



Research article

Biowastes to augment the essential oil production of *Leptospermum scoparium* and *Kunzea robusta* in low-fertility soil

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ABSTRACT

Biowastes are unwanted materials of biological origin. They include biosolids, dairy shed effluent, and sawdust. 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. We grew New Zealand native plants, mānuka (*Leptospermum scoparium* J.R. Forst & G. Forst) and kānuka (*Kunzea robusta* de Lange & Toelken) in series of greenhouse experiments in low-to-medium-fertility soils (Bideford clay loam, Lismore stony silt loam, and Pawson silt loam) amended with either biosolids (up to 13500 kg N ha⁻¹ equiv.), biosolids + sawdust (1:0.5–1250 kg N ha⁻¹ equiv.) and dairy shed effluent (200 kg N ha⁻¹ equiv.). Two types of biosolids from Kaikoura (KB) and Christchurch City Council (CB) were used in the experiments. CB (1500 kg N ha⁻¹ equiv.) and dairy shed effluent (200 kg N ha⁻¹ equiv.) increased the biomass of *L. scoparium* by up to 120% and 31%, and *K. robusta* by up to 170% and 34%, respectively. Adding sawdust to KB increased the biomass of *L. scoparium* and *K. robusta* although it offset the *L. scoparium* growth increase in the KB-only treatment. The growth response of *K. robusta* to biowastes was greater than *L. scoparium* with oil production in *K. robusta* increasing by up to 211% when 1500 kg N ha⁻¹ equiv. of CB was applied to Lismore stony silt loam. Generally, the treatments had a negligible effect on oil concentration in all the soil types, except for the KB + sawdust treatment, which increased the oil concentration by 82%. Most of the EOs' major components were unaffected by biowaste addition in the soils, although some components increased in the Bideford clay loam following KB and KB + sawdust application. Biosolids increased foliar concentrations of Zn, Cu, and Cd, but these were below risk-threshold concentrations. Applying CB (up to 1500 kg N ha⁻¹ equiv.) to low-fertility soils is recommended to establish ecosystems dominated by *L. scoparium* and *K. robusta* that annually would produce ca. 100 kg ha⁻¹ of EOs worth US\$ 26k and 24k, respectively. Adding sawdust to CB could have environmental benefits through reduction of N leaching. Field trials are warranted to elucidate critical ecological variables and production economics in biowaste management.

1. Introduction

Biowastes comprise unwanted material of biological origin and include the products of sewage treatment, animal effluents (Sanchez et al., 2009; Guo et al., 2014) as well as crop and silvicultural residues (Kim et al., 2015). Disposal of biowastes can be expensive, e.g. disposal to landfill (Güereca et al., 2006), or environmentally damaging, e.g. disposal in waterways or incineration (McLaughlin and Filmer, 2008). The application of biowastes to low-fertility or degraded soils could aid

the restoration of native or exotic ecosystems (Bolan et al., 2004; Esperschuetz et al., 2016).

Sewage treatment produces ca. 52 kg yr⁻¹ of biosolids per person, with a global output > 10 Mt yr⁻¹ (Bradley, 2008). Biosolids contain high concentrations of essential plant nutrients and organic matter, which can improve soil fertility (Obi and Ebo, 1995). However, biosolids can contain pathogens and contaminants (Krogmann et al., 1999; Singh and Agrawal, 2008). Disposal costs may be avoided by using biosolids to rebuild degraded soils (Daniels et al., 2003; Novak et al.,

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2009), a strategy that is used worldwide (Kowaljew et al., 2010; Martinez et al., 2003).

Dairy shed effluent (DSE) comprises bovine urine and feces collected during the milking of dairy cattle (Zaman et al., 2002). DSE contains elevated concentrations of N, K, P, and S, as well as pathogens and xenobiotics (Roach et al., 2001). DSE is rich in organic matter and improves soil water holding capacity, aeration, and drainage as well as reducing soil compaction and erosion (Rahmani and Tabaei-Aghdaei, 2014). Over-application of DSE results in excessive leaching of nitrates and can contaminate ground and surface waters (Houlbrooke et al., 2004).

In 2018, some 5% of New Zealand (1.3 M ha) was covered by *Pinus radiata* forests (NZGEO, 2018). The exploitation of these forests produces waste residues (sawdust and off-cuts) that are often stored in large piles that leach tannins (Robinson et al., 2007). These sawdust piles often occur on or near harvested sites, where the land has become degraded due to the loss of topsoil (Paramashivam et al., 2017). These soils are low in both organic matter and plant nutrients (Chirino et al., 2010). Sawdust has been demonstrated to reduce nitrate leaching from high N-content biowastes such as biosolids (Paramashivam et al., 2016). Moreover, sawdust could be mixed with biosolids to reduce plant uptake of contaminants from biosolids-amended soils (Esperschuetz et al., 2017).

