446)^{a}	6868 (574)^{a}	5113 (1101)^{a}	9984 (668)^{a}	3644 (192)	114 (22)^{a}	4.7 (1.5)^{a}	≤ 3*10^{-4}
	CB (13500)	---	---	---	---	---	---			
Exp. 4	**Control (0)**	12 (0.4)^{b}	1906 (196)^{b}	4727 (290)^{b}	1665 (112)^{b}	6710 (384)^{b}	3183 (341)	52 (4.7)^{b}	3.3 (0.4)	≤0.08*
	CB (1500)	24 (1.0)^{a}	2966 (226)^{a}	7106 (609)^{a}	2853 (139)^{a}	9191 (529)^{a}	3177 (213)	139 (39)^{a}	3.9 (0.5)	0.03 (0.02)

*Only one sample was evaluated, and mean calculation and statistical comparison was not possible.

Table 5-3 Elemental concentrations of *L. scoparium* and *K. robusta* leaves in the Duvauchelle field experiment (Exp. 7). Numbers in in the treatments represent the concentration of N equiv. (kg ha^{-1}) of biowastes applied to the soils. Different letters (a, b, c, d) represent significant differences between the treatments (based on Fisher's Least-Significant-Difference test at P ≤ 0.05). Standard errors are given in the parentheses. TMW represents the Treated Municipal Wastewater.

	Treatment (kg N ha^{-1} equiv.)	N	P	K	S	Ca	Mg	As	Zn	Cu	Cd
L. scoparium	**Control (0)**	12 (0.5)^{b}	1054 (116)^{b}	3644 (98)^{b}	1147 (48)^{b}	6532 (494)	1720 (78)	0.29 (0.06)	13.4 (1.1)	4.1 (0.4)	0.007 (0.003)
	TMW (30)	15 (0.8)^{a}	1351 (69)^{a}	4241(113)^{a}	1351 (43)^{a}	7015 (548)	1641 (98)	0.35 (0.03)	16.6 (0.9)	4.3 (0.2)	0.013 (0.005)
K. robusta	**Control (0)**	15 (0.8)	1458 (66)^{b}	4210 (152)	1434 (47)	4991 (265)	1663 (103)	0.15 (0.07)	28.2 (2.0)	4.9 (0.5)	0.020 (0.007)
	TMW (30)	17 (0.9)	1761 (124)^{a}	4112 (187)	1587 (66)	5075 (277)	1696 (70)	0.24 (0.08)	34.0 (6.4)	5.4 (0.6)	0.006 (0.002)

Table 5-4 Elemental concentrations (g kg $^{-1}$ dry matter for N and C and mg kg^{-1} for other elements) of *Leptospermum scoparium* and *Kunzea robusta* leaves in Exp. 8 (Field study). Standard errors of the means (5 > n < 7) are given in parentheses. Significant differences between the treatments at p ≤ 0.05 are indicated by capital letters (A, B, C) for *L. scoparium* and lower-case letters (a, b, c) for *K. robusta* within the plant species.

		N	P	K	S	Ca	Mg	Zn	Cu	Cd
		g kg^{-1} dry matter	mg kg^{-1} dry matter							
L. scoparium	**Nikau Gully**	9.7 (0.3)^{B}	761 (103)^{B}	2945 (96)^{C}	934 (81)^{B}	6600 (677)^{B}	2603 (286)	10 (0.6)^{B}	2.2 (0.1)^{B}	≤3*10^{-4}
	Quail Island	15.2 (0.4)^{A}	1431 (232)^{A}	4427 (253)^{A}	1749 (75)^{A}	8548 (1440)^{AB}	2662 (317)	18 (3)^{A}	4.0 (0.4)^{A}	≤3*10^{-4}
	Yarrs Flat	14.4 (0.4)^{A}	757 (66)^{B}	3644 (304)^{B}	1648 (68)^{A}	11218 (1199)^{A}	2902 (138)	12 (1.4)^{A}	1.5 (0.5)^{B}	≤3*10^{-4}
K. robusta	**Nikau Gully**	11.5 (0.8)^{b}	1413 (189)^{b}	3958 (196)	1249 (108)	4518 (309)^{b}	2740 (104)^{b}	21 (2)^{b}	2.8 (0.3)^{b}	≤3*10^{-4}
	Quail Island	12.8 (1.4)^{ab}	1741 (234)^{ab}	3990 (182)	1364 (146)	6880 (913)^{a}	2716 (223)^{b}	51 (7)^{a}	2.7 (0.4)^{b}	≤3*10^{-4}
	Bridle Path	14.2 (0.6)^{a}	2220 (283)^{a}	3790 (105)	1395 (55)	7788 (267)^{a}	3464 (202)^{a}	40 (4)^{a}	5.5 (0.4)^{a}	≤3*10^{-4}

Table 5-5: Elemental concentrations of *L. angustifolia* leaves in Exp. 2 (n=3) and Exp. 3 (n=5). Numbers in in the treatments represent the concentration of N equiv. (kg ha^{-1}) of biowastes applied to the soils. Different letters (a, b, c, d) represent significant differences between the treatments of each experiment (based on Fisher's Least-Significant-Difference test at P ≤ 0.05). Standard errors are given in parentheses. KB and CB represent Kaikoura Biosolids and Christchurch City Council biosolids, respectively.

	Treatment (kg N ha^{-1} equiv.)	N	P	K	S	Ca	Mg	Zn	Cu	Cd
		g kg^{-1} dry matter	mg kg^{-1} dry matter							
Exp. 2	**Control (0)**	15 (0.3)^{b}	1646 (104)^{c}	6341 (643)^{b}	2649 (300)^{b}	18663 (521)^{b}	5715 (172)^{ab}	58 (15.1)	4.9 (0.8)^{b}	≤ 3*10^{-4}
	KB (2800)	16 (0.7)^{b}	2738 (106)^{a}	6804 (317)^{b}	4228 (405)^{a}	20890 (386)^{a}	6497 (539)^{a}	58.9 (3.1)	14 (1.5)^{a}	≤ 3*10^{-4}
	Dairy shed effluent (200)	18 (0.4)^{a}	2115 (50)^{b}	9329 (319)^{a}	3139 (200)^{b}	16647 (632)^{c}	4747 (261)^{b}	47.2 (4.4)	8.6 (0.8)^{b}	≤ 3*10^{-4}
Exp. 3	**Control (0)**	15 (0.5)^{d}	1950 (76)^{c}	12433 (539)^{a}	3151 (89.3)^{c}	17145 (324)^{b}	6383 (106)	38.1 (2.2)	4.5 (0.4)	≤ 3*10^{-4}
	CB (500)	18 (0.7)^{c}	2742 (252)^{b}	8556 (247)^{bc}	4011 (260)^{b}	18500 (862)^{b}	6612 (301)	44.9 (1.5)	7.7 (0.6)	≤ 3*10^{-4}
	CB (1500)	20 (0.2)^{b}	2756 (93)^{b}	7636 (449)^{c}	3930 (128)^{b}	21457 (402)^{a}	6413 (238)	47 (1.5)	5.6 (0.3)	≤ 3*10^{-4}
	CB (4500)	25 (0.5)^{a}	3780 (192)^{a}	9912 (1027)^{b}	4643 (172)^{a}	21901 (1436)^{a}	6506 (447)	78.7 (3.6)	4.4 (0.2)	≤ 3*10^{-4}
	CB (13500)*	26	4236	6404	4502	24622	5706	115.5	4.2	≤ 3*10^{-4}

*Only one sample thrived in this treatment. Therefore, mean calculation and statistical comparison was not possible.

Table 5-6: Elemental concentrations of *R. officinalis* leaves in Exp. 2 (n=3) and Exp. 3 (n=5). Numbers in in the treatments represent the concentration of N equiv. (kg ha^{-1}) of biowastes applied to the soils. Different letters (a, b, c, d) represent significant differences between the treatments of each experiment (based on Fisher's Least-Significant-Difference test at P ≤ 0.05). Standard errors are given in parentheses. KB and CB represent Kaikoura Biosolids and Christchurch City Council biosolids, respectively.

	Treatment (kg N ha^{-1} equiv.)	N	P	K	S	Ca	Mg	Zn	Cu	Cd
		g kg^{-1} dry matter			mg kg^{-1} dry matter					
Exp. 2	**Control (0)**	11 (2.6)	1000 (214)^{b}	7412 (1061)^{b}	1511 (259)^{b}	13032 (803)^{ab}	2225 (282)	56 (22)	2.8 (1.1)^{b}	≤ 3*10^{-4}
	KB (2800)	11 (0.8)	1969 (351)^{a}	7054 (80)^{b}	2897 (256)^{a}	14095 (626)^{a}	2928 (535)	54 (9.6)	11 (0.4)^{a}	≤ 3*10^{-4}
	Dairy shed effluent (200)	16 (0.8)	1444 (42)^{ab}	16980 (2159)^{a}	2019 (163)^{b}	10397 (1406)^{b}	1824 (76)	39 (4.1)	4.8 (0.2)^{b}	≤ 3*10^{-4}
Exp. 3	**Control (0)**	11 (0.2)^{d}	2011 (112)^{c}	10056 (471)^{a}	2923 (188)^{bc}	11747 (848)^{b}	2399 (169)^{bc}	38 (3.0)	2.0 (0.1)	≤ 3*10^{-4}
	CB (500)	13 (0.7)^{c}	2601 (123)^{b}	6577 (416)^{b}	2788 (168)^{c}	10562 (574)^{b}	2310 (123)^{c}	33 (2.2)	6.0 (0.4)	≤ 3*10^{-4}
	CB (1500)	16 (0.5)^{b}	2518 (131)^{bc}	6300 (349)^{b}	3369 (139)^{b}	10962 (666)^{b}	2717 (86.4)^{b}	39 (0.4)	7.6 (0.6)	≤ 3*10^{-4}
	CB (4500)	23 (1.0)^{a}	3246 (331)^{a}	11392 (1686)^{a}	3981 (125)^{a}	15658 (1275)^{a}	3465 (92)^{a}	80 (7.5)	6.2 (0.5)	≤ 3*10^{-4}

Table 5-7: Elemental concentrations of *T. vulgaris* leaves in Exp. 2 (n=3). Numbers in in the treatments represent the concentration of N equiv. (kg ha^{-1}) of biowastes applied to the soils. Different letters (a, b, c, d) represent significant differences between the treatments (based on Fisher's Least-Significant-Difference test at P ≤ 0.05). Standard errors are given in parentheses. KB represents the Kaikoura Biosolids.

	Treatment (kg N ha^{-1} equiv.)	N	P	K	S	Ca	Mg	Zn	Cu	Cd
		g kg^{-1} dry matter			mg kg^{-1} dry matter					
Exp. 2	**Control (0)**	11 (0.9)	1167 (115)	7048 (323)	1162(96.2)	9768 (517)^{b}	2012 (193)	63 (22)	2.3 (0.3)^{b}	≤ 3*10^{-4}
	KB (2800)	11 (0.5)	1610 (52)	6926 (195)	1224 (85.5)	10562 (340)^{ab}	2596 (422)	66 (13)	4.5 (0.1)^{a}	≤ 3*10^{-4}
	Dairy shed effluent (200)	14 (2.4)	1580 (226)	11826 (3495)	1396 (123)	11889 (856)^{a}	2204 (85)	51 (6.6)	4.0 (0.9)^{ab}	≤ 3*10^{-4}

Table 5-8 Elemental concentrations in the commercial essential oils of the *L. scoparium*, *K. robusta*, *L. angustifolia*, *R. officinalis* and *T. vulgaris* (mg kg^{-1}).

	Al	As	B	Ca	Cd	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	P	S	Zn
L. scoparium	0.20	0.0005	0.02	0.34	≤ 0.0003	≤ 0.0004	0.0003	0.01	0.07	0.01	0.00008	0.22	≤ 0.001	0.010	0.08	0.05
K. robusta	0.61	0.0003	≤ 0.001	0.03	≤ 0.0003	≤ 0.0004	0.0008	0.01	0.00	0.00	0.00003	0.01	≤ 0.001	0.008	0.03	0.00
L. angustifolia	0.01	0.0004	0.02	0.08	≤ 0.0003	≤ 0.0004	0.0002	0.01	0.07	0.01	0.00006	0.21	≤ 0.001	0.008	0.11	0.03
R. officinalis	0.01	0.0003	0.02	0.06	≤ 0.0003	≤ 0.0004	0.0006	0.01	0.08	0.01	0.00006	0.21	≤ 0.001	0.008	0.08	0.03
T. vulgaris	0.01	≤0.002	0.02	0.05	≤ 0.0003	≤ 0.0004	0.0006	0.01	0.08	0.01	0.00004	0.23	0.001	0.008	0.07	0.03

Note: Appendix B provides supplementary data for concentrations and detection limits of the elements in all plant leaves.

5.3 Discussion

An application rate of biosolids at or around 1500 kg N ha^{-1}, resulted in significant increases in the concentrations of foliar N, P and S in most plants and most soils, while concentrations of K, Ca, and Mg were largely unaffected. The mass (and hence uptake) of all the macronutrients is likely to have increased because of the significant increase in biomass resulting from the application of biosolids. Based on these data alone, it is not possible to determine whether one or more of these elements were limiting. I had hypothesised (Chapter 4) that the surface application of biosolids would result in lower increases in the immobile nutrients (P, Ca, Mg, Zn, Cu), however, there were only small and inconsistent differences between the surface applied biosolids and the biosolids incorporated into the soil. One possible explanation is root foraging. Reis et al. (2017) demonstrated that the roots of *L. scoparium* forage patches of biosolids in low-fertility soil. I did not measure the distribution of roots in the soil, however, it is possible that the roots of *L. scoparium* proliferated into the surface-applied biosolids.

When considering the effects of mixing biosolids with sawdust, there was strong evidence that N was limiting in the BCL. This is because sawdust offset the biomass increase resulting from biosolids addition and also offset the increase in foliar N following biosolids addition. However, there were no significant differences in the concentrations of P, S, K, Ca and Mg in the sawdust+KB treatment compared to the KB alone treatment. This is inconsistent with these elements being limiting for plant growth.

Unlike biosolids, the application of DSE and TMW significantly increased foliar K concentrations in some plants. Elevated K in these effluents is likely responsible for reducing the plant uptake of Ca and Mg (McLaren and Cameron, 1996). It is unlikely that repeated applications of these effluents will cause significant accumulation of K because the soil concentrations are at least one hundredfold greater than the amount being added (Table 3-1). Nevertheless, Elevated concentrations of K and Na (Appendix B) in irrigation waters are concerning because accumulation of sodium can lead to aggregate instability and reduced permeability of soil (Tanji, 1997).

The increase in foliar Zn and Cu was consistent with the findings of Dickinson et al. (2015) and Gartler et al. (2013). The plant TE concentrations were lower than the limits that can pose a risk to human health and animals, which was similar to the previous study on *L. scoparium*, *K. robusta* (Esperschuetz et al., 2017) and *R. officinalis* (Cala et al., 2005b).

Scora and Chang (1997) showed that the TE concentration in *Mentha piperita* grown in soil treated with sewage sludge was same as control (<1mg L^{-1}). Zheljazkov et al. (2006) research showed that

Mentha piperita and *Mentha arvensis* would grow in soils enriched with Cd, Pb, and Cu without risk for excessive TE transfer into the EOs and without significant alternation of EO composition that may invalidate marketability. Even in some situations the EO quality of the plants like *Vetiveria zizanoides* in the soil treated with moderate concentrations of Cr, Cd, Pb and Ni would increase because of the increase in some important components of the EO (e.g. khuzimol) (Prasad et al., 2014b).

Although there are some concerns about the accumulation of biowastes-borne TEs in soil (Natal-da-Luz et al., 2012), there is less concern about the effects of biowastes contaminating TEs in the EOs. This is because TEs are less concentrated in the EOs compared to the leaves (Table 7-8). Bağdat and Eid (2007), Street (2012) and Zheljazkov et al. (2008) demonstrated the safe cultivation of medicinal plants to produce EOs in soils contaminated by TEs including Cu and Zn.

The application of biowastes to high fertility soil is unlikely to increase nutrient uptake as much as in low-fertility soils. It also could increase the concentration of some elements to the levels that are toxic for the plants (Morgan and Connolly, 2013). Increasing the mobile nutrients like N, would cause N leaching (Cogger et al., 2001, White et al., 2011). Some elements such as P, Ca, Mg and TEs micronutrients are relatively immobile in the soil may therefore accumulate upon repeated applications (MU, 2018).

5.4 Conclusions

In most cases, the biowaste addition increased the concentrations of plant macronutrients in all species. These increases were greatest in soils with the lowest fertility. There is limited evidence that N was the most important limiting nutrient. None of the biowaste additions resulted in TE concentrations in excess of guideline values, although for some TEs, particularly Zn, there were significant increases in many of the biosolids treatments. This indicates that repeated additions may eventually result in phytotoxic concentrations, or cause exceedances of threshold values for contaminants. Unexpectedly, there was little difference in macronutrient or TE concentrations in *L. scoparium* receiving surface applied biosolids compared to biosolids that were incorporated into the soil.

Chapter 6

Effect of biowastes on essential oil concentration and yield

6.1 Introduction

The EO concentration, composition and production of aromatic plants can be affected either positively or negatively by biowastes application depending on the ratio and amount of nutrients added to soil (Chrysargyris et al., 2016). Hadipour et al. (2013) showed that applying 180 kg N ha^{-1} of urea would increase the EO concentration of *Lavandula angustifolia*. The EO content of *Rosmarinus officinalis* increased following the N and K (150 and 100 kg ha^{-1} yr^{-1}) application (Puttanna et al., 2010). Baranauskiene et al. (2003) reported that applying 135 kg N ha^{-1} to *Thymus vulgaris* did not affect the EO yield. The N status of the soil prior to fertilisation was unclear. The addition of urea (60 kg ha^{-1}) increased the Feverfew (*Tanacetum parthenium*) EO production (Hamisi et al., 2012). El Gendy et al. (2015) showed that applying N and K fertilizers (up to 180 and 120 kg ha^{-1}, respectively) increased the EO production of *Anthriscus cerefolium*.

My experiments showed that biowastes application generally did not change the concentrations of EOs in the plants. In most cases the biomass of the plants were increased following the biowastes application (Chapter 4). Considering the EO concentration and plant biomass as the paramount factors in the EO production yield, generally, biowastes application increased the total EO production of the plants.

6.2 Mānuka (*Leptospermum scoparium* J.R. Forst & G. Forst) and kānuka (*Kunzea robusta* de Lange & Toelken.)

The EO concentration of *L. scoparium* (controls) in Exp. 1 – 6 ranged from 0.52% - 0.94% F.W. that is equivalent to 5.2- 9.4 mg g^{-1} F.W. (Figure 6.1, Figure 6.3 and Figure 6.5) in the greenhouse experiments. This concentration was 0.71% (7.1 mg g^{-1} FW) for the field experiment (Exp. 7). The highest EO concentrations occurred in the LSL (Exp. 2), however, a direct comparison of the soils is not possible because the experiments were conducted at different times.

None of the biowastes treatments in Exp. 1-Exp. 7 caused a significant reduction in EO concentration of *L. scoparium* and *K. robusta* (Figure 6.1, Figure 6.3 and Figure 6.5). The sawdust+KB treatment not only increased the biomass (Figure 4.3 A) but also increased the EO concentration in *L. scoparium* by 82% (Figure 6.1 A).

When considering the total EO yield (concentration x biomass), the total *L. scoparium* EO extracted of the greenhouse experiments was either not significantly different from the control or significantly increased (Figure 6.2 and Figure 6.4). Among the first four experiments the greatest increase of *L. scoparium* EO production (164%) occurred in the BCL soil when sawdust+KB was applied on the soil surface (Figure 6.2 A) followed by the PSL soil with 156% increase by application of 1500 kg N ha^{-1} equiv. CB (Figure 6.2 D). Generally, adding sawdust did not increase the effect of biosolids-only treatments in terms of EO production. Treating the soil with DSE did not increase the EO production.

Results of Exp. 5 showed a similar EO production by *L. scoparium* when biosolids were either surface-applied or mixed with the CSL soil at the rates of 630 and 5700 kg N ha^{-1} equiv. However, 1900 kg N ha^{-1} equiv. of CB surface application significantly increased the EO production compared to same rate of biosolids mixed with the soil that resulted in the highest EO production (68.3 kg ha^{-1}) in Exp. 5. Increase of the EO production were for the surface application of 1900 > mixing 630> surface application of 630 kg N ha^{-1} equiv. biosolids with the increase of 12-fold, 10-fold and 9-fold, respectively (Figure 6.4 E).

Result of the rhizoboxes (Exp. 6) was consistent with findings of the Exp. 5, which shows there is no significant difference in EO production when the same rate of biosolids is applied either on top or homogeneously mixed with the CSL. Increase of the EO production trend were related to mixing > surface application of 3000 kg N ha^{-1} equiv. CB, which increased the EO production by 29-fold and 26-fold ($p \leq 0.006$) (Figure 6.4 F). These experiments showed that either surface application or mixing the CB (by maximum 3000 kg N ha^{-1} equiv.) with the CSL would increase the EO production of *L. scoparium*.

TMW application in the Exp. 7 (field experiment) did not change the *L. scoparium* EO concentration (Figure 6.5). EO analysis of the *L. scoparium* from the field survey (Exp. 8) showed that there was a large variation between sites. The EO concentration of samples taken from Nikau Palm Gully and Quail Island were twice higher than the concentration found at Yarrs flat (Figure 6.6). Hence the collection site had significant effect on *L. scoparium* EO concentration. The average EO concentration of *L. scoparium* (no treatment) in all greenhouse experiments was 6.9 mg g^{-1} F.W. This concentration was significantly lower than the Nikau Palm Gully and Quail Island and higher than the Yarrs Flat. Calculation of the *L. scoparium* EO yield for Exp. 7 and Exp. 8 was not possible as the plants biomass was not available.

The EO concentration of *K. robusta* (controls) in Exp. 1 – 4 (that *K. robusta* was tested) ranged from 0.50 – 1.02% F.W. that is equivalent to 5.0- 10.2 mg g^{-1} F.W. (Figure 6.1). The EO concentration of *K. robusta* in the field experiment (Exp. 7) was 0.75% that is equivalent to 7.5 mg g^{-1} F.W. (Figure 6.5). The highest EO concentrations occurred in the BCL, however, as with *L. scoparium* a direct comparison

of the soils is not possible because the experiments were conducted at different times. None of the biowastes treatments caused a significant reduction in EO concentration. Unlike *L. scoparium,* the sawdust+KB treatment did not increase the EO concentration of *K. robusta*.

With respect to EO yield, the maximum increase of *K. robusta* EO production was observed in 1500 kg N ha^{-1} equiv. CB application (211%) in Eyrewell LSL (Figure 6.2 C). Mixing biosolids with soil increased the EO production of *K. robusta* (except when 4500 kg ha^{-1} N equiv. of CB were applied to the soil) (Figure 6.2 B-D) while surface application did not. Figure 6.6 shows that the EO concentration of wild *K. robusta* plants taken from Nikau Palm Gully, Quail Island and Bridle Path were similar and sampling site did not have effect on the EO concentration.

The average concentration of *K. robusta* EO in all greenhouse experiments was 6.9 mg g^{-1} F.W. This concentration was significantly lower than the EO concentration of the all the natural samples.

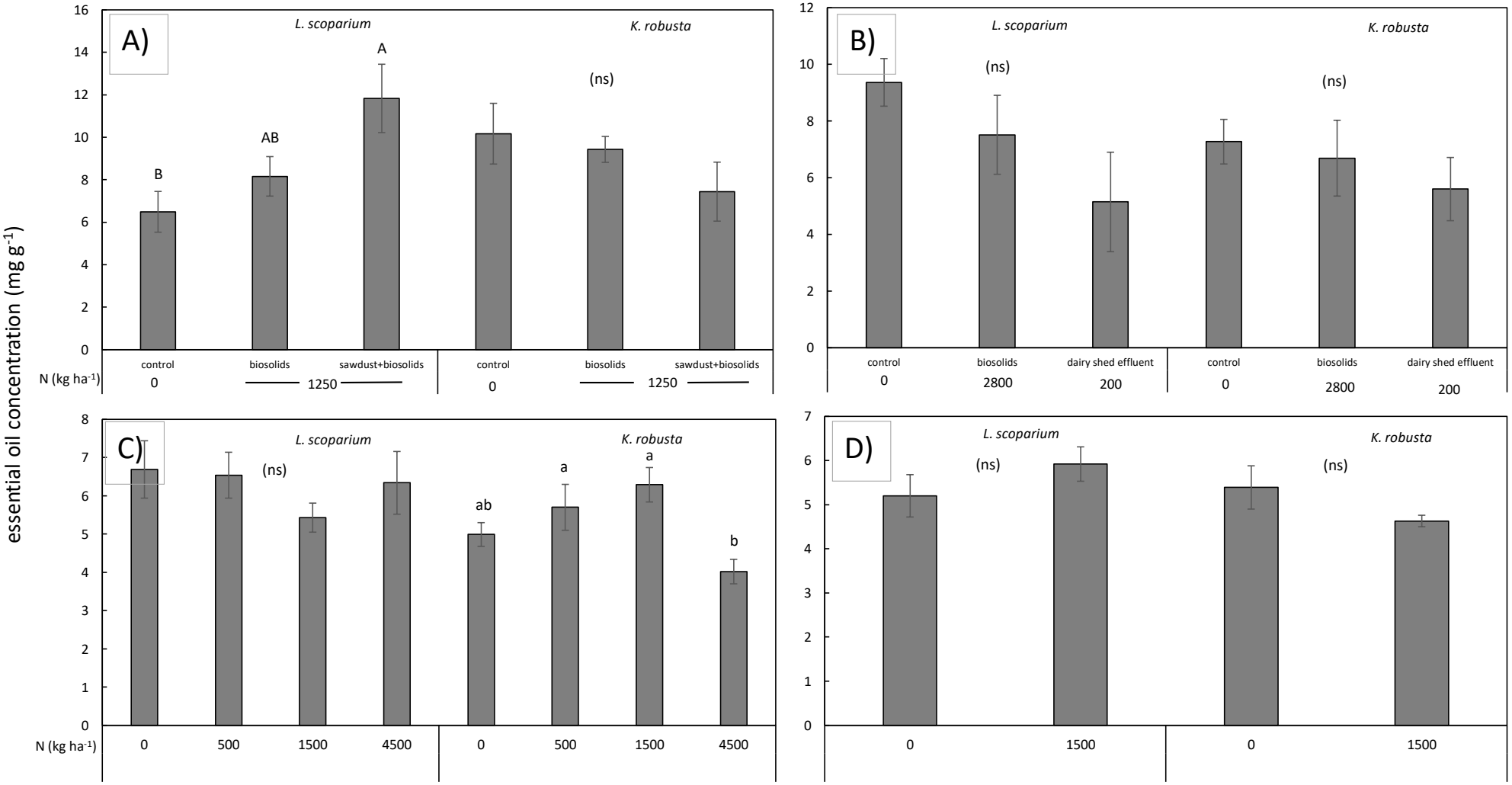


Figure 6.1: Average essential oil concentration (mg g^{-1} FW) of *L. scoparium* and *K. robusta* in A- Exp. 1 (n=4 ± se), B- Exp. 2 (n=3 ± se), C- Exp. 3 (n=5 ± se) and D- Exp. 4 (n=5 ± se). Significant differences between the treatments at p ≤ 0.05 are indicated by capital letters (A, B, C) for *L. scoparium* and lower-case letters (a, b, c) for *K. robusta* within the plant species.

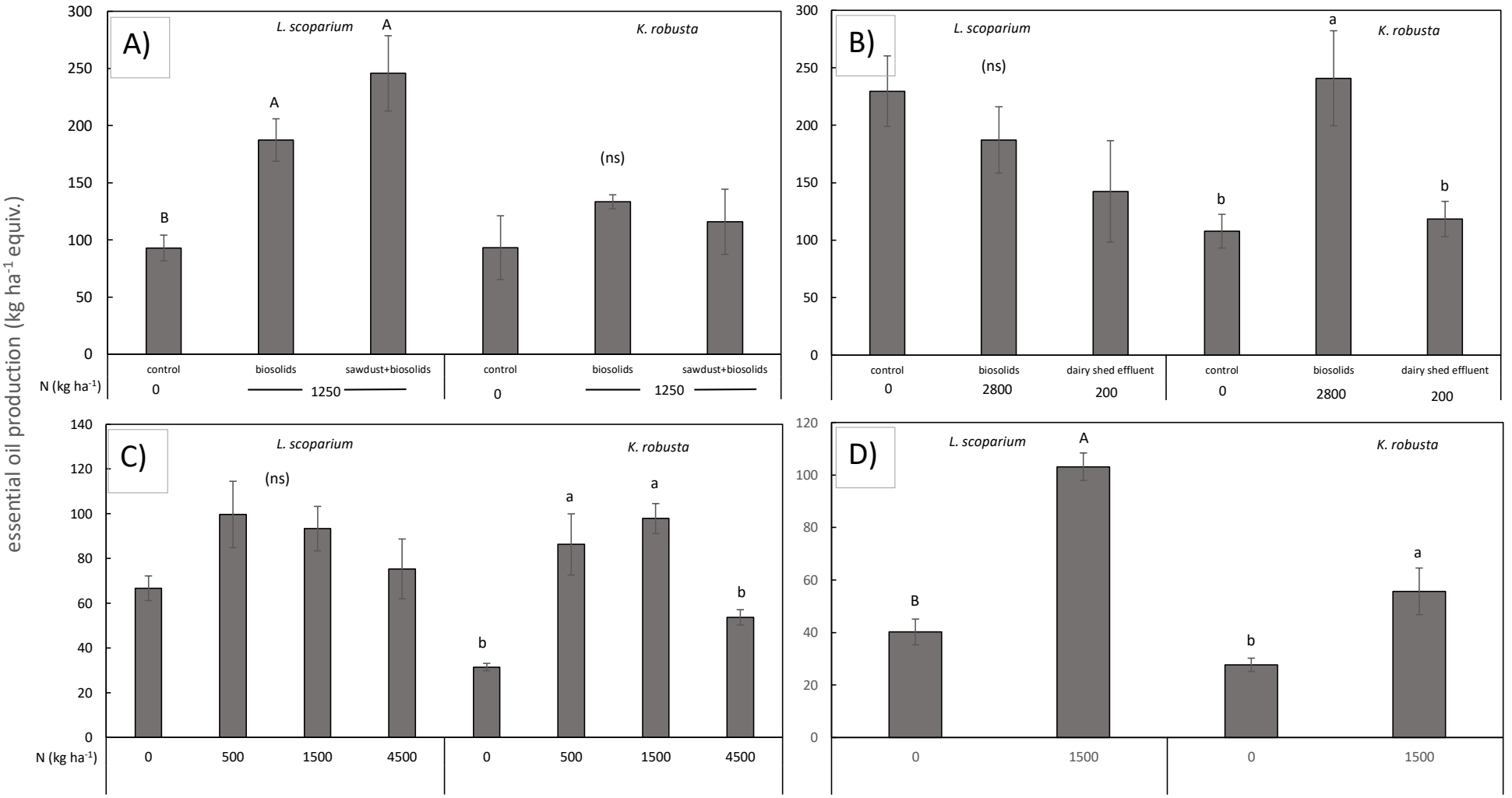


Figure 6.2: Average essential oil production (kg ha^{-1}) of *L. scoparium* and *K. robusta* in A- Exp. 1 (n=4 ± se), B- Exp. 2 (n=3 ± se), C- Exp. 3 (n=5 ± se) and D- Exp. 4 (n=5 ± se). Significant differences between the treatments at p ≤ 0.05 are indicated by capital letters (A, B, C) for *L. scoparium* and lower-case letters (a, b, c) for *K. robusta* within the plant species.

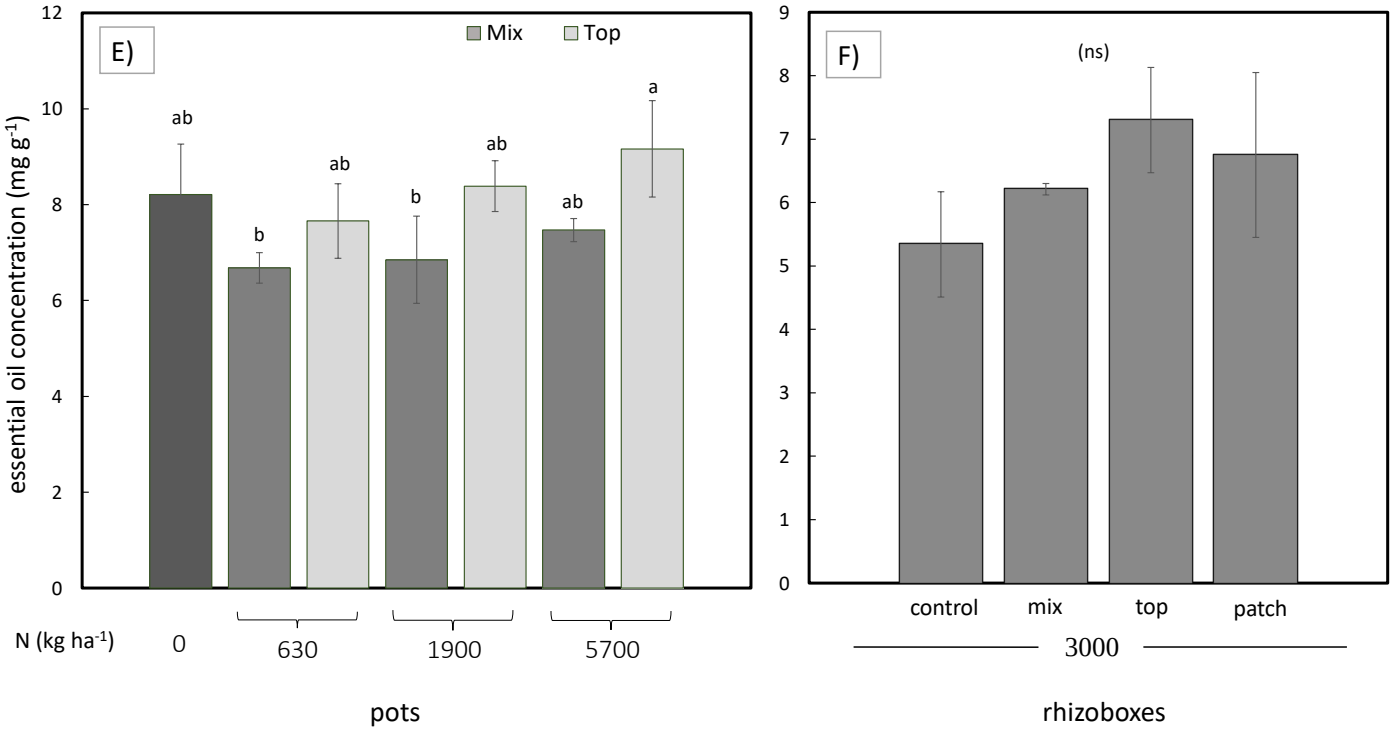


Figure 6.3 Average essential oil concentration (mg g^{-1} FW) of *L. scoparium* in E- Exp. 5 (n=5 ± se) and F- Exp. 6 (n=3 ± se). Different letters (a, b, c) indicate significant differences between the treatments at p ≤ 0.05.

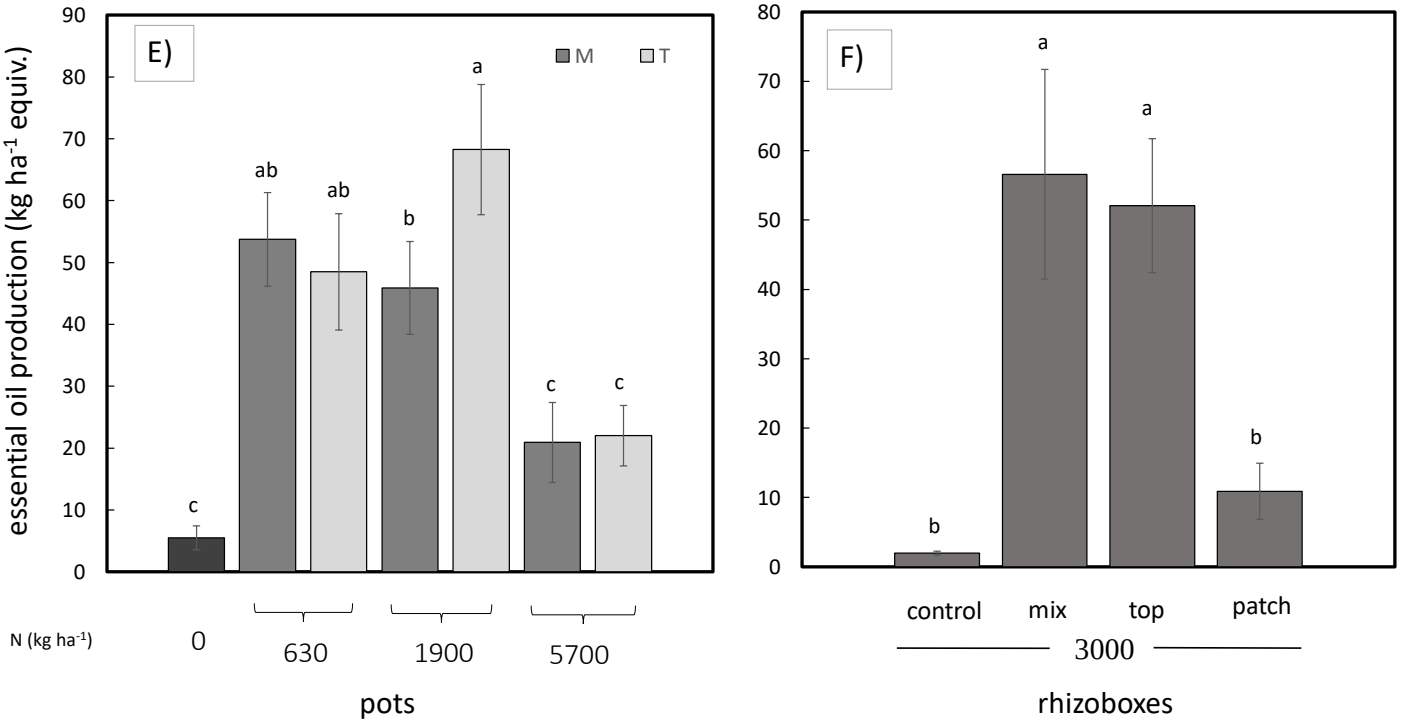


Figure 6.4: Average essential oil production (kg ha^{-1}) of *L. scoparium* in E- Exp. 5 (n=5 ± se) and F- Exp. 6 (n=3 ± se). Different letters (a, b, c) indicate significant differences between the treatments at p ≤ 0.05.

