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Mānuka (*Leptospermum scoparium*) roots forage biosolids in low fertility soil

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ABSTRACT

Potentially, biosolids could be applied to low fertility or degraded soils to establish mānuka (*Leptospermum scoparium*), an economically important plant species used for honey and essential oil production. Given that this pioneering species is adapted to low-fertility soils, it is unclear whether it would respond positively to biosolids. We aimed to determine the growth, root morphology and elemental uptake of *L. scoparium* in contrasting soils amended with biosolids, distributed either homogeneously or heterogeneously. Pot and rhizobox experiments revealed that the roots of *L. scoparium* morphologically foraged patches of biosolids in soil. This finding is in contrast to previous reports that foraging is uncommon in plants adapted to low fertility soils. In a low-fertility sand, biosolids increased the growth 40-fold, irrespective of the distribution of biosolids. This increase was lower (60%) in an orthic brown soil. In the biosolids-amended soils, the foliar concentrations of N, P, K, S, Mg and Ca were above 2%, 1.5 g kg^{-1}, 0.8%, 2.0 g kg^{-1}, 1.7 mg kg^{-1} and 0.8% respectively, which is within the range of concentrations found in native species in their natural habitat. In the control soils, foliar concentrations of N, P & S were significantly lower, indicating that these elements may be limiting. The maximum concentration of Mn (660 mg kg^{-1}), Zn (211 mg kg^{-1}), and Cd (1.5 mg kg^{-1}) in leaves of plants growing in biosolids-amended soil should not cause concern to plant health, but it should be taken into account for their potential effect on trophic networks. Further experiments should focus on the design of field-scale applications of biosolids for improving *L. scoparium* growth and determine the effect of biosolids distribution on nutrient losses.

1. Introduction

Countries with sewage treatment plants produce ca. 27 kg (dry matter) of biosolids per person per year, with global production exceeding 10 million tons per year (Bradley, 2008). Although land application of biosolids is commonplace (Lu et al., 2012; Singh and Agrawal, 2008) due to their potential as fertilizers and soil conditioners (Salazar et al., 2012; Speir et al., 2004; Tian et al., 2013), only 30% of the biosolids produced in New Zealand are applied to land (ANZBP, 2016). This figure stands in contrast to New Zealand's strategy that requires 95% of biosolids to be beneficially reused. The reuse of biosolids could reduce the reliance on inorganic fertilizers such as superphosphate, which have been associated with elevated soil concentrations of Cd, F, (Loganathan et al., 2003) Pb and As (Jiao et al., 2012).

In New Zealand, just 5.5% of the country's soils have sufficient natural fertility and value for food production (Hewitt, 2010). The remaining low-fertility soils require heavy fertilisation or low-nutrient demanding land uses such as forestry or extensive grazing. Historically, pine plantations have received biosolids to accelerate tree growth and improve the nutritional status of the soil and trees without negatively affecting the soil microbial communities or adding unacceptable amounts of trace elements to the soil or trees (Wang et al., 2013, 2004; Xue et al., 2015). However, due to the falling price of pine timber, there is little incentive to replant these low fertility soils in pine forests. Alternatively, these lands could be returned to native vegetation that generates valuable natural products (NZTE, 2016), which could provide environmental and social benefits (Roberts et al., 2015).

Mānuka (*Leptospermum scoparium* J.R.Forst. & G.Forst.) is a strong candidate for planting in marginal lands. Of all the native species in New Zealand, *L. scoparium* has the greatest economic potential with honey and essential oil production being highly profitable. However, since this species is mostly found in low-

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fertility and poorly drained environments (Stephens et al., 2005), it is unclear whether amending poor soils with nutrient-rich biosolids will be beneficial for its growth.

There are various options for the application of biosolids to low-fertility soils with the goal of enhancing *L. scoparium* production including ploughing, surface application, or strip-tilling. Each of these methods has distinct advantages and disadvantages, dependent on the soil environment. The method of biosolids application can affect not only the efficacy of biosolids as a fertilizer and soil conditioner, but also the soil properties and processes due to tillage activities (Alvarez and Steinbach, 2009). Many reports detail the efficacy of the incorporation or surface-application of biosolids. However, these studies are usually based on plant growth, yield, and the fate of nutrients or contaminants (Barbarick et al., 2012; Castillo et al., 2011). No studies have investigated the response of plant roots to the heterogeneous soil environment that biosolids application generates.