Gibbs and Salmon (2015) estimated that there are some six million hectares of degraded land worldwide. Potentially, some of these lands may be restored to native ecosystems using biowastes. These ecosystems may contain species that generate non-food products such as essential oils (EOs), which are unlikely to expose humans to biowaste-borne contaminants (McLaughlin et al., 2007). There is international interest in New Zealand *L. scoparium* EO due to its high β -trienone levels (Douglas et al., 2004), which significantly contributes to antimicrobial properties (Maddocks-Jennings et al., 2005). *L. scoparium* EO currently sells for US\$890 per liter (EONZ, 2014; NZMB, 2014). Likewise, *K. robusta* EO that contains high levels of α -pinene (> 50%) (Porter and Wilkins, 1999) is sold for US\$680 per liter (Lotus oils, 2016).

The quantity and quality of EOs change in response to environmental conditions, such as salinity, nutrient-deficiency, drought or heavy metals (Abdelmajeed et al., 2013). Biowastes may alleviate nutrient deficiency but increase plant stress through elevated concentrations of heavy metals and salts (Bai et al., 2012). Prasad et al. (2014) reported that soil concentrations of 25–50 mg kg⁻¹ Pb, Cr, Cd or Ni increased the EO concentration and khusimol content in *Vetiveria zizanioides*. However, Zheljzakov and Nielsen (1996) reported that heavy metals decreased the EO production of *Mentha piperita* and *Mentha arvensis*, but did not affect EO quality.

Puttanna et al. (2010) reported that *Rosmarinus officinalis* EO yield increased by the application of 150 kg ha⁻¹ year⁻¹ N (urea) and 100 kg ha⁻¹ year⁻¹ K (muriate of potash). Applying N (up to 135 kg ha⁻¹) to *Thymus vulgaris* did not affect the EO yield (Baranauskiene et al., 2003). 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 (ammonium nitrate) and K (potassium sulphate) fertilizers (up to 180 and 120 kg ha⁻¹) increased the EO production of *Anthriscus cerefolium*.

We hypothesized that the addition of biowastes to *L. scoparium* and *K. robusta* would change both the quantity and quality of the EOs through the actions of plant nutrients, contaminants, and physical alteration of the substrate. We aimed to test this hypothesis using contrasting biosolids, DSE and sawdust added to low-to-medium-fertility soils. Specifically, we sought to measure the biomass and key components of the EOs as well as potential contaminants, namely As, Cd, Cr, Cu, Mn, Ni, Pb, and Zn in the above-ground biomass.

Table 1A

Parameters of the soils used in the Exp. 1– Exp. 4. 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
Soil type	Bideford Clay Loam (BCL)	Lismore Stony Silt Loam (LSL)	Pawson Silt Loam (PSL)
N.Z. Soil Classification	Brown Soil	Pallic Firm Brown soil	Pallic Firm Brown soil
pH	6.1	5.2 (0.01)	4.9 (0.01)
CEC (me 100 g ⁻¹)	21	13	12.8
C (T) (%)	6.5	4.5 (0.2)	3.1 (0.07)
N (T) (%)	0.50	0.23 (0.01)	0.38 (0.05)
C/N	14	20 (0.4)	10 (0.1)
NH ₄ ⁺ - N (mg kg ⁻¹ FW)	2	3.5 (0.11)	6.8 (0.2)
NO ₃ ⁻ - N (mg kg ⁻¹ FW)	0.6	28 (1.6)	25 (1.26)
Olsen - P	11	13	15
P (T)	544 (5)	383 (7.3)	812 (14.7)
K (T)	1886 (46)	4468 (37)	2929 (39)
S (T)	405 (2)	210 (5.5)	375 (8.2)
Ca (T)	4063 (67)	2472 (41)	4448 (45)
Mg (T)	1962 (22)	3768 (33)	2580 (11)
Fe (T)	15461 (108)	22293 (270)	18876 (52)
Mn (T)	133 (3)	288 (2.8)	577 (4)
Cu (T)	4.2 (0.0)	3.4 (0.3)	8.1 (0.43)
Na (T)	207 (5)	268 (4.1)	182 (1.4)
Ni (T)	4.1 (0.0)	7.3 (0.1)	7.9 (0.1)
Zn (T)	29 (0.0)	75 (2.6)	51 (1)
Pb (T)	8.3 (0.1)	14 (0.1)	11.7 (0.15)
Cd (T)	0.05 (0.00)	0.43 (0.01)	≤ 3 × 10 ⁻⁴
Cr (T)	14.0 (0.2)	22 (0.3)	14.2 (0.16)

2. Materials and methods

2.1. Soils and biowastes

This study comprised four experiments (Exp. 1 – Exp. 4). For Exp. 1 (2013), a clay loam soil was taken from Bideford, New Zealand (40°45'56"S 175°54'42"E). The soil of Exp. 2 and 3 (2014 and 2015) was a Lismore stony silt loam that collected from Eyrewell Forest (43°43'87"S, 172°45'31"E), which formerly was under *Pinus radiata* cultivation. In Exp. 4 (2015), a Pawson silt loam was taken from Banks peninsula (43°47'31"S, 172°58'18"E). All soils were taken from the top 15 cm, after removing the surface vegetation. Based on the soils C and N (Table 1A) concentrations that are of overriding importance in soil fertility (Milne et al., 2015; Troeh and Thompson, 2005), the fertility of the soils used in the experiments was classified as Bideford clay loam > Lismore stony silt loam and Pawson silt loam.

