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Biowastes promote essential oil production on degraded soils

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ABSTRACT

Biowastes (wastes of biological origin) can improve soil fertility but may render the land unsuitable for food production because they introduce contaminants, including heavy metals, pathogens and xenobiotics. We investigated whether sewage waste (pond sludge from Kaikoura and anaerobically-digested biosolids from Christchurch) and Dairy Shed Effluent (DSE) could improve degraded soils for the production of essential oils (EOs). We grew lavender (*Lavandula angustifolia* Mill.), rosemary (*Rosmarinus officinalis* L.) and thyme (*Thymus vulgaris* L.) in two greenhouse experiments in Lismore stony silt loam soil (LSL) amended with pond sludge or biosolids (500–4500 kg N ha^{-1} equiv.) or DSE (200 kg N ha^{-1} equiv.). Pond sludge application (2800 kg N ha^{-1} equiv.) increased the biomass of *L. angustifolia* and *T. vulgaris* by 60 % and 62 %, respectively. Christchurch biosolids application up to 1500 kg N ha^{-1} equiv. to *L. angustifolia* and *R. officinalis* increased the biomass of both plant species by up to 86 % and 80 %, respectively. The effect of treatments on EO concentration was insignificant in most cases except for DSE (200 kg N ha^{-1} equiv.) and Christchurch biosolids at rates > 1500 kg N ha^{-1} equiv., which decreased the EO concentrations in *R. officinalis* and *L. angustifolia*. This decrease in EO concentration offset some of the increase in EO production resulting from the increased biomass of the biowaste-amended plants. The ideal EO production increase occurred when Christchurch biosolids were applied at 1500 kg N ha^{-1} equiv. The benefits of biowaste additions to degraded soils are greater than would occur if they were added to high-fertility soils. Heavy metal concentrations in all treatments were below food safety standards. Biowastes could rebuild degraded soils and produce valuable EOs, thereby reducing the economic and environmental costs of biowaste disposal, while improving soil fertility and generating revenue from otherwise underproductive land.

1. Introduction

Biowastes are unwanted materials of biological origin including the products of sewage treatment (Sanchez et al., 2009; Guo et al., 2014) and animal effluents (Colleran, 2000). Biowaste disposal e.g., into landfill or waterways can damage the environment (McLaughlin and Filmer, 2008) and may incur a significant economic cost (Güereca et al., 2006). 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).

Sewage treatment facilities generate, on average, 52 kg yr^{-1} of biosolids per person, resulting in the global production of > 10 Mt yr^{-1} Bradley et al. (2008). Biosolids boost up soil fertility as they contain high levels of important plant nutrients and organic matter (Obi and Ebo, 1995). Nevertheless, biosolids can also contain contaminants and pathogens (Krogmann et al., 1999; Singh and Agrawal, 2008). Biosolids disposal incurs costs, which would be reduced by using them to rebuild soils that have become degraded due to forestry, mining or intensive cropping (Daniels et al., 2003; Novak et al., 2009). Biosolids could also be applied to soils contaminated with HMs to reduce their bioavailability to plants and soil biota by sorption of the HMs onto organic matter exchange sites (Black et al., 2010).

Animal effluents can cause environmental harm (BPDNZ, 2011) such as the degradation of water quality and increasing greenhouse gas emissions (Baskaran et al., 2009). Dairy Shed Effluent (DSE) comprises bovine urine and faeces (Zaman et al., 2002) and contains organic matter, plant nutrients as well as pathogens (Roach et al., 2001).

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Discharge of DSE into the environment can result in nitrate leaching and infect ground and surface waters with pathogens (Houlbrooke et al., 2004). Land-application of DSE reduces the need for fertilizers (Cameron et al., 1995; Bolan et al., 2004), however DSE-borne pathogens can cause human illness (Jiang, 2008). When DSE is applied to a degraded soil, it can improve the productivity, water holding capacity, aeration and drainage as well as make the soil less vulnerable to compaction and loss through erosion (Rahmani and Tabaei-Aghdaei, 2014).

In countries with a substantial land area of plantation forestry, post-harvest soil degradation is commonplace (Paramashivam et al., 2017). Such degraded soils contain low levels of organic matter and plant nutrients (Chirino et al., 2010). There is ca. six Mha of degraded land worldwide (Gibbs and Salmon, 2015). These lands could be improved physically, chemically and biologically by applying biowastes (Rahmani and Tabaei-Aghdaei, 2014; Zebarth et al., 1999; Singh and Agrawal, 2008) and restored to productive ecosystems. These biowaste-restored environments could be used for non-food products generation by cultivation of essential oils (EOs) producing plants. Production of non-food crops reduces the concerns of human exposure to biowaste-borne contaminants (McLaughlin et al., 2007). Various studies showed the possibility of EO production in the contaminated lands by HMs (including Cu and Zn), as these metals are not distributed into secondary metabolites (Bağdat and Eid, 2007; Street, 2012; Zheljazkov et al., 2008). The EO production, not only enables profit generation from degraded land, but also divert biowastes from expensive or environmental-damaging disposal. Moreover, replanting the degraded lands with EO producing species refreshes the landscape to green, increases the biodiversity, pollinator services and possibly creates value through other industries such as honey production or tourism (Fontaine et al., 2005; Beyene and Verschuur, 2014).