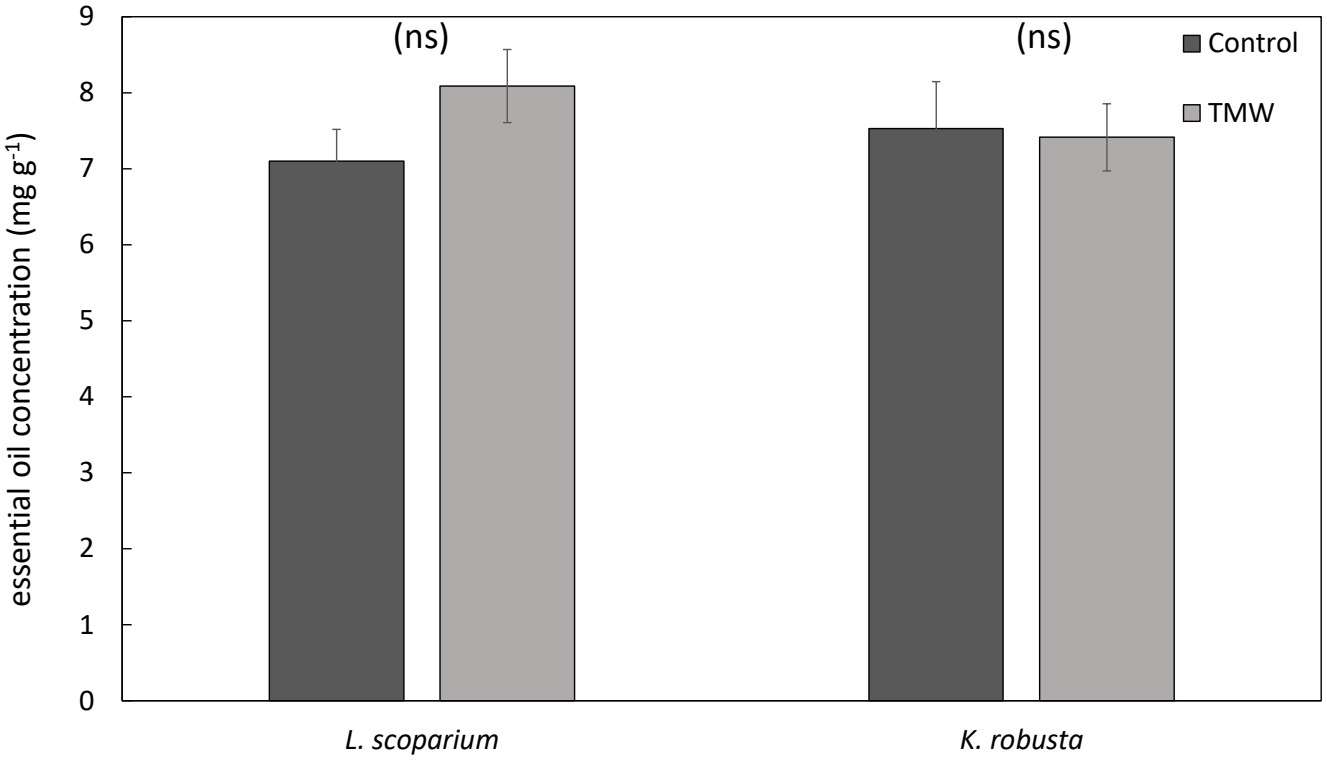


Figure 6.5: Average essential oil concentration (mg g^{-1} FW) of *L. scoparium* and *K. robusta* in Duvauchelle field experiment (Exp. 7) (n=15 ± se).

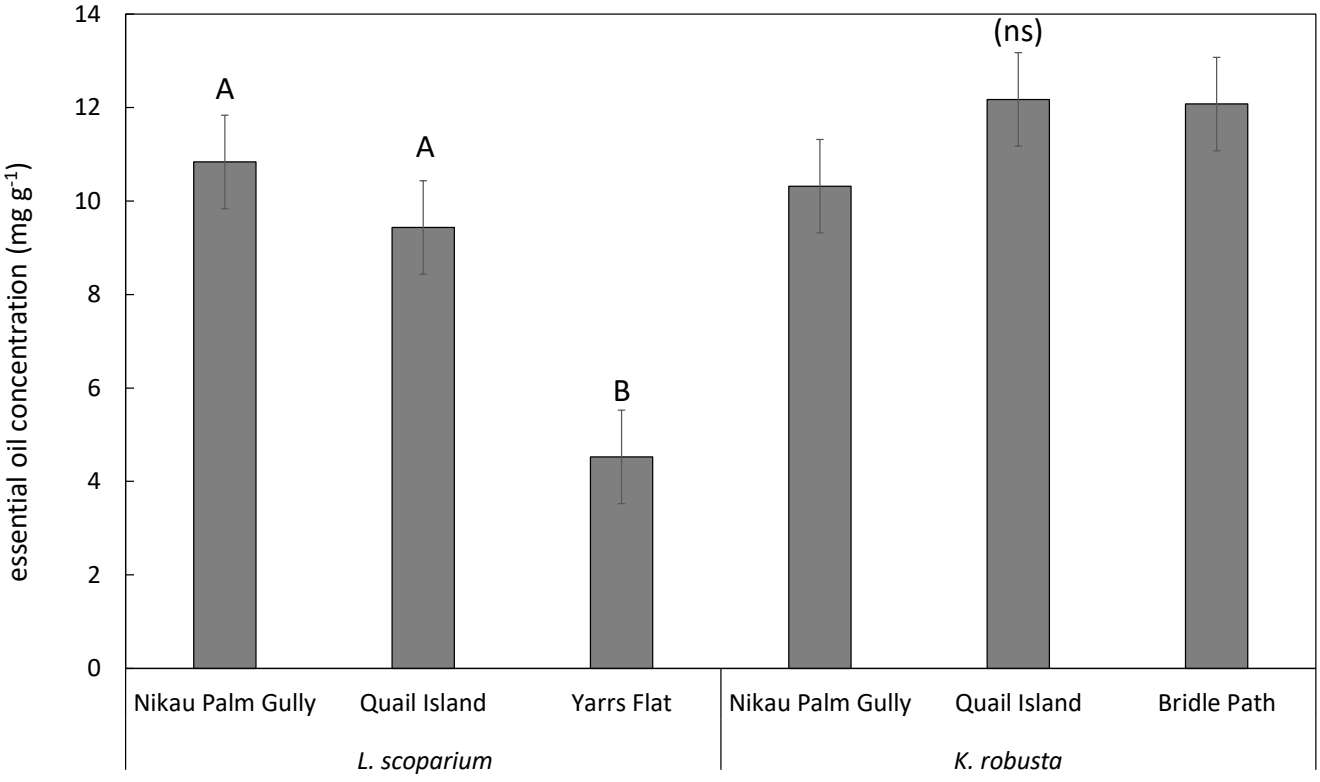


Figure 6.6 Average essential oil concentration (mg g^{-1} FW) of field sampled *L. scoparium* and *K. robusta* plants from Nikau Palm Gully (n=7± se), Quail Island (n=7± se), Yarrs Flat (n=5± se) and Bridle Path (n=7± se). Different letters (a, b, c) indicate significant differences between the treatments of same plant species at p ≤ 0.05.

6.3 Lavender (*Lavandula angustifolia* Mill.), rosemary (*Rosmarinus officinalis* L.) and thyme (*Thymus vulgaris* L.)

In Exp. 2 and Exp. 3, the EO concentration in the controls of *L. angustifolia* and *R. officinalis* ranged from 0.48%-0.83% (4.8-8.3 mg g^{-1} F.W.) and 0.60%-0.84% (6.0-8.4 mg g^{-1} F.W.), respectively (Figure 6.7). The concentration of *T. vulgaris* EO was 0.34% (3.4 mg g^{-1} F.W.). The EO concentrations of *R. officinalis* significantly decreased by ca. 29% when DSE (200 kg N ha^{-1} equiv.) was applied to LSL. Mixing more than 1500 kg N ha^{-1} equiv. CB with LSL significantly decreased (>8%) the *L. angustifolia* EO concentration (Figure 6.7 D). None of the other biowaste treatments changed the EO concentrations. There was a significant negative correlation between the *L. angustifolia* EO concentration and the level of N in CB applied to the soil.

The EO production of *L. angustifolia*, *R. officinalis* and *T. vulgaris* was increased by biosolids application but not the DSE. Applying 2800 kg N ha^{-1} equiv. of KB and 1500 kg N ha^{-1} equiv. of CB to LSL significantly increased the EO production of *L. angustifolia* by 74% and 69% in Exp. 2 and Exp. 3, respectively (Figure 6.8 A and D). The application of 1500 kg N ha^{-1} equiv. CB to LSL significantly increased the *R. officinalis* EO production (by 60%) in Exp. 3 (Figure 6.8 E) while KB had no effect on the EO production (Figure 6.8 B). Note that although the dry biomass of *R. officinalis* was highest in the 500 kg N ha^{-1} equiv. CB application (Figure 4.7 E), considering the water content of the plant the maximum oil production was related to 1500 kg N ha^{-1} biosolids application.

Biowastes application did not change the EO production by *T. vulgaris* in Eyrewell LSL (Exp. 2) (Figure 6.8 C). Maximum EO production increase was related to *L. angustifolia*. The EO production increased by 74% in Exp. 2 and 71% in Exp. 3 when 2800 kg N ha^{-1} equiv. of KB and 1500 kg N ha^{-1} equiv. of CB were applied to LSL (Figure 6.8 A and D).

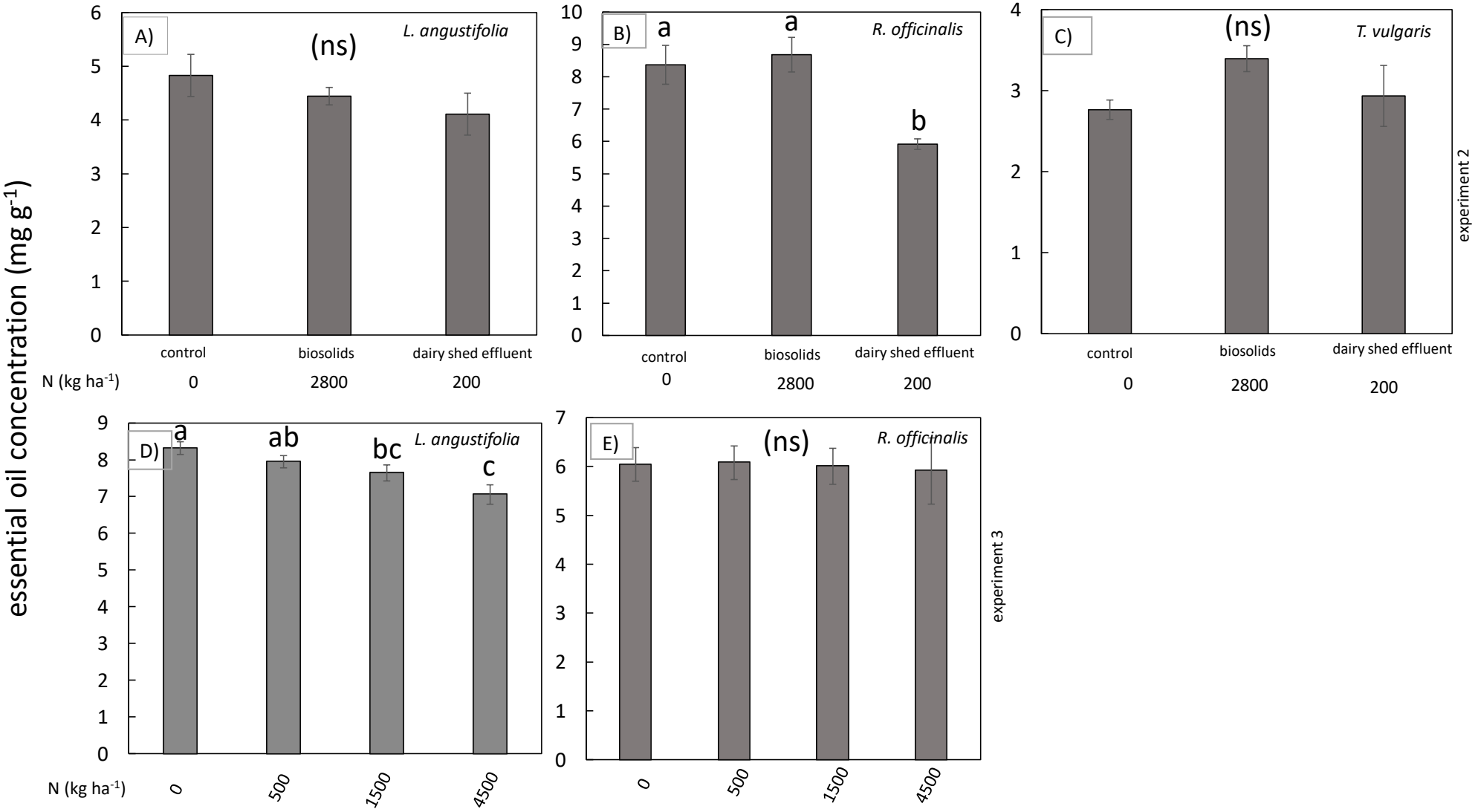


Figure 6.7: Average essential oil concentration (mg g^{-1} FW) of *L. angustifolia*, *R. officinalis* and *T. vulgaris* in A, B and C- Exp. 2 (n=3 ± se) in addition to D and E- Exp. 3 (n=5 ± se), respectively. Significant differences between the treatments at p ≤ 0.05 are indicated by different letters (a, b, c) within the plant species.

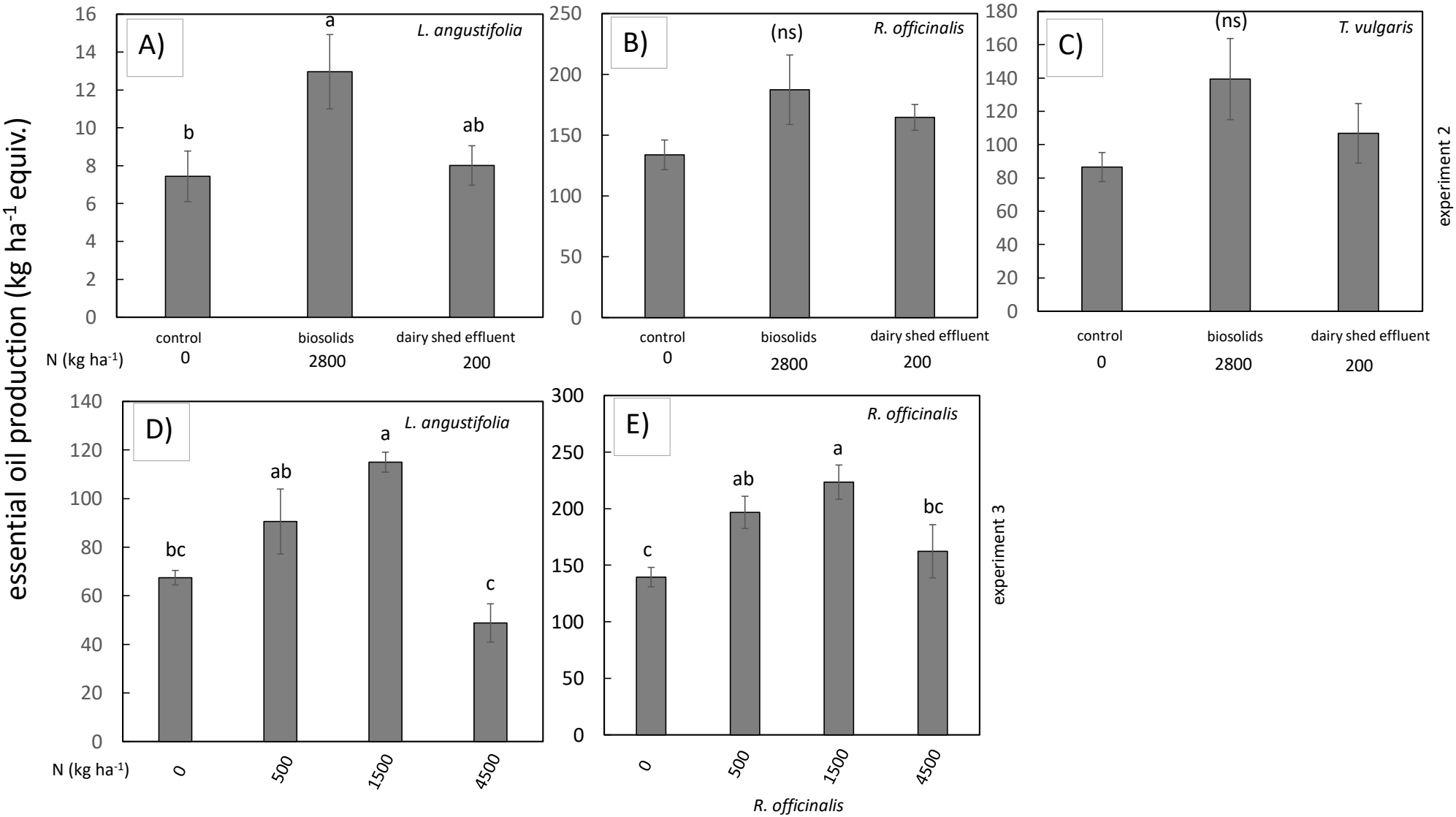


Figure 6.8: Average EO production (kg ha^{-1}) of *L. angustifolia*, *R. officinalis* and *T. vulgaris* in A, B and C- Exp. 2 (n=3 ± se) and D and E- Exp. 3 (n=5 ± se). Significant differences between the treatments at p ≤ 0.05 are indicated by different letters (a, b, c) within the plant species.

6.4 **Discussion**

The total EO concentrations of *L. scoparium* and *K. robusta* in the experiments were similar to those reported in other studies (Maddocks-Jennings et al., 2005b, Porter et al., 1998, C&F-Research, 2000). This indicates that the EO concentrations obtained in this study were similar to what may be found in commercially grown EO crops. The total EO concentrations of *L. angustifolia*, *R. officinalis* and *T. vulgaris* in the experiments were less than the average EO production of the commercially grown plants that usually is obtained by distillation (Esoteric-Oils, 2014). Therefore, the commercial production yield could be higher than the concentrations reported in this study.

For *L. scoparium, K. robusta* and *T. vulgaris* the increases in biomass resulting from biowastes application were not offset by a reduction in the plant oil concentration. Therefore, the addition of biowastes is likely to increase the total yield of EOs. For *R. officinalis* and *L. angustifolia*, some of the biowaste treatments significantly reduced the oil concentration (Figure 6.7 B & D), thereby offsetting some of the gain resulting from increased growth (Figure 4.7 B &D).

The sawdust+KB treatment not only increased the biomass production of *L. scoparium* but also significantly increased the oil concentration in the plant, thereby significantly increasing the oil yield. Blending biosolids with sawdust has demonstrable environmental benefits in terms of N losses (Paramashivam et al., 2016), and such mixtures may be an effective means of establishing high EO-yielding crops of *L. scoparium* on former pine forest soils.

Various studies showed the plants biomass increase affected by the different biowastes application (e.g. biosolids and DSE) (Monterumici et al., 2015, Esperschuetz et al., 2016a, Massa et al., 2016, Gutiérrez-Ginés et al., 2017). It was expected that EO concentration would either decrease because of the high concentration of plant essential nutrients that make an optimum condition for the plants growth (Stevović et al., 2011, Abdelmajeed et al., 2013, Obi and Ebo, 1995) or increase due to the presence of heavy metals in the biowastes, which increase the stress for the plants (Prasad et al., 2014b). For example N increases the photosynthesis rate, which promotes the growth and biomass, but it would decline the EO content (Shabahang et al., 2016), although some research show different results.

Shabahang et al. (2016) showed that the application of up to 100 kg N ha^{-1} equiv. urea decreased the EO content of *Nigella sativai* and *Foeniculum vulgare*. Milthorpe et al. (1994) found no change in the EO concentration and yield of *Eucalyptus polybmctea* following 30 kg ha^{-1} application of N and P fertilizers. Close et al. (2004) found higher EO level in *E. globulus* and *E. nitens* (Myrtaceae family) when fertilizer (N: P: K 20:2.2:6.6; solution concentration 1 g L^{-1}) was applied twice a week than once a week.

In general, the EO production by *L. scoparium*, *K. robusta*, *L. angustifolia*, and *R. officinalis* was increased by biosolids application as it was originally hypothesized. EO production of *T. vulgaris* did not change by biosolids and DSE application. The findings of Scavroni et al. (2005) are consistent with the results of this experiment. They found no change in the *Mentha piperita* EO quality but increase in the EO quantity in presence of 28 t ha^{-1} biosolids application. Other studies also reported the EO production increase by biowastes application. Results of an experiment on *Rosa damascena* showed that EO production increases by 59% when cow manure was applied at the rate of 15 t ha^{-1} (Rahmani and Tabaei-Aghdaei, 2014). Similarly, Anwar et al. (2005) showed that EO production of O*cimum basilicum* significantly increased by applying 10 t ha^{-1} farmyard manure (1.28% N, 2.14% P, and 0.95% K). Kumar and Patra (2012) showed that mixing organic wastes with fly ash and garden soil increases the EO production of *Mentha piperita*. Darvishi et al. (2010) also showed a 17% EO production increase by irrigating the *Ocimum basilicum* with treated domestic wastewater.

Applying up to 2800 kg N ha^{-1} equiv. of the biosolids in Exp. 2 and Exp. 3 significantly increased the EO production of *L. angustifolia* by more than 70% in both experiments (Figure 6.8 A and D). Results of Hadipour et al. (2013) supports the results gained from this study. They showed applying 180 kg N ha^{-1} increases EO content in *L. angustifolia*. There are other cases of EO yield increasing by N utilization. For example, 60 kg ha^{-1} application of urea increased the Feverfew (*Tanacetum parthenium*) EO production (Hamisi et al., 2012). Similarly, *R. officinalis* EO yield would significantly increase by applying N and K (150 and 100 kg ha^{-1} yr^{-1}) (Puttanna et al., 2010) that is parallel with the results of Exp. 3 (Figure 6.8 E). In this experiment application of up to 1500 kg N ha^{-1} equiv. of CB increased the EO production of *R. officinalis* by 60%. Other study showed that application of mutton manure besides phosphate-solubilizing bacterium and nitroxin increases the biomass and EO production of *T. vulgaris* (Yadegari and Mosadeghzad, 2012).

Time of the sampling for the EO production is also important in the quality and quantity of the produced EO. Figueiredo et al. (2008) and Hussain et al. (2008) demonstrated that the EO composition and yield of *Achillea millefolium* and *Ocimum basilicum* significantly changed during the season. During the vegetative period, *Achillea millefolium* EO had higher sesquiterpene hydrocarbons, while in the flowering season, the monoterpene hydrocarbons were dominant. *Ocimum basilicum* showed higher EO content (0.8%) in winter than summer (0.5%).

Plant age affects both EO quantity and composition. The EO concentrations and production of *L. scoparium and K. robusta* grown in Exp. 2 (longest period experiment) were relatively higher in unamended soil compared to the other experiments. The general higher concentration of the EO in

the field survey (older plants) compared to the greenhouse (younger plants) is consistent with this observation. Differences between the greenhouse and plants from the field may also arise from their being more environmental stress (wind, temperature extremes and drought) in the field than in the greenhouse (Bettaieb et al., 2009, Petropoulos et al., 2008, Singh-Sangwan et al., 1994). The only natural *L. scoparium* sample that had lower concentration compared to the greenhouse plants was related to the Yarrs Flat (Figure 6.6). In this place plants were a) taller than other sampling sites (>3.5m) b) grown in a swampy medium, high in N, C and available P (Table 3-6). This resulted less radiation to the lower parts of the plant that was possible to reach for the sampling and optimum growth situation for the plants. Therefore, these plants may have experienced less stress, which would result in lower EO concentrations (Abdelmajeed et al., 2013).

6.5 Conclusions

In most cases, the addition of biowastes did not reduce the EO concentrations in the plants. Therefore, the increased biomass resulting from biowaste addition (Chapter 4) would result in an increased EO yield. As with the previous Chapters, the addition of biosolids at or around 1500 kg N ha^{-1} equiv. would result in the largest increases in EO yield, while not resulting in excessive N-leaching or TE accumulation in the above-ground portions. The application of DSE significantly reduced the EO concentration in *R. officinalis* and should not be used on this species for EO production. Future work is needed to compare the application of same rates of elements (e.g. N, P, K, Ca and Mg) by mineral fertilizers and biowastes in terms of EOs. The effect of higher N concentration or other type of biosolids application on *T. vulgaris* EO production has not been evaluated, which could be the subject of future research.

Chapter 7

Effect of biowastes on essential oil composition

7.1 Introduction

The application of biowastes affect the concentration of macronutrients and TEs in the plants (Chapter 5). Essential oils are made from secondary metabolites (Figueiredo et al., 2008). Any change in the concentrations of macronutrients may alter the secondary metabolites (Chen et al., 2011). Moreover, all metabolism activities in the plants are run by enzymes (Evans et al., 2003). As TEs act as co-factor for enzyme activity (Morgan and Connolly, 2013), any change in the TEs concentration would change the secondary metabolites including the EO components. Likewise, TEs concentrations above the optimum level for the plants would cause metabolic disorders and induce changes in the essential oil composition (Zheljazkov et al., 2006).

There are conflicting results about the effect of macronutrients and TEs on the EO content of different plants. Biesiada et al. (2008) showed that N application (>100 kg ha^{-1} ammonium nitrate) diminishes the quality and antioxidant activities of *Lavandula angustifolia* EO. Chrysargyris et al. (2016) reported that some *L. angustifolia* EO components (1.8-cineole, borneol, camphor and α-terpineol) were affected (no constant pattern of increase or decrease) by increasing the N and P levels in a hydroponic experiment. Other research showed that applying 300 kg N ha^{-1} did not affect the *Thymus vulgaris* EO quality (thymol content) (Omidbaigi and Arjmandi, 2002). Prasad et al. (2014a) reported that application of 25 – 50 mg kg^{-1} Pb, Cr, Cd or Ni to the soil increased the EO concentration and khusimol content in *Vetiveria zizanioides*. However, Zheljazkov and Nielsen (1996a) reported that high concentrations of TEs decreased the EO production of *Mentha piperita* and *Mentha arvensis,* but did not affect EO quality. Zheljazkov and Nielsen (1996b) also showed that TEs did not change the EO quality and quantity of *L. angustifolia*. Furthermore, the authors reported that although *L. angustifolia* clusters accumulated different amounts of heavy metals, the EO was not contaminated.

In the experiments of this thesis, biowastes application affected the EO composition of mānuka (*Leptospermum scoparium*), kānuka (*Kunzea robusta*), lavender (*Lavandula angustifolia*), rosemary (*Rosmarinus officinalis*) and thyme (*Thymus vulgaris*). The changes in the EOs composition were generally small, nevertheless the pattern of components alteration was not consistent. Furthermore, as there is no international standard for the concentration

of individual components of the EOs, it is not possible to announce these minor changes positive or negative.

7.2 Mānuka (*Leptospermum scoparium* J.R. Forst & G. Forst) and kānuka (*Kunzea robusta* de Lange & Toelken.)

The addition of all biowastes except TMW resulted in significant changes in *L. scoparium* EO composition in some of the treatments. However, these changes were relatively small (<20%) in most cases. Table 7-1 shows the all EO evaluated components of *L. scoparium* in Exp. 1 –Exp. 5 (greenhouse pot experiments). This table also shows the number of components that were affected by biowastes application. Generally, Table 7-1 shows that mixing biosolids with soil would affect a greater number of *L. scoparium* EO components compared to surface application of biosolids and other biowastes.

In Exp. 1, five of 13 evaluated EO components of *L. scoparium* showed significant difference to the control when biowastes were applied. This number was 4/18, 8/23 and 2/19 for the Exp. 2-Exp. 4 (Figure 7.1). Results shows that applying 2800 kg N ha^{-1} of KB and up to 1500 kg N ha^{-1} CB would affect a small number of the *L. scoparium* EO components. Figure 7.1 also shows that generally the effect of sawdust+KB on *L. scoparium* components was positive, while this effect was negative by DSE application. In both Exp. 5 and Exp. 6, the application method (surface applied or incorporated) of CB did not change the EO concentration in *L. scoparium* (Figure 6.3) although the application had small but significant effect on some components (Figure 7.2 and Figure 7.3).

Figure 7.2 shows that in the Exp. 5, the surface application and mixing a same rate of biosolids with the soil mostly had similar effect on the EO components. Although in some rates of CB application the concentrations of limonene, linalool, α-terpineol, β-caryophyllene, α-farnesene, calamenene, spathulenol and ledol in the solvent extracts were higher when CB were surface-applied compared to the same rate of CB incorporated into the soil. Figure 7.3 shows that in the rhizoboxes experiment (Exp. 6), the same rate of CB application in different methods had similar effect on the components concentration except for β-ocimene that was higher and α-farnesene, which was lower in the solvent extract when CB were applied in patches compared to the surface application of CB.

The Number of evaluated *L. scoparium* EO components in the field survey was 26, 22 and 21 for Nikau Palm Gully, Quail Island and Yarrs Flat, respectively. The common major components

in the extracted EOs were β-elemene, caryophyllene, δ-cadiene, β-selinene and α-selinene (Table 7-2 and Appendix C). These components (except for caryophyllene) were more than twice concentrated in Nikau Gully and Quail Island samples than the Yarrs Flat. Traces of nor-triketones have been detected in *L. scoparium* samples, but as the chromatography peaks were indistinct, the quantity calculation was not possible.

Table 7-3 shows the five major EO components of *L. scoparium*, expressed as a percentage contribution in the Exp. 1-Exp. 7. Results showed that none of the *L. scoparium* major components were affected by KB (up to 2800 kg N ha^{-1} equiv.), DSE (200 kg N ha^{-1} equiv.) and TMW (30 kg N ha^{-1} equiv.) application. In Exp. 1 only β- Elemene was positively affected by sawdust+KB and not with the KB treatment alone. In Exp. 3, β-Elemene and β-Selinene were affected by the treatments. These components contribute ca. 4%-13% of the EO concentration. β-elemene was significantly decreased when 500 kg N ha^{-1} equiv. CB were applied to the soil, but the higher application rates did not change the concentration compared to the control. β-selinene was significantly decreased by 1500 kg N ha^{-1} equiv. CB application and neither less, nor higher application rates changed its concentration. In Exp. 4, calamenene was higher when 1500 kg N ha^{-1} equiv. CB were applied to the soil. In Exp. 5, surface application of 630 and 1900 kg N ha^{-1} equiv. CB and incorporation of 1900 kg N ha^{-1} equiv. CB decreased the concentration of α-selinene and incorporation of 630 kg N ha^{-1} equiv. CB decreased the calamenene concentration. The remainder of the components did not change compared to the control in this experiment. In this experiment, the highest biosolids application rate (5700 kg N ha^{-1}) made no change in the concentration of major components. The maximum *L. scoparium* EO component concentration in Exp. 6 was related to β-Elemene that was unaffected by the biosolids application methods. Incorporation of CB with soil and applying patches of CB decreased the β-Selinene concentration in Exp. 6. Moreover, all the treatments negatively affected the α-selinene concentration.

Table 7-1 All evaluated essential oil components range of *L. scoparium* in the greenhouse pot experiments. Standard errors are given in parenthesis. Existence of the significant difference at p ≤ 0.05 is indicated by "*". Concentrations are mg L^{-1} in the solvent extract.

Rank	Component name	Control range	Biosolids range (mixed)	Biosolids range (surface applied)	Dairy shed effluent	sawdust+ biosolids
1	**α-pinene**	0.3 (0.1)- 0.4 (0.1)	0.18 (0.03)-0.55 (0.22)	0.20 (0.03)-0.40 (0.08)	-	-
2	**β-pinene**	0.16 (0.03)	5.1 (4.9)	-	0.4 (0.3)	-
3	**β-myrcene**	0.3 (0.1)-0.6 (0.3)	0.13 (0.04)-2.4 (2.0) *	0.3 (0.1)-0.7 (0.37) *	-	0.8 (0.2) *
4	**limonene**	0.14 (0.04)-0.79 (0.48)	0.04 (0.00)-0.6 (0.3) *	0.12 (0.02)-1.2 (0.3) *	0.12 (0.09)	0.29 (0.05)
5	**1,8-cineole**	0.21 (0.03)-5.7 (2.8)	0.10 (0.09)-6.1 (2.2)	3.8 (2.0)-11 (2.7) *	0.02 (0.01) *	0.18 (0.05)
6	**β. ocimene**	0.05 (0.02)-0.13 (0.05)	0.07 (0.04)-1.7 (1.6) *	0.5 (0.4)	-	-
7	**γ-terpinene**	0.2 (0.1)-0.6 (0.2)	0.2 (0.1)-0.8 (0.4) *	0.2 (0.1)-0.5 (0.3)	-	-
8	**linalool**	0.7 (0.4)-6.2 (1.9)	0.5 (0.3)-5.4 (2.1) *	1.1 (0.4)- 4.5 (1.4) *	2.3 (0.8)	3.1 (1.9)
9	**α-terpineol**	0.7 (0.5)-2.1 (1.1)	0.06 (0.01)-1.6 (0.6) *	1.1 (0.6)-2.8 (0.8)	-	-
10	**α-cubebene**	10 (0.9)-18 (5.2)	6.7 (4.9)-15 (2.7)	14 (2.7)-15 (2.2)	11 (3.7)	18 (4.3)
11	**α-copaene**	1.2 (0.5)-10 (1.8)	1.6 (1.3)-3.6 (1.4) *	3.4 (0.6)- 4.4 (0.8)	4.2 (2.2) *	-
12	**β-elemene**	30 (5.2)-66 (32.0)	23 (4.0)-67 (11)	44 (18)-54 (4)	39 (4.3)	87 (20) *
13	**α-gurjunene**	0.9 (0.2)-4.1 (1.3)	1.7 (0.3)-3 (0.7) *	1.5 (0.3)- 4.9 (0.9)	1.5 (0.4)	1.9 (0.2) *
14	**caryophyllene**	6.3 (2.8)-36 (12)	4.6 (3.2)-29 (3.7)	17 (2.5)- 31 (4.0)	24 (5.5)	45 (19)
15	**aromadendrene**	0.6 (0.2)-5.0 (2.8)	1.1 (0.5)-5.6 (1.4)	1.1 (0.6)-13 (7.5)	1.8 (1.4)	2.6 (2.0)
16	**delta-cadiene**	6.7 (1.3)-13 (4.6)	4.5 (4.0)-11 (4.6)	9.2 (2.4)-14 (2.3)	-	-
17	**α-humulene**	1.5 (0.3)-34 (2.4)	1.6 (0.2)-3.1 (0.3)	2.5 (0.6)-40 (4.9)	1.7 (0.5) *	41 (16)
18	**alloaromadendrene**	0.5 (0.1)-2.7 (0.3)	1.4 (0.5)-2.4 (0.4)	2.0 (0.4)- 4.0 (1.6)	1.0 (0.6) *	3.3 (0.9)
19	**β-selinene**	19 (3.2)-40 (19)	10 (1.0)-29 (3.5) *	13 (3.1)- 23 (10.8)	24 (13)	23 (5.8)
20	**α-selinene**	7.9 (2.1)-52 (14)	22(3.5)- 40 (8.5) *	14 (3.1)-50 (10) *	27 (13)	22 (7.2) *
21	**α-muurolene**	1.0 (0.3)	0.2 (0.1)-0.7 (0.4) *	0.5 (0.3)-0.9 (0.3)	0.4 (0.1)	-
22	**α-farnesene**	6.7 (1.9)-24 (3.5)	3.7 (1.5)-27 (15)	6.4 (1.8)-11 (3.2) *	9.6 (2.1)	18 (2.5) *
23	**calamenene**	8.2 (1.9)-25.2 (4.5)	11 (8.6)-19 (2.3) *	24 (2.5)-29 (3.3)	13 (3.6)	29 (5.9)
24	**spathulenol**	0.2 (0.1)-1.3 (0.4)	0.2 (0.1)-1.1 (0.4) *	0.17 (0.02)-0.8 (0.3) *	-	-
25	**caryophyllene epoxide**	0.7 (0.2)-4.7 (3.9)	0.7 (0.2)	1.2 (0.4)	1.3 (1.2)	2.0 (1.2)
26	**ledol**	0.7 (0.1)	0.4 (0.2)-0.5 (0.1) *	0.6 (0.2)-0.9 (0.3)	-	-

Table 7-2 Essential oil components concentration of the field sampled *L. scoparium* from Nikau Palm Gully, Quail Island and Yarrs Flat. Concentrations are mg L^{-1} in the solvent extract. Standard errors are given in parenthesis. Different letters (a, b, c) indicate significant differences between the sampling sites at $p \leq 0.05$.