The biosolids (KB) used in Exp. 1 and 2 were collected from Kaikoura Regional Treatment Works, Kaikoura, Canterbury, New Zealand (42°21'37.40"S, 173°41'27.35"E). These biosolids had minimal industrial input and were stockpiled in the oxidation pond and weathered. For Exp. 3 and 4, biosolids (CB) were collected from the Christchurch City Council (CCC) Wastewater Treatment Plant. These biosolids had moderate industrial input and had undergone anaerobic digestion (Ccc, 2018). *Pinus radiata* sawdust was collected from Kaikoura Wastewater Treatment Plant, New Zealand (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). Soil, biosolids, and sawdust were sieved (≤ 10 mm), mixed and homogenized before application. Table 1A and B give the chemical properties of the soils and biowastes.

2.2. Plant material

In Exp. 1, *L. scoparium* and *K. robusta* seedlings were obtained from Waiora Nursery Ltd, Christchurch, New Zealand (<http://www.waioralandscapes.co.nz/pages/nursery/>). For Exp. 2–4, seedlings

Table 1B

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 dairy shed effluent unless otherwise indicated. n.a. = not applicable. KB, CB, LUDF and FW represent Kaikoura biosolids, Christchurch City Council Biosolids, Lincoln University Dairy Farm and fresh weight, respectively.

	KB	CB	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	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	42 (1)	17 (0.4)
K	3653 (34)	2164	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	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	0.6 (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)	≤3 × 10 ⁻³	≤3 × 10 ⁻³
Cd	3.97 (0.07)	1.6 (0.01)	≤3 × 10 ⁻⁴	≤3 × 10 ⁻⁴
Cr	47.6 (0.8)	127	0.2 (0.0)	≤4 × 10 ⁻⁴

were purchased from Motukarara Native Plant Nursery, Department of Conservation, Canterbury, New Zealand (<http://www.doc.govt.nz/our-work/motukarara-conservation-nursery/>). All seedlings were sourced from the Canterbury province in New Zealand. Before planting, the roots were washed with tap water to remove any potting mix.

2.3. Greenhouse experiments

All experiments were performed at the Lincoln University Plant Growth Unit (43°38'42"S, 172°27'41"E) from 2013 to 2016. Each of the treatments described below was conducted with both *L. scoparium* and *K. robusta*. The pots in all experiments were placed in a randomized block design.

2.3.1. Exp. 1

Pots (25 cm diameter x 29 cm high) were filled with 2 kg of pea gravel at the bottom to facilitate drainage. Soil (Bideford clay loam) was filled into pots. The treatments comprised 245 g dry weight of KB (1250 kg N ha⁻¹ equiv.) and the same rate of KB combined with sawdust at a ratio of 1:0.5. The controls received no KB or sawdust. Each treatment was replicated four times. Planting occurred in September 2013 and allowed establish for six weeks before applying the treatments. The experiment continued for 18 weeks in the greenhouse. Average night and day temperatures during the experiment were 14.5 °C and 21 °C (minimum 9 °C and maximum 28 °C). An automatic irrigation system was installed, and supplementary manual irrigation occurred as required.

2.3.2. Exp. 2

Pots of the same dimensions as Exp. 1 were filled with 2 kg of gravel at the bottom overlain with Lismore stony silt loam soil mixed with 1000 g of KB (2800 kg N ha⁻¹ equiv.). The DSE treatment comprised Lismore stony silt loam soil and a total of 200 kg N ha⁻¹ equiv. The DSE was added in ten weeks (500 mL per week) from three months after the

plants were established. There were three replicates of each treatment. The experiment was conducted for 24 weeks from September 2014. Average night and day temperature during the experiment 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.

2.3.3. Exp. 3

Pots (22.5 cm diameter x 17 cm high) were filled with Lismore stony silt loam soil. Dried CB were mixed with the soil at rates of 0, 50, 150, 450 and 1350 g pot⁻¹, equivalent to 0, 500, 1500, 4500 and 13500 kg N ha⁻¹. There were five replicates of each treatment. The experiment was conducted for 16 weeks from October 2015 to February 2016. Average night and day temperature during the experiment were 17 °C and 22.3 °C (minimum 9.5 °C and maximum 32 °C). Pots were irrigated once per day to field capacity.

2.3.4. Exp. 4

Pots of the same dimensions as used in Exp. 3 were filled with Pawson silt loam soil mixed with the dried CB at rates of 0 and 150 g (equivalent to 0 and 1500 kg N ha⁻¹). There were five replicates of each treatment. The experiment was conducted from November 2015 to February 2016 (12 weeks). Average night and day temperature during the experiment in the greenhouse were 17 °C and 22 °C (minimum 8.7 °C and maximum 43 °C). Pots were irrigated once per day to field capacity.