Biowastes have been demonstrated to increase the growth of some EO producing plant species including Lavender (*Lavandula angustifolia* Mill.), rosemary (*Rosmarinus officinalis* L.) and thyme (*Thymus vulgaris* L.) (Yadegari and Mosadeghzad, 2012; Agulló et al., 2011). The EO of *L. angustifolia* is commonly used because of its antibacterial and anti-inflammatory properties (Hajiali et al., 2016). The EO of *T. vulgaris* also has shown antibacterial activity (Dorman and Deans, 2000) that is broadly used. When biosolids and DSE are applied to the soil cultivated with EO producing plant species, they 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). It is unclear what role individual species of bacteria and fungi have on EO production.

Hadipour et al. (2013) showed that applying 180 kg N ha^{-1} of urea would increase the EO concentration of *L. angustifolia*. However, excessive N application (> 100 kg ha^{-1} ammonium nitrate) diminishes the quality and antioxidant activities of *L. angustifolia* EO (Biesiada et al., 2008). 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. The EO content of *R. 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 soil did not change the EO yield of *T. vulgaris*. Other research showed that applying 300 kg ha^{-1} of mineral N did not affect the *T. vulgaris* EO quality (thymol content) (Omidbaigi and Arjmandi, 2002).

The environmental conditions, such as nutrient-deficiency, drought, salinity or HMs would affect the quantity and quality of EOs (Abdelmajeed et al., 2013). Although application of biowastes to degraded soil can improve plant growth by alleviating nutrient deficiency, it may increase plants stress by increasing concentrations of salts and HMs (Bai et al., 2012). Prasad et al. (2014a), showed that soil concentrations of 25–50 mg kg^{-1} Pb, Cr, Cd or Ni increased the EO concentration and khusimol content in *Vetiveria zizanioides*. Zheljazkov and Nielsen (1996a) demonstrated that HMs decreased the *Mentha piperita* and *Mentha arvensis* EO production but did not diminish the EO quality.

The effect of biowastes addition on the *L. angustifolia, R. officinalis and T. vulgaris* EOs is unknown. We hypothesised that the biomass increases of the plants following the application of biowastes would dilute the EO concentration and change the EO quality either because of the change in macronutrients and micronutrients uptake that would alter the secondary metabolites or the plants' enzyme activity (Cingöz and Karakaş, 2016; Morgan and Connolly, 2013). It also was assumed that the biowastes application would increase the plants' EO production due to the biomass increase that would compensate the possible decline of EO concentration. Therefore, we aimed to determine the effect of biowastes on the quality and concentration of the EOs and to quantify the potential increase in the biomass and above-ground concentrations of HMs, namely Cu and Zn, following the contrasting biosolids and DSE application to a degraded soil.

2. Material and methods

2.1. Soils and biowastes

Two different experiments (Experiment 1: Exp. 1 and Experiment 2: Exp. 2) were designed and conducted in this research. In Exp. 1 two biowastes including biosolids and DSE were applied to a degraded soil. In this experiment three different species including *L. angustifolia*, *R. officinalis* and *T. vulgaris* were planted. In the next experiment (Exp. 2) the more profitable biowaste (biosolids) and EO producing species (*L. angustifolia* and *R. officinalis*) of Exp. 1 were used to be treated with different rates of biosolids.

A Lismore stony silt loam (LSL) soil was collected from the top 15 cm of Eyrewell Forest (43°43′87″S, 172°45′31″E) for the experiments. The forest was formerly under *Pinus radiata* cultivation. In Exp. 1, biosolids (KB) were collected from Kaikoura Regional Treatment Works, Kaikoura, Canterbury, New Zealand (42°21′37.40″S, 173°41′27.35″E). The KB were stockpiled in the oxidation pond and had least industrial input. For Exp. 2, the Christchurch City Council (CCC) supplied biosolids (CB) from municipal wastewater treatment plant. The biosolids were anaerobically digested and had a moderate industrial input (CCC, 2018). DSE used in Exp. 1 was provided from Lincoln University Dairy Farm (LUDF) (43° 38′ 38.07″ S, 172° 26′ 1.96″ E). The soil and biosolids were sieved (≤10 mm), mixed and homogenized prior to application. The chemical properties of the soils and biowastes are presented in Table 1.

2.2. Plant material

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. All the seedlings roots were fully washed with tap water to eliminate the potting mix before establishing.

2.3. Greenhouse experiments

The experiments were conducted at Lincoln University Plant Growth Unit (43°38′42″S, 172°27′41″E) from 2014 to 2016 and pots were placed in a completely randomized block design. In Exp. 1, 2 L pots of 15 cm diameter and 15 cm height were used for *L. angustifolia*, *R. officinalis* and *T. vulgaris*. Pots were filled with Eyrewell LSL soil

Table 1
Properties of the soil and biowastes used in the experiments. LUDF = Lincoln University Dairy Farm. CCC = Christchurch City Council, n.a = not applicable and FW = Fresh Weight.