	Nikau Palm Gully	Quail Island	Yarrs Flat
α-thujene	1.1 (0.4)	*	*
α-pinene	4.1 (2.5)	4.1 (1.9)	*
β-myrcene	0.4 (0.2)	3.1 (2.9)	2.0 (1.0)
α -terpinene	0.10 (0.03)	*	*
cymene	1.3 (0.6)	0.4 (0.1)	0.7 (0.2)
limonene	0.2 (0.1)[a]	0.12 (0.05)[ab]	0.08 (0.02)[b]
1,8-cineole	2.1 (2.0)	0.7 (0.6)	0.3 (0.1)
β. ocimene	0.6 (0.2)	0.18 (0.02)	*
γ-terpinene	3.8(1.5)[a]	0.4 (0.3)[b]	0.9 (0.4)[b]
linalool	8.8 (4.7)	1.1 (0.7)	1.5 (0.6)
terpinen-4-ol	0.2 (0.1)	*	0.11 (0.03)
α-terpineol	0.8 (0.68)	*	*
α-copaene	7.1 (1.2)[a]	4.2 (0.9)[b]	1.2 (0.4)[c]
β-elemene	32.1 (7.7)[ab]	52.0 (10.3)[a]	13.9 (4.7)[b]
α-gurjunene	6.7 (0.9)[a]	3.8 (0.5)[b]	2.0 (0.3)[b]
caryophyllene	22.7 (2.5)	16.2 (3.0)	16.7 (4.0)
aromadendrene	3.6 (0.9)[a]	4.5 (1.3)[a]	1.4 (0.8)[b]
δ-cadiene	10.1 (3.3)[a]	21.0 (3.4)[a]	3.0 (0.4)[b]
α-humulene	3.5 (0.4)[a]	2.7 (0.4)[ab]	1.8 (0.1)[b]
alloaromadendrene	3.6 (0.2)[a]	3.5 (0.8)[a]	1.0 (0.2)[b]
β-selinene	22.2 (4.9)[a]	25.2 (3.9)[a]	8.0 (1.7)[b]
α-selinene	31.5 (4.4)[b]	50.3 (7.0)[a]	11.3 (1.3)[c]
α-farnesene	11.9 (2.7)	7.7 (2.7)	*
calamenene	42 (2.0)[a]	31 (2.5)[b]	21.6 (3.5)[c]
spathulenol	1.9 (0.6)	6.2 (2.2)	1.7 (0.6)
caryophyllene-epoxide	*	*	2.1 (1.5)
ledol	1.6 (0.3)[a]	1.3 (0.3)[a]	0.4 (0.1)[b]

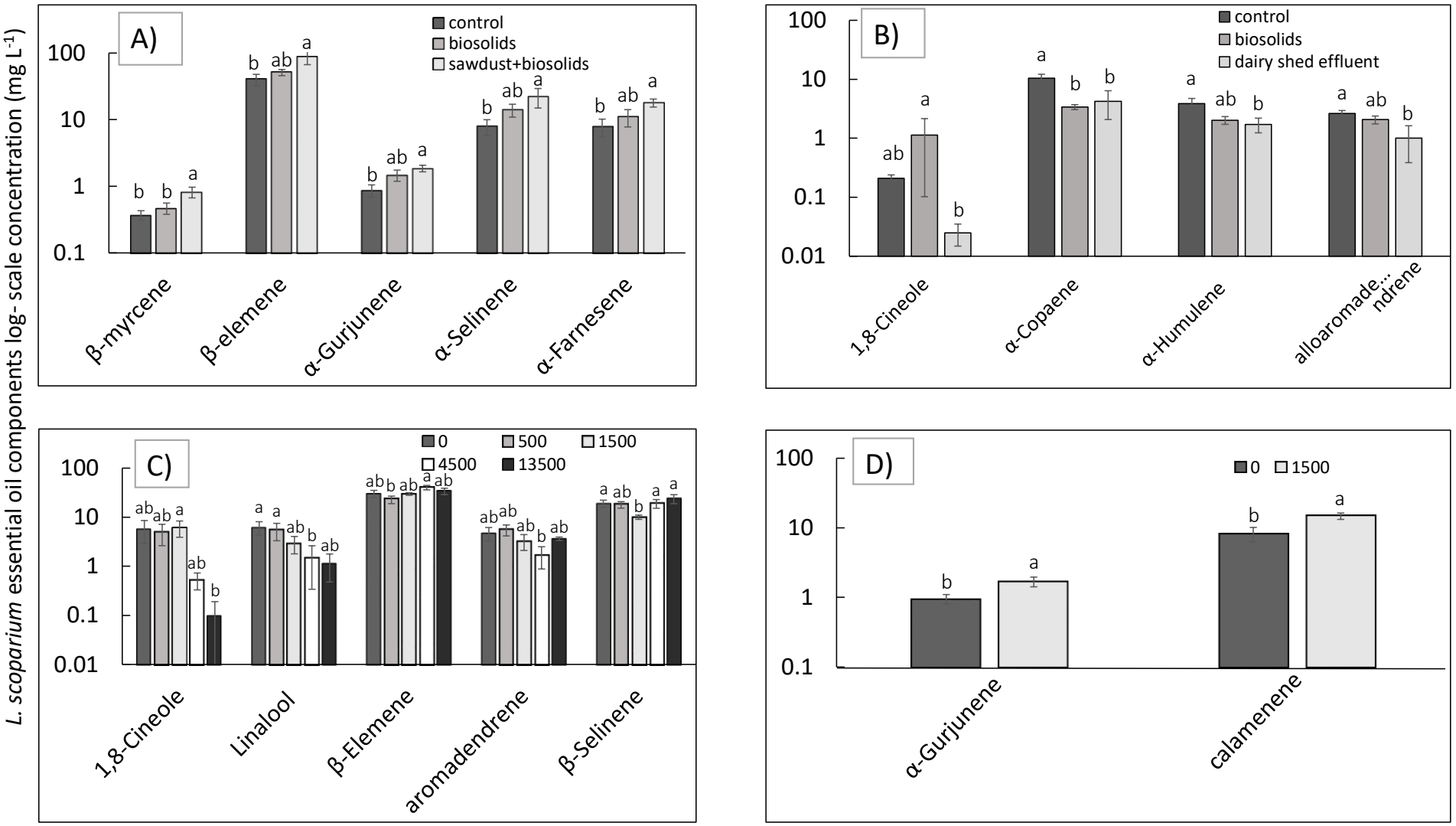


Figure 7.1 Significantly different *L. scoparium* essential oil components ($p \leq 0.05$) in Exp. 1- Exp. 4 (A-D). For Exp. 3, five components out of the eight significantly different ones, that had highest concentrations (>0.1 mg L^{-1}) are illustrated (for clarity). The concentrations are mg L^{-1} in the solvent extract.

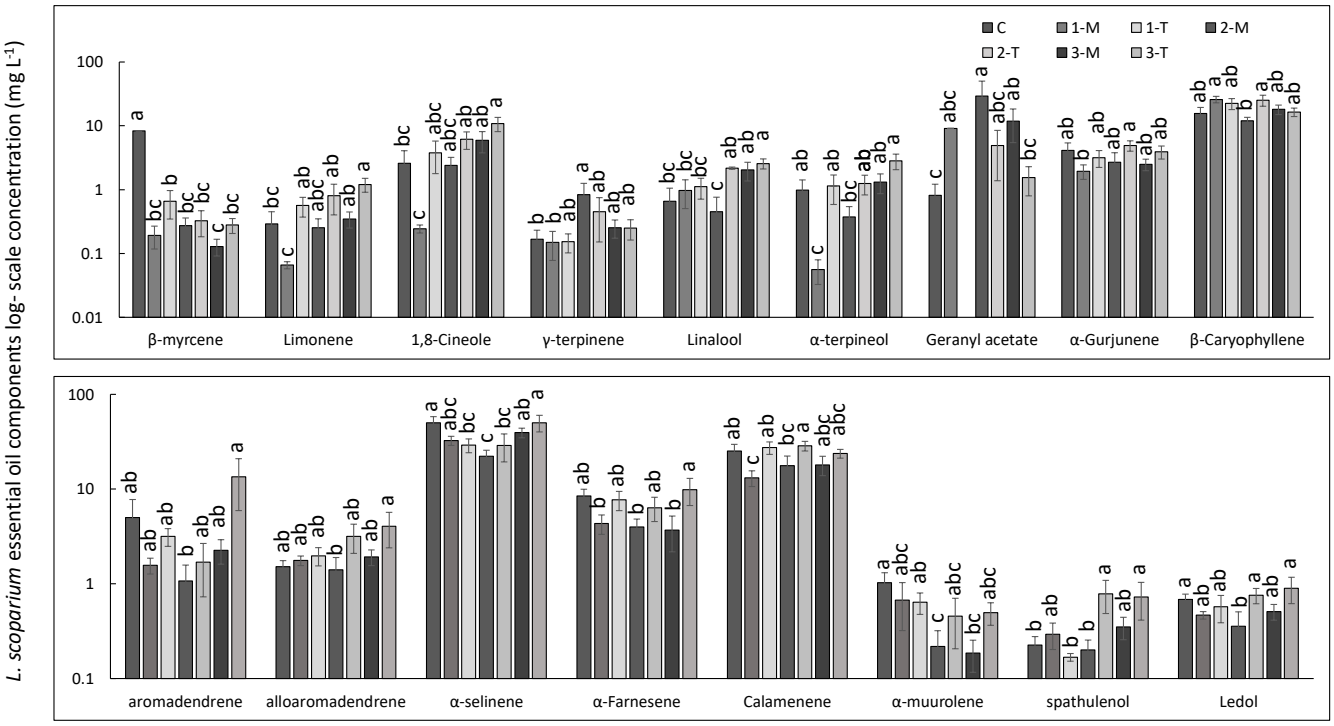


Figure 7.2 Significantly different *L. scoparium* essential oil components (p ≤ 0.05) in Exp. 5. The concentrations are mg L^{-1} in the solvent extract.

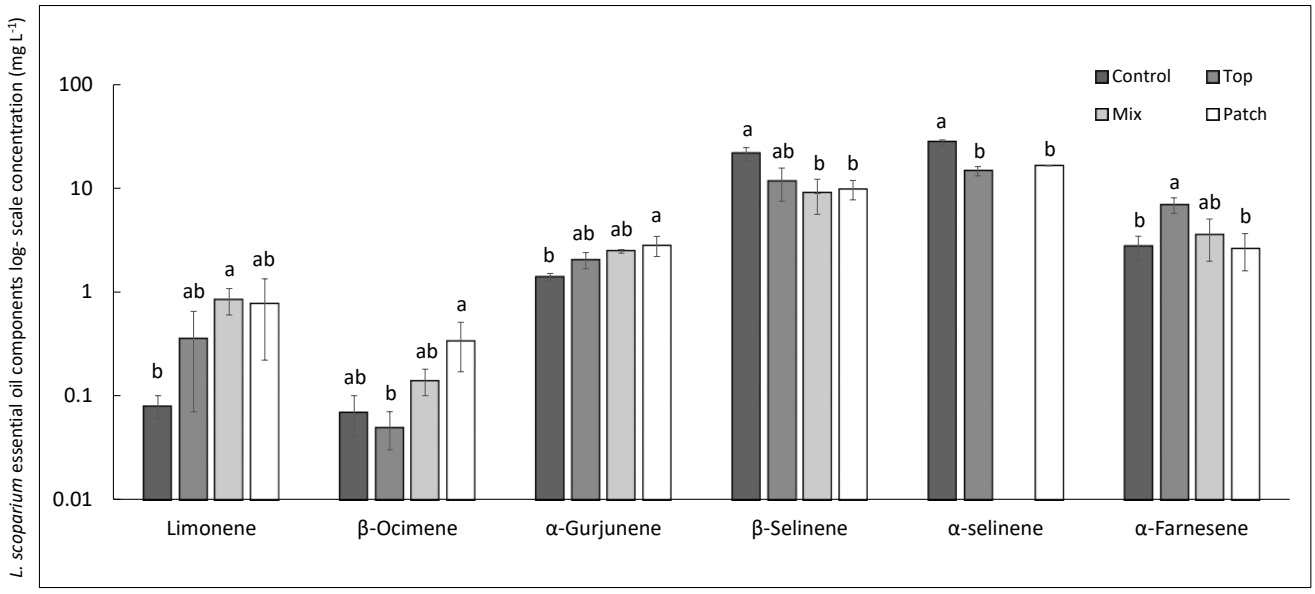


Figure 7.3 Significantly different *L. scoparium* essential oil components (p≤ 0.05) in Exp. 6. The concentrations are mg L^{-1} in the solvent extract.

Table 7-3 *L. scoparium* five major essential oil components, expressed as a percentage contribution in Exp. 1-7 (n=4, 3, 5, 5, 4, 3 and 15, respectively). Standard errors are given in parentheses. Numbers below the treatments show the N equiv. applied (kg ha^{-1}). Different letters (a, b, c) indicate significant differences between the treatments at p ≤ 0.05. Statistical analysis is based on the concentrations. KB, CB, DSE and TMW represent Kaikoura Biosolids, Christchurch City Council Biosolids, Dairy Shed Effluent and Treated Municipal Wastewater, respectively.

	Component	**Control (0)**	**KB (1250)**	**Sawdust+KB (1250)**				
Exp. 1	**β- elemene**	12 (1.6)^{b}	13 (1.8)^{ab}	15 (3.5)^{a}	-	-	-	-
	caryophyllene	8.5 (1.2)	7.6 (0.4)	7.7 (2.5)	-	-	-	-
	α-humulene	11 (2.1)	9.8 (0.6)	7.7 (3.2)	-	-	-	-
	β-selinene	2.6 (0.6)	3.6 (0.6)	3.7 (0.5)	-	-	-	-
	calamenene	5.9 (0.7)	6.2 (0.2)	5.1 (1.2)	-	-	-	-
		Control (0)	**KB (2800)**	**DSE (200)**	-	-	-	-
Exp. 2	**β- elemene**	15 (7.6)	18 (0.9)	17 (3.3)	-	-	-	-
	β-selinene	7.9 (3.1)	6.2 (1.1)	8.3 (3.5)	-	-	-	-
	α-selinene	11 (1.9)	11 (1.2)	9.9 (3.9)	-	-	-	-
	α-farnesene	5.3 (1.1)	6.3 (2.4)	4.0 (0.6)	-	-	-	-
	calamenene	4.6 (1.5)	4.4 (0.7)	5.0 (0.3)	-	-	-	-
		Control (0)	**CB (500)**	**CB (1500)**	**CB (4500)**	**CB (13500)**	-	-
Exp. 3	**β-elemene**	9.0 (1.4)^{ab}	7.3 (1.5)^{b}	11 (0.8)^{ab}	13 (1.7)^{a}	13 (4.8)^{ab}	-	-
	β-selinene	5.8 (1.0)^{a}	5.5 (0.7)^{ab}	3.7 (0.3)^{b}	5.9 (0.6)^{a}	8.5 (0.2)^{a}	-	-
	α-selinene	9.0 (0.2)	8.6 (0.8)	-	8.2 (0.8)	12 (1.7)	-	-
	α-farnesene	2.0 (0.4	1.8 (0.3)	3.3 (1.4)	3.4 (1.7)	6.0 (0.9)	-	-
	calamenene	5.7 (0.6)	5.6 (0.3)	5.2 (0.4)	4.6 (0.3)	3.4 (2.2)	-	-
		Control (0)	-	**CB (1500)**	-	-	-	-
Exp. 4	**β-elemene**	18 (2.4)	-	12 (1.4)	-	-	-	-
	β-selinene	10 (2.1)	-	9.8 (0.8)	-	-	-	-
	α-selinene	12 (2.0)	-	12 (0.7)	-	-	-	-
	α-farnesene	2.9 (0.9)	-	4.8 (1.9)	-	-	-	-
	calamenene	3.3 (1.0)^{b}	-	5.0 (0.5)^{a}	-	-	-	-
		Control (0)	**CB-M (630)**	**CB-T (630)**	**CB-M (1900)**	**CB-T (1900)**	**CB-M (5700)**	**CB-T (5700)**
Exp. 5	**β-elemene**	12 (3.0)	18 (2.0)	14 (1.9)	13 (2.3)	9.9 (3.5)	11 (1.3)	10 (3.6)
	β-caryophyllene	3.7 (0.8)^{ab}	6.0 (1.7)^{a}	5.7 (0.8)^{ab}	3.3 (1.0)^{b}	6.2 (1.4)^{a}	4.9 (0.9)^{ab}	3.6 (0.4)^{ab}
	β-selinene	8.2 (2.2)	5.0 (0.8)	3.1 (0.9)	4.5 (1.0)	2.4 (0.8)	7.3 (1.2)	4.6 (1.8)
	α-selinene	12 (1.2)^{a}	9.8 (1.2)^{abc}	7.8 (1.2)^{bc}	7.5 (1.4)^{c}	6.9 (2.1)^{bc}	11 (1.3)^{ab}	11 (1.4)^{a}
	calamenene	6.1 (0.6)^{ab}	4.0 (0.8)^{c}	5.5 (1.4)^{ab}	4.9 (0.6)^{bc}	6.8 (0.6)^{a}	4.8 (1.0)^{abc}	4.5 (1.3)^{abc}
		Control (0)	**CB-Top (3000)**	**CB-Mix (3000)**	**CB-Patch (3000)**	-	-	-
Exp. 6	**β-elemene**	18 (1.5)	19 (2.8)	20 (4.4)	17 (3.5)	-	-	-
	caryophyllene	6.8 (1.5)	4.3 (1.7)	7.1 (3.1)	4.6 (1.5)	-	-	-
	β-selinene	8.1 (0.5)^{a}	3.5 (1.6)^{ab}	3.9 (0.2)^{b}	3.1 (0.7)^{b}	-	-	-
	α-selinene	11 (1.0)^{a}	4.2 (0.9)^{b}	*	5.4*	-	-	-
	calamenene	5.9 (0.7)	5.0 (0.6)	5.6 (0.6)	7.1 (0.7)			-
		Control (0)	**TMW (30)**	-	-	-	-	-
Exp. 7	**β-elemene**	6.1 (1.4)	6.4 (0.9)	-	-	-	-	-
	caryophyllene	3.6 (0.4)	4.4 (0.7)	-	-	-	-	-
	β-selinene	4.3 (0.6)	3.6 (0.6)	-	-	-	-	-
	α-selinene	6.4 (0.7)	6.3 (0.7)	-	-	-	-	-
	calamenene	6.2 (0.5)	6.1 (0.4)	-	-	-	-	-

The addition of biosolids (KB and CB) and sawdust+KB resulted in significant changes in *K. robusta* EO composition in some of the treatments. Among the first four experiments, only in Exp. 1 and Exp. 3 the concentrations of the EO components significantly changed and as with *L. scoparium*, these changes were small (<20%). DSE and TMW application did not change the EO composition. Table 7-4 compares *K. robusta* EO components concentrations in the solvent extract of all pot experiments for all treatments. This table shows mixing biosolids (KB and CB) with the soil had a greater effect on the *K. robusta* EO quality compared to other treatments (sawdust+KB, surface application of KB and DSE).

The major component of *K. robusta* EO is α-pinene, which was unaffected by all the biowaste treatments. This component comprised 31%-47% of the EO in the greenhouse experiments. The number of *K. robusta* EO evaluated components in Exp. 1- Exp. 4, were 13, 14, 20 and 20, respectively. In Exp. 1 only p-cymene, which has ca.2-7% contribution to the EO components, was decreased by KB application and sawdust+KB decreased the α-terpineol (Figure 7.4 A). Out of the eight components that were significantly affected by the treatments in Exp. 3, only linalool, alloaromadendrene and spathulenol were affected when CB were applied up to 1500 kg N ha^{-1} equiv. and all three were increased by this treatment (Figure 7.4 B). CB application in higher rates negatively affected the other components illustrated in Figure 7.4 B.

The number of evaluated EO components were 23 for Nikau Palm Gully and Quail Island and 24 for Bridle Path. Analysis of the naturally grown *K. robusta* EO samples showed that as other *K. robusta* plants from different regions of NZ, α-pinene is the major EO component. After that, cymene, γ-terpinene, calamenene and ledol mainly contribute in the *K. robusta* EO and other components were negligible (Table 7-5). The concentration of α-pinene was not substantially different in the samples of the three sites, but some variations has been monitored in the minor components (generally lower in Nikau Palm Gully compared to Bridle Path).

Table 7-6 shows three major components of the *K. robusta* EO and their contribution percentage in Exp. 1- Exp. 4 and Exp. 7. In these experiments the major components of *K. robusta* EO were α-pinene, p-cymene, 1,8-cineole, γ-terpinene and ledol. In Exp. 7 (field experiment), the application of TMW affected neither the total concentration nor the composition of the EOs (Figure 6.5, Table 7-6 and Appendix C).

Table 7-4 All evaluated essential oil components range of *K. robusta* in the greenhouse experiments. Standard errors are given in parenthesis except for the numbers that have been detected once. Existence of the significant difference at p ≤ 0.05 is indicated by "*". Concentrations are mg L^{-1} in the solvent extract.

Rank	Component name	Control range	Biosolids range (mixed)	Biosolids (surface applied)	Dairy shed effluent	Sawdust+ KB
1	**α-thujene**	1.7 (0.7)-3.8 (1.9)	1.9 (0.3)-3.4 (0.9)	-	-	-
2	**α-pinene**	104 (7)-1707 (29)	90 (7)-110 (12)	161 (9)	93 (9.0)	124 (28)
3	**α-terpinene**	0.06 (0.01)	0.11 (0.02)-0.20 (0.09)	-	-	-
4	**β-pinene**	1.0 (0.1)-1.6 (0.2)	0.8 (0.2)	2.2 (0.3)	0.8 (0.2)	1.6 (0.7)
5	**β-myrcene**	0.6 (0.1)	-	0.82 (0.04)	-	0.5
6	**cymene**	9.3 (3.3)-26 (6.6)	3.3 (0.9)-14 (7.5)	10 (2.0)*	13 (12)	23 (4.6)
7	**limonene**	1.8 (0.6)-4.3 (0.6)	1.4 (0.2)-2.6 (0.8)*	4.5 (0.7)	2.0 (0.7)	8.4 (4.1)
8	**1,8-cineol**	5.0 (2.7)-28 (7.0)	4.0 (1.1)-26.2 (3.6)*	14 (5.5)	26 (2.2)	6.4 (4.4)
9	**β-ocimene**	0.5 (0.2)-1.1 (0.3)	0.5 (0.3)-2.2 (1.8)	-	-	-
10	**γ-terpinene**	13 (4.6)-28 (14)	15 (4.8)-26 (6.7)	-	-	-
11	**α-terpinolene**	2.1 (0.6)-3 (1.5)	2.1 (0.6)-4.8 (1.4)	-	-	-
12	**linalool**	2.7 (0.5)-13 (3.7)	2.2 (0.3)-7.1 (1.2)*	8.1 (1.2)	1.5 (0.9)	9.0 (2.4)
13	**terpinen-4-ol**	0.8 (0.2)-3.0 (1.0)	0.36 (0.03)-1.3 (0.52)	1.8 (0.3)	0.7 (0.6)	1.8 (0.4)
14	**α-terpineol**	1.3 (0.6)-7.6 (1.5)	1.0 (0.3)-6.6 (1.5)*	2.6 (0.8)	5.5 (1.4)	1.4 (0.6)*
15	**α-copaene**	0.4 (0.1)-0.8 (0.2)	0.26 (0.04)-1.3 (0.6)*	-	0.6 (0.1)	-
16	**α-gurjunene**	0.8 (0.2)-0.8 (0.1)	0.8 (0.1)-1.2 (0.2)	-	-	-
17	**caryophyllene**	0.3 (0.1)-1.1 (0.7)	0.4 (0.1)-0.6 (0.1)	0.8 (0.1)	-	0.3 (0.2)
18	**alloaromadendrene**	0.5 (0.1)-0.7 (0.2)	0.4 (0.1)-1.0 (0.2)*	-	0.7 (0.2)	-
19	**delta-cadinene**	0.6 (0.1)-0.8 (0.2)	0.7 (0.1)-0.8 (0.2)	-	-	-
20	**calamenene**	3.2 (0.8)-9.2 (0.8)	1.12 (0.03)-9.4 (3.2)*	4.7 (0.8)	8.3 (1.5)	3.7 (0.8)
21	**viridiflorol**	11 (3.3)	-	14 (4.5)	-	14 (4.5)
22	**spathulenol**	0.7 (0.2)-2.4 (1.0)	0.3 (0.1)-2.5 (0.5)*	-	4.1 (0.8)	-
23	**caryophyllene, epoxide**	0.3 (0.1)-0.6 (0.1)	0.2 (0.1)-0.4 (0.1)	-	0.9 (0.4)	-
24	**globulol**	1.8 (0.7)-6.0 (2.4)	7.2 (3.7)	2.1 (0.6)	6.1 (2.1)	0.7 (0.1)*
25	**ledol**	3.4 (0.9)-4.6 (0.6)	5.2 (1.0)-7.0 (1.9)	-	-	-

Table 7-5 Essential oil components concentration of the field sampled *K. robusta* from Nikau Palm Gully, Quail Island and Bridle Pass. Concentrations are mg L^{-1} in the solvent extract. Standard errors are given in parenthesis. Different letters (a, b, c) indicate significant differences between the sampling sites at $p \leq 0.05$.

	Nikau Palm Gully	Quail Island	Bridle Path
α-thujene	4.7 (1.5)	4.6 (1.7)	5.6 (1.2)
α-pinene	185 (4)	205 (16)	204 (30)
β-pinene	1.9 (0.4)^{b}	0.6 (0.1)^{b}	3.4 (0.5)^{a}
α-terpinene	*	0.12 (0.09)	0.14 (0.04)
cymene	17.3 (4.2)	38.9 (9.6)	25.5 (4.8)
limonene	3.9 (0.5)	4.4 (0.4)	4.0 (0.2)
1,8-Cineol	8.5 (2.3)^{b}	15.5 (4.0)^{a}	6.9 (2.8)^{b}
β-ocimene	1.4 (0.5)	0.6 (0.2)	1.8 (0.9)
γ-terpinene	32.5 (10.5)	20.6 (9.5)	38.3 (9.1)
α-terpinolene	5.6 (1.9)	4.0 (2.1)	6.0 (1.4)
linalool	7.6 (2.4)	7.7 (1.7)	9.8 (2.4)
terpinen-4-ol	2.1 (0.5)	2.2 (0.4)	2.4 (0.6)
α-terpineol	2.7 (0.67)^{b}	3.9 (0.8)^{a}	2.3 (0.5)^{b}
α-cubebene	0.4 (0.1)	*	*
α-copaene	1.9 (0.2)^{b}	2.4 (0.3)^{ab}	2.8 (0.4)^{a}
α-gurjunene	2.7 (0.6)	2.4 (0.7)	4.4 (0.3)
caryophyllene	1.4 (0.5)^{b}	1.27 (0.47)^{ab}	3.3 (0.5)^{a}
α-humulene	0.6 (0.1)^{b}	0.87 (0.27)^{ab}	1.0 (0.1)^{a}
alloaromadendrene	2.9 (0.5)	3.47 (0.6)	3.9 (0.6)
δ-cadinene	1.9 (0.3)^{b}	1.6 (0.1)^{b}	3.0 (0.3)^{a}
α-muurolene	*	*	1.4 (0.1)
calamenene	10.3 (1.2)^{b}	19.5 (2.7)^{a}	20.9 (2.4)^{a}
spathulenol	5.5 (1.3)^{ab}	8.3 (1.4)^{a}	2.5 (1.2)^{b}
caryophyllene, epoxide	1.2 (0.2)^{b}	1.9 (0.4)^{ab}	2.2 (0.3)^{a}
ledol	21.5 (2.6)^{b}	27.0 (4.0)^{ab}	41.1 (5.2)^{a}

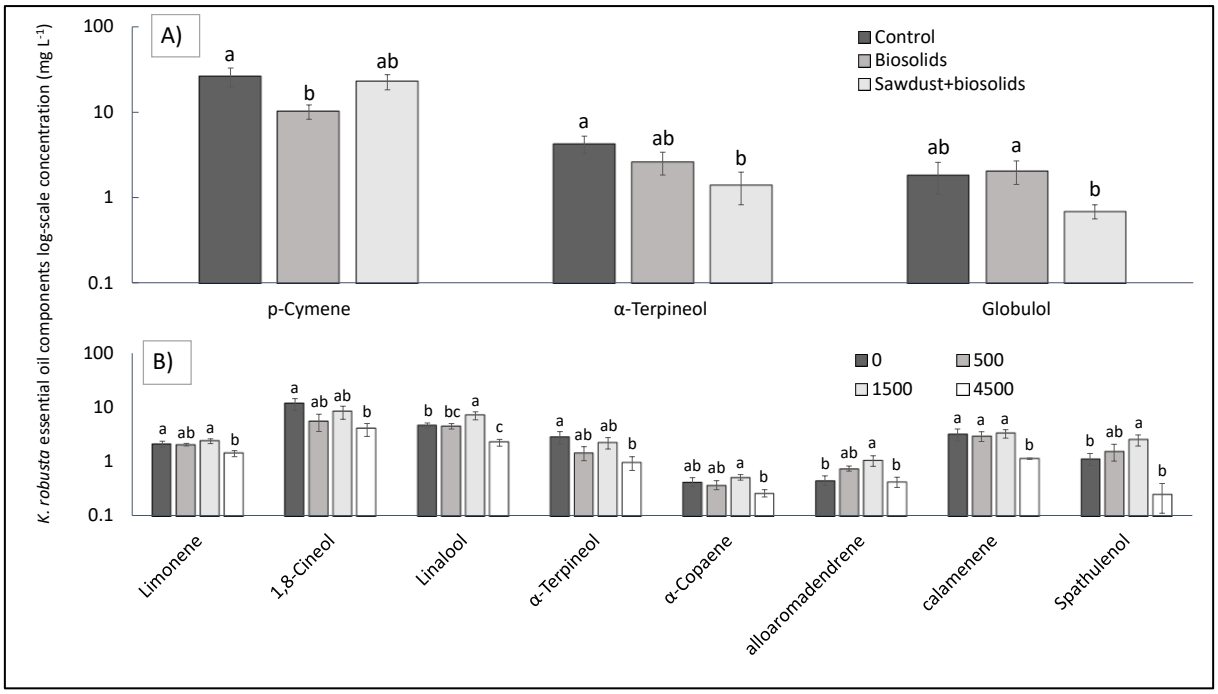


Figure 7.4 Significantly different *K. robusta* essential oil components ($p \leq 0.05$) in Exp. 1 (A) and Exp. 3 (B). The concentrations are mg L^{-1} in the solvent extract.

Table 7-6 *K. robusta* three major essential oil components, expressed as a percentage contribution in Exp. 1- Exp. 4 and Exp. 7 (n=4, 3, 5, 5 and 15, respectively). Standard errors are given in parentheses. Numbers below the treatments show the N equiv. applied (kg ha^{-1}). Different letters (a, b, c) indicate significant differences between the treatments at $p \leq 0.05$. Statistical analysis is based on the concentrations. KB, CB, DSE and TMW represent Kaikoura Biosolids, Christchurch City Council Biosolids, Dairy Shed Effluent and Treated Municipal Wastewater, respectively.

	Component	**Control (0)**	**KB (1250)**	**Sawdust+ KB (1250)**	**-**
Exp. 1	**α-pinene**	33 (2.8)	34 (0.5)	33 (3.5)	-
	p-cymene	5.5 (1.6)[a]	2.2 (0.5)[b]	7.3 (2.4)[ab]	-
	1,8-cineole	2.8 (1.2)	2.9 (1.0)	1.5 (1.2)	-
Exp. 2		**Control (0)**	**KB (2800)**	**DSE (200)**	-
	α-pinene	33 (2.2)	31 (2.5)	35 (3.4)	-
	p-cymene	3.0 (2.1)	2.7 (1.8)	3.5 (3.0)	-
	1,8-cineole	8.3 (2.6)	8.1 (0.6)	9.6 (1.2)	-
Exp. 3		**CB (0)**	**CB (500)**	**CB (1500)**	**CB (4500)**
	α-pinene	44 (3.2)	34 (2.4)	35 (2.5)	47 (2.0)
	p-cymene	3.6 (1.3)	3.9 (0.9)	4.6 (2.4)	1.6 (0.4)
	γ-terpinene	5.5 (2.0)	9.5 (2.6)	8.1 (2.4)	8.0 (0.8)
Exp. 4		**CB (0)**	-	**CB (1500)**	-
	α-pinene	40 (5.5)	-	39 (2.1)	-
	p-cymene	6.1 (1.6)	-	4.6 (1.6)	-
	γ-terpinene	8.8 (4.1)	-	6.5 (2.1)	-
Exp. 7		**Control (0)**	**TMW (30)**	-	-
	α-pinene	32 (1.4)	32 (1.1)	-	-
	calamenene	2.4 (0.2)	2.5 (0.2)	-	-
	ledol	4.3 (0.5)	4.2 (0.4)	-	-

7.3 Lavender (*Lavandula angustifolia* Mill.), rosemary (*Rosmarinus officinalis* L.) and thyme (*Thymus vulgaris* L.)

Biosolids (KB and CB at rates up to 2800 and 1500 kg N ha^{-1}) and DSE application affected small number of the EO components in *L. angustifolia*, *R. officinalis* and *T. vulgaris* (Table 7-7-Table 7-11).

Results in Table 7-7 shows the concentration range of *L. angustifolia* EO components in the solvent extract for biosolids (CB and KB) and DSE treatments in Exp. 2 and Exp. 3. The table shows that mixing biosolids with the soil has greater impact on the *L. angustifolia* EO quality than the DSE application. There were some significant differences at high application rates. The number of the evaluated EO components for *L. angustifolia* was 15 and 18 in Exp. 2 and Exp. 3, respectively. Figure 7.5 shows the EO components that were affected by biowastes application in Exp. 2 and Exp. 3. The biosolids application (KB and CB at rates up to 2800 and 1500 kg N ha^{-1}) did not change the concentration of most of the major EO components of *L. angustifolia* (Table 7-7, Figure 7.5 and Appendix C). Although in Exp. 3, five components including α-ocimene, β- ocimene, linalool, lavandulol and borneol were decreased when biosolids were applied up to 1500 kg N ha^{-1} equiv. to the soil but nine more components showed reduction in higher application rate (Figure 7.5 B). Table 7-8 shows the five major components of *L. angustifolia* EO in the experiments. This table showed that DSE application in Exp. 2, (200 kg N ha^{-1} equiv.) only decreased α-ocimene, which contributes to 2.4%-3.9% of the *L. angustifolia* EO components. In Exp. 3, linalool and borneol were decreased when 1500 kg N ha^{-1} equiv. CB or more were applied to the soil. These components contributes in ca. 25% and 5.5% of the EO.

Table 7-9 compares the *R. officinalis* EO components concentration ranges of the solvent extract in Exp. 2 and Exp. 3. This table shows that mixing biosolids (KB or CB) with the soil and DSE application would affect some components of the *R. officinalis* EO. The number of the evaluated EO components for *R. officinalis* was 15 and 25 for Exp. 2 and Exp. 3, respectively.

Figure 7.6 shows the *R. officinalis* EO components that were affected by biowastes application in Exp. 2 and Exp. 3. The results showed that KB application in Exp. 2 did not alter the EO composition *R. officinalis* while DSE application reduced the concentration of β-myrcene, 1,8-cineole, α-terpineol and verbenone (Figure 7.6 A). In Exp. 3, applying CB up to 4500 kg N ha^{-1} equiv. to *R. officinalis* either increased or did not change the concentration of EO components compared to control (Figure 7.6 B). Table 7-10 shows the five components that had highest concentration in *R. officinalis* EO and their contribution percentage in Exp. 2 and Exp. 3. Results of both experiments show that the major components of the EO were unaffected by the application of biowastes. The only reduced component

was verbenone. This component was affected by DSE application and contributes to only 3-6.5% of the *R. officinalis* EO.

In Exp. 2, ten components were identified in the solvent extract of *T. vulgaris*. Figure 7.7 shows that *T. vulgaris* EO was unaffected by KB application, while DSE decreased cymene content of the EO. This component is one of the top five concentrated components of the EO with 11-17 % contribution to the EO components (Table 7-11). Table 7-11 also shows that thymol that is the major component of *T. vulgaris* EO, was unaffected by biowastes application. This component contributes to ca. 36-39% of the *T. vulgaris* EO.