2.4. Plant harvest

For all experiments, plants were harvested and analyzed for the chemical elements, biomass and EO quality and quantity. Some 0.1 g of fresh leaves from each plant was taken randomly over the plant from both young and old parts. Samples were plunged in liquid nitrogen immediately after harvesting and kept at -80 °C until solvent extraction of the EO. Shoot biomass of the plants was weighted immediately after harvest and washed with deionized water. Aboveground portions of the plants were oven dried (at 60 °C to a consistent weight) to calculate the moisture content and oven dried equivalent of the plants. Oven dried leaves of the plants were used to study the nutrient and trace element status of the plants.

2.5. Essential oil extraction and GC-MS analysis

Following the micro-scale technique described by Brophy et al. (1989), 0.1 g of leaf sample was soaked in 2 mL of the ethanol + dichloromethane (1:1) solvent in glass vials for 18–20 h at room temperature. One mL of the extract was transferred to GC vials, and the internal standard (eicosane- C20- 125 mg L⁻¹) was added. The analysis of volatile organic compounds (VOCs) from EO plant extracts was performed by Gas Chromatography Mass Spectrometry (GC/MS) (Supplementary methods 1). Samples were interpreted according to commonly found components detected in studies on *L. scoparium* and *K. robusta* EOs throughout New Zealand (Porter and Wilkins, 1999; Maddocks-Jennings et al., 2005).

2.6. Elemental analysis

Element concentrations were determined in the acid digests using ICP-OES (Varian 720 ES). Wageningen (ISE 921, IPE 100) and NIST (1573a) Certified Reference Materials were analyzed in the same sample sets. Recoveries ranged from 91 to 112%. A CNS-2000 Element Analyser (LECO Australia Pty Ltd., Australia) was used to determine the total C and N in ground soils and plants. Soil nitrate (NO₃⁻) and ammonium (NH₄⁺) concentrations were determined using a KCl extraction from frozen soil following the method of (Blackmore et al., 1987). These methods have been described in detail in the supplementary

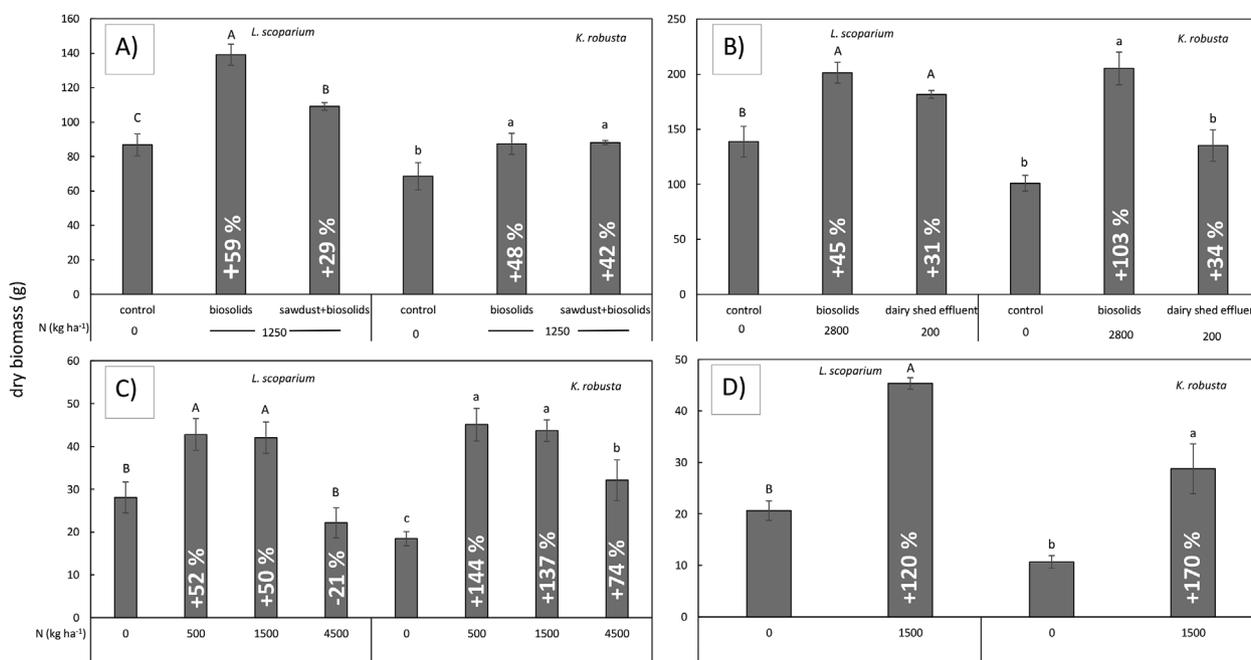


Fig. 1. Aboveground biomass (g DW) in A- Exp. 1 ($n = 4 \pm se$), B- Exp. 2 ($n = 3 \pm se$), C- Exp. 3 ($n = 5 \pm se$) and D- Exp. 4 ($n = 5 \pm se$). Significant differences between the treatments at $p \leq 0.05$ are indicated by capital letters (A, B, C) for *L. scoparium* and lower-case letters (a, b, c) for *K. robusta* within the plant species. Numbers in the bars represent the percentage of changes caused by the treatments compared to the control.

material.