	Lismore stony silt loam (Pallic Firm Brown soil) mg kg^{-1} dry matter	Kaikoura biosolids	CCC Biosolids	LUDF dairy effluent mg kg^{-1} fresh matter
pH	5.2	4.5	6.8	7.5
CEC [me $100g^{-1}$]	13.0	17.1	36.5	n.a.
Total C [%]	4.5	27	30	0.1
Total N [%]	0.23	2.6	4	0.02
C/N	20	11	8	6
NH4- N (mg kg^{-1} FW)	3	101	2375	82
NO3- N (mg kg^{-1} FW)	25	305	3.6	0.05
P	383	5941	16247	17
K	4468	3653	2164	143
S	210	8681	14029	19
Ca	2472	6331	30493	65
Mg	3768	3005	5022	15
Fe	22293	14534	22356	3.2
Mn	288	185	411	0.6
Cu	3.4	891	291	0.12
Na	268	202	648	27
Ni	7.3	20.7	27.5	0.01
Zn	75	1073	993	0.28
Pb	14	151	54	≤ 3*10-3
Cd	0.43	4.0	1.6	≤ $3*10^{-4}$
Cr	22	47.6	127	≤ $4*10^{-4}$

(1.35 kg) mixed with 0.15 kg of KB (2800 kg N ha^{-1} equiv.). The amount of biosolids was converted from kg pot^{-1} to kg N ha^{-1} by using the percentage of total N in biosolids and surface area of the pots. 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 to optimise the soil pH (0.015 kg for control and DSE treatments and 0.0135 kg for the biosolids treatments). Daily irrigation of the pots to field capacity were performed. DSE application started in January 2015. A total of 2 L DSE was applied to the pots in portions of 50 mL (four days a week) for ten weeks. Note that the average greenhouse temperatures were not the same for *L. angustifolia, R. officinalis,* and *T. vulgaris* because the experimental periods were different. Details are given below.

L. angustifolia plants were grown for 16 weeks 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 had been cut one time before final harvest. 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. 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 replicates of each treatment for all the plants in Exp. 1, though three replicates were used for the analysis that were selected at random.

In Exp. 2, 5 L pots of 22.5 cm diameter and 17 cm high were used. Pots were filled with mixture of Eyrewell LSL soil and biosolids (CB) at rates of (0, 50, 150, 450 and 1350) g pot^{-1}, equivalent to 0, 500, 1500, 4500 and 13,500 kg N ha^{-1}. *L. angustifolia* and *R. officinalis* seedlings were cultivated in the prepared pots. One seedling per pot was planted and each treatment replicated five times. Lime was added to the pots at the rate equivalent of 1 % of the soil weight for increasing the soil pH and make the optimum element uptake by the plants. This experiment was continued from November 2015 to February 2016 intended for 11 weeks and 15 weeks for *L. angustifolia* and *R. officinalis*, 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.

2.4. Plant harvest

Aboveground parts of the plants were harvested by a cutter and used for the biomass, elemental composition and EO quality and quantity evaluation. Fresh EO producing part of the plants (leaves for *R. officinalis* and *T. vulgaris* as well as flowers for *L.* angustifolia) were sampled randomly. Immediately after harvest samples were plunged in liquid nitrogen and kept at −80 °C for EO extraction. Aboveground biomass of the plants was weighed instantly after harvest and washed using deionized water. Portions of the plants shoot were oven dried at 60 °C to reach a constant weight for calculating the whole plants' moisture content and oven dried equivalent weight. After grinding the oven dried leaves, they were digested for analysis of nutrient and trace element status.

2.5. Essential oil extraction

Samples of the essential oil producing organs of the plants (clusters for *L. angustifolia* and leaves for *R. officinalis* and *T. vulgaris*) were accurately weighted (0.1 g) and soaked in 2 mL of a solvent in glass vials at room temperature. Different solvents and soaking times were tested for extracting the plants EOs to have optimum chromatographs. The solvents and soaking times used for EO extraction of *L. angustifolia, R. officinalis* and *T. vulgaris* were hexane for 19 h, hexane + ethanol (9:1) for three hours and hexane + ethanol (9:1) for 18 h[1], respectively. After soaking the samples, one mL of the extracts and 100 µL of internal standard (eicosane- C20- 125 mg L^{-1}) were transferred to vials that are

[1] The solvents mixtures and soaking time for *L. angustifolia*, *R. officinalis* and *T. vulgaris* were selected by evaluating different solvents including hexane, diethyl ether, petroleum ether and ethanol. Results showed that using ethanol more than the concentrations that are used in the experiments, extracted various unwanted components that caused difficulties in the chromatography interpretation. Different times of soaking was evaluated by Gas Chromatography Mass Spectrometry (GC/MS) to have the best chromatograph.

designed for Gas Chromatography Mass Spectrometry (GC/MS). Analysis of the solvent extracts were performed by GC/MS. *L. angustifolia, R. officinalis* and *T. vulgaris* main EO components were selected and analysed using previous researches on these plants (Sahraoui et al., 2008; da Silva Bomfim et al. (2015d), Baranauskienė et al., 2003).