Table 7-7 All evaluated essential oil components range of *L. angustifolia* in the greenhouse experiments. Standard errors are given in parenthesis except for the numbers that have been detected once. Existence of the significant difference at p ≤ 0.05 is indicated by "*". Concentrations are mg L^{-1} in the solvent extract. The only alive plant in the highest biosolids rate application (13500 kg N ha^{-1}) has not been considered in this table.

Rank	Component name	Control range	Biosolids range (mixed)	Dairy shed effluent
1	**α-thujene**	0.19 (0.01)	0.15 (0.01)-0.19 (0.01)*	-
2	**α-pinene**	0.4 (0.4)-1.3 (0.1)	0.4 (0.1)-1.4 (0.1)	0.3 (0.1)
3	**camphene**	1.26 (0.04)	1.1 (0.1)-1.3 (0.1)	-
4	**β-pinene**	0.7 (0.2)	0.8 (0.3)	0.5 (0.1)
5	**3-octanone**	4.2 (0.8)	2.9 (0.3)	4.9 (0.3)
6	**β-myrcene**	1.8 (0.3)-2.2 (0.1)	1.7 (0.1)-1.9 (0.1)*	1.5 (0.4)
7	**p-cymene**	0.15 (0.02)	0.17 (0.01)	0.14 (0.01)
8	**limonene**	1.5 (0.5)-3.2 (0.2)	1.5 (0.1)-3.0 (0.2)*	1.6 (0.5)
9	**1,8-cineole**	1.2 (0.2)-22 (0.4)	1.2 (0.2)-26 (1.0)*	0.9 (0.1)
10	**α-ocimene**	6.7 (0.5)-9.5 (0.9)	4.1 (0.4)-7.8 (1.6)*	4.9 (1.1)*
11	**β-ocimene**	1.4 (0.1)-11 (2.0)	0.99 (0.04)-9.7 (1.5)*	6.7 (1.6)
12	**linalool**	73 (4.2)-109 (5.3)	71(4.5)-98 (3.6)*	73 (4.9)
13	**camphor**	40 (0.7)	35 (8.6)-50 (1.2)	-
14	**lavandulol**	2.4 (0.3)-2.8 (0.2)	0.9 (0.1)-2.8 (0.4)*	2.7 (0.1)
15	**borneol**	25 (1.3)	19 (0.6)-23 (0.7)*	-
16	**terpinen-4-ol**	7.2 (0.1)-20 (0.7)	6.6 (0.7)-20 (1.3)*	7.7(0.2)
17	**α-terpineol**	2.0 (0.1)	2.0 (0.1)-2.3 (0.1)	-
18	**linalyl acetate**	68 (6.2)-95 (1.7)	58 (3.6)-88 (2.3)*	48 (7.5)
19	**lavandulyl acetate**	9.9 (1.1)-15 (1.1)	8.2 (0.9)-15 (0.9)*	7.8 (2.1)
20	**neryl acetate**	0.42 (0.02)	0.38 (0.02)-0.41 (0.03)	-
21	**caryophyllene**	6.0 (0.4)-6.7 (0.8)	4.5 (0.4)-6.3 (0.7)*	3.7 (1.2)

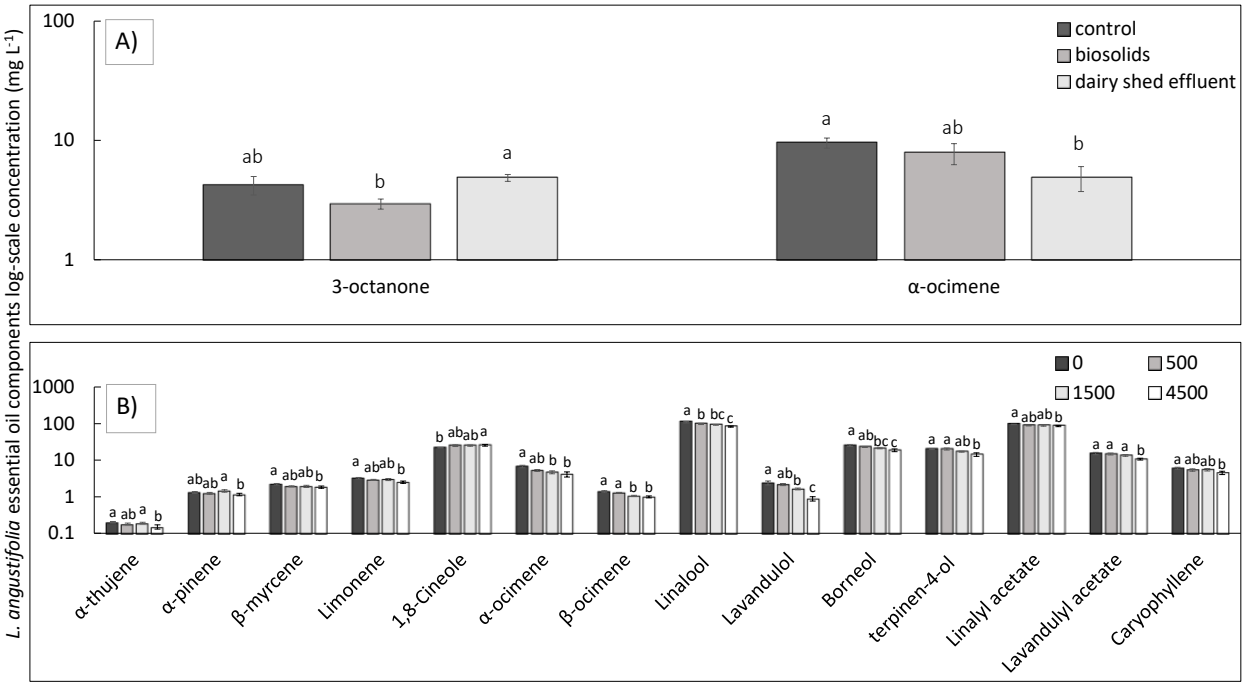


Figure 7.5 Significantly different *L. angustifolia* essential oil components (p ≤ 0.05) in Exp. 2 and 3 (A and B). Concentrations are mg L^{-1} in the solvent extract.

Table 7-8 *L. angustifolia* five major essential oil components, expressed as a percentage contribution in Exp. 2 (n=3) and Exp. 3 (n=5). Standard errors are given in parenthesis. Numbers below the treatments show the N equiv. applied (kg ha^{-1}). Different letters (a, b, c) indicate significant differences between the treatments at p ≤ 0.05. Statistical analysis is based on the concentrations. KB, CB and DSE represent Kaikoura Biosolids, Christchurch City Council Biosolids and Dairy Shed Effluent, respectively.

		Control (0)	**KB (2800)**	**DSE (200)**	**-**	**-**
Exp. 2	**β-ocimene**	4.3 (0.5)	4.4 (0.6)	3.2 (0.5)	-	-
	α-ocimene	3.9 (0.1)^{a}	3.5 (0.7)^{ab}	2.4 (0.5)^{b}	-	-
	linalool	30 (1.3)	32 (1.7)	36 (2.6)	-	-
	linalyl acetate	28 (0.6)	26 (0.7)	23 (1.5)	-	-
	lavandulyl acetate	4.1 (0.2)	3.7 (0.4)	3.7 (0.6)	-	-
		CB (0)	**CB (500)**	**CB (1500)**	**CB (4500)**	**CB* (13500)**
Exp. 3	**1,8-Cineole**	5.3 (0.2)^{b}	6.3 (0.3)^{ab}	6.5 (0.2)^{ab}	7.2 (0.3)^{a}	7.6
	linalool	26 (0.9)^{a}	25 (0.5)^{b}	24 (0.7)^{bc}	23 (0.4)^{c}	23
	camphor	9.7 (0.3)	8.6 (2.1)	11 (0.3)	11 (0.3)	12
	borneol	6.0 (0.2)^{a}	5.8 (0.1)^{ab}	5.5 (0.2)^{bc}	5.3 (0.4)^{c}	6.0
	linalyl acetate	23 (0.3)^{a}	22 (0.7)^{ab}	23 (0.5)^{ab}	24 (0.7)^{b}	23

*Result of one sample. This treatment has not been considered for the statistical analysis.

Table 7-9 All evaluated essential oil components range of *R. officinalis* in the greenhouse experiments. Existence of significant differences at p ≤ 0.05 is indicated by "*". Standard errors are given in parenthesis. Concentrations are mg L^{-1} in the solvent extract.

Rank	Component name	Control range	Biosolids range (mixed)	Dairy shed effluent
1	**α-pinene**	44 (1.8)-48 (1.3)	43 (3.7)-52 (4.3)	37 (3.0)
2	**camphene**	25 (3.2)-31 (5.8)	21 (2.3)-32 (5.4)	25 (3.5)
3	**β-pinene**	15 (2.1)-17 (3.6)	12 (2.3)-23 (4.0)	14 (2.0)
4	**3- octanone**	0.06 (0.02)-9.9 (6.0)	0.09 (0.01)-14 (1.2)*	1.8 (1.5)
5	**β-myrcene**	16 (3.0)-25 (6.0)	11 (1.3)-18 (1.2)	9.1 (4.6)*
6	**α-fellandrene**	0.8 (0.1)	0.9 (0.1)-1.07 (0.03)*	-
7	**cymene**	1.2 (0.3)-2.2 (1.6)	0.8 (0.1)-3.3 (1.4)*	1.5 (0.3)
8	**limonene**	11 (0.8)	11 (1.1)-12 (0.7)	-
9	**1,8-cineole**	33 (1.1)-76 (2.7)	33 (4.4)-87 (2.8)	46 (16)
10	**γ- terpinene**	5.4 (0.9)	3.9 (0.5)-5.6 (0.3)	-
11	**α-terpinolene**	2.1 (0.2)	2.2 (0.3)-2.5 (0.2)	-
12	**linalool**	5.4 (1.1)-7.4 (1.6)	3.5 (0.5)-7.3 (1.6)	4.2 (0.2)
13	**camphor**	20 (4.7)-64 (19)	13 (4.7)-60 (1.0)	53 (8.3)
14	**borneol**	13 (2.4)-14 (2.1)	6.9 (0.8)-21 (5.1)	13 (4.0)
15	**α- terpineol**	4.2 (0.2)-9.3 (0.4)	4.2 (0.4)-11 (0.3)	4.7 (1.1)*
16	**verbenone**	13 (0.9)-27 (3.7)	12 (2.0)-20 (0.6)*	8.3 (4.7)*
17	**bornyl- acetate**	8.2 (5.4)-27 (2.6)	11 (6.9)-30 (7.6)	13 (3.6)
18	**eugenol**	0.13 (0.01)	0.09 (0.01)-0.15 (0.01)	-
19	**α-copaene**	1.0 (0.1)	1.2 (0.2)-1.4 (0.2)	-
20	**caryophyllene**	12 (1.4)-18 (2.1)	14 (3.4)-18 (1.1)	17 (5.9)
21	**α-humulene**	1.9 (0.2)-7.3 (2.2)	1.8 (0.1)-5.3 (2.2)	3.7 (1.9)
22	**α-muurolene**	0.6 (0.1)	0.6 (0.2)-0.7 (0.2)	-
23	**δ -cadinene**	2.7 (0.2)	3.2 (0.5)-3.5 (0.4)	-
24	**α-cadinene**	0.22 (0.02)	0.27 (0.04)-0.28 (0.05)	-
25	**caryophyllene oxide**	3.8 (0.9)	2.1 (0.2)-3.4 (0.6)	-

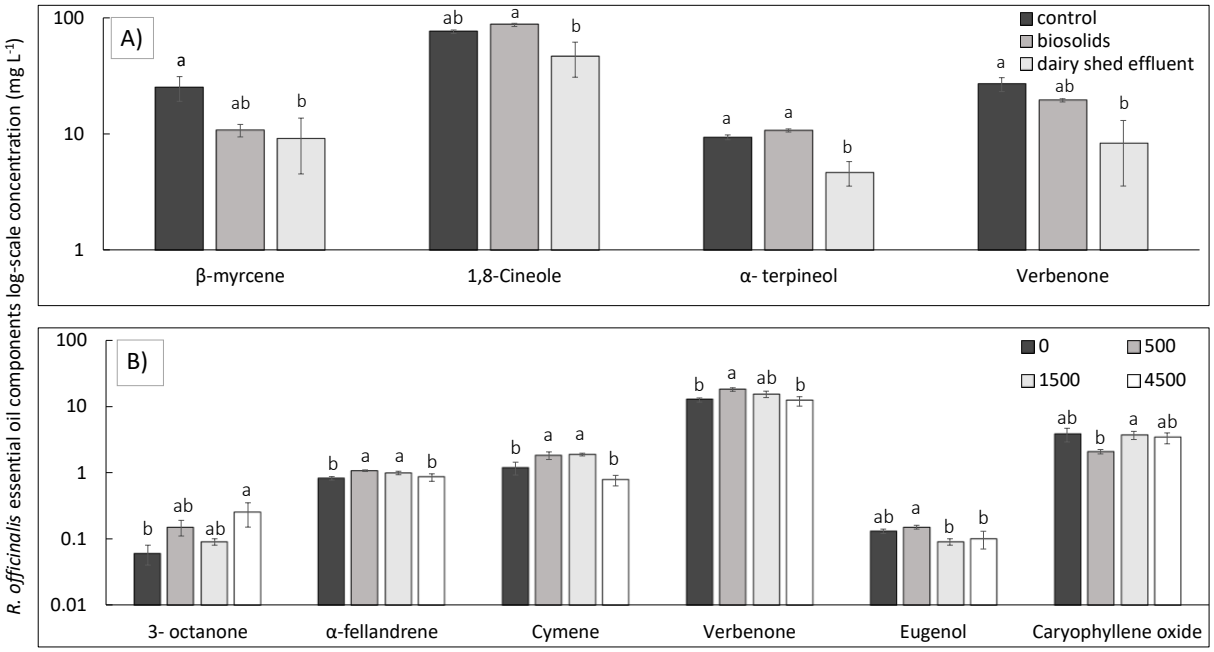


Figure 7.6 Significantly different *R. officinalis* essential oil components ($p \leq 0.05$) in Exp. 2 and 3 (A and B). Concentrations are mg L^{-1} in the solvent extract.

Table 7-10 *R. officinalis* five major essential oil components, expressed as a percentage contribution in Exp. 2 (n=3) and Exp. 3 (n=5). Standard errors are given in parentheses. Numbers below the treatments show the N equiv. applied (kg ha^{-1}). Different letters (a, b, c) indicate significant differences between the treatments at $p \leq 0.05$. Statistical analysis is based on the concentrations. KB, CB and DSE represent Kaikoura Biosolids, Christchurch City Council Biosolids and Dairy Shed Effluent, respectively.

		Control (0)	**KB (2800)**	**DSE (200)**	**-**
Exp. 2	**α-pinene**	12 (1.0)	11 (1.4)	12 (0.81)	-
	camphene	7.2 (0.96)	7.2 (0.86)	8.3 (1.0)	-
	1,8-cineole	18 (2.1)^{ab}	20 (1.6)^{a}	16 (5.3)^{b}	-
	camphor	15 (3.9)	14 (0.64)	18 (2.5)	-
	verbenone	6.5 (1.0)^{a}	4.5 (0.28)^{ab}	2.8 (1.6)^{b}	-
		CB (0)	**CB (500)**	**CB (1500)**	**CB (4500)**
Exp. 3	**α-pinene**	15 (1.3)	17 (0.60)	15 (0.32)	15 (0.89)
	camphene	8.2 (0.79)	7.3 (0.84)	8.7 (0.25)	7.2 (0.90)
	1,8-cineole	11 (0.71)	12 (0.37)	11 (0.44)	11 (0.56)
	camphor	6.5 (1.6)	5.9 (2.1)	8.3 (0.18)	5.1 (1.8)
	bornyl- acetate	8.8 (0.63)	7.5 (1.2)	7.5 (0.50)	9.7 (1.4)

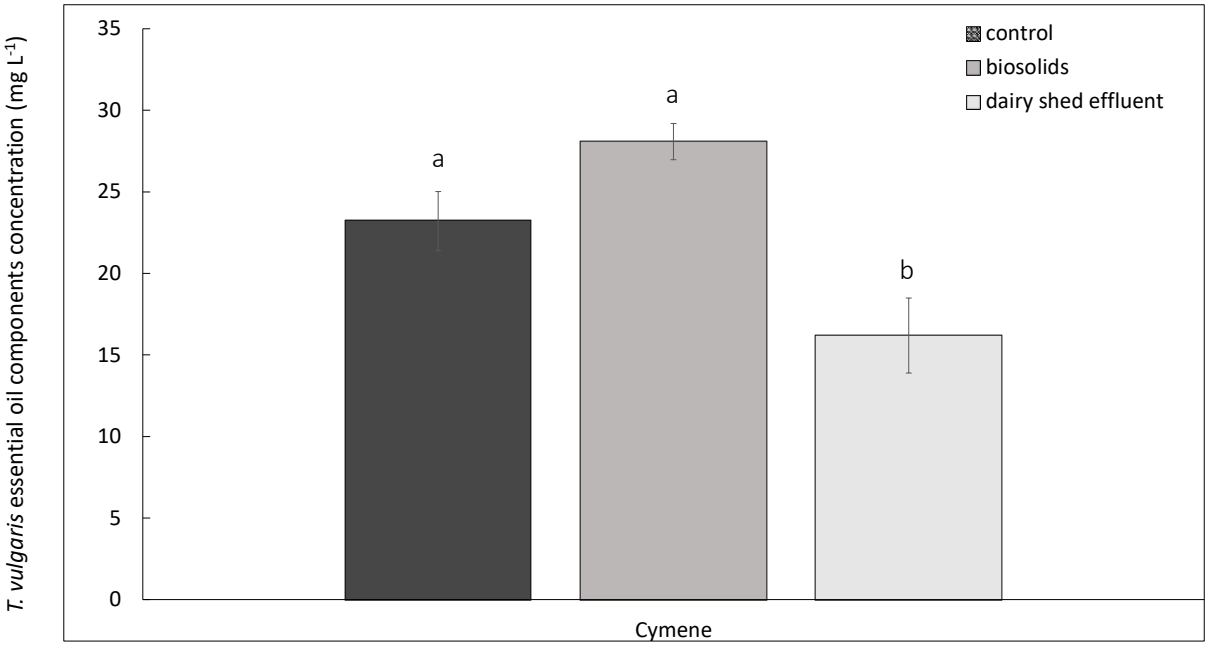


Figure 7.7 Significantly different *T. vulgaris* essential oil component ($p \leq 0.05$) in Exp. 2. Concentrations are mg L^{-1} in the solvent extract.

Table 7-11 *T. vulgaris* five major essential oil components, expressed as a percentage contribution in Exp. 2 (n=3). Standard errors are given in parentheses. Numbers below the treatments show the N equiv. applied (kg ha^{-1}). Different letters (a, b, c) indicate significant differences between the treatments at $p \leq 0.05$. Statistical analysis is based on the concentrations.

		treatments		
Number	**Compound name**	**Control (0)**	**Biosolids (2800)**	**Dairy shed effluent (200)**
1	**cymene**	17 (2.0)[a]	17 (1.2)[a]	11 (2.6)[b]
2	**γ- terpinene**	15 (1.5)	16 (2.5)	18 (1.7)
3	**linalool**	2.8 (0.31)	3.1 (0.46)	2.7 (0.23)
4	**thymol**	36 (2.1)	36 (2.4)	39 (2.1)
5	**carvacrol**	3.8 (0.21)	4.3 (0.25)	3.6 (0.19)

Table 7-12 shows the correlation between the elements of *L. scoparium, K. robusta, L. angustifolia* and *R.officinalis* leaves and EO components. This table shows that higher uptake of some elements including N, P, K, S and Cd would either negatively or positively affect some of the components in the EO that play valuable role in the EO properties.

Table 7-12 The correlations between leaves elements and essential oil components of *L. scoparium*, *K. robusta*, *L. angustifolia* and *R. officinalis* (0.001 ≥ p ≤ 0.01) in greenhouse experiments.

	L. scoparium		***K. robusta***		***L. angustifolia***		***R. officinalis***	
Leaf element	**Correlation status (-/+)**	**Affected component**	**Correlation status (-/+)**	**Affected component**	**Correlation status (-/+)**	**Affected component**	**Correlation status (-/+)**	**Affected component**
N	-ve (r=0.46, p<0.001) -ve	caryophyllene α-humulene	-ve (r=0.49, p<0.001) -ve	calamenene caryophyllene-epoxide	-ve (r=0.49, p<0.01) -ve -ve -ve -ve	α-ocimene β-ocimene lavandulol borneol caryophyllene	-ve (r=0.49, p<0.01)	eugenol
C	+ve (r=0.46, p<0.001) +ve	β-elemene α-farnesene	+ve (r=0.49, p<0.001) +ve +ve +ve	α-copaene calamenene caryophyllene-epoxide globulol	-ve (r=0.49, p<0.01) +ve +ve	β-pinene lavandulol borneol	+ve (r=0.49, p<0.01) +ve +ve	γ- terpinene camphor
P	*	*	-ve (r=0.49, p<0.001)	caryophyllene-epoxide	-ve (r=0.49, p<0.01) -ve -ve -ve -ve	α-thujene 3-octanone α-ocimene lavandulol borneol	-ve (r=0.49, p<0.01) -ve	camphor a-humulene
K	-ve (r=0.46, p<0.001)	α-humulene	-ve (r=0.49, p<0.001) +ve +ve	limonene globulol viridiflorol	+ve (r=0.49, p<0.01) +ve	3-octanone linalool	+ve (r=0.49, p<0.01) -ve -ve	β-pinene α-fellandrene verbenone
S	-ve (r=0.46, p<0.001) -ve	linalool α-terpineol	+ve(r=0.49, p<0.001) -ve -ve -ve	β-pinene calamenene caryophyllene-epoxide globulol	-ve (r=0.49, p<0.01) -ve	lavandulol borneol	-ve (r=0.49, p<0.01) +ve	camphor bornyl acetate
Cd	*	*	-ve (r=0.82, p<0.001) +ve	β-myrcene viridiflorol	*	*	*	*

In field sampled plants, there was a negative correlation between N and S of the naturally grown *L. scoparium* plants and calamenene (at $p \leq 0.01$). The plant P concentration was positively correlated with δ-cadiene. Plant K was not correlated with the main EO components. There was a negative correlation between the soil total N, calamenene and δ-cadiene. The soil NH_4^+-N was not correlated to the major EO components, while NO_3^--N was positively correlated with α-selinene. There was a negative correlation between the soil available P (Olsen P) and calamenene.

The N, P, K and S of *K. robusta* plants were not correlated with the major EO components. There was no correlation between soil available N and P (NH_4^+-N, NO_3^--N and Olsen P) and the *K. robusta* main EO components.

7.4 Discussion

An increase in total EO yield does not necessarily indicate that more profit will be generated from a crop if the quality of the EOs is diminished. The experiments showed that there were small but significant changes in the EO composition in many of the biowaste treatments. Given the low magnitude (<20%) of most changes in the EO components (specially the major ones) (Table 7-1, Table 7-4, Table 7-7, Table 7-9 and Table 7-11), it is unlikely that biowaste addition will significantly reduce oil quality. There is no information in the public domain about the economic importance of the individual EO components for any of the species tested. However, it is likely that a reduction in some of these components would correspondingly reduce the value of the EO. The importance of any change in the EO components is dependent on the ultimate use of the EO. For example, antimicrobial properties are less important if the EO is to be used in a perfume.

Biowaste addition to low-fertility soils increased foliar macronutrient concentrations in most treatments (Chapter 5). There were significant negative correlations between foliar macronutrients and a suite of EO components in *L. scoparium*, *K. robusta*, *L angustifolia,* and *R. officinalis* (Table 7-12). Abdelmajeed et al. (2013) reported a reduction in plant stress (for example through increased nutrient acquisition), would reduce the EO components that attract pollinators or repel herbivores. In *L. scoparium* the negative correlations between N, K, S and caryophyllene, α-humulene, caryophyllene-epoxide, linalool and α-terpineol could affect the anti-inflammatory, anticancer and analgesic properties of the EO (Fernandes et al., 2007, Fidyt et al., 2016). Conversely, in *K. robusta*, the positive correlation between K, globulol and viridiflorol could increase the antimicrobial and hormonal balancing properties of the EO (Hafsa et al., 2016, Ahmadvand et al., 2014). Calamenene and limonene were negatively correlated with the N and K in *K. robusta* leaves. Both of these compounds have anti-

cancer properties (Takei et al., 2006, Miller et al., 2011). In the leaves of *K. robusta,* Cd was negatively correlated with β-myrcene, which has anti-inflammatory properties (Abbaszadegan et al., 2015).

The major antimicrobial ingredient of *L. angustifolia* EO is linalool (Cavanagh and Wilkinson, 2002, Özek et al., 2010) that was positively correlated with the leaf K. lavandulol, borneol, α-ocimene and 3-octanone did not show a constant status of correlation with the leaf elements (Table 7-12). In *R. officinalis,* the negative correlation between the P and some EO components (camphor besides α-humulene and K with α-fellandrene besides verbenone), may reduce the pain killing, sleep inducing (Panesar, 2017), anti-inflammatory (Fernandes et al., 2007), anti-cancer (Lin et al., 2014) and antimicrobial properties of the EO (Panizzi et al., 1993). There was a positive correlation between K in the *R. officinalis* leaves and β-pinene that would improve the antimicrobial properties of the EO (Silva et al., 2012). This may be particularly important when *R. officinalis* is irrigated with K-rich biowastes such as TMW or DSE.

T. vulgaris total EO concentration and the thymol content of the plant were unaffected by the biowastes treatments. Baranauskienė et al. (2003) also reported that different concentrations of N fertilizers (0-135 kg ha^{-1}) did not affect the *T. vulgaris* EO components. This result is similar to the findings of other studies, which application of N (up to 135 kg ha^{-1}) (Baranauskiene et al., 2003) and NP (300 and 250 kg ha^{-1}) (Omidbaigi and Arjmandi, 2002) did not alter the thymol content of *T. vulgaris* that is consistent with the results of this research.

Other studies have reported the effect of geographical variations on *L. scoparium* and *K. robusta* EO composition (Maddocks-Jennings et al., 2005b, Douglas et al., 2004, Porter and Wilkins, 1999). The difference between the EO composition and the consequence results should be considered if they are used for therapeutic aims. Perry et al. (1997a) showed that the *L. scoparium* EO composition is mainly genetically controlled. In all the samples caryophyllene, β-elemene, α-selinene, β- selinene and calamenene had higher concentrations in the EO than the other components. This could be because of the vast number oblate *L. scoparium* seeds that can blow away in the wind to a large area, which leads to growing genetically similar plants (Tepapaonline, n.d.). The similarity between the EO major components of the natural *K. robusta* samples was supported by NZCFRL (2000) study that explained this plant has relatively similar EO throughout NZ.

7.5 Conclusions

Biowastes can be used to establish *L. scoparium* and *K. robusta* on low fertility soils with the goal of EO production because the biowastes significantly increased the EO yield, and only result in small changes

in the oil quality. Former pine forest soils would be ideal for such biowaste applications because blending the biosolids with sawdust increases oil concentration while reducing N-mobility. While biowastes improve the growth of *R. officinalis* and *L. angustifolia*, there may be significant reductions in the plant oil concentration as well as changes in the oil quality. These experiments were conducted over a shorter time than would normally be used for an EO crop cultivation.

In the field sampled plants, the composition of *L. scoparium* was more variable than the *K. robusta.* The concentration of *L. scoparium* and *K. robusta* EOs were positively affected by the environmental stress, and they showed greater potential for EO production in the nature compared to the greenhouse. Therefore, field testing of the most promising biowaste treatments, namely ca. 1500 kg N ha^{-1} equiv. biosolids with and without sawdust addition, is warranted to determine whether the effects found in this study are translatable to the field.

Chapter 8

Summarising conclusions

This thesis has shown that biowastes, including biosolids, Dairy Shed Effluent (DSE), sawdust+biosolids and Treated Municipal Wastewater (TMW) can be beneficially applied to low-fertility soils to increase EO production. The conclusions of this research are summarized in the context of the five specific objectives of the research:

a) To evaluate the growth response of Leptospermum scoparium, Kunzea robusta, Lavandula angustifolia, Rosmarinus officinalis and Thymus vulgaris growing in four contrasting medium-to low-fertility soil to addition of two types of biosolids and sawdust+biosolids mixtures, DSE, and TMW.

In general, the growth of all species was increased by the addition of biowastes. The increase in growth was inversely proportional to the fertility of the soil as indicated by the C and N content. These EO producing plants could be established on low-fertility soils with an application of biosolids (up to 1500 kg N ha^{-1} equiv.), biosolids+ sawdust (1250 kg N ha^{-1} equiv.) and TMW (30 kg N ha^{-1} equiv.). While DSE (200 kg N ha^{-1} equiv.) did not change the production of *L. angustifolia* flower clusters, *K. robusta* and *T. vulgaris* biomass, other treatments consistently showed positive effect in terms of biomass production. Other studies have demonstrated that the biowastes application at these rates are unlikely to result in excessive NO_3^- leaching (Robinson et al., 2017, Esperschuetz et al., 2017).

b) To evaluate how the biowastes affected the accumulation of the nutrients and contaminants by the plants.

The addition of biowastes increased the concentrations of some macronutrients in the plant tissue, especially N, P and S. There is limited evidence that N was the most limiting nutrient in the soils tested. Concentrations of TEs, particularly Zn, were increased by the application of biosolids, but unaffected by the other biowastes. Concentrations of TEs, including Cd, were always below threshold values: I used Food Safety Standards (FSS) as a conservative benchmark (ANZFSC, 2015). While the partitioning of contaminants, such as Cd, into the oils was not determined, analyses of commercial EOs revealed TE concentrations significantly lower than the foliar concentrations in this study, including the controls. This indicates that even if foliar TE levels were raised above FSS, the TE concentrations in the EOs would remain at safe levels.

c) To determine how the biowastes affected the concentration and therefore the yield of EOs in the plants.

For the most part, the addition of biowastes did not change the EO concentration in the plants. Therefore, it would be expected that EO yields would be increased in proportion to the biomass increase of the plants. The exceptions were the addition of DSE to *R. officinalis* and CB at the rates higher than 1500 kg N ha^{-1} equiv. to *L. angustifolia* where EO concentrations were decreased, which would offset the growth benefits.

Results of this research and other studies showed that nutrients and TEs in the biowastes would not negatively change the EO concentration and quality of the plants (Hadipour et al., 2013, Puttanna et al., 2010, Baranauskienė et al., 2003, Hamisi et al., 2012, Prasad et al., 2014b).

d) To determine the extent to which biowastes affected the quality of the EOs.

The addition of biowastes resulted in small (mostly < 20%) but significant changes in components of the EOs. With *L. angustifolia*, *R. officinalis* and *T. vulgaris,* the quality of the oil, as determined by evaluated components, showed both small increases and decreases when the plants were grown in soils amended with biowastes. Certainly, the addition of biowastes would not jeopardize the oil quality of these species. For *L. scoparium*, *K. robusta*, there is no information relating the EO composition to quality of value, it is therefore not possible to speculate how the changes resulting from biowaste addition would likely affect the value of the crop. Nevertheless, as with the other species tested, biowastes had only a small effect on the EOs composition of *L. scoparium* and *K. robusta.* Surface application of biosolids had less impact on EO composition compared to incorporating the biosolids into the soil. Moreover, although DSE affected the EO yield, it caused trivial changes in the EO composition.

The pattern of relation between plants' elements (e.g. N, K, S and Cd) and the EO components was not consistent. The results showed that nutrients would be positively or negatively correlated with some of the EO components that would affect the oil properties (e.g. antimicrobial, anti-inflammatory, anticancer and analgesic).

e) *To evaluate quality of EOs in natural populations of L. scoparium and K. robusta and compare this to the greenhouse trials.*

The EO major components evaluation showed higher variability in *L. scoparium* than *K. robusta* in the field sampled plants. This result is consistent with other studies on these plant species (Perry et al., 1997b, C&F-Research, 2000). Most of the field sampled plants of *L. scoparium* and *K. robusta* from the Canterbury region had significantly higher EO concentrations that the plants in the greenhouse study (which were also sourced from the Canterbury region). These differences are likely due to environmental factors rather than genetic differences between the plants. Other studies also have shown the positive effect of environmental stress like radiation and drought on EO production (Bettaieb et al., 2009, Petropoulos et al., 2008, Singh-Sangwan et al., 1994, Lima et al., 2017, Abdelmajeed et al., 2013).

Economic feasibility of growing essential oil producing plants in low-fertility soils

Considering the 1500 kg N ha^{-1} equiv. CB application as an optimum rate evaluated in the studies of this research for EO production, this treatment could increase the gross income of the EO crops as below. Clearly, a part of the revenue is spent on labour, planting, biowastes application and other production costs.

a) *L. scoparium*: 42600 kg of the plant fresh material produced 93.4 kg ha^{-1} of EO that would be worth NZ$87000 ha^{-1} (40% increase) in LSL.

43600 kg of the plant fresh material produced 103 kg ha^{-1} of EO that would make NZ$96000 ha^{-1} (156% increase) in PSL.

b) *K. robusta*: 39000 kg of the plant fresh material produces 98 kg ha^{-1} of EO that would be worth NZ$85700 ha^{-1} (211% increase) in LSL.

30000 kg of the plant fresh material produces 56 kg ha^{-1} of EO that would make NZ$49000 ha^{-1} (100% increase) in PSL.

c) *L. angustifolia*: 15730 kg of the fresh lavender clusters produces 115 kg ha^{-1} of EO that would be worth NZ$21500 ha^{-1} (70% increase) in LSL.

d) *R. officinalis*: 37300 kg of the rosemary fresh plant material produces 220 kg ha^{-1} of EO that would be worth NZ$50000 ha^{-1} (60% increase) in LSL.

These results show that *L. scoparium* or *K. robusta* could be good choices for revegetating low fertility lands (e.g. former *Pinus radiata* forests) in New Zealand. While *L. scoparium* would likely result in higher economic returns than *K. robusta*, ecological conditions, such as drought and the presence of plant pathogens, may make *K. robusta* a better choice. Both species could generate significant revenue through EO (and maybe honey) production besides conservation of local character and enhancing the biodiversity.

Knowledge gaps and potential application of this research in the field

Applying this research in the field requires that further ecological and social aspects of biowastes be considered. While biowastes increase the growth of the EO species tested, they may also increase the growth of competing weed species (Zimdahl, 2007). This may be particularly important for the NZ native species *L. scoparium* and *K. robusta*, which are adapted to low-fertility soils: the growth of exotic weeds that are adapted to high-fertility soils may be much greater than the NZ natives resulting in increased competition.

Many EO species have important symbiotic relationships with soil microbes, particularly mycorrhizal fungi (Bell et al., 2013). By providing high concentrations of macronutrients such as P, the biowastes may supress the growth of these essential microorganisms (Čapek et al., 2016, Liu et al., 2015). In later years, as fertility drops, reduced numbers of beneficial microbes in biowastes-amended soils may result in lower growth rates of EO species.

Similarly, the social acceptance of using EO's produced on soils amended with biowastes is unclear. There is a demonstrable negative public perception of foods that have been grown on biosolids-amended soils (Robinson et al., 2012). However, it is unclear whether this also relates to EOs. As individual cultures differ between countries and even regions, numerous studies would be required to resolve this issue.

Potentially, EO production could be linked with other environmental uses of the plants, such as combining EO production with riparian protection or on degraded lands such as mine spoil (Gutierrez Gines et al., 2017, Maddocks et al., 2004). In these cases, an investigation is required to determine the economic feasibility of harvesting EOs from small areas. Data are also needed on the effects of removing part or all of the EO-plants on their environmental function.

Further research is also required to:

1) Compare the effect of using different types of biosolids (fresh, aged, non-treated and treated) at the same time, by using the same soil type and plant on the EO production.

2) Compare the effect of same concentration of nutrients in mineral fertilizers and biosolids on the EO production by the plants.

3) Compare the effect of same biosolids and soil type on the plants EO production in the greenhouse and field (growing at the same time).

Appendix A

Supplementary information for Chapter 3

Methods development for essential oil extraction of mānuka (*Leptospermum scoparium*), kānuka (*Kunzea robusta*), lavender (*Lavandula angustifolia*), rosemary (*Rosmarinus officinalis*) and thyme (*thymus vulgaris*).

Essential oil extraction of *L. scoparium* and *K. robusta* were followed by a designed method from the University of Waikato (Senanayake, 2006b). In this method plants' leaves were soaked in ethanol + dichloromethane (1:1) for 18-20 hours. Although different ratios of ethanol + dichloromethane solvent mixture including (8:2), (6:4), (4:6) and (2:8) and soaking times of 6 and 12 hrs were tried, results showed that the original method had the best extraction of the most important EO components from *L. scoparium* and *K. robusta*.

The methods for *L. angustifolia*, *R. officinalis* and *T. vulgaris* EO extraction were selected by soaking the EO producing organ of the plants in different solvents for varied time lengths to principally extract the EO components and least possible unwanted materials. The tested solvents were hexane, diethyl ether, petroleum ether and ethanol.