2.7. Statistical analysis

Minitab® 16 was used for ANOVA analysis and Fisher's Least-Significant-Difference ($P < 0.05$) as a post-hoc test to compare means. Data were tested for normality, and non-normally distributed data were log-transformed before analysis.

3. Results

3.1. Plant biomass

The maximum dry biomass increase of *L. scoparium* and *K. robusta* occurred in the lowest fertility soil (Pawson silt loam- Exp. 4) by CB application ($1500 \text{ kg N ha}^{-1}$ equiv.) up to 120% and 170%, respectively (Fig. 1). This result was consistent with the findings of Reis et al. (2017), who showed a 40-fold increase of *L. scoparium* growth in the low-fertility sand by applying 90 t ha^{-1} equiv. fresh biosolids. The relative biomass increase of each species was inconsistent, with the *L. scoparium* having larger biomass increases in Exp. 1 while *K. robusta* had larger biomass increase in Exp. 2, 3 and 4. Blending KB with sawdust offset some of the biomass increase in *L. scoparium* but not *K. robusta* (Fig. 1-A). DSE added at 200 kg N ha^{-1} equiv. had a similar beneficial effect to KB added at $2800 \text{ kg N ha}^{-1}$ in *L. scoparium*, but the DSE had a smaller beneficial effect than the KB in *K. robusta* (Fig. 1-B). The results show that negative effects of any contaminants present in the biowastes were more than offset by the plant nutrients, except in the $4500 \text{ kg N ha}^{-1}$ CB treatment (*L. scoparium*), which was not significantly different from the control. Application of more than $4500 \text{ kg N ha}^{-1}$ equiv. CB reduced plant growth and most of the plants died (Fig. 1-C).

3.2. Essential oils

Biowastes had only small effects on the EO concentrations. However, the total EO produced (biomass x concentration) often significantly increased (Fig. 2A–D). The largest increase of *L. scoparium* EO production (164%) occurred in the Bideford clay loam soil when

KB + sawdust was applied (Fig. 2A) followed by the Pawson silt loam soil with 156% increase by application of $1500 \text{ kg N ha}^{-1}$ equiv. CB (Fig. 2D). The maximum increase of *K. robusta* EO production was observed in $1500 \text{ kg N ha}^{-1}$ equiv. CB application (211%) in Lismore stony silt loam (Fig. 2C). Biosolids significantly increased the EO production of *K. robusta* in Exp. 2, 3 and 4 treatments except for applying $4500 \text{ kg N ha}^{-1}$ equiv. of CB (Fig. 2B–D). Applying DSE did not increase the EO production of *L. scoparium* and *K. robusta*.

The addition of KB + sawdust, but not KB alone, to the Bideford clay loam soil, significantly increased (by 82%) the EO concentration in *L. scoparium* (Fig. 3A), as indicated by the concentrations of beta-myrcene, beta-elemene, alpha-gurjunene, alpha-selinene and alpha-farnesene (Fig. S1A). In the other three experiments, significant differences occurred in some components (Figs. S1B and S2), but the difference did not affect the total concentration of the EO (Fig. 3). None of the treatments affected the EO concentration of *K. robusta* (Fig. 3), except for the highest CB treatment ($13500 \text{ kg N ha}^{-1}$ equiv.), where the plants died. The main component of the EO in *K. robusta* is α -pinene, which was not affected by the treatments (Figs. S3 and S4). In Exp. 3, the concentration of α -pinene was not significantly different from the control (110 mg L^{-1}) when $1500 \text{ kg N ha}^{-1}$ equiv. CB were applied (Fig. S4C).

Generally, our results showed that $1500 \text{ kg N ha}^{-1}$ equiv. CB application was an optimal rate for plant growth. The concentration of key components of *L. scoparium* and *K. robusta* EOs did not significantly decrease and in many cases increased. The only exception was β -selinene, which decreased by 47% when $1500 \text{ kg N ha}^{-1}$ equiv. CB were applied (Exp. 3). Therefore, the quality of the EO would not be affected by biowaste application.

3.3. Effect of the biowastes on leaf elemental composition

The application of biowastes increased the concentration of N in both *L. scoparium* and *K. robusta* leaves in most treatments compared to the control. The concentration of heavy metals in the treated plants leaves remained below threshold concentrations for food and environmental safety (ANZFSC, 2015) (Tables 2A–B and Tables S1– S5).