Many plant species can respond to heterogeneous distribution of nutrients by increasing the nutrient uptake capacity and/or by proliferating the roots in patches with higher nutrient concentration (Hodge, 2004). However, there is a wide range of factors that affect these responses, including the type of nutrient present in the patch, the concentration of nutrients in the patch in relation with the soil background, the distribution and size of patches, and the plant species (ecological strategy of the plant). Trace elements or other contaminants in soils can also induce variations in root architecture (Arduini et al., 1994).

We aimed to determine the growth and the elemental composition of *L. scoparium* in two soils amended with biosolids either homogeneously or heterogeneously. We also sought to elucidate the behaviour of *L. scoparium* roots to contrasting distributions of biosolids in soil.

2. Materials and methods

2.1. Collection and preparation of soils, biosolids and plants

Soils (ca. 100 kg each) were collected from two degraded areas that could potentially receive biosolids for reforestation with *L. scoparium*. The first soil, S1 (Sandy Raw soil, Hewitt, (2010)), was a sand from an area of old dunes 5 km north of Kaikōura, Canterbury, New Zealand (42°21′37.7″S 173°41′28.1″E). The second soil, S2, (Orthic Brown Soil, Hewitt, (2010)) was sourced from the top 40 cm of a *Pinus radiata* plantation in Eyrewell forest, 26 km south east of Oxford, Canterbury (43°43′87.11″, 172°45′30.79″). Both soils were collected by removing any surface vegetation and sampling from the top 40 cm, as this represent the depth to which biosolids are incorporated during land application. The soils were homogenised and passed through a 20 mm sieve to remove stones while maintaining soil aggregates and structure. Subsamples of both soils were taken for chemical analyses (Table 1).

Biosolids were collected from a stockpile at the Kaikōura Regional treatment works, at Kaikōura, Canterbury. Before being stored in the stockpile, the biosolids went through initial treatment of sedimentation and anaerobic digestion in settlement ponds. The biosolids were collected from eight different locations across the pile and bulked. The biosolids were homogenised and passed through a 20 mm sieve. Subsamples were taken from the sieved bulk sample and further processed for subsequent chemical analysis (Table 1).

Approximately 100 *L. scoparium* seedlings were obtained in seedling trays from Wai-Ora nursery (Christchurch, New Zealand, http://www.waioralandscapes.co.nz). Seedlings ranged in above ground size from 4 cm to 6.5 cm when planted. The seedlings chosen for the trials had small roots, where the main root was of similar size to the above ground plant and had few laterals. Before

Table 1
Mean and standard deviation (n = 5) of the parameters determined in soils, biosolids and mixtures used in the pot experiment. Units are mg kg^{-1} unless otherwise indicated. T, pseudototal concentration; E, extractable concentration.