For *L.* angustifolia, hexane, diethyl ether and petroleum ether were tested with different soaking times of 7, 14, 19 and 24 hours. The best method was soaking the *L. angustifolia* flowers in hexane for 19 hours.

For *R. officinalis* and *T.* vulgaris, hexane and ethanol were tested either separately or mixed at different ratios of (9:1), (8:2), (7:3) and (1:1). Moreover, soaking times of 3, 6, 12, 18 and 24 hours were tested for all the mixtures. The best method was using the hexane+ ethanol (9:1) mixture and soaking the *R. officinalis* and *T.* vulgaris leaves in the solvent mixture for 3 and 18 hours, respectively. Results showed that using ethanol more than 10% of the ratio, extracted various unwanted components that caused difficulties in the chromatography interpretation. All the extractions were evaluated by Gas Chromatography and Mass Spectrometry (GC/MS) to have the best chromatograph.

GC/MS analysis of lavender (*Lavandula angustifolia*), rosemary (*Rosmarinus officinalis*) and thyme (*thymus vulgaris*).

The analysis of volatile organic compounds (VOCs) from EO plant extracts was performed by Gas Chromatography Mass Spectrometry (GC/MS) and followed the method described by (Brophy et al., 1989). In brief, a Shimadzu QP2010 Ultra (GC/MS) fitted with a Restek RTX-5 ms capillary column (30m x 0.25mm i.d x 0.25 µm film thickness) was used to provide chromatographic separation, with helium carrier gas set to a constant linear velocity of 44.3 cm sec^{-1}. A CTC-Combipal autosampler was used to inject 1 µL of sample extracts into the injection port operated in splitless high-pressure injection mode (168 kPa) at a temperature of 250 °C for 40 seconds. The GC column oven was set to an initial temperature of 45.0 °C and held for 1.33 minutes before being ramped to 65.0 °C at 10.0 °C min^{-1} with a final ramp to 285.0 °C at 6.0 °C min^{-1} held for 10 minutes to release high boiling point components such as flavonoids and wax hydrocarbons.

The mass spectrometer was operated in electron impact mode (EI) at an ionization energy of 70eV and a mass scanning range of 33.0 to 500 m/z. The ion source and interface temperatures were set to 200 °C and 280 °C respectively. Compounds were identified by comparing acquired mass spectral data with those held in NIST11 and Wiley10 mass spectral libraries and confirmed through the use of published linear retention indices and the retention times of purchased standards. Compounds were tentatively quantified by comparing the amount of each compound identified to that of the internal standard added to each sample extract.

Shimadzu software GCMS solution version 2.72 was used to both acquire and process the chromatographic data.

Appendix B

Supplementary data for Chapter 5

Table 8-1 Elemental concentrations (g kg $^{-1}$ dry matter for N and C and mg kg^{-1} for other elements) of *Leptospermum scoparium* and *Kunzea robusta* leaves in Exp. 1. Standard errors are given in parenthesis. Significant differences between the treatments at p ≤ 0.05 are indicated by capital letters (A, B, C) for *L. scoparium* and lower-case letters (a, b, c) for *K. robusta* within the plant species.; results according to Esperschuetz et al. (2017). Numbers below the treatments show the N equiv. applied (kg ha^{-1}). KB=Kaikoura Biosolids and DSE= Dairy Shed Effluent.

	Leptospermum scoparium			*Kunzea robusta*		
	Sawdust+ KB (1250)	**KB (1250)**	**Control (0)**	**Sawdust+KB (1250)**	**KB (1250)**	**Control (0)**
N	8.21 (0.33)^{AB}	8.64 (0.42)^{A}	7.27 (0.11)^{B}	8.36 (0.95)^{a}	9.58 (0.53)^{a}	8.66 (0.85)^{a}
C	504 (2.1)^{A}	497 (2.5)^{AB}	496 (2.2)^{B}	487 (5.3)^{a}	487 (2.6)^{a}	479 (3.8)^{a}
B	34.8 (2.9)^{A}	39.6 (2.5)^{A}	37.3 (1.8)^{A}	39.7 (13.5)^{a}	33.2 (11.4)^{a}	54.6 (5.2)^{a}
Ca	12427 (500)^{A}	11746 (1190)^{A}	14359(738)^{A}	6997 (2361)^{ab}	5678 (1985)^{b}	10465 (573)^{a}
Cd	≤ 3*10^{-4}	≤ 3*10^{-4}	≤ 3*10^{-4}	0.06 (0.06)^{a}	0.02 (0.02)^{a}	0.02 (0.02)^{a}
Cu	2.31 (0.16)^{A}	2.49 (0.27)^{A}	2.26 (0.25)^{A}	1.53 (0.62)^{a}	2.25 (0.76)^{a}	2.34 (0.79)^{a}
Fe	40 (2.2)^{A}	50 (3.4)^{B}	41 (0.9)^{A}	35 (13.5)^{a}	44 (15)^{a}	61 (4.0)^{a}
K	3528 (140)^{A}	3248 (42)^{A}	3458 (92)^{A}	2531 (870)^{a}	2715 (929)^{a}	3290 (82)^{a}
Mg	1459 (57)^{A}	1410 (28)^{A}	1761 (185)^{A}	1075 (360)^{a}	1030 (349)^{a}	1913 (160)^{b}
Mn	72.5 (11.8)^{A}	62.4 (12.4)^{A}	69.4 (18.4)^{A}	94.7 (31.7)^{ab}	72.7 (27.4)^{a}	160.7 (15.0)^{b}
Na	587 (50)^{A}	725 (98)^{A}	662 (38)^{A}	697 (258)^{a}	723 (243)^{a}	928 (119)^{a}
P	1055 (44)^{B}	915 (6.9)^{AB}	783 (93)^{A}	1123 (378)^{a}	1563 (539)^{a}	2106 (348)^{a}
S	1200 (67)^{A}	1171 (30)^{A}	1112 (67)^{A}	1279 (447)^{a}	1457 (490)^{a}	2090 (298)^{a}
Zn	14.4 (1.4)^{AB}	17 (1.2)^{B}	12.9 (0.23)^{A}	32 (10.9)^{b}	37 (13.7)^{b}	22.8 (2.1)^{a}

Table 8-2 Elemental concentrations (g kg $^{-1}$ dry matter for N and C and mg kg^{-1} for other elements) of *Leptospermum scoparium* and *Kunzea robusta* leaves in Exp. 2. Standard errors are given in parenthesis. Significant differences between the treatments at p ≤ 0.05 are indicated by capital letters (A, B, C) for *L. scoparium* and lower-case letters (a, b, c) for *K. robusta* within the plant species. Numbers below the treatments show the N equiv. applied (kg ha^{-1}). DSE = Dairy Shed Effluent.

	Leptospermum scoparium			*Kunzea robusta*		
	DSE (200)	**biosolids (2800)**	**control (0)**	**DSE (200)**	**biosolids (2800)**	**control (0)**
N	11.4 (0.38)	12.0 (0.53)	11.2 (0.37)	8.8 (1.09)	8.6 (0.56)	7.6 (0.45)
C	509 (3.2)^{B}	509 (3.9)^{B}	524 (2.4)^{A}	507 (3.4)	513 (1.7)	510 (5.4)
B	50.3 (7.8)	54.5 (11.9)	39.0 (3.5)	50.8 (5.6)	32.5 (4.2)	49.2 (7.2)
Ca	10929 (357)^{AB}	11636 (951)^{A}	9039 (520)^{B}	5836 (154)^{ab}	7216 (685)^{a}	4789 (369)^{b}
Cd	0.02 (0.01)	0.07 (0.04)	0.02 (0.01)	0.02 (0.01)^{b}	0.31 (0.09)^{a}	0.01 (0.00)^{b}
Cu	3.3 (0.15)^{A}	3.4 (0.29)^{A}	2.3 (0.16)^{B}	1.5 (0.23)^{b}	2.3 (0.17)^{a}	1.3 (0.28)^{b}
Fe	60 (12)	71 (19)	44 (1.9)	72 (16)	47 (5.0)	95 (42)
K	6556 (414)	5713 (302)	5875 (579)	5915 (374)^{b}	5927 (86)^{b}	7221 (389)^{a}
Mg	3065 (307)	3236 (243)	2799 (223)	2226 (168)	1886 (239)	1828 (176)
Mn	180 (47)	315 (84)	168 (69)	503 (64)^{ab}	683 (102)^{a}	399 (49)^{b}
Na	478 (169)	607 (78)	549 (138)	1021 (165)	1623 (220)	1181 (142)
P	1096 (182)	1323 (125)	916 (46)	1672 (371)	1747 (101)	1523 (170)
S	1173 (82)	1142 (51)	1183 (69)	962 (94)^{b}	1310 (117)^{a}	995 (65)^{ab}
Zn	11.2 (2.3)^{B}	68.2 (21.5)^{A}	10.2 (1.2)^{B}	40.9 (8.5)^{b}	118.8 (5.8)^{a}	29.8 (6.7)^{b}

Table 8-3 Elemental concentrations (g kg $^{-1}$ dry matter for N and C and mg kg^{-1} for other elements) of *L. scoparium* leaves in Exp. 3. Standard errors are given in parenthesis. Different letters (a, b, c, d) represent significant differences between the treatments (p ≤ 0.05). Numbers below the treatments show the N equiv. applied (kg ha^{-1}). CB = Christchurch City Council Biosolids.

	Leptospermum scoparium				
	CB (0)	**CB (500)**	**CB (1500)**	**CB (4500)**	**CB (13500)**
N	11.2 (0.58)^{d}	16.3 (0.54)^{c}	20.3 (0.90)^{b}	25.5 (0.75)^{a}	21.9*
C	496 (6.2)^{a}	493 (2.3)^{a}	485 (5.1)^{a}	483 (3.9)^{a}	478*
Al	69.4 (13.6)^{ab}	44.2 (7.4)^{b}	52.9 (5.0)^{b}	89.9 (19.8)^{a}	78.0 (5.0)^{ab}
B	49.6 (3.6)^{ab}	38.8 (3.4)^{b}	45.3 (2.1)^{ab}	57.3 (6.2)^{a}	53.7 (7.2)^{ab}
Ca	11919 (909)^{cd}	11782 (685)^{d}	13929 (386)^{bc}	15775 (657)^{ab}	18267 (998)^{a}
Cd	≤ 3*10^{-4}	≤ 3*10^{-4}	≤ 3*10^{-4}	≤ 3*10^{-4}	≤ 3*10^{-4*}
Cr	0.03 (0.01)^{a}	≤0.01*	0.11 (0.1)^{a}	0.22 (0.16)^{a}	≤0.08*
Cu	2.53 (0.45)^{c}	3.07 (0.17)^{c}	3.97 (0.17)^{b}	6.82 (0.60)^{a}	4.23 (0.13)^{b}
Fe	123 (24)^{a}	170 (37)^{a}	124 (5.8)^{a}	228 (57)^{a}	112 (25)^{a}
K	4146 (183)^{a}	4535 (109)^{a}	4588 (196)^{a}	4165 (190)^{a}	4432 (391)^{a}
Mg	3042 (333)^{b}	3023 (162)^{b}	3728 (199)^{ab}	3959 (348)^{a}	4295 (21)^{a}
Mn	350 (37)^{b}	560 (74)^{a}	612 (55)^{a}	675 (17)^{a}	672 (119)^{a}
Na	1060 (191)^{ab}	1003 (89)^{ab}	858 (99)^{b}	839 (123)^{b}	1451 (303)^{a}
P	1003 (186)^{c}	1194 (46)^{bc}	1476 (87)^{b}	2515 (109)^{a}	2310 (84)^{a}
S	913 (66)^{d}	1638 (129)^{c}	2306 (106)^{b}	3182 (194)^{a}	3200 (481)^{a}
Zn	23.5 (3.6)^{c}	32.6 (3.2)^{bc}	42.5 (5.2)^{b}	69.3 (6.0)^{a}	102 (45)^{a}

* Just one sample was evaluated.

Table 8-4 Elemental concentrations (g kg $^{-1}$ dry matter for N and C and mg kg^{-1} for other elements) of *Kunzea robusta* leaves in Exp. 3. Standard errors are given in parenthesis. Different letters (a, b, c, d) represent significant differences between the treatments (p ≤ 0.05). Numbers below the treatments show the N equiv. applied (kg ha^{-1}). CB = Christchurch City Council Biosolids.

	Kunzea robusta				
	CB (0)	**CB (500)**	**CB (1500)**	**CB (4500)**	**CB (13500)**
N	9.7 (0.93)^{d}	14.7 (0.87)^{c}	20.6 (0.65)^{b}	24.8 (2.2)^{a}	---
C	483 (0.98)^{a}	489 (4.4)^{a}	487 (2.6)^{a}	472 (3.0)^{b}	---
Al	145 (62)^{a}	40 (7.2)^{b}	33 (5.4)^{b}	70 (27)^{ab}	---
B	51 (5.3)^{a}	35 (3.4)^{b}	33 (3.0)^{b}	36 (2.5)^{b}	---
Ca	6714 (705)^{b}	7813 (965)^{ab}	9212 (819)^{a}	9984 (668)^{a}	---
Cd	≤ 3*10^{-4}	≤ 3*10^{-4}	≤ 3*10^{-4}	≤ 3*10^{-4}	---
Cr	0.09* (0.02)^{a}	0.07[1]	0.02[1]	0.01 (0.00)^{a}	---
Cu	1.7 (0.41)^{b}	3.0 (0.52)^{ab}	2.8 (0.57)^{ab}	4.7 (1.5)^{a}	---
Fe	131 (36)^{a}	102 (7.2)^{a}	179 (39)^{a}	480 (342)^{a}	---
K	4944 (216)^{b}	5087 (216)^{b}	4848 (75)^{b}	6868 (574)^{a}	---
Mg	2979 (338)^{a}	3073 (346)^{a}	3131 (288)^{a}	3644 (192)^{a}	---
Mn	328 (53)^{b}	532 (52)^{ab}	713 (75)^{a}	615 (138)^{a}	---
Na	1168 (30)^{a}	1368 (118)^{a}	1270 (128)^{a}	1824 (430)^{a}	---
P	1540 (298)^{b}	2056 (240)^{ab}	1904 (120)^{ab}	2729 (446)^{a}	---
S	1302 (126)^{d}	1687 (89)^{c}	2350 (166)^{b}	5113 (1101)^{a}	---
Zn	76 (11)^{a}	84 (5.2)^{a}	114 (16)^{a}	114 (22)^{a}	---

Table 8-5 Elemental concentrations (g kg $^{-1}$ dry matter for N and C and mg kg^{-1} for other elements) of *Leptospermum scoparium* and *Kunzea robusta* leaves in Exp. 4. Standard errors are given in parenthesis. Different letters (a, b, c, d) represent significant differences between treatments of the same plant species (p ≤ 0.05). Numbers below the treatments show the N equiv. applied (kg ha^{-1}). CB = Christchurch City Council Biosolids.

	Leptospermum scoparium		*Kunzea robusta*	
	CB (0)	**CB (1500)**	**CB (0)**	**CB (1500)**
N	10.8 (0.33)^{B}	23.9 (1.23)^{A}	11.5 (0.39)^{b}	23.9 (0.98)^{a}
C	495 (2.0)^{A}	495 (3.0)^{A}	494 (1.8)^{a}	483 (3.2)^{b}
Al	69.7 (8.0)^{A}	50.5 (6.4)^{A}	52.6 (6.5)^{a}	38.8 (7.2)^{a}
B	54.8 (4.0)^{A}	52.2 (1.4)^{A}	52.3 (4.8)^{a}	37.6 (3.5)^{b}
Ca	12687 (794)^{A}	12630 (888)^{A}	6710 (384)^{b}	9191 (529)^{a}
Cd	≤ 3*10^{-4}	≤ 3*10^{-4}	≤ 3*10^{-4}	≤ 3*10^{-4}
Cr	0.05 (0.00)	≤0.05*	≤0.08*	0.03 (0.02)
Cu	2.97 (0.50)^{A}	5.83 (0.19)^{A}	3.28 (0.36)^{a}	3.95 (0.54)^{a}
Fe	89 (6.0)^{B}	151 (19.5)^{A}	72 (4.6)^{b}	94 (7.9)^{a}
K	4836 (179)^{A}	5028 (149)^{A}	4727 (290)^{b}	7106 (609)^{a}
Mg	3082 (171)^{A}	3515 (253)^{A}	3183 (341)^{a}	3177 (213)^{a}
Mn	373 (30)^{B}	541 (42)^{A}	520 (43)^{b}	1000 (106)^{a}
Na	1609 (166)^{A}	1021 (168)^{B}	1885 (138)^{a}	1633 (180)^{a}
P	1136 (84)^{B}	2898 (403)^{A}	1906 (196)^{b}	2966 (226)^{a}
S	1192 (54)^{B}	2995 (272)^{A}	1665 (112)^{b}	2853 (139)^{a}
Zn	20 (1.4)^{B}	68 (9.2)^{A}	52 (4.7)^{b}	139 (39)^{a}

* Only in one sample was detected.

Table 8-6 Elemental concentrations (g kg $^{-1}$ dry matter for N and mg kg^{-1} for other elements) of *Leptospermum scoparium* leaves in Exp. 5. Standard errors are given in parenthesis. Different letters (a, b, c, d) represent significant differences between treatments (p ≤ 0.05). Numbers below the treatments show the N equiv. applied (kg ha^{-1}). CB = Christchurch City Council Biosolids.

	Exp. 5						
	Control (0)	**CB-1T (630)**	**CB-2T (1900)**	**CB-3T (5700)**	**CB-1M (630)**	**CB-2M (1900)**	**CB-3M (5700)**
N	20.3 (0.4)^{b}	24.3 (0.6)^{ab}	26.0 (0.3)^{a}	27.2 (1.7)^{a}	22.2 (1.0)^{b}	26.6 (0.5)^{a}	28.0 (2.6)^{a}
Ca	14100 (950)^{ab}	8900 (350)^{d}	11900 (630)^{bc}	10600 (500)^{cd}	9680 (650)^{cd}	14200 (670)^{ab}	17000 (2180)^{a}
Cd	0.02 (0.02)^{bc}	0.01 (0.01)^{c}	0.13 (0.01)^{ab}	0.15 (0.01)^{ab}	0.01 (0.01)^{c}	0.12 (0.04)^{ab}	0.22 (0.03)^{a}
Cr	0.6 (0.09)^{ab}	0.81 (0.29)^{ab}	0.63 (0.16)^{ab}	0.49 (0.25)^{b}	0.55 (0.05)^{ab}	1.12 (0.19)^{a}	0.68 (0.15)^{ab}
Cu	6.3 (0.8)^{a}	5.3 (0.5)^{ab}	4.3 (0.4)^{bc}	3.7 (0.7)^{c}	3.2 (0.4)^{c}	5.5 (0.5)^{ab}	5.6 (0.6)^{ab}
Fe	170 (9.5)^{ab}	185 (52)^{ab}	144 (28)^{ab}	126 (53)^{b}	119 (11)^{b}	216 (35)^{a}	146 (14)^{ab}
K	12400 (530)^{a}	8950 (780)^{b}	9040 (900)^{b}	6140 (700)^{c}	8520 (410)^{bc}	9130 (1700)^{b}	6050 (450)^{c}
Mg	4080 (230)^{a}	1900 (120)^{d}	2450 (120)^{bc}	2650 (200)^{b}	2080 (210)^{cd}	2610 (50)^{b}	3650 (380)^{a}
Mn	292 (27)^{d}	267 (21)^{d}	720 (54)^{bc}	803 (42)^{ab}	241 (32)^{d}	578 (57)^{c}	977 (99)^{a}
Na	1050 (120)^{a}	1000 (80)^{a}	852 (74)^{ab}	847 (76)^{ab}	724 (111)^{b}	1000 (110)^{a}	853 (106)^{ab}
P	1360 (41)^{cd}	1490 (99)^{bcd}	2040 (130)^{a}	1810 (200)^{abc}	1180 (93)^{d}	1890 (180)^{ab}	2110 (220)^{a}
S	3180 (66)^{abc}	3100 (150)^{abc}	3040 (120)^{bc}	2910 (170)^{cd}	2540 (180)^{d}	3440 (140)^{ab}	3480 (160)^{a}
Zn	61 (16)^{bc}	36 (2.3)^{d}	42 (3.7)^{cd}	41 (4.3)^{cd}	40 (5.7)^{cd}	62 (9.4)^{b}	88 (9.3)^{a}

Table 8-7 Elemental concentrations (g kg $^{-1}$ dry matter for N and mg kg^{-1} for other elements) of *Leptospermum scoparium* leaves in Exp. 6. Standard errors are given in parenthesis. Different letters (a, b, c, d) represent significant differences between treatments (p ≤ 0.05). Numbers below the treatments show the N equiv. applied (kg ha^{-1}). CB = Christchurch City Council Biosolids.

	Control (0)	Mixed (3000)	Patch (3000)	Top (3000)
N	n.a.*	23.3 (0.6)	23.4 (0.9)	22.3 (0.9)
B	24 (1.5)	30 (4.2)	23 (3.1)	23 (2.6)
Ca	17800 (1300)^{a}	15300 (1400)^{a}	10500 (460)^{b}	10400 (490)^{b}
Cd	≤ 3*10^{-4}	0.03 (0.03)^{a}	0.04 (0.00)^{b}	0.01 (0.01)^{b}
Cr	0.08 (0.03)^{c}	0.99 (0.24)^{a}	0.55 (0.07)^{ab}	0.46 (0.14)^{bc}
Cu	7.1 (0.9)^{a}	5.2 (0.2)^{b}	5.6 (0.3)^{ab}	4.4 (0.3)^{b}
Fe	94 (16)^{b}	209 (43)^{a}	130 (21)^{ab}	162 (37)^{ab}
K	8230 (910)	8000 (1430)	8000 (470)	6660 (1040)
Mg	4690 (390)^{a}	2590 (70)^{b}	2470 (200)^{bc}	2020 (140)^{c}
Mn	185 (2.7)^{b}	397 (46)^{a}	255 (15)^{b}	403 (49)^{a}
Na	1800 (640)^{a}	850 (50)^{b}	1040 (170)^{ab}	1020 (60)^{ab}
P	2450 (180)^{a}	1380 (40)^{b}	2260 (150)^{a}	1030 (60)^{b}
S	3000 (390)	2730 (110)	2920 (110)	2360 (60)
Zn	41 (4.8)^{a}	42 (7.9)^{a}	29 (2)^{ab}	21 (0.7)^{b}

* There was insufficient biomass for the determination.

Table 8-8 Elemental concentrations (g kg $^{-1}$ dry matter for N and C and mg kg^{-1} for other elements) of *Leptospermum scoparium* and *Kunzea robusta* leaves in Exp. 7. Standard errors are given in parenthesis. Significant differences between the treatments at p ≤ 0.05 are indicated by capital letters (A, B, C) for *L. scoparium* and lower-case letters (a, b, c) for *K. robusta* within the plant species. Numbers below the treatments show the N equiv. applied (kg ha^{-1}). TMW = Treated Municipal Wastewater

	Leptospermum scoparium		*Kunzea robusta*	
	Control (0)	TMW (30)	Control (0)	TMW (30)
N	12.0 (0.5)^{B}	15.0 (0.8)^{A}	14.8 (0.8)	17.1(0.9)
C	494 (2)	498 (2)	484(2)	488(2)
Al	140 (12)^{B}	182 (15)^{A}	119(7)	135(10)
As	0.29 (0.06)	0.35 (0.03)	0.15(0.07)	0.24(0.08)
B	30.9 (2)	37.3 (3.7)	48.6(1.8)	45.2(1.8)
Ca	6532 (494)	7015 (548)	4991(265)	5075(277)
Cd	0.007 (0.003)	0.013 (0.005)	0.020(0.007)	0.006(0.002)
Cr	0.29 (0.09)^{A}	0.13 (0.02)^{B}	0.25(0.06)	0.30(0.07)
Cu	4.1 (0.4)	4.3 (0.2)	4.9(0.5)	5.4(0.6)
Fe	219 (19)^{B}	290 (21)^{A}	188(11)^{b}	233(16)^{a}
K	3644 (98)^{B}	4241(113)^{A}	4210(152)	4112(187)
Mg	1720(78)	1641(98)	1663(103)	1696(70)
Mn	162(28)	125(17)	441(47)^{a}	285(33)^{b}
Na	183(83)^{B}	2142(88)^{A}	2114(92)	2475(174)
P	1054(116)^{B}	1351(69)^{A}	1458(66)^{b}	1761(124)^{a}
Pb	0.28(0.06)	0.17(0.03)	0.12(0.05)	0.25(0.06)
S	1147(48)^{B}	1351(43)^{A}	1434(47.1)	1587(66)
Zn	13.4(1.1)	15.6(0.9)	28.2(2.0)	34(6.4)

Table 8-9 Elemental concentrations (g kg $^{-1}$ dry matter for N and C and mg kg^{-1} for other elements) of *Leptospermum scoparium* and *Kunzea robusta* leaves in Exp. 8 (Field study). Standard errors of the means (5 > n < 7) are given in

parentheses. Significant differences between the treatments at p ≤ 0.05 are indicated by capital letters (A, B, C) for *L. scoparium* and lower-case letters (a, b, c) for *K. robusta* within the plant species.

Specie	*Leptospermum scoparium*			*Kunzea robusta*		
	Nikau Gully	**Quail Island**	**Yarrs Flat**	**Nikau Gully**	**Quail Island**	**Bridle Path**
N	9.7 (0.3)^{B}	15.2 (0.4)^{A}	14.4 (0.4)^{A}	11.5 (0.8)^{b}	12.8 (1.4)^{ab}	14.2 (0.6)^{a}
C	545 (4)^{A}	531 (3)^{B}	524 (4.6)^{B}	519 (2)^{a}	518 (5)^{a}	505 (3.8)^{b}
Al	141 (18)^{A}	83 (18)^{B}	78 (10)^{B}	81 (6)^{b}	112 (15)^{b}	494 (103)^{a}
B	40 (3)	43 (7)	44 (5)	38 (3)	39 (4)	47 (4)
Ca	6600 (677)^{B}	8548 (1440)^{AB}	11218 (1199)^{A}	4518 (309)^{b}	6880 (913)^{a}	7788 (267)^{a}
Cr	0.08 (0.01)^{A}	0.03 (0.01)^{B}	0.03 (0.00)^{B}	0.12*	0.03 (0.02)^{b}	0.5 (0.1)^{a}
Cu	2.2 (0.1)^{B}	4.0 (0.4)^{A}	1.5 (0.5)^{B}	2.8 (0.3)^{b}	2.7 (0.4)^{b}	5.5 (0.4)^{a}
Fe	115 (14)^{A}	77 (11)^{B}	92 (8)^{AB}	78 (5)^{b}	106 (12)^{b}	551 (137)^{a}
K	2945 (96)^{C}	4427 (253)^{A}	3644 (304)^{B}	3958 (196)	3990 (182)	3790 (105)
Mg	2603 (286)	2662 (317)	2902 (138)	2740 (104)^{b}	2716 (223)^{b}	3464 (202)^{a}
Mn	281 (67)^{B}	396 (44)^{AB}	598 (68)^{A}	352 (78)	511 (131)	341 (89)
Na	1959 (232)	1518 (122)	1909 (57)	2008 (163)	2043 (130)	2309 (316)
P	761 (103)^{B}	1431 (232)^{A}	757 (66)^{B}	1413 (189)^{b}	1741 (234)^{ab}	2220 (283)^{a}
S	934 (81)^{B}	1749 (75)^{A}	1648 (68)^{A}	1249 (108)	1364 (146)	1395 (55)
Zn	10.0 (0.6)^{B}	18 (3)^{A}	12.0 (1.4)^{AB}	21 (2)^{b}	51 (7)^{a}	40 (4)^{a}

* Only in one sample was detected.

Table 8-10 Elemental concentrations (g kg $^{-1}$ dry matter for N and C and mg kg^{-1} for other elements) of *L. angustifolia, R. officinalis* and *T. vulgaris* leaves in Exp. 2. Standard errors are given in parenthesis. Different letters (a, b, c, d) represent significant differences between treatments of the same plant species ($p \leq 0.05$). Numbers below the treatments show the N equiv. applied (kg ha^{-1}). KB=Kaikoura Biosolids and DSE= Dairy Shed Effluent.

	Lavandula angustifolia			*Rosmarinus officinalis*			*Thymus vulgaris*		
	Control (0)	**Biosolids (2800)**	**DSE (200)**	**Control (0)**	**Biosolids (2800)**	**DSE (200)**	**Control (0)**	**Biosolids (2800)**	**DSE (200)**
N	14 (0.3)^{b}	15 (0.7)^{b}	18 (0.4)^{a}	11 (2.6)	11 (0.8)	15 (0.8)	11 (0.9)	11 (0.5)	14 (2.4)
C	482 (0.7)^{ab}	477 (5.4)^{b}	489 (1.4)^{a}	495 (10)	489 (6.3)	506 (5.7)	447 (0.5)	446 (1.7)	452 (2.9)
Al	61 (8.5)	42 (5.5)	45 (11)	30 (6.4)	20 (3.6)	31(4.1)	165 (59)	51 (14)	78 (23)
B	41 (1.3)	38 (1.8)	42 (2.0)	36 (4.5)	40 (2.8)	32 (5.4)	23 (0.5)	26 (2.3)	28 (2.9)
Ca	18663 (521)^{b}	20890 (386)^{a}	16647 (632)^{c}	13032 (803)^{ab}	14095 (626)^{a}	10397 (1406)^{b}	9767 (517)^{b}	10562 (340)^{ab}	11889 (856)^{a}
Cr	0.67 (0.03)	0.73 (0.08)	1.3 (0.7)	0.46 (0.04)	0.46 (0.07)	0.7 (0.3)	2.1 (0.7)	1.1 (0.4)	1.14 (0.12)
Cu	4.9 (0.8)^{b}	14 (1.5)^{a}	8.5 (0.8)^{b}	2.8 (1.1)^{b}	11 (0.4)^{a}	4.8 (0.2)^{b}	2.3 (0.3)^{b}	4.45 (0.12)^{a}	4.0 (0.9)^{ab}
Fe	68 (4.5)	61 (4.3)	68 (9.2)	39 (9.9)	33 (2.6)	48 (2.7)	127 (34)	60 (11)	94 (2.4)
K	6341 (643)^{b}	6804 (317)^{b}	9329 (319)^{a}	7412 (1061)^{b}	7054 (80)^{b}	16980 (2159)^{a}	7048 (323)	6926 (195)	11826 (3495)
Mg	5715 (172)^{ab}	6497 (539)^{a}	4747 (261)^{b}	2225 (282)	2928 (535)	1824 (76)	2012 (193)	2596(422)	2204 (85)
Mn	79 (6.2)	92 (15)	80 (4.3)	78 (15)	61 (9.7)	61 (8.8)	85 (7.1)^{b}	71 (6.9)^{b}	204 (30)^{a}
Mo	0.78 (0.16)^{b}	2.7 (0.3)^{a}	0.37 (0.01)^{c}	1.4 (0.4)^{b}	10 (1.2)^{a}	0.93 (0.14)^{b}	0.21 (0.11)^{b}	1.4 (0.4)^{a}	0.230 (0.004)^{b}
Na	244 (16)	214 (30)	173 (19)	1636 (390)^{a}	1208 (270)^{ab}	466 (143)^{b}	169 (45)	105 (58)	69 (11)
Ni	0.24 (0.03)	0.340 (0.002)	0.50 (0.25)	0.20 (0.05)	0.32 (0.05)	0.43 (0.25)	0.75 (0.28)	0.50 (0.11)	0.5 (0.1)
P	1646 (103)^{c}	2738 (106)^{a}	2115 (50)^{b}	1000 (214)^{b}	1969 (351)^{a}	1444 (42)^{ab}	1167 (115)	1610 (52)	1580 (226)
S	2649 (300)^{b}	4228 (405)^{a}	3139 (200)^{b}	1511 (259)^{b}	2897 (256)^{a}	2019 (163)^{b}	1162 (96)	1224 (85)	1396(123)
Zn	58 (15)	59 (3.1)	47 (4.4)	55.61 (21.5)	53 (9.6)	39 (4.1)	63 (22)	66 (13)	51 (6.6)

Table 8-11 Elemental concentrations (g kg $^{-1}$ dry matter for N and C and mg kg^{-1} for other elements) of *L. angustifolia* and *R. officinalis* leaves in Exp. 3. Standard errors are given in parenthesis. Different letters (a, b, c, d) represent significant differences between treatments of the same plant species ($p \leq 0.05$). Numbers below the treatments show the N equiv. applied (kg ha^{-1}). CB= Christchurch City Council Biosolids.

	Lavandula angustifolia					*Rosmarinus officinalis*			
	CB (0)	**CB (500)**	**CB (1500)**	**CB (4500)**	**CB (13500)***	**CB (0)**	**CB (500)**	**CB (1500)**	**CB (4500)**
N	15 (0.5)	18 (0.7)	20 (0.2)	25 (0.5)	26.25	11 (0.2)	13 (0.7)	16 (0.5	23 (1.0)
C	487 (1.7)	482 (1.0)	483 (0.9)	472 (2.2)	470	494 (3.2)	492 (1.2)	489 (3.3)	485 (3.6)
Al	192 (63)	115 (36)	51 (13)	55 (17)	33	49 (13)	30 (2.9)	16 (2.4)	22 (1.5)
B	51 (0.8)	40 (0.5)	34 (1.3)	35 (0.9)	41	30 (1.2)	26 (1.2)	26 (0.9)	30 (0.9)
Ca	17145 (324)	18500 (861)	21457 (402)	21901 (1436)	24622	11747 (848)	10561 (574)	10962 (665)	15658 (1275)
Cr	0.6 (0.4)	0.2 (0.1)	0.06 (0.02)	0.060 (0.001)	0.00	0.03 (0.01)	0.15 (0.13)	0.11*	0.04*
Cu	4.5 (0.4)	7.7 (0.6)	5.6 (0.3)	4.4 (0.2)	4.12	2.0 (0.1)	6.0 (0.4)	7.6 (0.6)	6.2 (0.5)
Fe	188 (70)	126 (20)	102 (8.3)	104 (9.5)	81	53 (8.4)	47 (3.9)	43 (1.5)	67 (3.2)
K	12433 (539)	8556 (246)	7636 (449)	9912 (1027)	6404	10056 (470)	6577 (416)	6300 (349)	11392 (1686)
Mg	6382 (106)	6612 (301)	6413 (238)	6506 (447)	5706	2398 (169)	2310 (123.09)	2717 (86)	3465 (92)
Mn	71 (3.4)	78 (4.5)	87 (5.7)	116 (8.1)	241	39 (1.9)	38 (3.2)	45 (3.9)	120 (5.2)
Na	172 (9.6)	168 (12)	193 (8.6)	211 (15)	146	1092(132)	1096 (67)	941 (67)	1106 (143)
P	1950 (76)	2742 (252)	2756 (93)	3780 (192)	4236	2011 (112)	2601(123)	2518 (131)	3246 (331)
S	3151 (89)	4011 (260)	3930 (128)	4643 (172)	4502	2922 (188)	2788 (168)	3369 (139)	3981 (125)
Zn	38 (2.2)	45 (1.5)	47 (1.5)	79 (3.6)	115	38 (3.0)	33 (2.2)	39 (0.4)	80 (7.5)

*Only one plant thrived in this treatment.

Inductively Coupled Plasma Optical Emission Spectrometry (ICP) Detection limits
The minimum concentrations that is detected by ICP (detection limits).

	mg kg^{-1} / mg L^{-1}
Ag	0.000395441
Al	0.002131047
As	0.001504422
B	0.000585293
Bi	0.008
Ca	0.000313316
Cd	0.000258139
Co	0.001
Cr	0.000
Cu	0.000559437
Fe	0.000653585
K	0.007290669
Li	0.000
Mg	0.000374433
Mn	0.000
Na	0.00107476
Ni	0.001169069
P	0.004546571
Pb	0.003265581
S	0.007442794
Sr	1.62923E-05
Zn	0.00015

Appendix C

Supplementary data for Chapter 7

Table 8-12 The most important component groups of *L. scoparium*, *K. robusta*, *L. angustifolia*, *R. officinalis* and *T. vulgaris* essential oils.

Plant Specie	Oil Producer Organ	Yield	Major Component Groups of the Essential Oils	References
L. scoparium	Leaves (occasionally flowers and branches)[a]	0.8 % [b] According to the season from 0.2- 1%[c]	sesquiterpenes, B- triketones and monoterpene compounds [b]	[a](Maddocks-Jennings et al., 2005b) [b](G Porter, 2003b) [c](C&F-Research, 2000)
K. robusta	Leaves (and occasionally branches)[a]	0.3- 2.1 %[a]	monoterpenes, sesquiterpenes, monoterpenoids[b]	[a](C&F-Research, 2000) [b](Maddocks-Jennings et al., 2005b)
L. angustifolia	flowering tops	1.4 -1.6%	monoterpenes, sesquiterpenes, monoterpenoids	(Esoteric-Oils, 2014).
R. officinalis	Fresh flowering tops	1.0 -2.0%	monoterpenes, terpinoids,	(Esoteric-Oils, 2014).
T. vulgaris	Fresh or partly dried flowering tops	0.7-1.0%	monoterpenes, sesquiterpenes, monoterpinoids	(Esoteric-Oils, 2014).