2.6. GC–MS analysis

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). In brief, a Shimadzu QP2010 Ultra (GC/MS) fitted with a Restek RTX-5 ms capillary column (30 m x0.25 mm i.d x0.25 µm film thickness) was used to provide chromatographic separation, with helium carrier gas set to a constant linear velocity of 2658 cm min^{-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 0.67 min. The GC column oven was set to an initial temperature of 45 °C and held for 1.33 min before being ramped to 65 °C at 10 °C min^{-1} with a final ramp to 285 °C at 6 °C min^{-1} held for 10 min 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 33–500 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.

To evaluate the EOs of *L. angustifolia*, *R. officinalis* and *T. vulgaris*, standard commercial EOs were analysed by GC/MS. The chromatographs showed that the EO components of these plants elute early from the column. Therefore, the components that eluted before internal standard (eicosane- C20) were considered as volatile EO constituents.

2.7. Elemental analysis

Total elemental analysis of P, K, S, Ca, Mg, Na, B, Cu, Fe, Mn, Zn, Cd, Ni, Cr and Pb carried out using microwave digestion. Samples were digested in 8 mL of Aristar ™ nitric acid (± 69 %), filtered using pre-leached Whatman 52 filter paper, and diluted with milliQ water to a volume of 25 mL.

An Inductively coupled plasma optical emission spectrometry (ICP-OES Varian 720 ES-USA) was used to evaluate the elements concentrations in the acid digested samples. Certified Reference Materials of Wageningen (ISE 921, IPE 100) and NIST (1573a) were tested in the same sample sets. The range of recoveries was from 91 to 112 %. Total C and N of plants and soils were determined using A CNS-2000 Element Analyser (LECO Australia Pty Ltd., Australia).

2.8. Extractable inorganic- N species (NH_4^+-N and NO_3^--N) from the soil and biowastes

The concentrations of nitrate (NO_3^-) and ammonium (NH_4^+), which are mineral nitrogen content, were analysed following the KCl extraction method of Blackmore et al. (1987) from frozen samples. Forty mL of 2 M KCl was added to 4 *g* of samples 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).

2.9. pH

The soil and biosolids pH were measured using the method of Rayment and Lyons (2011) and Blackmore et al. (1987). To evaluate the pH, 10 ± 0.05 g of air-dried samples was mixed with 25 mL of deionised water (DIW), stirred and left to stabilise for one day. The pH meter (Mettler Toledo, Columbus, OH, USA) was calibrated by pH 4 and pH 7 buffers then the samples pH was measured.

2.10. Electrical conductivity (EC)

For reading the EC the method of Rayment and Lyons (2011) was used. The quantity of 10 ± 0.05 kg of air-dried samples was mixed with 50 mL DIW in the centrifuge tube. The samples were mixed using an end over end shaker for 30 min and left for 30 min to settle. The conductivity meter was calibrated by 1413 µS cm^{-1} standard and EC was recorded using the EC meter.

2.11. 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 75×10^{-3} kg thiourea in 1.5 L of DIW in a 5000 mL volumetric flask. A magnetic stirrer used for mixing. Silver nitrate (8.49×10^{-3} kg) was dissolved in 2500 mL of DIW. The silver nitrate solution was then slowly added to the thiourea solution and made up to a final volume of 5000 mL.

2.12. Statistical analysis

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

3. Results and discussion

3.1. Plants biomass

Fig. 1 shows that 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. The maximum biomass increase for *L. angustifolia* clusters occurred when CB (1500 kg N ha^{-1} equiv.) were added to the LSL. The higher rates of application did not significantly increase growth and the highest rate of application (13,500 kg N ha^{-1} equiv.) killed the plants. The KB application of 2800 kg N ha^{-1} equiv. to the LSL significantly increased the dry biomass of *L. angustifolia* by 60 %. The application of 200 kg N ha^{-1} equiv. of DSE to the LSL increased the growth of *R. officinalis* by 60 % compared with the control. However, DSE application did not increase the biomass of the other two species.

While the rates of biosolids application that significantly increased the plants' growth in the experiments exceed the regulatory threshold for annual application of N (Gibbs, 2003) most of the biosolids N is present as organic form. When the organic matter in biosolids oxidises N is released slowly, therefore the high biosolids application rates would not result in excessive N leaching (Paramashivam, 2015). Increasing the CB application rate increased both biomass and foliar N concentration of the plants, a process that is called luxury uptake (McLaren and Cameron, 1996a).