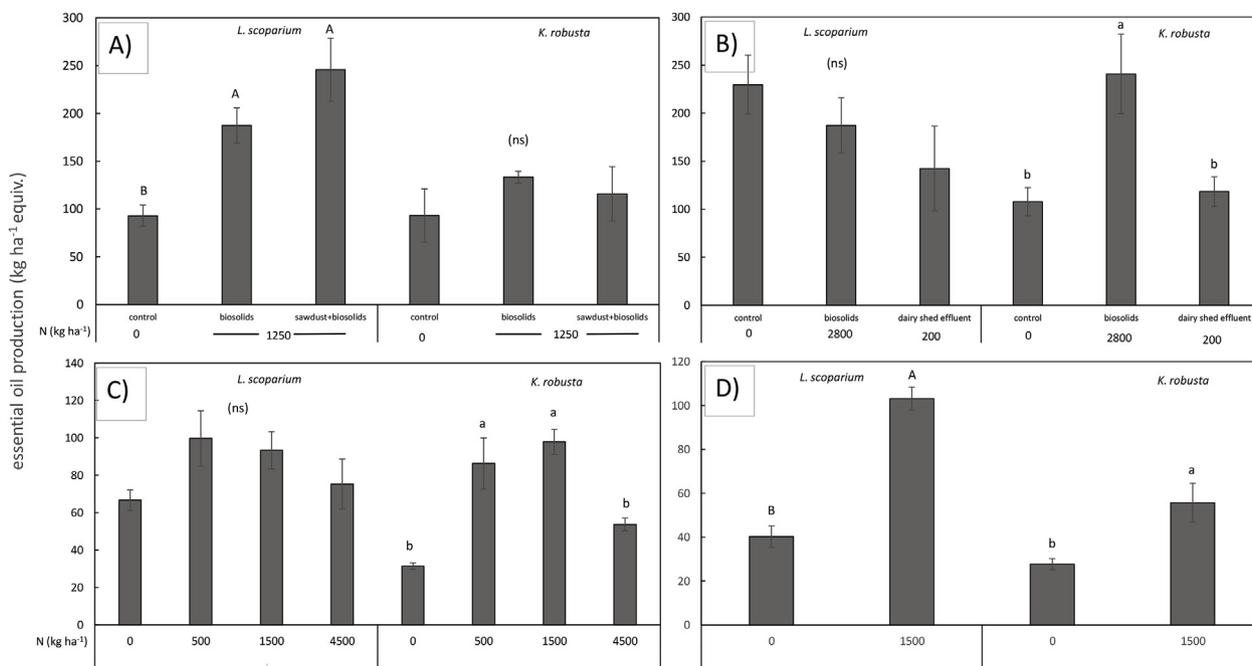


Fig. 2. Plants average essential oil production (kg ha^{-1}) in A- Exp. 1 ($n = 4 \pm \text{se}$), B- Exp. 2 ($n = 3 \pm \text{se}$), C- Exp. 3 ($n = 5 \pm \text{se}$) and D- Exp. 4 ($n = 5 \pm \text{se}$). Significant differences between the treatments at $p \leq 0.05$ are indicated by capital letters (A, B, C) for *L. scoparium* and lower-case letters (a, b, c) for *K. robusta* within the plant species.

4. Discussion

In the experiments the maximum biomass increase was observed in the lowest fertility soil (Pawson silt loam- Exp. 4). The positive effect of biowastes on the growth of *L. scoparium* and *K. robusta* is consistent with previous studies using low-fertility soil (Reis et al., 2017; Esperschuetz et al., 2017). It is likely that biomass increases would be smaller if biowastes were added to higher-fertility soils. The EO production increase of *L. scoparium* was high in both highest and lowest

fertile soils of the experiments (Bideford clay loam and Pawson silt loam), and maximum EO production increase of *K. robusta* occurred in the Lismore stony silt loam soil. A direct comparison of the soil types in terms of EO quality and quantity is not possible because the experiments were conducted at different times. 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, it is unlikely that biowaste addition will significantly reduce oil quality. Biowaste addition to high-fertility soils may decrease