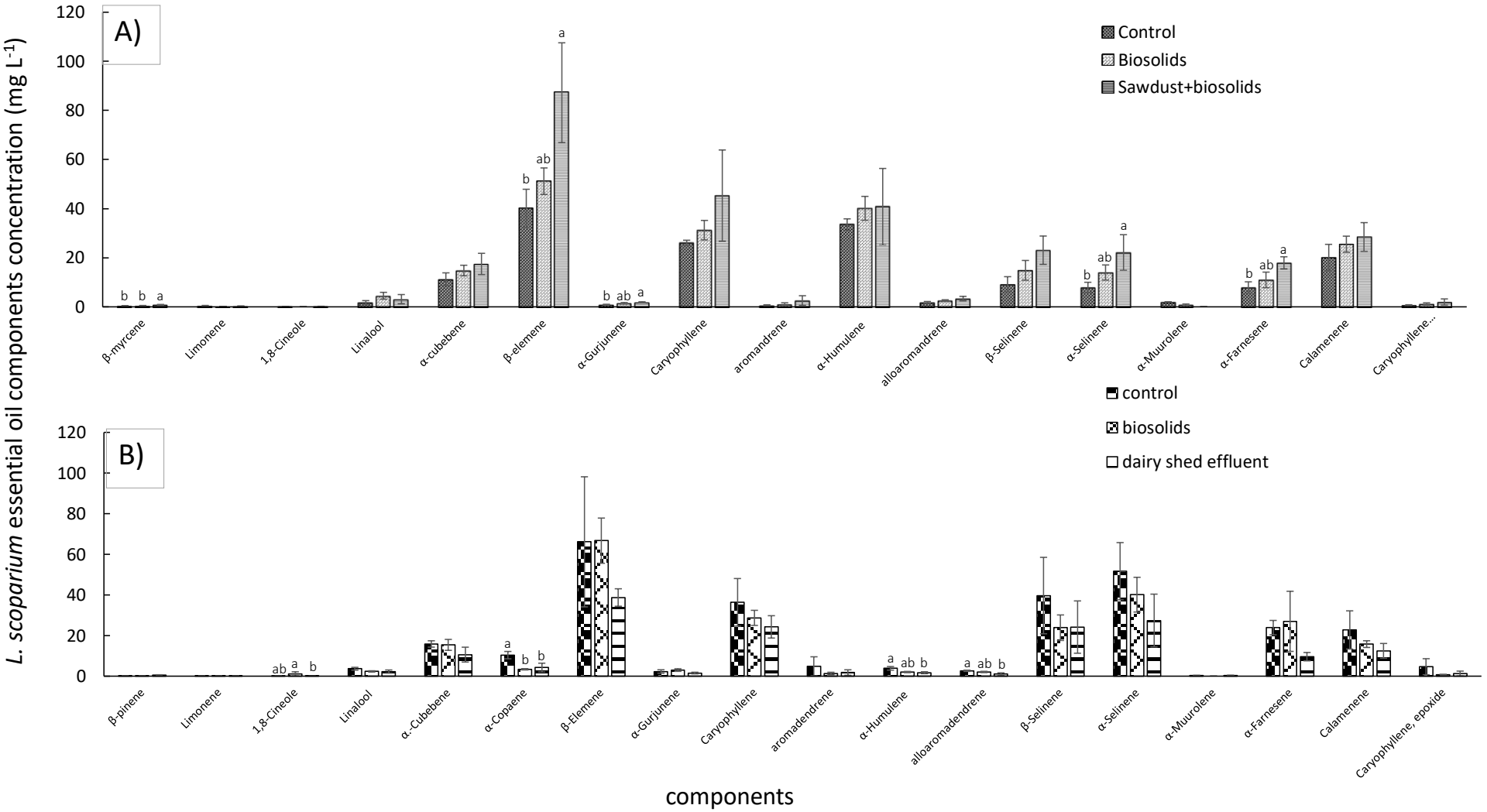


Figure 8.1 *L. scoparium* average essential oil components concentrations in A- Exp. 1 (n=4 ± se) and B- Exp. 2 (n=3 ± se). Concentrations are mg L^{-1} in the solvent extract. Significant differences between the treatments at $p \leq 0.05$ are indicated by different letters (a, b, c).

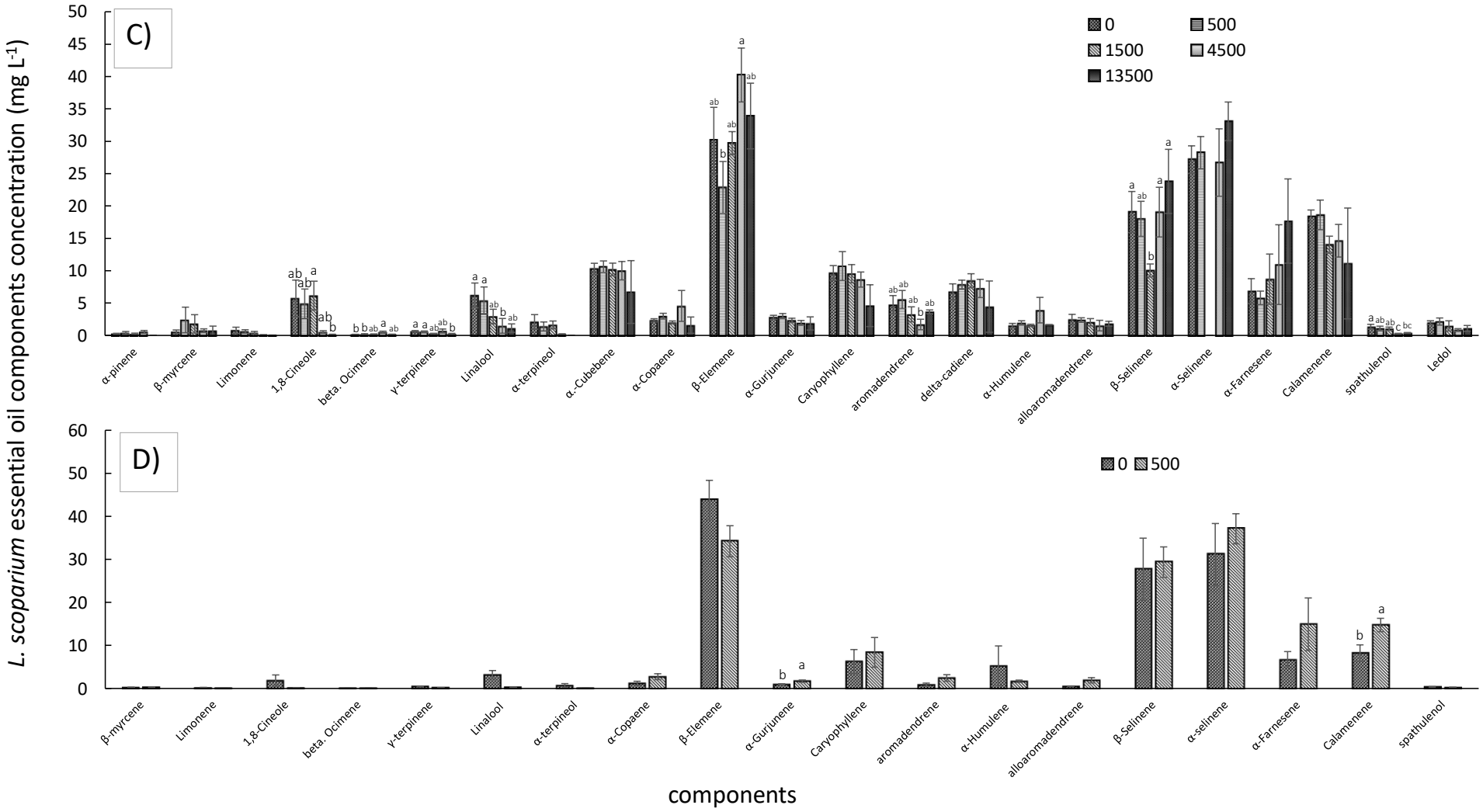


Figure 8.2 *L. scoparium* average essential oil components concentrations in C- Exp. 3 (n=5 ± se) and D- Exp. 4 (n=5 ± se). Concentrations are mg L^{-1} in the solvent extract. Significant differences between the treatments at $p \leq 0.05$ are indicated by different letters (a, b, c).

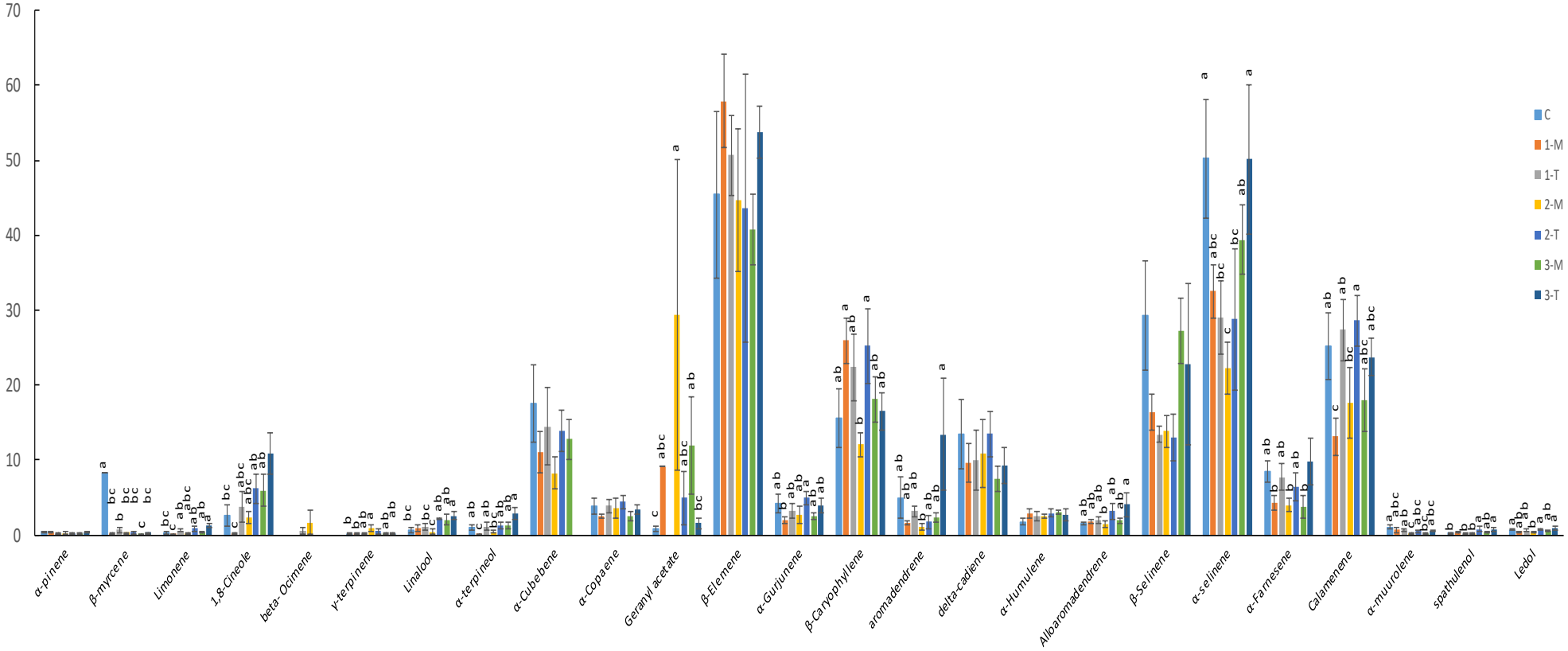


Figure 8.3 *L. scoparium* average essential oil components concentrations in Exp. 5 (n=5 ± se). Concentrations are mg L^{-1} in the solvent extract. Significant differences between the treatments at p ≤ 0.05 are indicated by different letters (a, b, c).

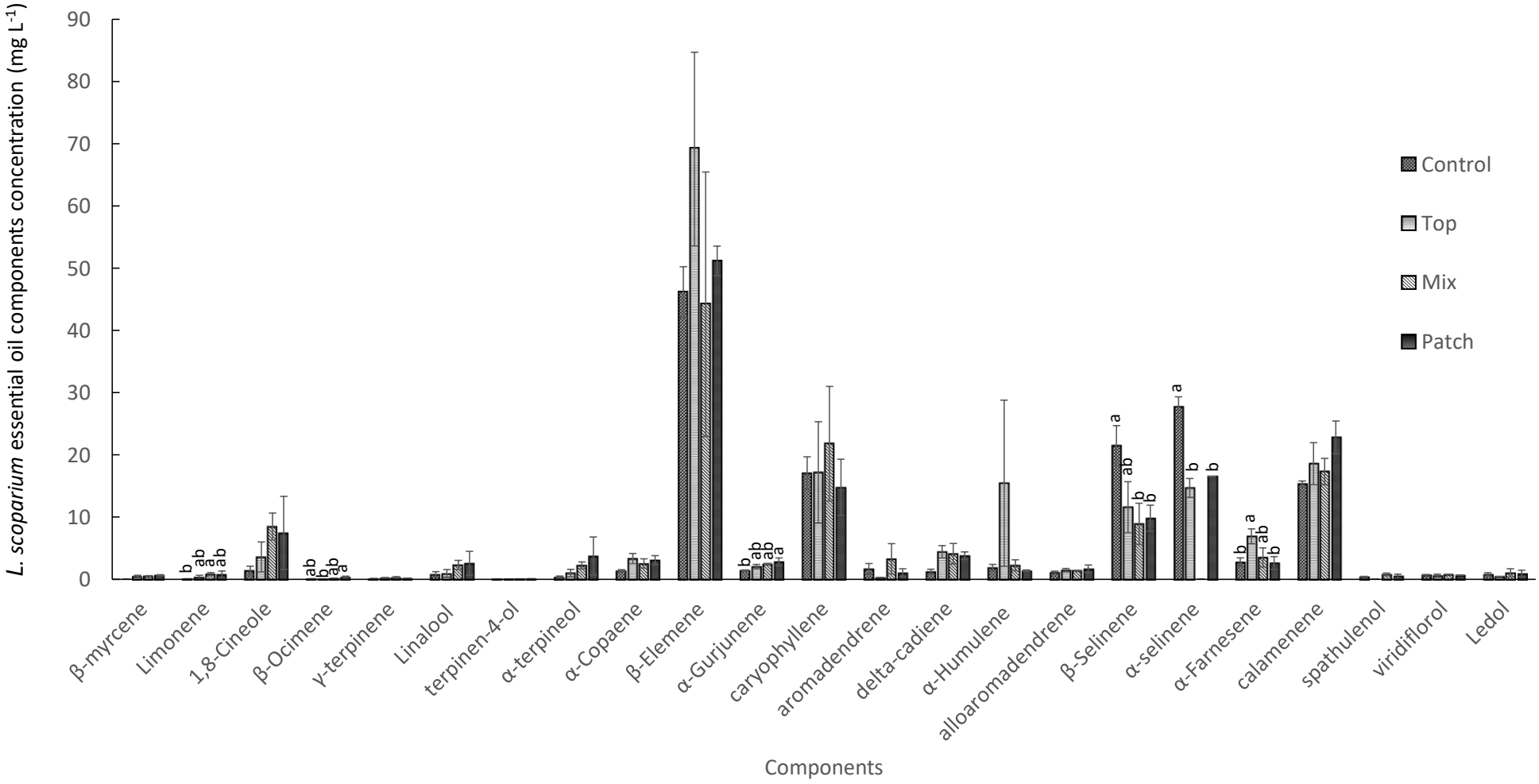


Figure 8.4 *L. scoparium* average essential oil components concentrations in Exp. 6 (n=3 ± se). Concentrations are mg L^{-1} in the solvent extract. Significant differences between the treatments at $p \leq 0.05$ are indicated by different letters (a, b, c).

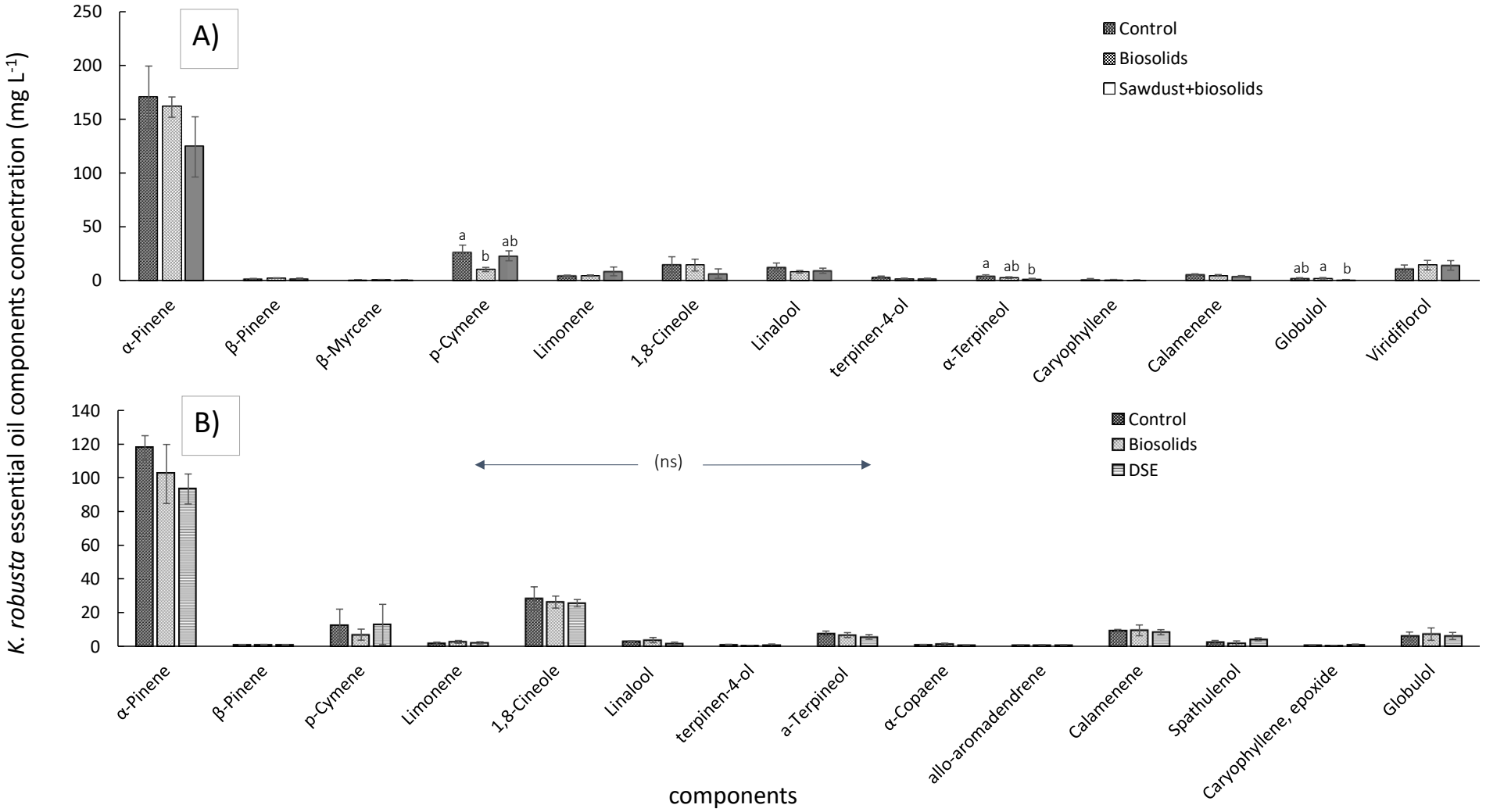


Figure 8.5 *K. robusta* average essential oil components concentrations in A- Exp. 1 (n=4 ± se) and B- Exp. 2 (n=3 ± se). Concentrations are mg L^{-1} in the solvent extract. Significant differences between the treatments at $p \leq 0.05$ are indicated by different letters (a, b, c).

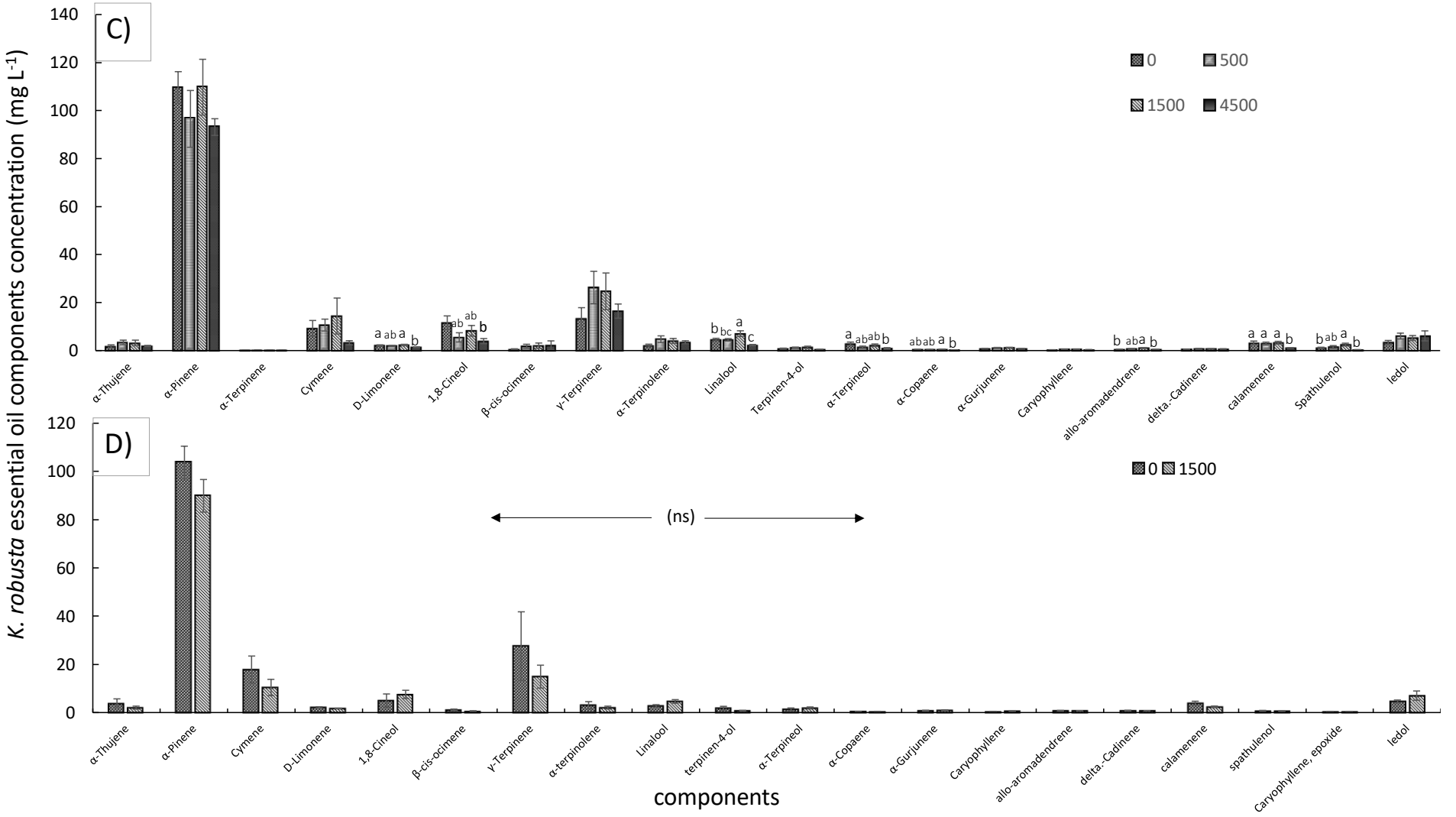


Figure 8.6 *K. robusta* average essential oil components concentrations in C- Exp. 3 (n=5 ± se) and D- Exp. 4 (n=5 ± se). Concentrations are mg L^{-1} in the solvent extract. Significant differences between the treatments at p ≤ 0.05 are indicated by different letters (a, b, c).

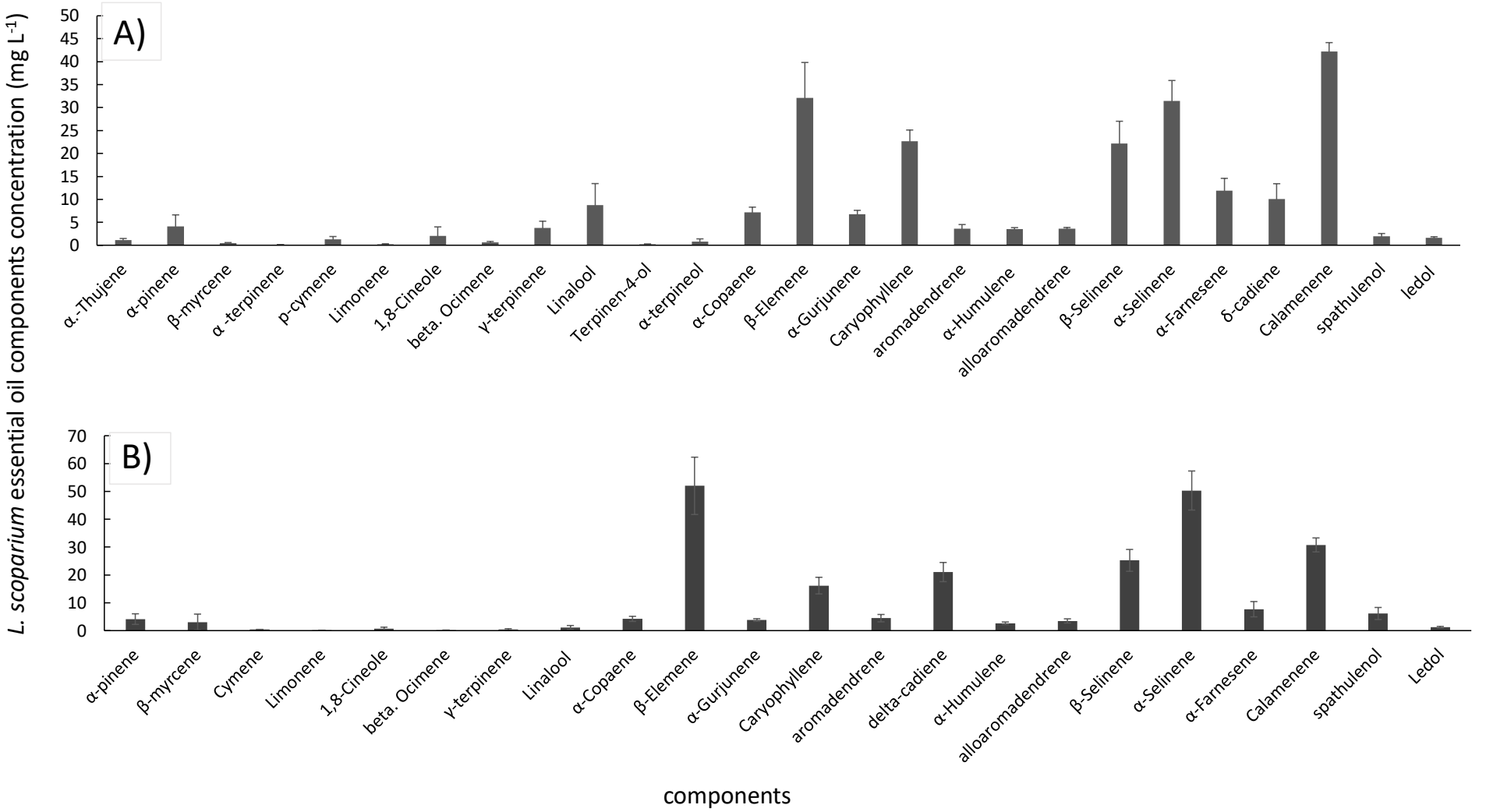


Figure 8.7 *L. scoparium* average essential oil components concentrations of the samples taken from A- Nikau Gully B- Quail Island. Concentrations are mg L^{-1} in the solvent extract.

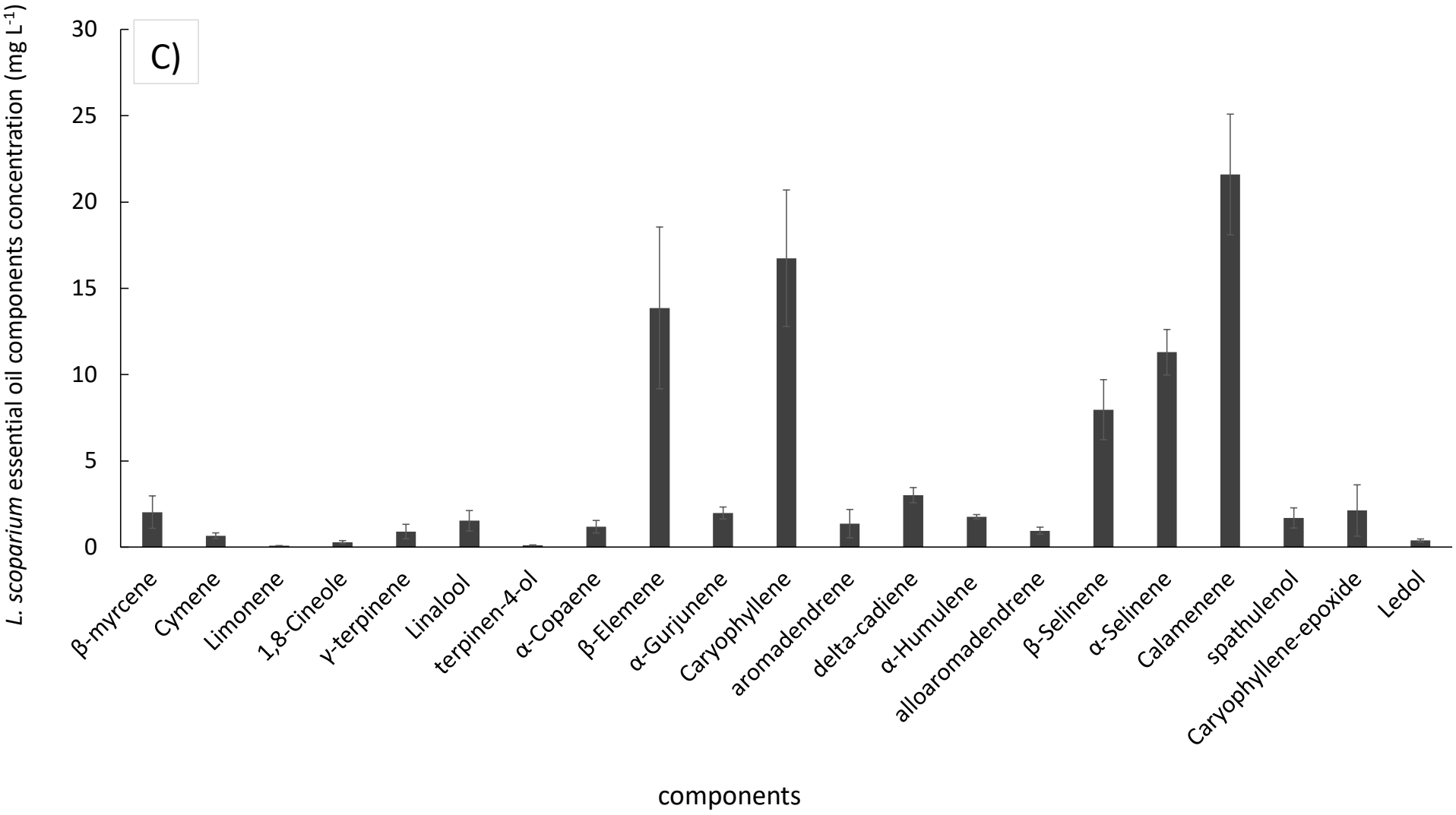


Figure 8.8 *L. scoparium* average essential oil components concentrations of the samples taken from Yarrs Flat. Concentrations are mg L^{-1} in the solvent extract.

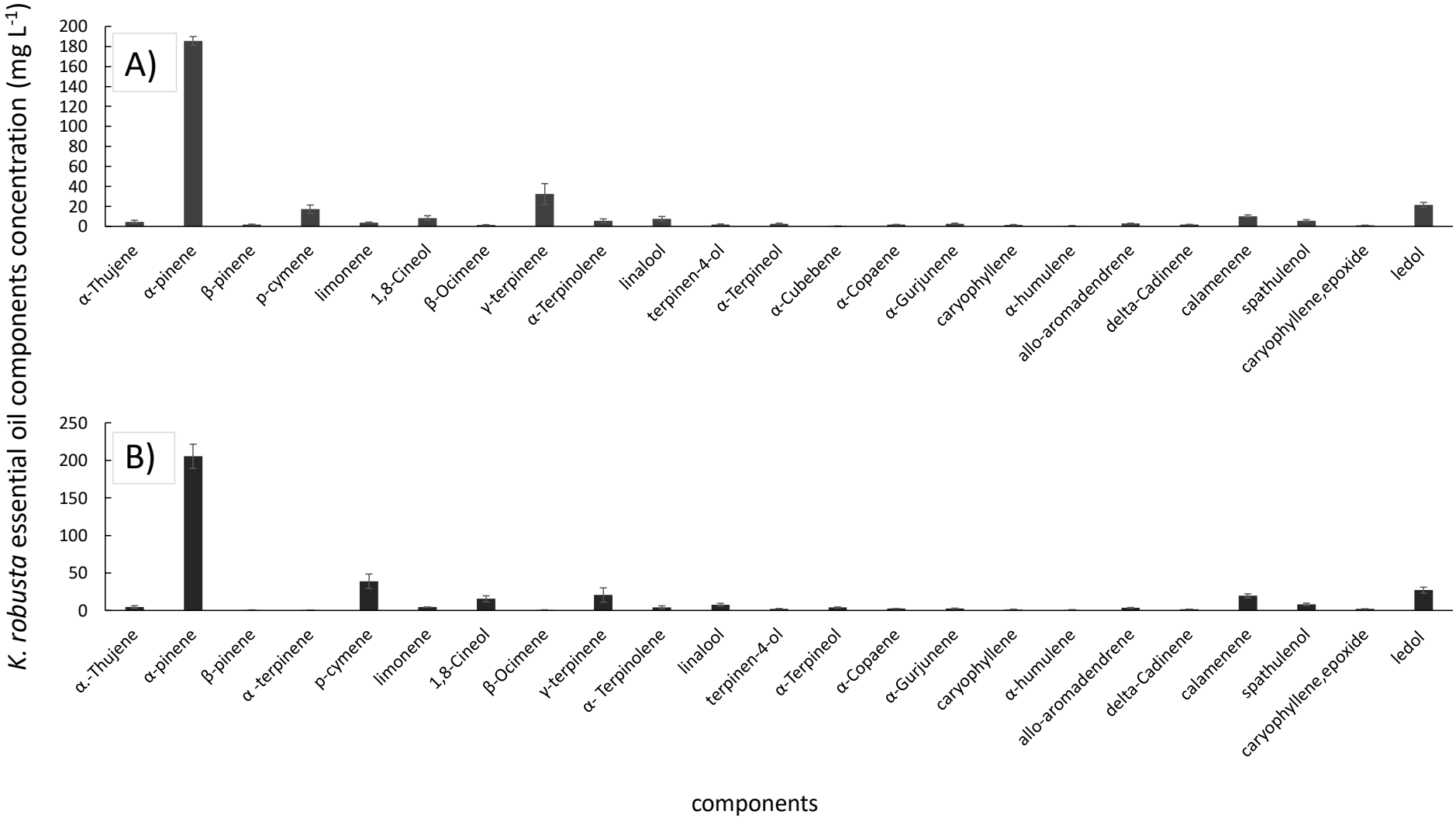


Figure 8.9 *K. robusta* average essential oil components concentrations of the samples taken from A-Nikau Gully B-Quail Island. Concentrations are mg L^{-1} in the solvent extract.