Parameter		Soil 1	Soil 2	Biosolids	Soil 1 + biosolids	Soil 2 + biosolids
pH		8.8 ± 0.1	4.9 ± 0.0	4.3 ± 0.0	5.2 ± 0.1	4.3 ± 0.0
EC	($\mu S\,cm^{-1}$)	24 ± 1.3	97 ± 3.2	2634 ± 52	282 ± 25	397 ± 21
C	(%)	0.13 ± 0.03	3.9 ± 0.2	23 ± 1.5	0.33 ± 0.03	4.9 ± 0.32
N	(%)	< 0.05	0.20 ± 0.01	2.3 ± 0.1	0.11 ± 0.02	0.97 ± 0.24
NH_4^+ –N		0.0 ± 0.0	3.1 ± 0.5	504 ± 25	17 ± 4.7	18 ± 2.5
NO_3^- –N		0.0 ± 0.0	23.3 ± 3.4	634 ± 57	35 ± 7.6	87 ± 26
S	T	110 ± 13	210 ± 3.7	9010 ± 200	550 ± 47	830 ± 102
	E	32 ± 50	32 ± 51	1500 ± 200	150 ± 20	180 ± 16
P	T	480 ± 36	370 ± 6.9	5660 ± 230	610 ± 19	690 ± 66
Olsen P		0.7 ± 0.1	42 ± 1.2	270 ± 5.6	47 ± 3.3	67 ± 2.1
Ca	T	8900 ± 520	2700 ± 84	11000 ± 320	8100 ± 250	3300 ± 130
K	T	3900 ± 220	4700 ± 60	3800 ± 50	4100 ± 170	4500 ± 130
	E	150 ± 110	98 ± 50	160 ± 20	49 ± 10	99 ± 10
Mg	T	6400 ± 170	4100 ± 54	3900 ± 91	6400 ± 190	4400 ± 99
	E	120 ± 12	140 ± 40	230 ± 13	82 ± 13	160 ± 13
Na	T	240 ± 12	220 ± 4.3	400 ± 14	220 ± 18	240 ± 11
	E	41 ± 4.3	25 ± 7.8	110 ± 9.1	29 ± 4.7	49 ± 4.9
Al	T	17700 ± 640	28700 ± 100	18500 ± 230	19200 ± 960	32700 ± 610
	E	0.2 ± 0.1	56 ± 16	24 ± 20	1.3 ± 0.4	58 ± 5.8
Mn	T	430 ± 11	340 ± 15	250 ± 7.2	430 ± 23	350 ± 13
	E	0.3 ± 0.0	12 ± 4	52 ± 12	4.6 ± 0.8	37 ± 2.8
Zn	T	50 ± 1.1	69 ± 1.6	1240 ± 45	120 ± 6.5	170 ± 13
	E	0.1 ± 0.1	0.4 ± 0.2	280 ± 54	20 ± 4.7	38 ± 3.9
Cu	T	11 ± 1.5	3.0 ± 0.2	610 ± 16	43 ± 4.0	42 ± 8.9
	E	0.01 ± 0.01	0.04 ± 0.06	3.0 ± 2.2	0.15 ± 0.04	0.18 ± 0.02
Cd	T	0.00 ± 0.00	0.01 ± 0.01	2.22 ± 0.14	0.06 ± 0.03	0.23 ± 0.10
	E	0.00 ± 0.00	0.01 ± 0.01	0.57 ± 0.35	0.03 ± 0.01	0.05 ± 0.01
Pb	T	14 ± 0.4	15 ± 0.8	120 ± 4.3	17 ± 0.5	21 ± 4.4
	E	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.06	0.00 ± 0.00	0.00 ± 0.00

planting, the roots were carefully washed with tap water to remove the potting mix.

2.2. Pot experiment setup

The pot experiment used 2.5 kg capacity pots and comprised three treatments on two soils, with four replicates of each treatment. Treatments were i) soil without biosolids (S1 and S2, for soils 1 and 2 respectively); ii) soil with biosolids homogeneously incorporated into the soil (+M); and iii) soil with top application of biosolids (+T). Biosolids were applied to soils at a proportion of 10% fresh weight (equivalent to 90 t/ha fresh biosolids, and to 45 t/ha of dry biosolids). For treatment +M (homogeneous application), biosolids were mixed by hand with soil for more than 20 min, and the pots were then filled with 2.5 kg of biosolids-amended soil. The chemical properties of the soil with mixed biosolids are shown in Table 1. For treatment +T (surface application), pots were filled with 2.25 kg of soil, and 250 g of fresh biosolids were added to the soil surface, immediately after planting the *L. scoparium* seedlings. One *L. scoparium* seedling was planted in each pot.

The 24 pots were placed in a randomised block design in the greenhouse for 12 weeks. They were watered daily with tap water to field capacity to ensure none of the plants were water stressed. The mean temperature during the experiment period was 20.2 °C with maximum temperature of 32 °C and minimum of 9.8 °C.