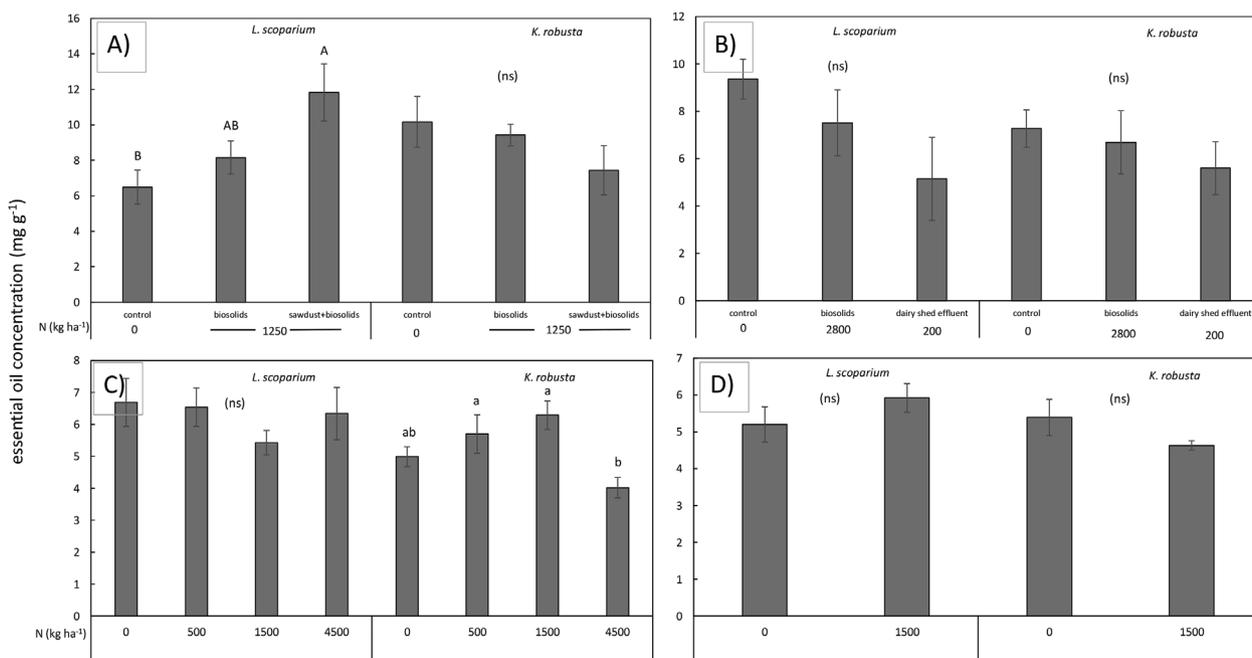


Fig. 3. Plants essential oil concentration (mg g^{-1}) in A- Exp. 1 ($n = 4 \pm \text{se}$), B- Exp. 2 ($n = 3 \pm \text{se}$), C- Exp. 3 ($n = 5 \pm \text{se}$) and D- Exp. 4 ($n = 5 \pm \text{se}$). Significant differences between the treatments at $p \leq 0.05$ are indicated by capital letters (A, B, C) for *L. scoparium* and lower-case letters (a, b, c) for *K. robusta* within the plant species.

Table 2A
Elemental concentrations of *L. scoparium* leaves in Exp. 1- Exp. 4. Numbers in front of 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.

Exp.	Treatment (kg N ha ⁻¹ equiv.)	N g kg ⁻¹ dry matter	P	K	S	Ca mg kg ⁻¹ dry matter	Mg	Zn	Cu	Cd
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) ^b	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) ^b	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

^{*}Only a few leaves of two plants survived in the 13500 kg N ha⁻¹ equiv. CB application rate.

^{**}There was insufficient material for N analysis of one of the plants. Cadmium was detected in one of the two samples, therefore, statistical analyses were not possible.

Table 2B
Elemental concentrations of *K. robusta* leaves in Exp. 1- Exp. 4. Numbers in front of 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.

Exp.	Treatment (kg N ha ⁻¹ equiv.)	N g kg ⁻¹ dry matter	P	K	S	Ca mg kg ⁻¹ dry matter	Mg	Zn	Cu	Cd
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) ^a	1310 (117) ^a	7216 (685) ^a	1886 (239)	119 (5.8) ^a	2.3 (0.2) ^a	0.31 (0.09) ^b
	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 getting average and statistical comparison was not possible.

both the quantity and quality of EOs, presumably due to phytotoxic components (Rahmani and Tabaei-Aghdaei, 2014; Petropoulos et al., 2009; Tabatabaie and Nazari, 2007).

EOs are plants' secondary metabolites (Dhifi et al., 2016). These products are biosynthesized from one or more primary metabolites through various pathways (Verpoorte, 2000), that are affected by nutrients in the soils and the amendments. Hassan (2012) demonstrated that increasing the soil N content would change the quality and quantity of the secondary metabolites, either positively or negatively. The generally positive effect of biowaste on EO production was similar to the study of Hadipour et al. (2013), who reported that applying 180 kg N ha⁻¹ (urea) increases EO content in *Lavandula angustifolia*. Hamisi et al. (2012) showed that 60 kg ha⁻¹ application of urea increases the *Tanacetum parthenium* EO production. *Rosa damascena* Mill EO production increased by cow manure application at the rate of 15 t ha⁻¹ (Rahmani and Tabaei-Aghdaei, 2014). 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) and by 300 kg N ha⁻¹ (ammonium nitrate) application (Sifola and Barbieri (2006). The application of mineral N and P (300 and 250 kg ha⁻¹) (Omidbaigi and Arjmandi, 2002) did not alter the Thymol content of *Thymus vulgaris*. Both macro and micronutrients affect the production of secondary metabolites (Hassan, 2012). The beneficial effects of N (ammonium sulphate) on the EO production of *Coriandrum sativum* L. were further increased upon the addition of micronutrients (Khalid, 2015).

Plants allocate C and N to produce secondary metabolites only after primary needs and growth requirements are met (Hassan, 2012). Therefore, if the N fertilisation is more than growth requirements, production of N-based secondary metabolites (e.g. alkaloids) should also be increased.