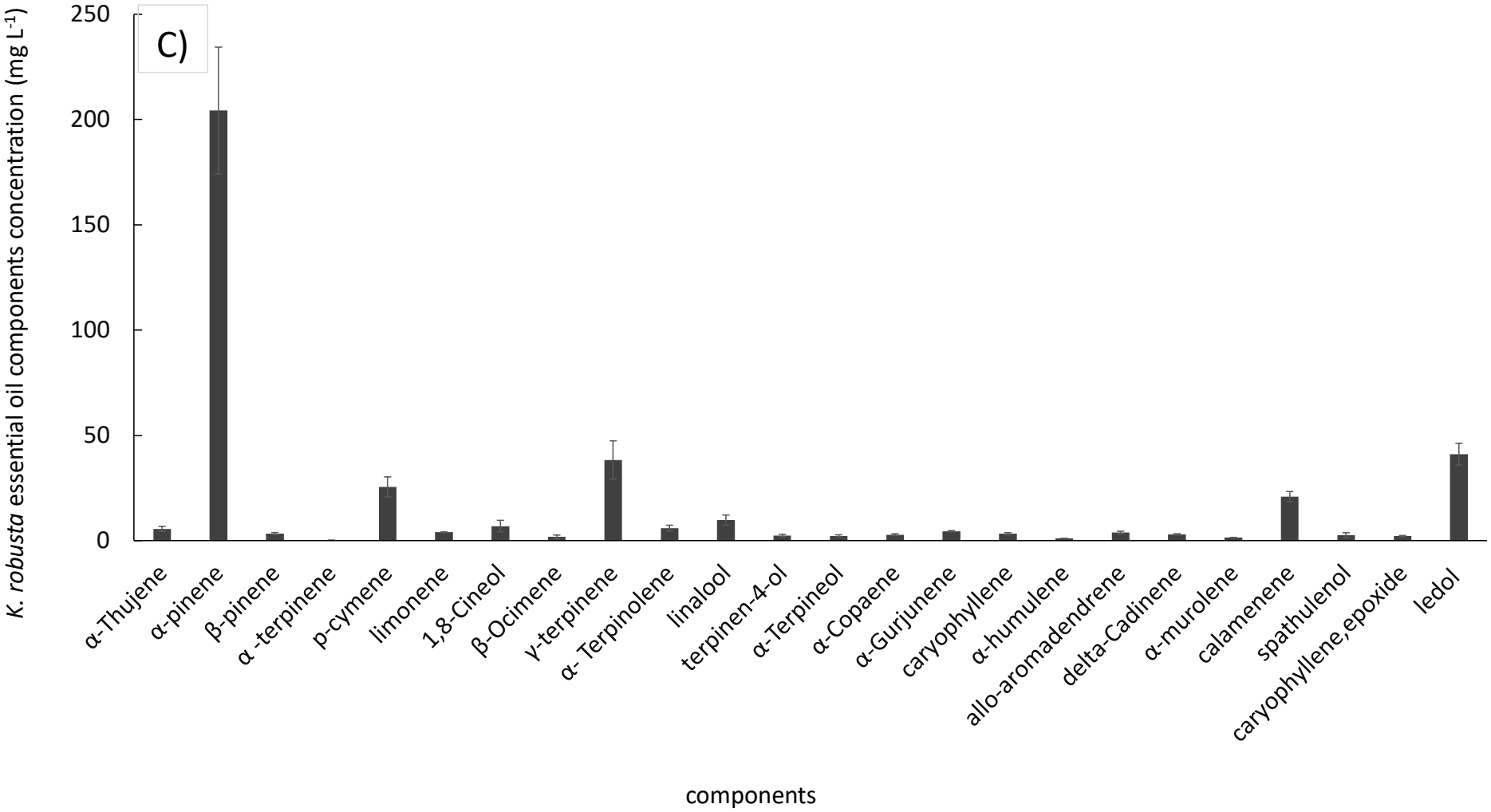


Figure 8.10 *K. robusta* average essential oil components concentrations of the samples taken from C- Bridle Path. Concentrations are mg L^{-1} in the solvent extract.

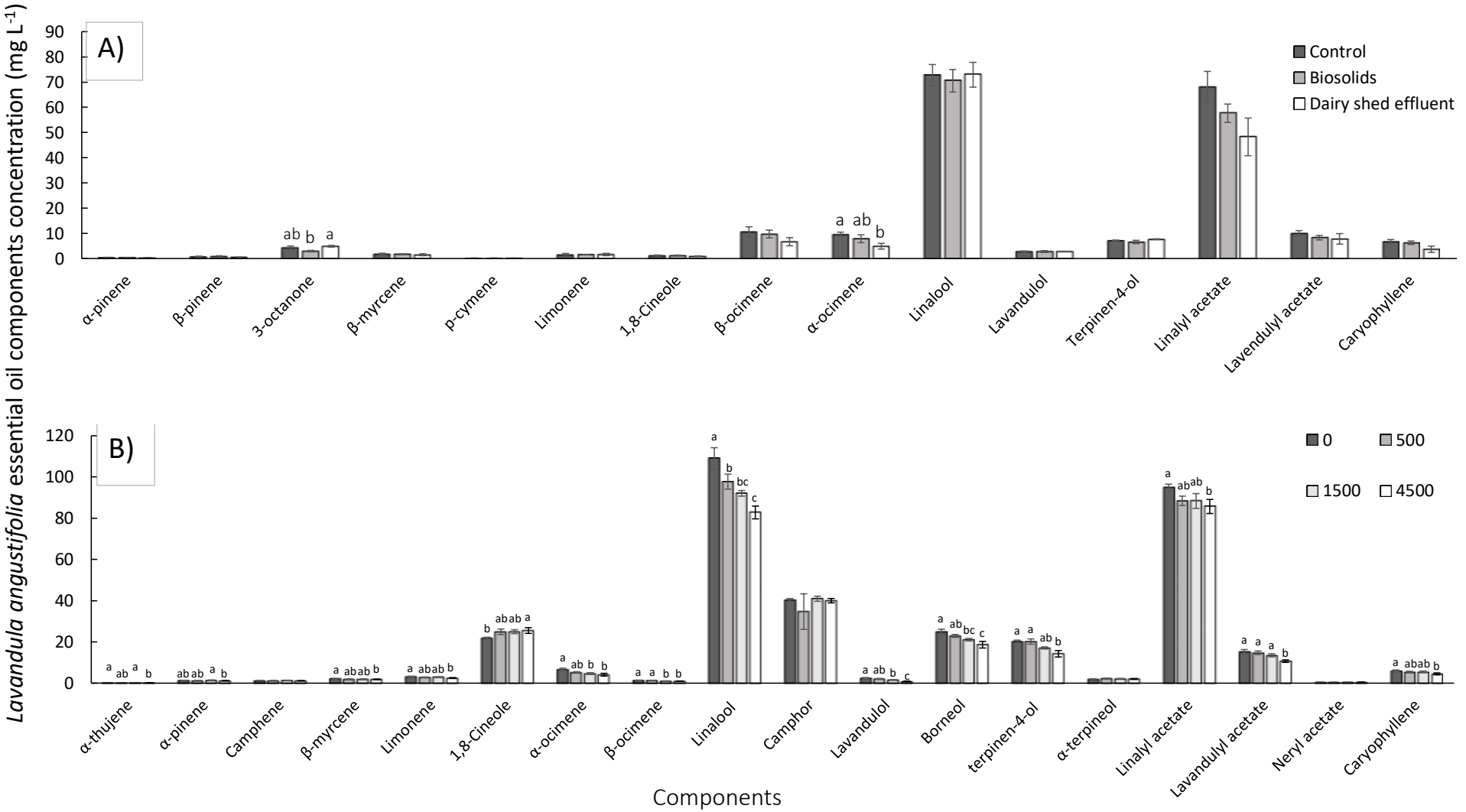


Figure 8.11 *L. angustifolia* average essential oil components concentrations in A- Exp. 2 (n=3 ± se) and B- Exp. 3 (n=5 ± se). Concentrations are mg L^{-1} in the solvent extract. Significant differences between the treatments at $p \leq 0.05$ are indicated by different letters (a, b, c).

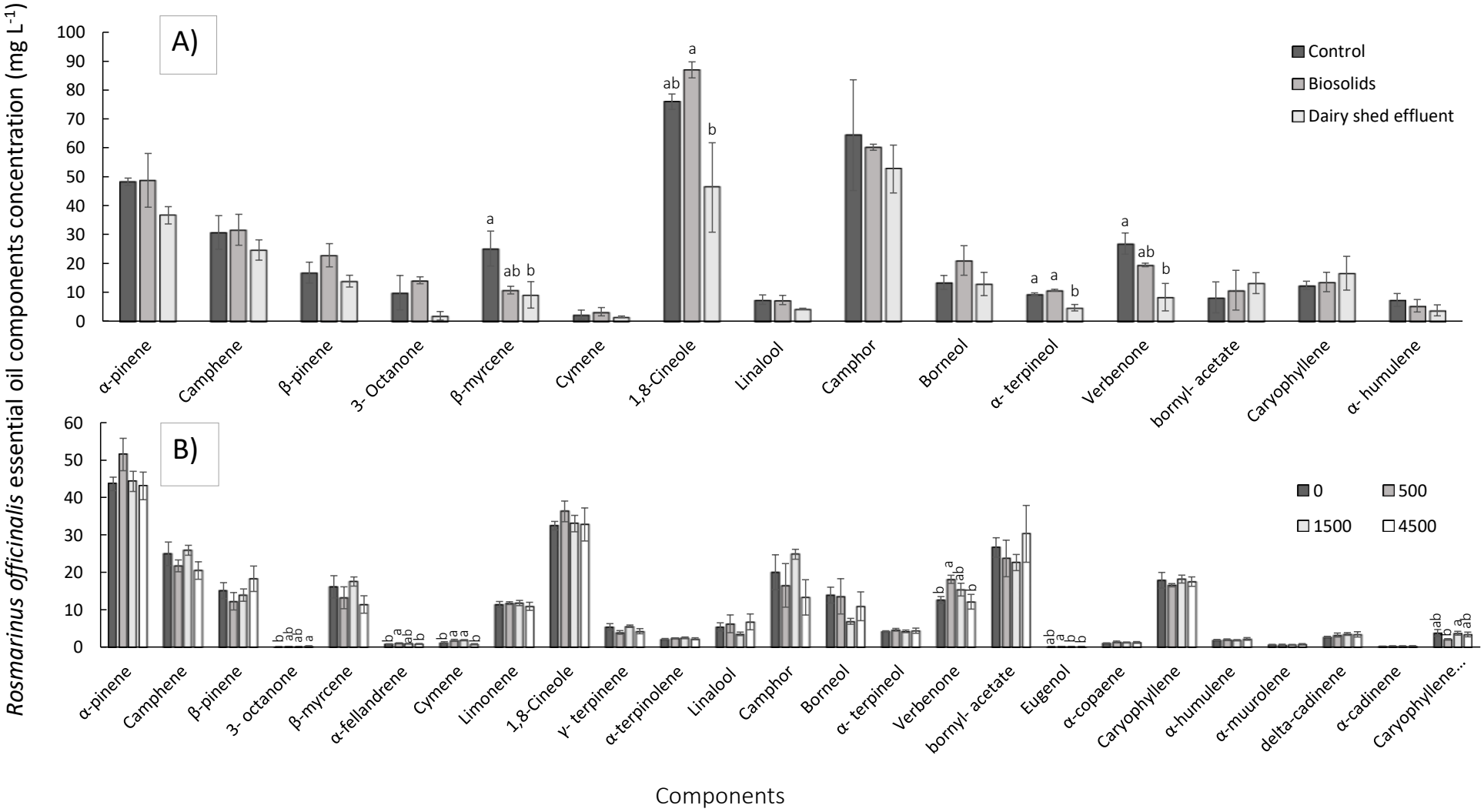


Figure 8.12 *R.officinalis* average essential oil components concentrations in A- Exp. 2 (n=3 ± se) and B- Exp. 3 (n=5 ± se). Concentrations are mg L^{-1} in the solvent extract. Significant differences between the treatments at $p \leq 0.05$ are indicated by different letters (a, b, c).

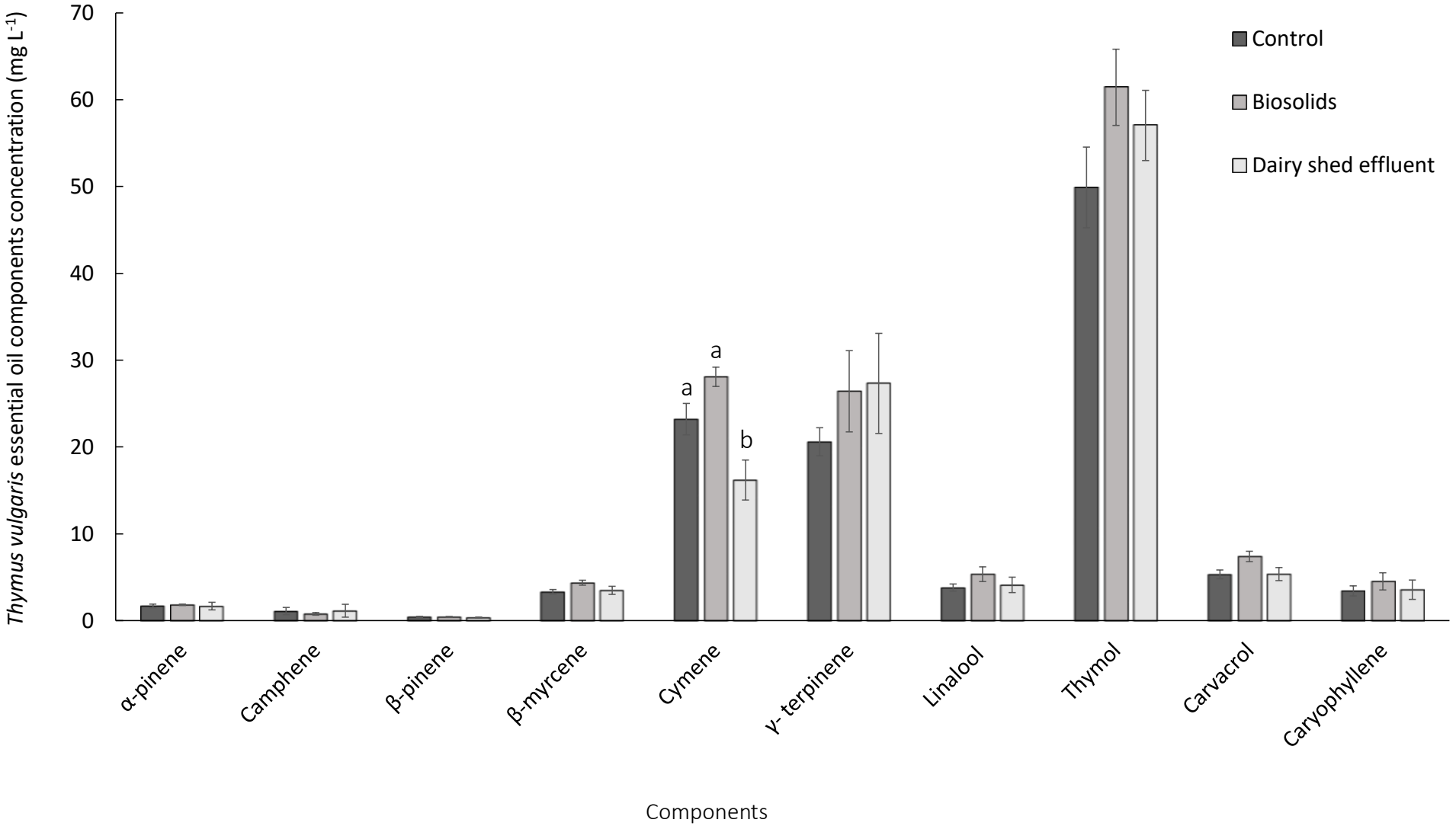


Figure 8.13 *T. vulgaris* average essential oil components concentrations in A- Exp. 2 (n=3 ± se). Concentrations are mg L^{-1} in the solvent extract. Significant differences between the treatments at p ≤ 0.05 are indicated by different letters (a, b, c).

Appendix D

Final report (June 2017): A lysimeter experiment and field trial to determine options for the beneficial reuse of wastewater from Duvauchelle and Akaroa, Banks Peninsula

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This report provides end-of-contract outcomes from lysimeter and field trials. This project has been the subject of postgraduate research by Cameron McIntyre, Saloomeh Seyedalikhani, Minakshi Mishra and Obed Lense. Their dissertations and related publications will be made available when they are complete.

Note that the field trials will continue until at least the 30th of June 2018. The field trials will be the subject of postgraduate research at the University of Canterbury and the Centre for Integrated Biowaste Research (CIBR). Updates will be provided on:

http://www.kiwiscience.com/duvauchelle.html

Executive summary

- In 2014, the Christchurch City Council (CCC) commissioned Lincoln University to determine options for the beneficial reuse of Treated Municipal Wastewater (TMW) from Duvauchelle and Akaroa, Banks Peninsula through a lysimeter experiment and a field trial.

- Following an initial assessment of the soils where the TMW would be applied, a lysimeter trial was set up at Lincoln University in December 2014. This trial comprised 18 50 cm x 70 cm lysimeters containing intact soil cores from the golf course at Duvauchelle (12 lysimeters) and an area between Takamatua and Akaroa (6 lysimeters). The soils from Duvauchelle and Takamatua were Barry's soil and a Pawson silt loam, respectively.

- From December 2014 until April 2015, these lysimeters were irrigated with 10 mm per day, resulting in all lysimeters draining approximately equal volumes. On the 22nd of April, treatments started with municipal wastewater from Duvauchelle. Treatments comprised a control (Duvauchelle, Akaroa), 440 mm/yr (Duvauchelle), 825 mm/yr (Duvauchelle, Takamatua) and 1650 mm/yr (Duvauchelle). These treatments continued until the 3rd of October 2016. The lysimeters were then deconstructed and analysed.

- All lysimeters drained freely and there was no ponding. Nitrogen leaching was negligible in all treatments, although mineral nitrogen accumulated in the soil profile of the 1650 mm/yr treatment. It is unlikely that phosphorus, potassium, sulphur, calcium and magnesium will cause problems with either fertility or environmental quality in a system irrigated with TMW.

- Sodium-induced degradation of soil structure is a major concern when using TMW as irrigation water. Sodium accumulated in the soil columns in all the TMW treatments. The rate of accumulation was not proportional to the TMW application rate, indicating that sodium was moving down through the soil profile and leaching. The sodium accumulation ratio of the TMW was 15, indicating that in the long term (>10 years) at a moderate irrigation rate (<1000 mm) the soil may need to amended with gypsum, lime or dolomite to maintain soil structure.

- Pasture growth in the lysimeters was significantly enhanced by the TMW throughout the entire experiment. There were no signs of toxicity.

- A field trial comprising 11 native species, namely *Leptospermum scoparium, Kunzea robusta, Olearia paniculata, Pseudopanax arboreus, Coprosma robusta, Podocarpus cunninghamii, Griselinia littoralis, Pittosporum eugenioides, Cordyline australis, Phormium tenax, Phormium colensoi* was established on ca. 1000 m^2 of land near Pipers Valley Road. 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 *Leptospermum scoparium, Olearia paniculata, Coprosma robusta, Podocarpus cunninghamii, Cordyline australis,* and *Phormium tenax.* The field trial will continue until at least June 2018.

- The use of TMW to produce valuable biomass such as cut-and-carry pasture, grazed pasture, or valuable native products such as manuka honey or essential oils constitutes the beneficial reuse of a valuable resource that is less environmentally damaging than disposal into the sea.

- It is recommended that the effluent be applied at a rate of 500 – 800 mm per year and that the soil is periodically monitored for aggregate stability. Gypsum, dolomite, or lime may need to be added periodically. A successfully designed system requires a hydrological and geotechnical assessment of the area to be irrigated.

Contents

Introduction

Land application of treated municipal wastewater

In New Zealand, the land application of Treated Municipal Wastewater (TMW) is the preferred option over discharge into waterways or the ocean (Sparling et al., 2006), where it can exacerbate eutrophication and / or toxic algal blooms (Sonune and Ghate, 2004). Compared to direct discharge into water, Irrigation of TMW onto land reduces the contaminants that enter waterways and therefore has positive effects on the water quality (Herath, 1997). The root-zones of plants remove nutrients contained in the TMW, mitigate pathogens (Mandal et al., 2007), and break down or immobilise contaminants (Chaudhry et al., 2005) that would otherwise degrade water bodies. TMW can reduce or eliminate the need for mineral fertilisers such as superphosphate, which contain elevated concentrations of toxic cadmium, fluorine and uranium that can accumulate in soil (Kim and Robinson, 2015). In many countries, including NZ, TMW is used to irrigate pasture, crops and forestry (Capra and Scicolone, 2004, Barton et al., 2005).

The application of TMW to land also carries risks that need to be mitigated for a successful operation. There are numerous examples of where land application of TMW has been discontinued because of environmental degradation. Excessive rates of TMW application to land can result in unacceptable nutrient leaching (Houlbrooke et al., 2003), runoff, soil instability and erosion, as well as accumulation of some components, such as sodium, in the topsoil (Cameron et al., 1997). High sodium concentrations can reduce plant growth through salinity and sodicity as well as degrade soil structure through the dispersion of clays (Mojid and Wyseure, 2013). The nature of the risks of the land application of TMW and therefore the design of a successful system is dependent on the quality of the TMW and the local environment. Therefore, every system needs to be specifically designed.

Potential for land application of TMW on Banks Peninsula

The successful application of TMW to land on Banks Peninsula requires particular attention to soil quality. Soils of the lowland areas of the peninsula where TMW could potentially be applied are mostly derived from loess with a relatively high clay content. They are often imperfectly drained and may contain a fragipan (a layer of impermeable soil). These soils present a higher risk of infiltration problems compared to free-draining soils and consequently an improperly designed TMW application system may be susceptible to surface runoff and erosion.

The Christchurch City Council seeks to reduce the direct disposal of TMW into Akaroa harbour. Several small communities now have their wastewater irrigated onto woodlots. There is now an on-going program of options analysis for alternatives to harbour disposal for the settlement of Duvauchelle. Potentially, some of the effluent produced in Akaroa could also be land-applied. Duvauchelle produces some 27600 m^3 of wastewater per year (based on 2016 data provided), which is currently discharged directly into the harbour through one long harbour outfall.

In 2014, the Christchurch City Council (CCC) approached Lincoln University regarding the possibility of irrigating TMW from Duvauchelle onto the local golf course. In subsequent discussions with stakeholders during public open days in 2015 and 2016, this brief was expanded to include cut-and-carry pasture as well as NZ native vegetation. While there are numerous examples of successful irrigation onto cut-and-carry pasture in NZ and elsewhere, there is a shortage of information on how

native species will interact with TMW. Potentially, TMW could be irrigated onto NZ native vegetation, with a view to increasing the production of valuable native products or the creation of zones of ecological value (Meurk, 2008; Franklin et al., 2015). Manuka (*Leptospermum scoparium*) is an obvious candidate species because of its associated high-value honey and essential oils. Moreover, mānuka has been shown to kill soil-borne pathogens (Prosser et al., 2016) and reduce nitrate leaching (Esperschuetz et al., 2017b).

Other potential valuable native species are kanuka (*Kunzea robusta*) for essential oil production, horopito (*Pseudowintera colorata*), which produces antifungal compounds, harakeke (*Phormium tenax*) for fibre production, and a whole suite of species, including kapuka (*Griselinia littoralis*) that may be a nutritious supplement due to tannins and trace elements (Dickinson et al., 2015).

It is unclear whether TMW would confer the same growth benefits to native vegetation as to pasture. Many NZ-native species, such as mānuka, are adapted to low-fertility soils and it may not respond well to the addition of high concentrations of plant macronutrients. Franklin et al., (2015) reported that some responded positively to N (200 kg/ha equiv.), but *Leptospermum scoparium* did not. Dickinson et al. (2015) reported that biosolids improved the growth of *Grisilinea littoralis* and *Kunzea robusta*, but not *Dodonaea viscosa*.

A native ecosystem receiving TMW would likely remain unharvested or have only a small fraction of the biomass removed. Therefore, unlike a cut-and-carry pasture receiving TMW, there would be no significant removal of nutrients or contaminants from the system. It is likely that nitrate leaching and phosphorous accumulation in the soil would therefore be greater.

Aims

We aimed to determine the suitability of soils from the Duvauchelle golf course and Takamatua peninsula to receive treated municipal wastewater from the Duvauchelle Wastewater Treatment Plant. Specifically, we sought to determine whether irrigation rates of up to and in excess of 1000 mm per year would result in ponding, excess nitrate leaching, accumulation or depletion of elements in soil, changes in pasture growth and quality, change in the survival and growth of NZ native vegetation.

Materials and methods

Site description

On the 28th of August 2014, a site visit was made to Duvauchelle Golf Course (Barry's soil) and the Takamatua Peninsula (Pawson silt loam). Soil pits were opened with a view to ascertain whether the soils would be suitable for lysimetry, namely that they would have an adequate permeability to allow significant through-flow of water. Soil pits revealed both soils to be imperfectly drained (some mottling) but no evidence of a fragipan, perched water, or impermeably (reduced iron). The mean (standard deviation) of the size fractions for these soils are: course sand 1.2 (0.2)%, fine sand 44.5 (0.9)%, silt 28.1 (2.1)% and clay 24.0 (2.2%) (Anon, 1939). Fig. 1 shows the locations of the experimental sites.

Fig. 1. Locations where the lysimeters were excavated and of the ongoing field trial where TMW is being irrigated onto NZ native vegetation.

Lysimeter experiment

Two intact lysimeters were collected from the golf course at Duvauchelle on the 18th of September 2014. These lysimeters were taken to Lincoln University and irrigated with water (10 mm per day) until drainage stabilised in late October 2014. This demonstrated that the intact cores would drain and therefore be suitable for the full experiment. In November 2014, a further 10 lysimeters were taken from the golf course in Duvauchelle (43°44'53.06"S, 172°55'41.44"E) and six were taken from a

paddock containing cattle (43°47'33.11"S, 172°57'16.96"E) between Takamatua and Akaroa (Fig. 1). Each lysimeter cylinder was placed on the soil surface, and gently tapped into the soil, while the soil surrounding the cylinder was excavated (Fig 2). Molten Vaseline was poured around the edge of the intact soil core before removal to the Lincoln University lysimeter facility.

The lysimeters, replete with intact soil cores, were installed at the Lincoln University lysimeter paddock (43°38'53.54"S, 172°28'7.69"E) in December 2014. The original vegetation was left upon the lysimeters. The Duvauchelle lysimeters were covered with a fescue / browntop mixture, while the Takamatua lysimeters were dominated by perennial ryegrass. A decision was taken not to remove and re-sow the pasture because this would have resulted in significant topsoil disturbance and consequent flush of nitrogen through the soil profile.

Between December 2014 and April 22nd 2015, the lysimeters were irrigated with 2 L (10 mm) of water per day. The lysimeters started to drain in February 2015 and by March 2015, similar volumes of leachate were obtained for all lysimeters. On the 22nd of April 2015, effluent application of the lysimeters began. Treated Municipal Wastewater (TMW) was collected by the Christchurch City Council (CCC) and delivered to Lincoln University in a 1000 L tank. Samples of the stored effluent were taken weekly. The tank was refilled as needed. There were three replicates of five treatments. Namely:

1) Barry's soil. Control (no effluent application)
2) Barry's soil. Wastewater added at ca. 500 mm / yr (0.4 L/day, 5x per week)
3) Barry's soil. Wastewater added at ca. 1000 mm / yr (0.75 L/day, 5x per week)
4) Barry's soil. Wastewater added at ca. 2000 mm / yr (1.5 L/day, 5x per week)
5) Pawson silt loam. Control.
6) Pawson silt loam. Wastewater added at ca. 1000 mm/yr (0.75 L/day, 5x per week)

Note that the actual annual rates were slightly less than anticipated. The actual annual rates for the 500 mm, 1000 mm, and 2000 mm treatments were 440 mm, 825 mm and 1650 mm per year. Drainage volumes were measured weekly or more often following high rainfall events. Pasture was harvested periodically, typically every three weeks, during the growing season. Fig. 3 shows the installed lysimeters, with PhD student, Minakshi Mishra measuring pasture growth and Dr Maria Jesus Gutierrez-Gines irrigating effluent and collecting drainage. On the 16th of November 2016, the lysimeters were deconstructed. Following a final harvest of the pasture, soil samples from 0-15 cm, 15-30 cm, 30-45 cm, and 45 – 60 cm were taken and stored for chemical analyses.

Fig. 2. Collecting lysimeters from the Takamatua peninsula, November 2014.

Fig. 3. Top: The installed lysimeters showing the six Pawson silt loam soil cores (front-left) and the 12 Barry's soil cores (rear-right). Centre left: Effluent application. Centre right: Drainage collection. Bottom: Destructive sampling of the lysimeters at the conclusion of the experiment. 16th of November, 2016.

Field trial

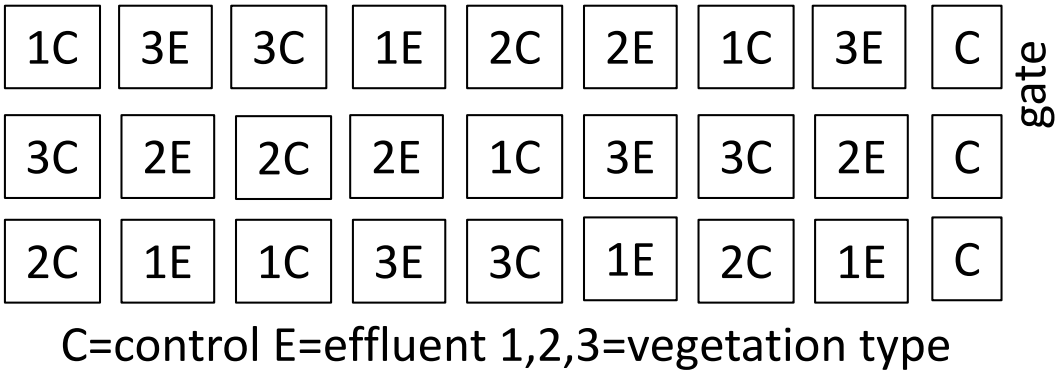
In July 2015, we planted 1350 native trees (Fig. 4), divided into 27 blocks of three different vegetation types (Table 1). Twelve of the 27 blocks are receiving treated municipal wastewater at a rate of 500 mm during the growing season (October – April), a similar rate to that used on an irrigated dairy farm in Canterbury. Effluent irrigation started in January 2016. Weeds were controlled using a lawnmower. An information board was installed near the roadside describing the aims of the experiment.

In May 2017 the survival of the plants was recorded along with the canopy volume of each individual plant. Soil and plant samples have been taken for chemical analysis. In June 2017, all areas within the plot that were not under native vegetation were planted with silver tussock (*Poa cita*). It is hoped that these tussocks will minimise the need for further weed control at the site.

Fig. 4. The field trial in Piper's valley road shortly after planting. The gate is at the top left of the picture.

Table 1. Composition of the thee vegetation types used in the experiment. The design of the field plot is shown below.

Vegetation type 1			**Vegetation type 2**			**Vegetation type 3**	
Mānuka	*Leptospermum scoparium*		Akiraho	*Olearia paniculata*		Kapuka	*Griselinia littoralis*
Kānuka	*Kunzea robusta*		Puahou	*Pseudopanax arboreus*		Tarata	*Pittosporum eugenioides*
			Karamu	*Coprosma robusta*		Tī kōuka	*Cordyline australis*
			Hall's tōtara	*Podocarpus cunninghamii*		Harakeke	*Phormium tenax*
						Whararikī	*Phormium colensoi*



Chemical analyses

Inorganic nitrogen species in soils were determined using an extraction on fresh soil (Blackmore et al., 1987). After adding 40 mL of a 2M KCl reagent to 4 g of soil, the solution was shaken on an end-over-end shaker for 1 h, centrifuged at 2000 rpm for 10 min and subsequently filtered through Whatman 41 filter paper. Extracted solutions, along with leachate and TMW samples were kept at -20°C until analysed. Nitrate-N (NO_3-N), nitrite-N,(NO_2-N) and ammonium-N (NH_4-N) were determined using a flow injection analyser (FIA FS3000 twin channel analyser, Alpkem, USA).

Soils were dried at 105 ^oC and sieved to <2mm using a Nylon sieve. Plant samples kept in labelled paper envelopes and left in an oven at 70°C until a constant weight was obtained (approximately one week). Paper envelopes were immediately transferred in sealed polythene sacks to prevent absorption of moisture from the air. After weighing and grinding, samples were placed in sealed plastic vials.

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 solid/water ratio of 1:2.5. The mixture was shaken, left to equilibrate for 24 hr before measurement and shaken again before determination with a pH meter (Mettler Toledo Seven Easy) (Blakemore, 1987). An Elementar Vario-Max CN Elementar analyser (Elementar ®, Germany) was used to analyse the total carbon and nitrogen content in the soil and plant samples.

Elemental analyses of plants, soils, and effluents were carried out using microwave digestion (MARSXPRESS, CEM Corporation, USA) of 0.5 g of sample in 8 mL of Aristar^{TM} 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 also digested.

Concentrations of Cd, B, Ca, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, P, S and Zn were determined using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES Varian 720 ES - USA) in soils (Kovács et al., 2000) and in plants (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 for all metals. Recoverable concentrations of the CRMs were within 93% - 110% of the certified values.

Statistical analysis

Data were analysed using Minitab® 17 (Minitab Inc, State College, Pennsylvania, USA) and Microsoft Excel 2013. The ANOVA with Fisher's Least-Significance-Difference post-hoc test was used to assess the effects of different treatments. The significance level for all statistical analyses was $P<0.05$.

Results and discussion

Characteristics of the wastewater and soils

Table 2 shows the characteristics of the Treated Municipal Wastewater (TMW) from the Duvauchelle Wastewater Treatment Plant. The composition of the TMW is similar to data provided by the Christchurch City Council (CCC) from various times the past five years (data not shown). Of note are the elevated concentrations of nitrate (above drinking water standard of 11.3 mg/L nitrate-N), phosphate, and sulphur. When discharged into water bodies such as Akaroa harbour, these nutrients can exacerbate algal blooms, which can damage fisheries and tourism. The TMW contains sodium at a concentration that may pose a "slight to moderate" risk if irrigated onto the foliage of sensitive crops (Ayers and Westcot, 1985). Most pasture species are not overly sensitive. Although, the sodium tolerance of NZ native vegetation has not been well quantified, salt tolerance is expected in coastal and seaside species.

The Sodium Adsorption Ratio (SAR) is the sodium concentration divided by the square root of half the calcium and magnesium concentrations. The SAR is used in combination with EC (Electrical Conductivity) to indicate the likelihood that irrigation water will 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. The SAR of the TMW is at a level that may cause aggregate instability if used over the long term (Ayers and Westcot, 1985). Soil quality can be maintained by the occasional application of gypsum, dolomite, or lime (FAO, 2017). The total concentration of Ca and Mg in the soil is relatively large compared to the irrigation water (Table 2), so it is likely that irrigation could occur for many years before remedial measures would need to be taken. Nevertheless, the fertility of both soils could be improved with liming and the pH of the Pawson Silt Loam from the Takamatua peninsula is below the range recommended for agricultural soil (McLaren and Cameron, 1996a).

Table 2. Characteristics of the Treated Municipal Wastewater used in the lysimeter experiment. Values in brackets represent the standard deviation of the mean (*geometric mean and standard deviation range). n=54 except trace elements n=9.

	Treated Municipal Wastewater	Barry's soil (Duvauchelle)	Pawson Silt Loam (Takamatua peninsula)
pH	7.5	5.2	4.8
EC (uS/cm)	423 (40)	-	
Total suspended solids (g/m3)	32	-	-
NH_4^+-N (mg/L)	0.49 (0.15 – 0.80)*	10.1 (7.5)	11 (6.8)
NO_3^--N (mg/L)	18 (7.5)	17.1 (13.2)	4.4 (1.1)
NO_2^--N (mg/L)	0.86 (0.09)	-	-
Total C (%)	-	4.4 (0.6)	5.4 (0.3)
Total N (%)	<25	0.38 (0.05)	0.48 (0.03)
Al (mg/L)	0.43 (0.11 – 1.7)*	32731 (1418)	34903 (3699)
B (mg/L)	0.10 (0.04)	-	
Ca (mg/L)	59 (12)	6770 (393)	5852(187)
Cd (mg/L)	<0.001	-	-
Cu (mg/L)	0.04 (0.03)	7.7 (0.2)	5.1 (1.4)
Fe (mg/L)	0.96 (0.25 – 3.6)*	20155 (2852)	16806 (4098)
K (mg/L)	22 (5.0)	4491 (346)	4008 (365)
Mg (mg/L)	19 (5.5)	4251 (76)	3575 (463)
Mn (mg/L)	0.06 (0.03)	624 (9)	496 (50)
Na (mg/L)	95 (21)	290 (10)	374 (30)
P (mg/L)	11 (5.0)	1046 (30)	599 (125)
S (mg/L)	25 (11)	490 (21)	430 (5)
Zn (mg/L)	0.17 (0.11)	68 (3)	62 (7)
Sodium Accumulation Ratio (SAR)	15 (2.6)	-	-

Table 3 shows the masses of the individual elements added if TMW were to be irrigated at 500 mm / yr. The annual mass of nitrogen added per hectare is approximately half of the maximum rate permitted in many jurisdictions (200 kg/ha/yr). Phosphorus and potassium are within the ranges that these nutrients would be added to maintain an intensively grazed pasture (DairyNZ, 2017a). However, the sulphur loading is more than double rates normally applied (20 – 50 kg/ha/yr). This excess is likely to leach because sulphur is poorly retained by most NZ soils, including the Banks Peninsula loess.

The values of the nutrients were calculated using the lowest cost fertiliser sold by Ballance Ltd. Note that the value of the nutrients is less than the sum of the individual elements because some fertilisers contain more than one element, for example, superphosphate contains both phosphorus and sulphur. The average cost of irrigation in NZ is $770 per ha/yr (Curtis, 2016). Combining the irrigation value with the savings from reduced fertiliser use give a total value of >$1178 /ha/yr.

Table 3. Mass and value of plant macronutrients added through irrigating treated municipal wastewater at a rate of 500 mm per year. The value was calculated from prices listed on http://www.ballance.co.nz/Our-Products/PriceListing. Accessed April 2017. Note that the total value of the nutrients is less than the sum of the individual elements because some fertilisers contain more than one element.

Element	Mass (kg/ha/yr)	Value of element in cheapest fertiliser (NZ$/ha/yr)
N	95	103
P	55	193
K	110	287
S	125	375
Mg	95	250
Ca	295	356

Lysimeter experiment

Irrigation with effluent visibly increased the vigour of the pasture in all the treatments (Fig. 5). Over the course of the experiment, there were significant increases in the biomass of nearly all the treatments (Table 4).