Increased biomass in *L. angustifolia* and *R. officinalis* following

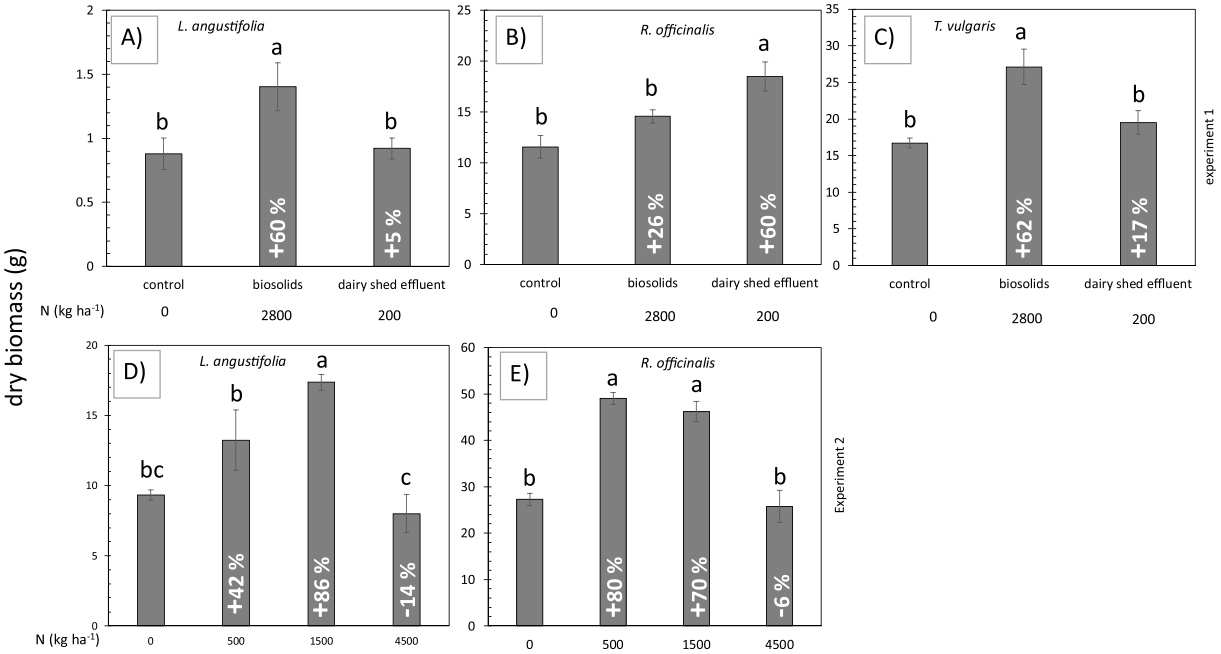

Fig. 1. Aboveground biomass (g DW) of *L. angustifolia*, *R. officinalis* and *T. vulgaris* in A, B and C- Exp. 1 (n = 3 ± se) in addition to D and E- Exp. 2 (n = 5 ± se). The biomass of the *L. angustifolia* is related to the plant clusters (oil producing part). Different letters (a, b, c) indicate significant differences between the treatments at $p \leq 0.05$ within the plant species. Numbers in the bars represent the percentage of changes caused by the treatments compared to the control.

biosolids application is consistent with the findings of Agulló et al. (2011); Cala et al. (2005a). Similarly, Yadegari and Mosadeghzad (2012) showed that manure application increased the biomass and EO production of *T. vulgaris*. Soils tested in the current study and the experiment of Cala et al. (2005a) were both degraded and had C and N content range of 0.56 %–4.5 % and 0.06 %-0.23 %, respectively. Nevertheless, 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, probably due to toxic agents within the biosolids. In this study 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 in *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).

Other studies have similarly reported biomass increase by biowastes application (Monterumici et al., 2015; Seyedalikhani et al., 2016; Massa et al., 2016; Gutiérrez-Ginés et al., 2017). The increased biomass of the plants grown in biowastes amended soils would reduce the albedo and the consequent microclimate change would affect the atmosphere. The positive effect of biowastes on plant growth could be due to their available nutrients including N, P, K and S. Biowastes, including biosolids and DSE contain organic material. Organic material supports the plants' growth and 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). 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 HMs in the soil (Black, 2010).

3.2. Effect of the biowastes on leaf elemental composition

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

Table 2
Elemental concentrations of *L. angustifolia* leaves in Exp. 1 (n = 3) and Exp. 2 (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 $P \leq 0.05$). 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. 1	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. 2	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 3
Elemental concentrations of *R. officinalis* leaves in Exp. 1 (n = 3) and Exp. 2 (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.)	g kg^{-1} dry matter			mg kg^{-1} dry matter					
Exp. 1	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. 2	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}

in *L. angustifolia* and *R. officinalis*, while concentration of Mg was largely unaffected (Tables 2–4). The concentration of Cu was increased following the application of KB (2800 kg N ha^{-1} equiv.) in all three plant species. The mass (and hence uptake) of all the macronutrients increased because of the significant rise in biomass resulting from the application of biosolids. The concentration of Cd in the plants was compared with food safety standards (ANZFSC, 2015). These standards are conservative indicator of HMs concentrations in the leaves that 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. The plants 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 the analysed plants were ≤ 3*10^{-4} mg kg^{-1}.