Table S6 shows the negative correlation between leaf N and two sesquiterpenes (caryophyllene and α -humulene) in *L. scoparium* that could affect the anti-inflammatory properties of the EO (Fernandes et al., 2007). In addition, calamenene and caryophyllene were negatively correlated with the N in *K. robusta* leaves. Both of these compounds have anti-cancer properties (Takei et al., 2006).

The leaf elemental composition (Tables 2A-B and S1-S5) showed that the application of biowastes to soil increased the concentrations of several heavy metals, which is consistent with findings of Dickinson et al. (2015) and Gartler et al. (2013). These levels were below risk thresholds, which was similar to the previous study on *L. scoparium*, *K. robusta* (Esperschuetz et al., 2017) and *Rosmarinus officinalis* (Cala et al., 2005). Several authors (Bağdat and Eid, 2007; Street, 2012; Zheljzakov et al., 2008) have demonstrated that heavy metals are not partitioned into secondary metabolites and have recommended the cultivation of medicinal plants to produce EOs, in soils contaminated by trace elements including Cu and Zn. Results of the Scora and Chang (1997) experiment showed that the composition of *Mentha piperita* EO was unaffected by metals in sludge-treated soils. Our results are consistent with Zheljzakov et al. (2006) that showed *Mentha piperita*, *Ocimum basilicum* and *Anethum graveolens* could grow in soils enriched with Cd, Pb, and Cu with no risk for metal transfer into the EOs and without significant alteration of the EO composition.

Some of the changes in plants growth and EO production may be related to the physical changes soil resulting from the addition of biowastes. In this study, we used three different soil textures. Soil type affects the plants' biomass (Quan and Liang, 2017) and EO quality and quantity (Hendawy et al., 2017). Hendawy et al. (2017) showed that *Rosmarinus officinalis* EO has higher quantity in loamy and better quality in sandy soil.

In the field, the growth improvement and EO production of *L. scoparium* and *K. robusta* may be greater than our results due to the improvement in the soils' water holding capacity following the application of biowastes (Quan and Liang, 2017). This effect was not measured in our experiments because the plants were well-watered. Our

experiments used young seedlings. Therefore, the effect of these biowastes on mature plants in the field may well be different (Porter et al., 1998). We used *L. scoparium* and *K. robusta* from the Canterbury Region in New Zealand. Perry et al. (1997) reported that *L. scoparium* and *K. robusta* from different locations have unique chemical profiles. Different ecotypes may have contrasting reactions to biowaste addition.

Biowastes alter the soil microbiota (Hossain et al., 2017; Oliveira and Ferreira, 2014) that may change the secondary metabolites and EO production of *L. scoparium* (Wicaksono et al., 2017). Biowastes can increase microbial activity (Kao et al., 2006), which in turn, may increase EO production (Banchio et al., 2008).

The results of Esperschuetz et al. (2017) showed negligible nitrate leaching (<2 kg ha⁻¹ equiv.) from soil following the application of 1250 kg N ha⁻¹ equiv. KB planted with *L. scoparium*, *K. robusta* and *Pinus radiata*. Moreover, *L. scoparium* has been shown to attenuate pathogens in soil (Prosser, 2011) and reduce the conversion of ammonium to nitrate (Downward, 2013). Therefore, it is unlikely that excessive nitrate leaching will be of concern following the rates of N applied in biosolids in these experiments.

In our experiment CB application of more than 1500 kg N ha⁻¹ equiv. decreased the fresh biomass and EO production of both *L. scoparium* and *K. robusta*. Similarly, the KB application (2800 kg N ha⁻¹ equiv.) reduced the EO concentration in *L. scoparium*, which was not offset by the biomass increase and resulted in the EO production decline (Figs. 1–3). Therefore, the application of CB at rates of ca. 1500 kg N ha⁻¹ equiv. to low-fertility soil is recommended to produce both higher biomass and EOs by *L. scoparium* and *K. robusta*.

5. Conclusion

Biowastes increased the growth of *L. scoparium* and *K. robusta* and the maximum increase was observed in the soil that had lowest fertility. The EO production of *L. scoparium* and *K. robusta* was either increased or did not change following the application of biowastes. Adding sawdust to biosolids increased the EO production of *L. scoparium* but not *K. robusta*. Although biosolids increased the concentration of Zn and Cd in the leaves of these species, their concentrations were below threshold values of these elements in foodstuffs, indicating that it is likely that they will not exceed guideline values in EOs. Nevertheless, the concentration of heavy metals in EOs should be monitored. Biowastes can be safely applied to stands of *L. scoparium* and *K. robusta* to augment EO production and aid the growth of these New Zealand-native species. This represents the beneficial reuse of wastes materials that would otherwise require costly disposal. Future work should investigate field-scale operations and determine the effects of the biowastes on the long-term growth and EO production of *L. scoparium* and *K. robusta*.

Authors' contributions

BR, ND, JE and SS designed the experiments. BR led the writing and all authors delivered feedback on the manuscript and final approval for publication.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://>

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