At the end of the experiment, the shoots were cut, rinsed with tap water and oven dried at 70 °C to a constant weight and weighed. Leaves were separated from stems and then weighed and sampled individually. The top 3 cm of the growing medium in the pot (consisting of soil, biosolids and root material) was cut to separate it from the remaining material in the pot below. The roots had all the soil gently brushed off and were washed in tap water before being individually sampled in the top 3 cm and the remaining base roots. Roots were oven dried at 70 °C to a constant weight and weighted.

2.3. Rhizobox experiment setup

Rhizoboxes are transparent perspex boxes that allow the visualisation of root growth (Moradi et al., 2010; Wenzel et al., 2001). We used 12 rhizoboxes with internal dimensions of $15 \times 30 \times 2.5$ cm, with 10 holes drilled through the back plate used for even irrigation across the box (Fig. 1).

The rhizobox trial used S1, allowing for a greater contrast than S2 between treatments with and without biosolids. For this experiment, soil material and biosolids were sieved to <2 mm. Three treatments with four replicates comprised: i) control soil without biosolids (S1), ii) biosolids homogeneously incorporated into soil at 10% fresh weight (S1+M, mixed), iii) same amount of biosolids concentrated in just one vertical band of the rhizobox (S1 +V, vertical). The third treatment aimed to provide an analogue of a possible soil environment following biosolids incorporation using strip tilling.

In the control treatment, 1.05 kg of S1 was packed horizontally in four layers of approximately 262 g (field moist) to maintain a similar bulk density (of approximately 0.93 g cm^3) along the whole rhizobox. The biosolids-incorporated treatment consisted of an evenly distributed mixture of biosolids and S1. Each rhizobox had 1.11 kg of the mixture S1 + biosolids, maintaining the biosolids at 10% concentration. In the vertical application treatment, the rhizobox was divided into two sections (one third and two thirds of width). A thin plastic barrier was positioned at the division point to help with the substrate allocation leaving 10 cm on one side and 5 cm on the other. In the 10 cm section, 1 kg of soil 1 was carefully added while the 5 cm section received the mixture soil 1 + biosolids at 10% of the total box weight. The barrier was then carefully removed.

L. scoparium seedlings were carefully transplanted from the seedling tray to the rhizobox after having all the potting mixture removed from the roots by gentle brushing. To ensure root growth along the transparent lid, the rhizoboxes were placed on a 45° angle, using a purpose-built stand (Fig. 1A). Black plastic was placed around the rhizoboxes to prevent light encouraging biofilm growth at the soil-rhizobox interfaces. The rhizoboxes were positioned on the stand in a randomised pattern and maintained in the greenhouse for 9 weeks. Watering occurred 6 days per week using a spray bottle for top applications and a syringe for applying water through the holes of the rhizoboxes. The watering maintained the rhizoboxes at field capacity with amounts applied varying according to evapotranspiration, ranging between 5 ml to 15 ml per day. After 40 days, the lid of the rhizobox was temporarily removed so the roots could be scanned for monitoring the root growth. This process occurred weekly for 4 weeks, using a Canon scanner (CanoScan LIDE 210).

At the end of the experiment, when the roots on the fastest growing treatment (vertical band) reached the edges of the rhizobox, the shoots were cut and rinsed with deionized water. For the root collection, the soil material from each rhizobox was divided in 9 equally-sized sectors (Fig. 1B), with the heterogeneous biosolids treatments encapsulated precisely within three sectors.