The increase in foliar Cu by KB application was consistent with the findings of Dickinson et al. (2015) and Gartler et al. (2013). The plants' HMs concentrations were lower than the limits that can pose a risk to human health and animals that is supported by results of previous experiments on *L. scoparium*, *K. robusta* Esperschuetz et al. (2017a) and *R. officinalis* Cala et al. (2005a). For instance, the toxic levels of Zn and Cu are 225 − 450 mg and 250 − 500 mg (Barceloux and Barceloux, 1999; Bingham et al., 2001), although could vary among the individuals. Scora and Chang (1997) showed that the HMs concentration in *Mentha piperita* grown in soil treated with sewage sludge was same as control (< 1 mg L^{-1}). Cultivation of *Mentha piperita* and *Mentha arvensis* in soils that contained high concentrations of Cd, Pb, and Cu showed that there is no risk of excessive HMs transfer into the EOs. Moreover, high HMs concentrations in the soil did not change the EO compositions to the extent that would invalidate marketability (Zheljazkov et al., 2006). The EO quality of *Vetiveria zizanoides* grown in soil treated with moderate concentrations of Cr, Cd and Ni (25 mg Cr, 25 and 50 mg of Cd, and 50 mg kg^{-1} soil) was improved due to increased concentrations of some key EO components, e.g. khuzimol Prasad et al. (2014a).

There are some concerns about the accumulation of biowastes-borne HMs in soil (Natal-da-Luz et al., 2012). However, since EOs are usually obtained through distillation process, HMs are less concentrated in the EOs compared with leaves (Supplementary Table S-4), there is less apprehension about the biowastes contaminating HMs transfer to the EOs. Bağdat and Eid (2007); Street (2012) and Zheljazkov et al. (2008) demonstrated that medicinal plants can be safely cultivated in soils contaminated by HMs (including Cu and Zn) for EO production. Moreover, biosolids for instance, show the potential of reducing the phytoavailability of heavy metals, like Pb and Cd in contaminated soils (Basta et al., 2001). It should be noted that the application of biowastes to high fertility soils is unlikely to increase nutrient uptake as much as in low-fertility ones. The benefits of increased nutrients may be offset by increases in some elements to levels that are toxic for the plants (Morgan and Connolly, 2013). Moreover, increasing the mobile nutrients like N following some of the biowastes application, can result in excessive N leaching (Cogger et al., 2001; White et al., 2011) and some elements such as P, Ca, Mg and HMs micronutrients that are relatively immobile in the soil may accumulate following repeated applications (MU, 2018).

3.3. Essential oils

The effect of biowastes application on the EO producing plants' biomass and elemental composition is equally important as the EO concentrations in terms of EO production. In this study 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 (Fig. 2). The average concentration of *T. vulgaris* EO in the control 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 (Fig. 2 D). None of the other biowaste treatments changed the EO concentrations. There was a significant negative correlation between the *L. angustifolia* EO concentration and

Table 4
Elemental concentrations of *T. vulgaris* leaves in Exp. 1 (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.)	g kg^{-1} dry matter			mg kg^{-1} dry matter					
Exp. 1	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}