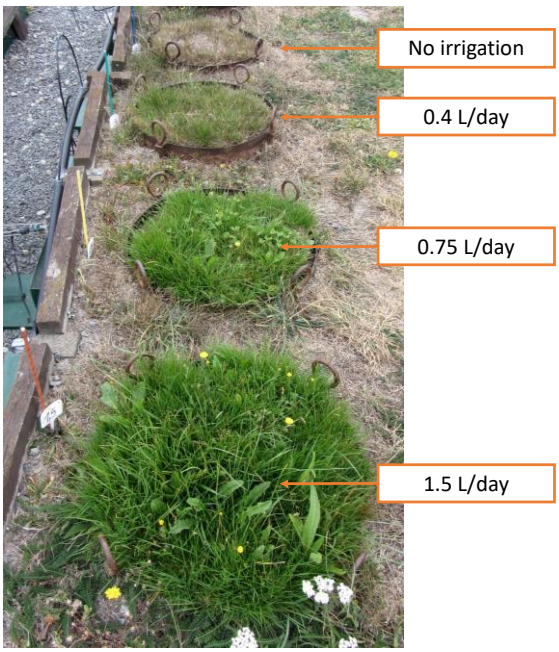


Fig. 5. Pasture growth on four lysimeters containing Barry's soil in February 2016. The numbers to the right of the picture indicate the volume of treated municipal wastewater that the lysimeter was receiving Monday – Friday.

Table 4. General parameters from the 21st of May 2015 until the 3rd of October 2016. Values in brackets represent the standard error of the mean (n=3).

Treatment	Total Irrigation (mm)	Total Rainfall (mm)	Total drainage (mm)	Total Evapotranspiration (mm)	Biomass production (t/ha equiv.)
Barry's soil					
Control	0	779	169 (22)[a]	610	5.4 (1.0)[a]
440 mm/yr	637		485 (23)[b]	931	6.3 (0.6)[a]
825 mm/yr	1190		736 (17)[c]	1233	8.9 (0.6)[b]
1650 mm/yr	2375		1375 (11)[d]	1779	12.3 (0.2)[c]
Pawson silt loam					
Control	0	779	148 (2)[a]	631	6.0 (0.3)[a]
825 mm/yr	1190		609 (32)[b]	1360	13.3 (0.7)[b]

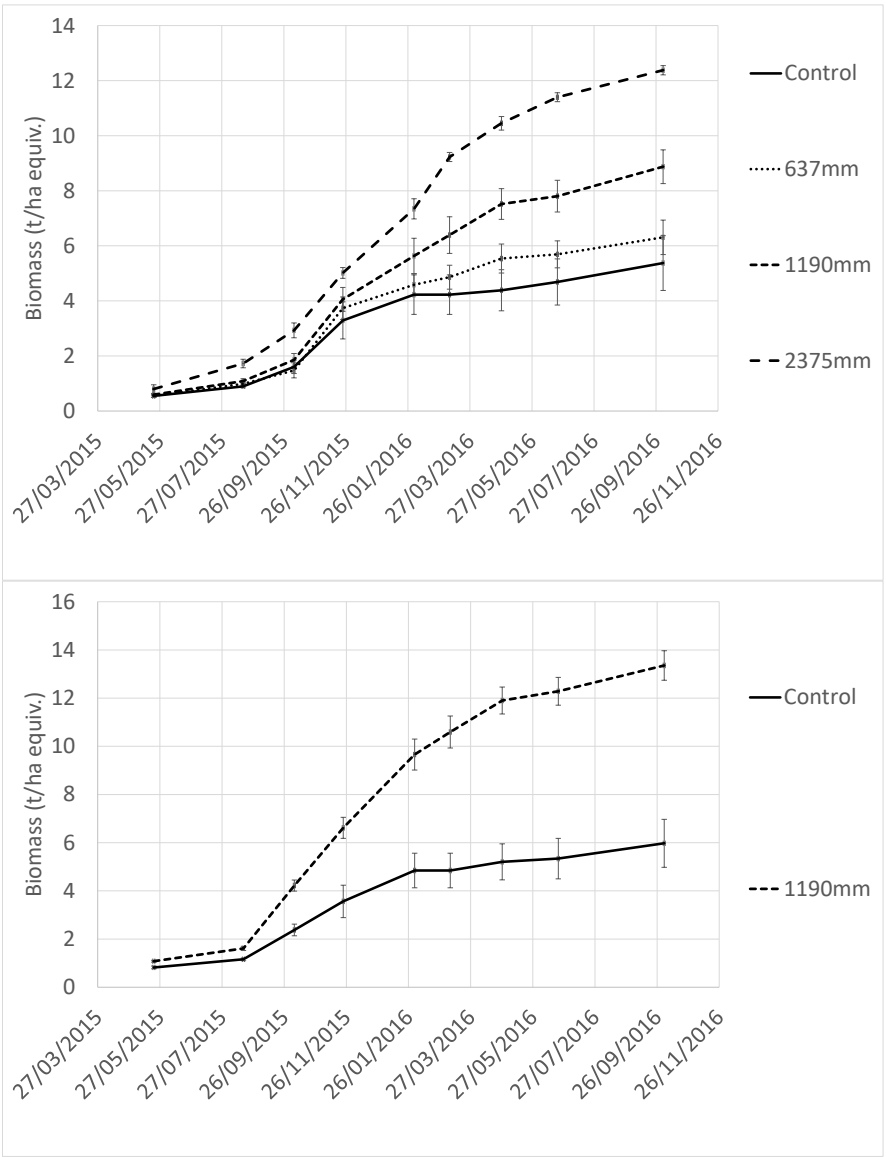


Fig. 6. Cumulative biomass production in the lysimeter experiment for the Barry's soil (top) and Pawson silt loam (bottom), expressed as tonnes per hectare equivalent. Bars represent the standard error of the mean (n=3).

Fig. 6 shows the cumulative biomass production for the pasture in the lysimeters. The biomass increase of the pasture in the treatments was greater than the controls for the whole duration of the experiment, even at the highest treatment rate. This indicates that increase in fertility resulting from the TMW application was maintained and that pasture growth was not significantly perturbed by any sodium or any other element in the TMW. The pasture growth in the Pawson silt loam lysimeters was significantly higher than in the lysimeters containing Barry's soil. This is most likely due to differences in the pasture composition as well as previous soil management. The Barry's soil lysimeters contained a fescue / browntop mixture, while the Pawson silt loam lysimeters were dominated by perennial ryegrass. Note that there were also other species present (Fig. 5), which were not removed so as not to disturb the soil. The Pawson silt loam was maintained as a graze pasture and possibly had historically received higher fertiliser additions than the Barry's soil, which was the fairway on the Duvauchelle Golf Course.

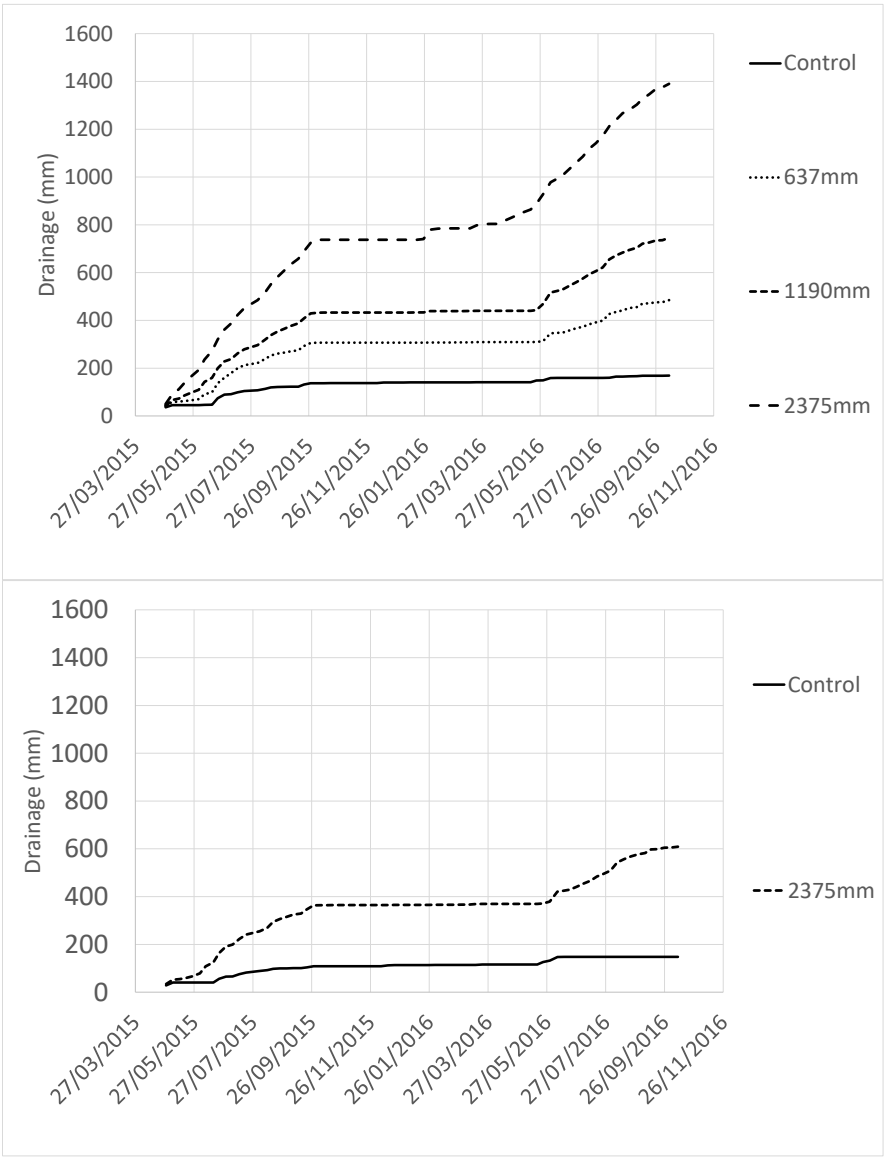


Fig. 7. Cumulative drainage from the lysimeters for the Barry's soil (top) and Pawson silt loam (bottom).

Drainage

All the lysimeters receiving TMW drained throughout the experiment, even at the highest application rate. There was no ponding or visible evidence that the soil structure had been degraded. Infiltration at various tensions forms part of an MSc degree by Cameron McIntyre. These data will be made available upon completion of his thesis, expected in late 2017.

Fig. 7 shows that all the treatments significantly increased drainage relative to the control. In a TMW application system on Banks Peninsula, drainage is unavoidable, irrespective of the vegetation type. Nevertheless, there would be marginally less drainage from a closed-canopy forest of high water-use trees because a significant portion of the incident rainfall is re-evaporated from the canopy before infiltration occurs (McNaughton and Jarvis, 1983). Unlike a dryland system, where deep rooted trees continue to transpire after pasture species have become dormant (Vogeler et al., 2001), rooting depth will have little impact on plant water use because the irrigation will ensure that the plants never become water stressed. Increased drainage does not necessarily imply that there will be unacceptable leaching of nitrogen or other potential contaminants. High levels of leaching requires both high drainage and a significant concentration of the contaminant in soil solution. If the contaminant is retained on the soil colloids, broken down, or taken up by the plant, then leaching will be minimal even under high drainage conditions.

Table 5. Mass of nitrogen (kg/ha equiv) in the treated municipal wastewater, pasture, soil and drainage water over the entire lysimeter experiment. Values in brackets represent the standard error of the mean (n=3). For each soil type, values with the same letter are not significantly different. The Barry's soil and Pawson silt loam were tested independently.

	Irrigation N (kg/ha equiv.)	Pasture N (%)	Pasture N (kg/ha equiv.)	Soil mineral N (kg/ha equiv.)	Leached N (kg/ha equiv.)
Barry's soil					
Control	<1	2.17 (0.13)^{ab}	115 (21)^{a}	71 (12)^{a}	0.32 (0.03)^{a}
637 mm	111	1.89 (0.12)^{b}	124 (14)^{a}	59 (7)^{a}	0.72 (0.08)^{b}
1190 mm	207	2.07 (0.09)^{ab}	193 (14)^{a}	87 (4)^{a}	1.09 (0.03)^{c}
2375 mm	415	2.47 (0.15)^{a}	288 (113)^{b}	149 (16)^{b}	1.97 (0.18)^{d}
Pawson silt loam					
Control	<1	2.66 (1.4)^{a}	151 (13)^{a}	72 (16)a	0.37 (0.06)^{a}
1190 mm	207	2.64 (1.4)^{a}	314 (11)^{b}	72 (17)a	1.05 (0.05)^{b}

Nitrogen

Irrigation with TMW had little effect on the pasture's nitrogen concentration (Table 5). This is environmentally important because grazing animals excrete excess nitrogen in their urine, which then subsequently leaches (Woods et al., 2016). Nevertheless, the TMW treatments significantly increased the amount of nitrogen that was extracted from the soil, primarily because of the increased pasture growth. This indicates that at least in part, nitrogen was limiting pasture growth in the lysimeters because under nitrogen sufficient conditions, additional nitrogen results in increase pasture concentration, a process called luxury uptake (McLaren and Cameron, 1996a). For TMW irrigation rates up to 825 mm/yr, the mass of nitrogen extracted by the pasture was similar to or greater than the nitrogen that was applied. Given that our lysimeter experiment comprised two winters and just one summer, relatively less nitrogen was extracted than would be the case if we included a second growing season. It is therefore likely that pasture could remove the nitrogen added with TMW at rates above 1000 mm/yr. In the highest treatment (1650 mm/yr), the mass of N added was significantly greater than that which was removed in the pasture. This additional nitrogen was found as mineral nitrogen principally (NH_4^+, NO_3^-) in the soil profile. None of the other treatments showed accumulation of nitrogen in the soil. The mass of nitrogen leached from all treatments was <2 kg/ha equiv., which is negligible compared to the nitrogen leached from a grazed pasture, which can be >40 kg/ha/yr (Menneer et al., 2004).

Phosphorus

The phosphorus applied to the lysimeters with the TMW was 5 – 7 fold greater than the phosphorus removed by the pasture (Table 6). This discrepancy is normal because of phosphorus fixation in soil, a process that renders this nutrient unavailable for plant uptake (McLaren and Cameron, 1996a). The strong adsorption of phosphorus in soil also results in negligible amounts of phosphorus being leached. Therefore, in a TMW irrigated soil, phosphorus will accumulate, just as it does in all NZ soils that receive phosphate fertilisers. Phosphorus can cause serious environmental issues when it enters waterways (Tilman et al., 2001). This could occur via runoff from a TME-irrigated area, particularly if it is accompanied by soil erosion. TMW irrigation onto a cut-and-carry pasture or NZ native vegetation will always be less than phosphorus losses from a grazed pasture (TMW irrigated or otherwise) because of the mechanical disturbance of soil by the animals' hooves (McDowell et al., 2003).

Table 6. Mass of phosphorus (kg/ha equiv) in the treated municipal wastewater, pasture, soil and drainage water over the entire lysimeter experiment. Values in brackets represent the standard error of the mean (n=3). For each soil type, values with the same letter are not significantly different. The Barry's soil and Pawson silt loam were tested independently.

	Irrigation P (kg/ha equiv.)	Pasture P (mg/kg)	Pasture P (kg/ha equiv.)	P leached (kg/ha equiv.)	Soil P (0 – 60 cm) (kg/ha equiv.)
Barry's soil					
Control	<1	2606 (36)^{a}	13 (2)^{a}	<1	3975 (495)^{a}
637 mm	77	2593 (165)^{a}	16 (2)^{a}	<1	3268 (598)^{a}
1190 mm	144	2648 (55)^{a}	25 (3)^{b}	<1	3154 (198)^{a}
2375 mm	289	3196 (82)^{b}	40 (1)^{c}	<1	3437 (339)^{a}
Pawson silt loam					
Control	<1	3651 (184)^{a}	20 (2)^{a}	<1	5808 (303)^{a}
1190 mm	144	3663 (8)^{a}	45 (2)^{b}	<1	4863 (425)^{a}

Potassium

As with phosphorus, more potassium was added with the TMW than was removed by the pasture (Table 7). Most of this potassium will accumulate in the soil, with only minor amounts leached. Leached potassium is relatively environmentally benign compared to nitrogen and phosphorus. The accumulation of potassium in soil is insignificant because the soil concentrations are at least one hundredfold greater than the amount being added. At the highest TMW application rate (1650 mm/yr), the pasture took up significantly more potassium than the controls. High potassium in animal feeds can induce magnesium deficiency in livestock, resulting in grass staggers. In extreme cases, this requires that the animals be supplemented with magnesium (DairyNZ, 2017b).

Table 7. Mass of potassium (kg/ha equiv) in the treated municipal wastewater, pasture, soil and drainage water over the entire lysimeter experiment. Values in brackets represent the standard error of the mean (n=3). For each soil type, values with the same letter are not significantly different. The Barry's soil and Pawson silt loam were tested independently.

	Irrigation K (kg/ha equiv.)	Pasture K (mg/kg)	Pasture K (kg/ha equiv.)	K leached (kg/ha equiv.)	Soil K (0 – 60 cm) (kg/ha equiv.)
Barry's soil					
Control	1	11624 (263)^{ab}	65 (12)^{a}	1 (0)^{a}	34597 (493)^{a}
637 mm	177	8990 (723)^{c}	68 (4)^{a}	2 (0)^{a}	34848 (785)^{a}
1190 mm	331	10349 (510)^{bc}	112 (8)^{a}	3 (0)^{a}	35627 (908)^{a}
2375 mm	662	13060 (1150)^{a}	179 (6)^{b}	4 (1)^{a}	35165 (1134)^{a}
Pawson silt loam					
Control	1	17252 (1847)^{a}	104 (15)^{a}	6 (2)^{a}	40824 (1322)^{a}
1190 mm	331	17933 (518)^{a}	229 (16)^{b}	21 (6)^{a}	37392 (3319)^{a}

Sulphur

Irrigation with TMW provided an excess of sulphur (Table 8), which will eventually leach through the soil profile to receiving waters. Sulphur leaching does not provoke eutrophication like nitrogen or phosphorus. There were no significant effects of the TMW irrigation on the sulphur concentration in the pasture or in the soil profile.

Table 8. Mass of sulphur (kg/ha equiv) in the treated municipal wastewater, pasture, soil and drainage water over the entire lysimeter experiment. Values in brackets represent the standard error of the mean (n=3). For each soil type, values with the same letter are not significantly different. The Barry's soil and Pawson silt loam were tested independently.

	Irrigation S (kg/ha equiv.)	Pasture S (mg/kg)	Pasture S (kg/ha equiv.)	S leached (kg/ha equiv.)	Soil S (0 – 60 cm) (kg/ha equiv.)
Barry's soil					
Control	<1	2376 (40)[a]	14 (3)[a]	7 (2)	2389 (169)[a]
637 mm	169	2653 (169)[a]	17 (2)[a]	21 (5)	2190 (168)[a]
1190 mm	317	2649 (113)[a]	24 (2)[b]	40 (13)	2065 (75)[a]
2375 mm	634	2676 (60)[a]	35 (2)[b]	67 (14)	2294 (124)[a]
Pawson silt loam					
Control	<1	2941 (164)[a]	17 (2)[a]	11 (1)	2275 (96)[a]
1190 mm	382	3111 (76)[a]	40 (0)[b]	45 (8)	1989 (196)[a]

Calcium and magnesium

The TMW provided net additions of magnesium and calcium to the soil (Tables 9 and 10). These elements are important in maintaining soil pH as well as offsetting the negative effects of sodium on soil structure (FAO, 2017). Despite being applied in excess of pasture requirements, neither element was taken up at higher concentrations in the TMW treatments. Potential increases in magnesium uptake may have been offset by the elevated potassium levels in the TMW (McLaren and Cameron, 1996a).

Table 9. Mass of calcium (kg/ha equiv) in the treated municipal wastewater, pasture, soil and drainage water over the entire lysimeter experiment. Values in brackets represent the standard error of the mean (n=3). For each soil type, values with the same letter are not significantly different. The Barry's soil and Pawson silt loam were tested independently.

	Irrigation Ca (kg/ha equiv.)	Pasture Ca (mg/kg)	Pasture Ca (kg/ha equiv.)	Mg leached (kg/ha equiv.)	Soil Ca (0 – 60 cm) (kg/ha equiv.)
Barry's soil					
Control	3	3879 (527)[a]	24 (5)[a]	20 (5)[a]	48351 (1620)[a]
637 mm	371	3373 (216)[a]	26 (4)[a]	55 (13)[a]	46775 (748)[a]
1190 mm	696	3350 (69)[a]	39 (3)[ab]	61 (10)[a]	47506 (1059)[a]
2375 mm	1392	3327 (170)[a]	51 (0)[b]	92 (18)[a]	48786 (1433)[a]
Pawson silt loam					
Control	<1	5581 (396)[a]	31 (2)[a]	22 (6)[a]	53218 (3475)[a]
1190 mm	696	4890 (183)[a]	68 (2)[b]	92 (5)[a]	49948 (4004)[a]

Table 10. Mass of magnesium (kg/ha equiv) in the treated municipal wastewater, pasture, soil and drainage water over the entire lysimeter experiment. Values in brackets represent the standard error of the mean (n=3). For each soil type, values with the same letter are not significantly different. The Barry's soil and Pawson silt loam were tested independently.

	Irrigation Mg (kg/ha equiv.)	Pasture Mg (mg/kg)	Pasture Mg (kg/ha equiv.)	Mg leached (kg/ha equiv.)	Soil Mg (0 – 60 cm) (kg/ha equiv.)
Barry's soil					
Control	<1	2065 (279)[a]	13 (3)[a]	6 (1)[a]	33017[a]
637 mm	124	1823 (110)[a]	15 (2)[a]	21 (7)[a]	32580[a]
1190 mm	232	1964 (52)[a]	23 (1)[ab]	23 (1)[a]	32074[a]
2375 mm	463	1960 (210)[a]	33 (3)[b]	50 (17)[a]	32469[a]
Pawson silt loam					
Control	<1	2481 (106)[a]	16 (1)[a]	5 (1)[a]	42274 (2734)[a]
1190 mm	463	2572 (78)[a]	38 (2)[b]	30 (2)[a]	40351 (2596)[a]

Sodium

Elevated concentrations of sodium in irrigation waters are concerning because accumulation of sodium can lead to aggregate instability and reduced permeability of soil (Tanji, 1997). Table 11 shows that significantly more sodium was added to soil than was taken up by the pasture. Some of this excess sodium leached, while the remainder accumulated in the soil profile (Fig 7). There were significantly higher sodium concentrations in the TMW-irrigated effluent on the Pawson silt loam, but surprisingly, not on the Barry's soil. This elevated sodium concentration indicates that TMW from Duvauchelle is not suitable for irrigation onto plants that are sensitive to sodic or saline conditions. Elevated concentrations of sodium in pasture increase its palatability to stock (Chiy et al., 1998) and farmers occasionally "fertilise" their pastures with sodium for this reason.

Fig. 8 shows the distribution of sodium within the soil profile of the control and TME-treated lysimeters. The TMW treatments had significantly higher sodium concentrations than the controls at the 0-15 cm and 15 – 30 cm depths. The greatest difference in soil sodium concentrations was between the control (ca. 285 mg/kg) and the 440 mm/yr treatment (ca. 375 mg/kg). Doubling the irrigation rate to 825 mm/yr only increased the sodium in the surface soil to ca. 405 mg/kg, and quadrupling the TMW irrigation rate increased sodium to ca. 420 mg/kg. This indicates that above ca. 400 mg/kg, sodium is not strongly retained by the soil and migrates down through the soil profile and will eventually be lost via leaching. This effect has been replicated in laboratory columns containing a Pawson silt loam, where sodium-spiked TMW (up to 260 mg/L) was irrigated (C. McIntyre, unpublished data). It is therefore unlikely that in the short-to-medium term (<10 years), sodium will accumulate to unacceptable levels in soils. Over the long term, the soils may require periodic amendments with gypsum or dolomite to maintain structure (FAO, 2017).

Table 11. Mass of sodium (kg/ha equiv) in the treated municipal wastewater, pasture, soil and drainage water over the entire lysimeter experiment. Values in brackets represent the standard error of the mean (n=3). For each soil type, values with the same letter are not significantly different. The Barry's soil and Pawson silt loam were tested independently.

	Irrigation Na (kg/ha equiv.)	Average Pasture Na (mg/kg)	Pasture Na (kg/ha equiv.)	Na leached (kg/ha equiv.)	Soil Na (0 – 60 cm) (kg/ha equiv.)
Barry's soil					
Control	5	2243 (475)[a]	10 (3)[a]	45 (6)[a]	2492 (76)[a]
637 mm	605	2256 (241)[a]	13 (3)[a]	159 (18)[b]	2840 (137)[ab]
1190 mm	1131	2651 (159)[a]	23 (3)[ab]	264 (23)[b]	2980 (106)[b]
2375 mm	2256	3109 (308)[a]	45 (6)[b]	412 (61)[b]	3113 (122)[b]
Pawson silt loam					
Control	5	2525 (198)[a]	13 (1)[a]	30 (0)[a]	2428 (181)[a]
1190 mm	1131	4038 (273)[b]	50 (2)[b]	232 (32)[b]	2610 (239)[a]

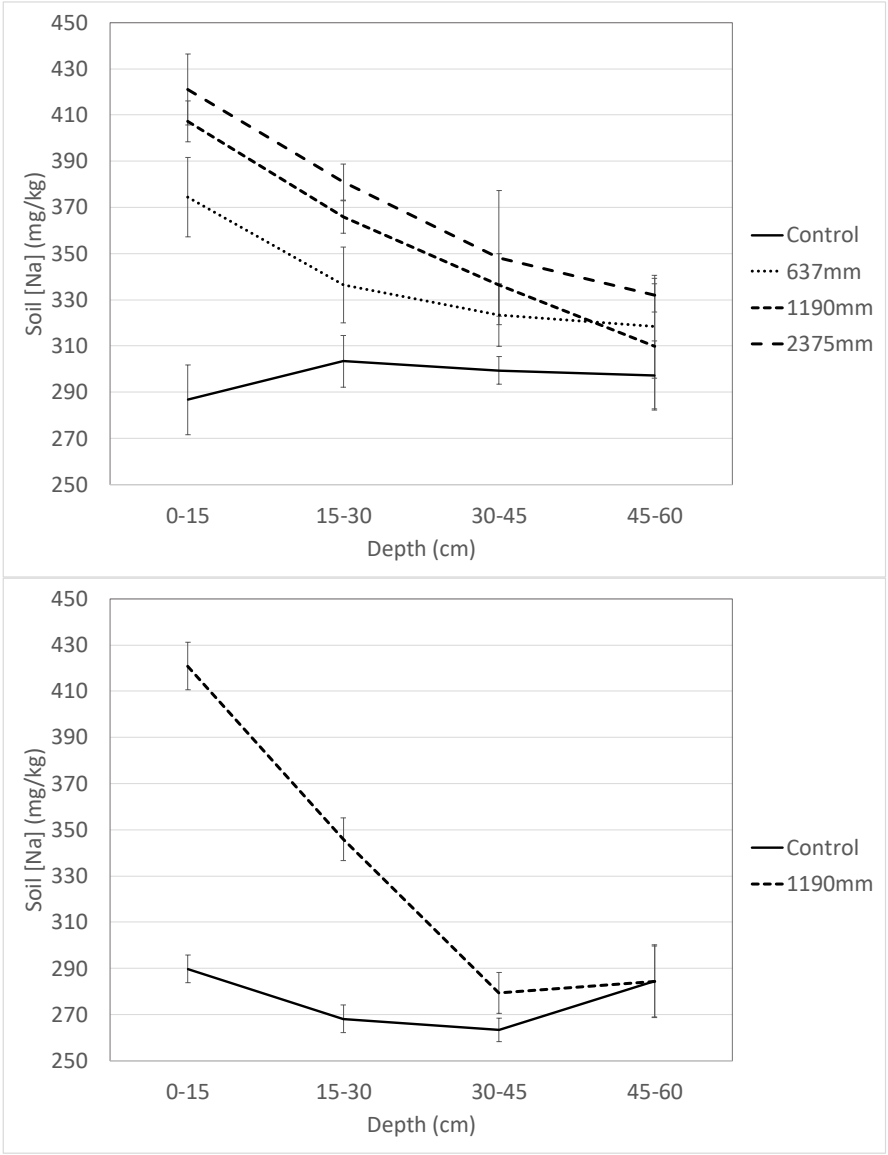


Fig. 8. Soil sodium concentration as a function of depth at the end of lysimeter experiment for the Barry's soil (top) and Pawson silt loam (bottom), expressed as tonnes per hectare equivalent. Bars represent the standard error of the mean (n=3).

Field trial

Plant survival

Fig. 9 shows the survival of individual species in the field plot as a percentage of the number planted. Most of the plant deaths occurred during the spring of 2015 - which was extraordinarily dry – before irrigation with TMW had started. Survival in March 2016 was similar to May 2017 (data not shown). As of May 2017, there were no significant differences between the irrigated and non-irrigated plots. Note that Fig. 9 does not include the additional control plots, at the Southern end of the field trial. These non-irrigated plots have a higher mortality, which we attribute to the soils, which are distinct (stonier) than the remainder of the field trial.

The only significant failure is *Pseudopanax arboreus*. This species has survived well in areas of the trial that are protected from the wind, but elsewhere survival is very poor. Potentially, this species could be used for wastewater treatment, but it should be planted in sheltered areas once the other species have become established.

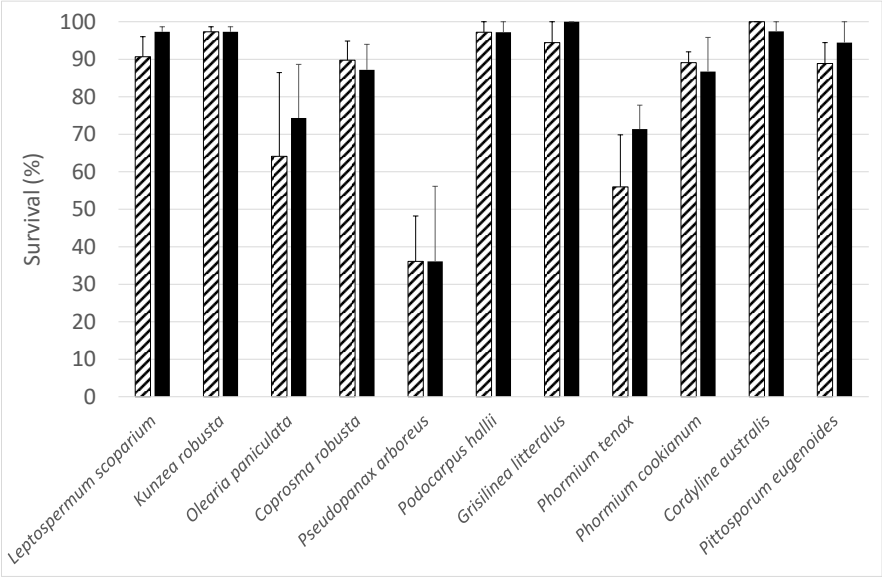


Fig. 9. Percentage survival of the plants in the field plot on Pipers Valley Road as of May 2017. There were no significant differences between the controls (striped bars) and treatments (black bars). Error bars represent the standard error of the mean of three plots, with each plot containing 5 – 25 plants.

Plant growth

Fig. 10 shows the field trial, along with the information board. Plants growing in the effluent-treated plots are visibly larger than the control plots. This observation is borne-out by measurement of the canopy volume (Fig. 11). Compared to the control, the canopy volume of all species in the TMW plots is either larger or not significantly different. There are no signs of toxicity or salt damage (burning of the leaves) on any of the plants. Nevertheless, there are stark differences between the species in how they respond to effluent. *Griselinia littoralis*, *Phormium cookianum*, and *Pittosporum eugenioides* are not significantly larger in the TMW-irrigated plots and are, in general, smaller than the other species in the trial.

Fig. 10. The field plot on Pipers Valley Road in June, 2017, showing the plant trial, information board, and boarders that were planted with *Poa picta* in May 2017.

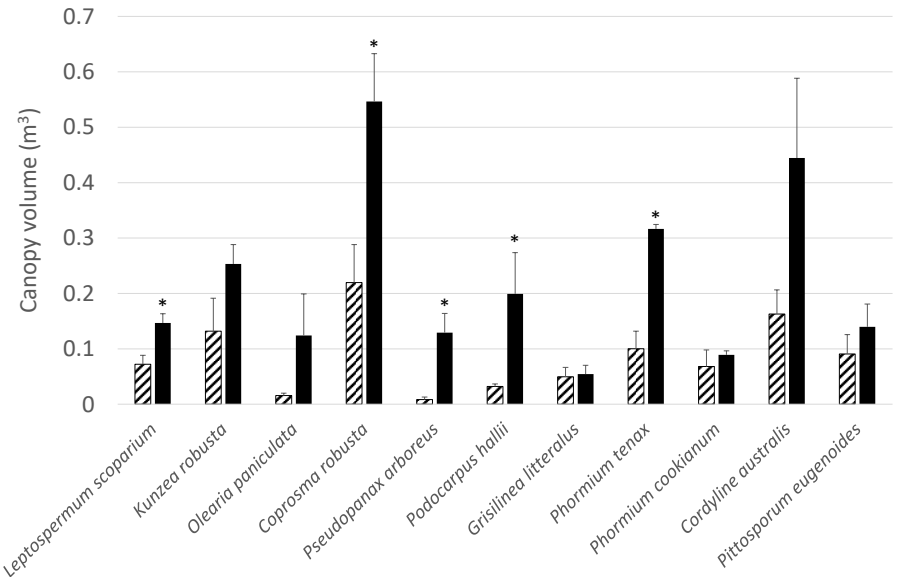


Fig. 11. Canopy volume of the plants in the field plot on Pipers Valley Road as of May 2017. Asterisks (*) signify significant differences between the controls (striped bars) and treatments (black bars). Error bars represent the standard error of the mean of three plots, with each plot containing 5 – 25 plants.

Plant stability in a wet area

One of the TMW-treated plots in the trial was established on a boggy area, as evidenced by waterlogging at the time of planting. Two trees have fallen over in this area (Fig 12). It is likely that TMW irrigation will reduce plant stability because the nutrients contained therein increase the shoot: root ratio of most plants (Agren and Franklin, 2003), thereby creating "top heavy" trees that are more likely to topple in soft substrates. *Cordyline australis* and *Phormium tenax* are more suited to grow in boggy patches.

Fig. 12. Fallen *Pittosporum eugenoides* and *Kunzea robusta* in the field plot at Pipers Valley Road in June, 2017.

In general, NZ native species will take up less water and nitrogen than pasture species from an irrigated shallow rooted environment. However, in the Banks Peninsula environment, the water flux through closed-canopy native vegetation and pasture may be similar because of the "umbrella effect", whereby a significant proportion of rainfall is re-evaporated from the canopy before it reaches the ground (McNaughton and Jarvis, 1983). A mature stand of irrigated native vegetation is likely to leach more nitrogen than irrigated cut-and-carry pasture because little nitrogen is being removed from the system.

Next steps

In each plot of the field trial, five soil samples have been taken and sub-samples from five replicates of each plant species have been analysed. Results from this sampling will be made available upon completion of the PhD theses of Obed Lense and Saloomeh Seyedalikhani. This is expected to occur in early 2018. The field plots will be monitored for various postgraduate projects for at least another three years.

Irrigation of treated municipal wastewater onto NZ native plants: beneficial reuse or disposal?

Disposal of TMW implies discharge into an environment with the aim of minimising negative environmental effects but not gaining value from the TMW. Examples of disposal include discharge to waterways, the ocean, and the application of TMW to land at rates that are far in excess of plant requirements for water and nutrients. This contrasts with beneficial reuse where the irrigation value and nutrient value of the TMW is used to produce valuable biomass, offsetting costs for fertilisers and irrigation that would otherwise have to be met by the landowner. Using this definition, irrigation of TMW to produce of cut-and-carry pasture or pasture for grazing is an example of beneficial reuse.

Clearly, TMW irrigation is not required to establish and grow NZ native plants on Banks Peninsula – nor is it required to grow pasture. Therefore, TMW-irrigation onto NZ native plants can only be considered

a beneficial reuse if it generates more value than would otherwise be realised on a non-irrigated system. Irrigating TMW onto mānuka (*Leptospermum scoparium*) ecosystems for the production of honey or essential oils would be an example of beneficial reuse of the water and nutrients contained within TMW because most of Banks Peninsula is too dry to support mānuka production (there are small pockets of mānuka in Nikau Palm Gully and on Quail Island). Moreover, mānuka has been demonstrated to be effective in reducing nitrogen losses from soil (Esperschuetz et al., 2017c). Using TMW to accelerate the production of any product derived from native plants is an example of beneficial reuse.

NZ native plants may have a role in the land application of TMW even if no valuable native product is realised. Native plants, including mānuka and kānuka, could be used on paddock margins of TMW-accelerated pasture (cut-and-carry or grazed) to reduce environmental impacts. There are innumerable examples of where NZ native plants have been used successfully to improve environmental outcomes on conventional farms. Replacing a conventional grazed pasture with a well-designed TMW-application system is likely to improve the water quality of the local streams.

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