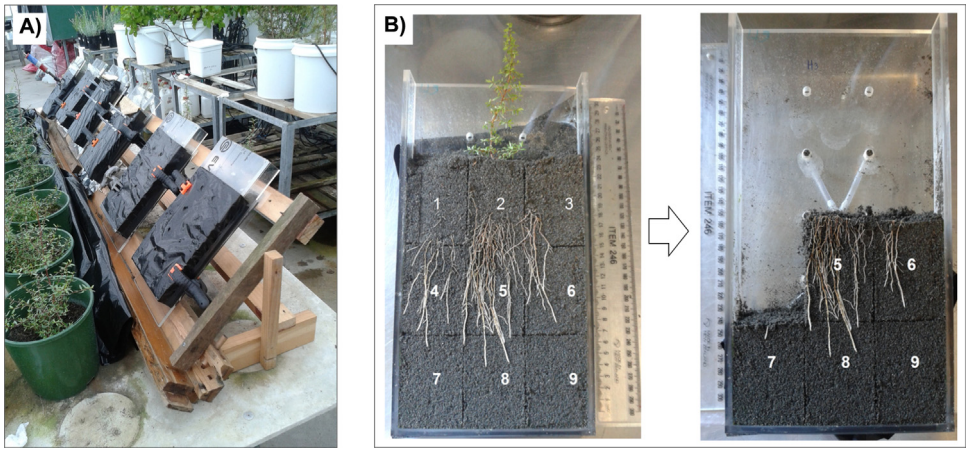

Fig. 1. A) Set up of the rhizoboxes in the greenhouse. B) Sampling of soil and root biomass in the different quadrats.

The roots were then collected from each sector, washed with tap water and sampled individually. All plant material was oven dried at 70 °C until constant weight was obtained.

2.4. Chemical analysis

The fresh samples of soils, biosolids, and amended soils were sieved (<2 mm), and ammonia and nitrate concentrations were determined on 2 M KCl extracts (Clough et al., 2001) and analysed with a Flow Injection Analyser (FOSS FIAstar 5000). Sieved samples of soil, biosolids and amended soils were then dried at room temperature and analysed for pH and electrical conductivity in a 1:2.5 (w:v) soil-water ratio (Blakemore et al., 1987). Pseudo-total elements of the soil, biosolids and biosolids-amended soil were extracted using the microwave CEM MARS Xpress acid digest technique (0.5 g substrate, 4.0 ml trace element grade nitric acid (69%) and 4.0 ml 30% hydrogen peroxide, according to the equipment specifications, (Al Mamun et al., 2016)). The exchangeable fraction of elements were extracted with 0.05 M $Ca(NO_3)_2$ (McLaren et al., 2005). The Olsen P was extracted with 0.5 M $NaHCO_3$ according to Olsen et al. (1954). Extracts of pseudototal, exchangeable elements and Olsen P were analysed by ICP-OES (Varian 720-ES). Total carbon and nitrogen concentration were determined using an Elementar Vario-Max CN Elemental Analyser.

L. scoparium leaves were analysed for N and C using the CN Elemental analyser. The nutrients (P, K, Ca, Mg and S) and trace elements (As, Cd, Cu, Pb and Zn) were extracted from plant samples using the microwave acid digestion technique (CEM MARS Xpress, using 0.3 g dried plant material 2.0 ml trace element grade nitric acid (69%) and 2.0 ml 30% hydrogen peroxide, according to the equipment specifications (Al Mamun et al., 2016)). Analysis of mineral nutrients and trace elements in the extracts of soil and plants were determined by the ICP-OES and expressed on a dry weight basis. Due to the small amount of leaves in the control treatments the minimum weight required for analysis was not reached in all the cases. Therefore, the rhizobox trial did not have the elemental composition analysed in the control treatment. The microwave extraction method was assessed using a reference soil (reference 981) and a reference plant sample (reference 952) from the WEPAL International Soil-Analytical Exchange (www.wepal.nl). We obtained recoveries between 85% and 120%.

2.5. Data analysis

The leaf elemental concentrations were corrected for the fraction of elements in the leaves originating from surface-deposited dust that may incorporate particles into the waxy layers of the leaves (Robinson et al., 2008). The mass fraction of the soil on the leaf sample, M_{soil} (mg/kg) was calculated as:

$$M_{soil} = (T_{plant} - R_{plant})/T_{soil} \quad (1)$$

where T_{plant} is the measured indicator element concentration in the plant tissue (mg kg^{-1}), R_{plant} is the baseline concentration of the indicator element that the plant has accumulated through the roots and translocated to the shoots (in this case Fe [mg kg^{-1}]), and T_{soil} is the concentration of the indicator element in the soil (mg kg^{-1}).