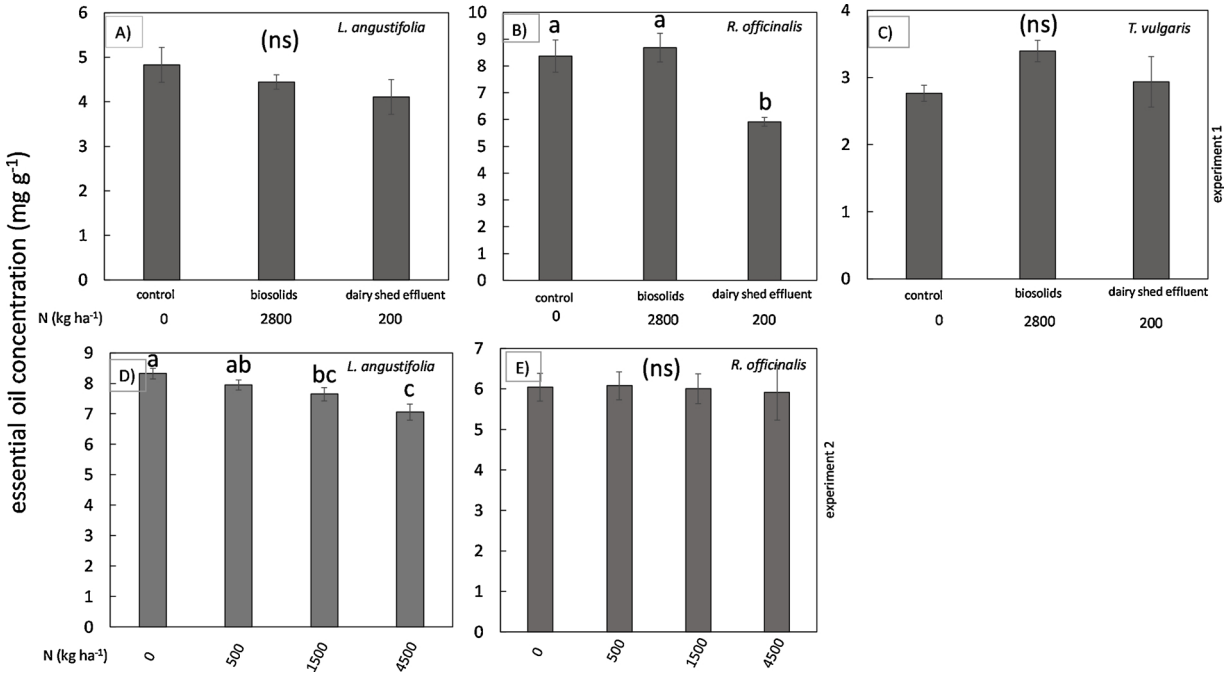

Fig. 2. Average essential oil concentration (mg g^{-1} FW) of *L. angustifolia*, *R. officinalis* and *T. vulgaris* in A, B and C- Exp. 1 (n = 3 ± se) in addition to D and E- Exp. 2 (n = 5 ± se), respectively. Significant differences between the treatments at $p \leq 0.05$ are indicated by different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

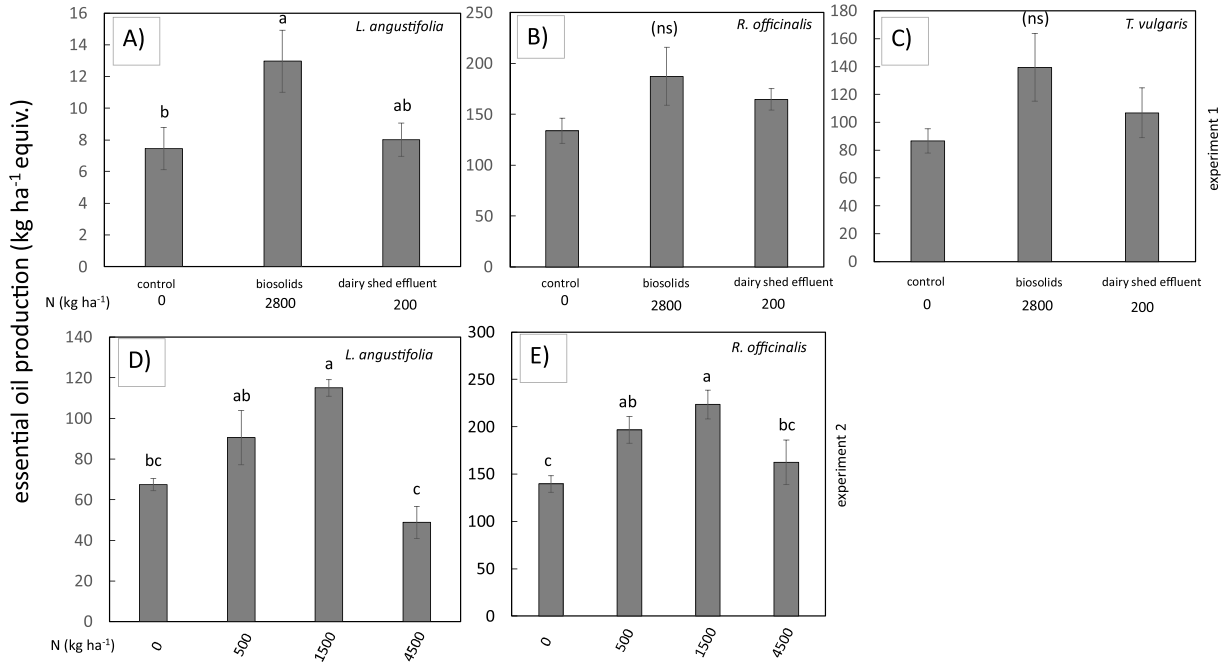

Fig. 3. Average EO production (kg ha^{-1}) of *L. angustifolia*, *R. officinalis* and *T. vulgaris* in A, B and C- Exp. 1 (n = 3 ± se) and D and E- Exp. 2 (n = 5 ± se). Significant differences between the treatments at $p \leq 0.05$ are indicated by different letters (a, b, c) within the plant species.

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. 1 and Exp. 2, respectively (Fig. 3 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. 2 (Fig. 3 E) while KB had no effect on the EO production (Fig. 3 B). Although the dry biomass of *R. officinalis* was highest in the 500 kg N ha^{-1} equiv. CB application (Fig. 1 E), the maximum oil production was related to 1500 kg N ha^{-1} biosolids application. This was because the fresh biomass and water content of the plants were higher when 1500 kg N ha^{-1} biosolids were applied to the soil. Biowastes application did not change the EO production by *T. vulgaris* in LSL (Exp. 1) (Fig. 3 C). The maximum EO production increase occurred with *L. angustifolia*. The EO production increased by 74 % in

Exp. 1 and 71 % in Exp. 2 when 2800 kg N ha^{-1} equiv. of KB and 1500 kg N ha^{-1} equiv. of CB were applied to LSL (Fig. 3 A and D) [2].

In our study the EO concentration[3] of *T. vulgaris* did not reduce when the biomass was increased following the application of biowastes (Fig. 2 C). For *R. officinalis* and *L. angustifolia*, some of the biowaste treatments significantly reduced the oil concentration (Fig. 2 B & D), thereby offsetting some of the gain resulting from increased growth (Fig. 1 B &D). 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 HMs in the biowastes, which increase the stress for the plants Prasad et al. (2014a). For example, N increases the rate of photosynthesis, which increases the growth and biomass, but it can decrease the EO components concentration (Shabahang et al., 2016), although the concentration changes are not consistent. Shabahang et al. (2016) showed that the application of up to 100 kg N ha^{-1} equiv. urea decreased the EO concentration 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 compared with once a week.

In our study, the EO composition of the plants were slightly affected by some of the biowastes treatments. However, the magnitude of the changes in the concentration of EO components were mostly < 20 %. The 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 %).

The EO production of *L. angustifolia* and *R. officinalis* was increased by biosolids application although *T. vulgaris* EO production did not change following the application of biosolids and DSE. 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 increased by 59 % when cow manure was applied at the rate of 15 t ha^{-1} (Rahmani and Tabaei-Aghdaei, 2014). Similarly, EO production of O*cimum basilicum* significantly increased following the application of 10 t ha^{-1} farmyard manure with 1.28 % N, 2.14 % P, and 0.95 % K (Anwar et al., 2005). Kumar and Patra (2012) showed that mixing organic wastes with fly ash and garden soil increases the EO content of *Mentha piperita*. Darvishi et al. (2010) also reported 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 significantly increased the EO production of *L. angustifolia* by more than 70 % in both experiments (Fig. 3 A and D). This result is supported by Hadipour et al. (2013) experiment that showed an increase in *L. angustifolia* EO content following 180 kg N ha^{-1} application. There are other cases that showed N application would enhance the production of EOs in various plants. For example, 60 kg ha^{-1} application of urea increased the *Tanacetum parthenium* EO production (Hamisi et al., 2012). Similarly, *R. officinalis* EO content significantly increased by applying N and K (150 and 100 kg ha^{-1} yr^{-1}) in forms of urea and muriate of potash (Puttanna et al., 2010). These findings are parallel with the results of Exp. 2 (Fig. 3 E) that application of up to 1500 kg N ha^{-1} equiv. of CB increased the EO production of *R. officinalis*.

4. Conclusions

The growth of *L. angustifolia*, *R. officinalis* and *T. vulgaris* was increased by the addition of biowastes to a low-fertility soil. Foliar concentrations of HMs, including Cd, were always below the food safety standards. In most cases, the addition of biowastes did not reduce the EO concentration and quality in the plants, hence, the increased biomass resulting from biowaste addition would result in an improved EO yield. This study showed that low-fertility or contaminated soils could be beneficially rebuilt using a single biosolids application equivalent to 1500 kg N ha^{-1} without the risk of human exposure to HMs. 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. Nitrogen could be applied through mineral fertilisers or alternatively through further applications of biowastes. Longer-term experiments would clarify the options for repeated applications with time. 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 EO production. The effect of higher N concentration or other type of biosolids application on *T. vulgaris* EO has not been evaluated, which could be the subject of future research. The biowastes effect on the EOs would be different in contrasting environments and based on the biowastes composition, soil type and plant species. However, the findings of the current research indicate that real benefits can be achieved through a combination of biosolids application and essential oil production on degraded land.

Authors' contributions

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

CRediT authorship contribution statement

S. Seyedalikhani: Conceptualization, Methodology, Writing - review & editing, Writing - original draft. **J. Esperschuetz:** Conceptualization, Methodology, Software, Formal analysis. **N.M. Dickinson:** Supervision, Methodology, Writing - review & editing. **R. Hofmann:** Supervision, Methodology, Writing - review & editing. **J. Breitmeyer:** Conceptualization, Methodology, Software, Formal analysis. **J. Horswell:** Supervision, Methodology. **D. Paramashivam:** Conceptualization, Methodology, Software, Formal analysis. **B.H. Robinson:** Conceptualization, Methodology, Writing - review & editing, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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[2] Supplementary figures and tables provide further information about the affected components and the major components of the EOs.

[3] It should be clarified that the total EO concentrations of *L. angustifolia*, *R. officinalis* and *T. vulgaris* in our experiments were less than the average of commercially grown plants that usually is obtained by distillation (Esoteric-Oils, 2014). The EO concentration in untreated low-fertility soil used in this research only reached 50% of the commercial production rate.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.indcrop.2020.112108.

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