Therefore, the corrected plant concentration of the target element, $C_{plant}{}^{*}$ (mg kg^{-1}) was calculated by:

$$C_{plant}{}^{*} = C_{plant} - M_{soil}.C_{soil}\, M_{soil}.C_{soil} \quad (2)$$

where C_{plant} and C_{soil} are the measured concentrations (mg kg^{-1}) of the target element in the plant and soil.

Plant dry weight, percentage of roots in the base part of the pots, shoot: root ratio and concentration of elements were compared between treatments in the two experiments separately with analysis of variance (ANOVA) and post hoc multiple comparison testing (LSD test). Average dry weight of roots in the left and right sides of the rhizoboxes were compared using t-test with paired results. Element concentrations in leaves of *L. scoparium* in the two treatments of rhizobox experiment were also compared with a t-test. When the hypothesis of normality and homoscedasticity were not fulfilled, data was log transformed. Standardized data of dry weight and N, S, P, Ca, Mg, Na, K, Al, Mn, Zn, Cu, Cd plant concentration was used to do a Principal Component Analysis. Calculations were done with Statgraphics Centurion XVII.

3. Results

3.1. Plant biomass

The addition of biosolids to both soils and in the two experiments resulted in a significant increase of total shoot and root dry weight (Fig. 2). However, the response of *L. scoparium* to biosolids addition depended on the soil type, being more pronounced in S1, which was the soil with the lowest fertility (Table 1). In the pot experiment, shoot dry weights were 30 and 40 times higher in the S1+M and S1+T treatments respectively, compared to S1, while the root weights were 9 and 13 times higher. In the rhizobox experiment, even though the biomass production

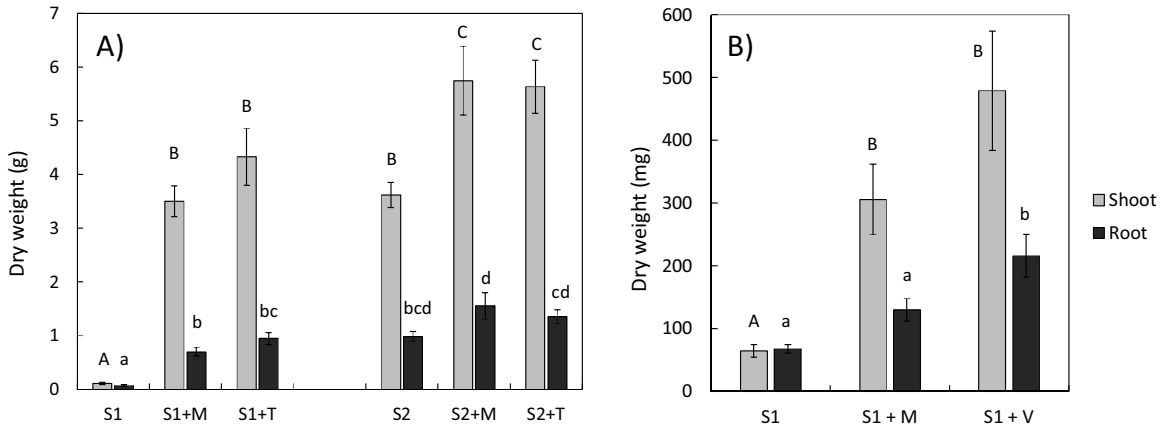

Fig. 2. Total shoot and root biomass in the pot experiment (A) and in the rhizobox experiment (B). Where S1 and S2 are soils 1 and 2, respectively; +M = biosolids homogeneously incorporated into the soil; +T = top application of biosolids; +V = vertical band of biosolids-soil mixture. Bars represent standard errors. Different letters indicate significant differences (95%) between soils and treatments.

was ca. tenfold lower than in the pots, shoot dry weights were, respectively, 5 and 7 times higher in the S1+M and S1+V treatments compared to S1, and the root weights were 2 and 3 times higher respectively. The shoot: root ratio in this soil type in both experiments increased in S1+M, +T, +V compared with S1.

In S2 in the pot experiment, there was a significant increase in shoot dry weight in S2+M and S2+T (ca. 60%), but there was no significant change in root biomass compared to the control. The treatments did not significantly affect the shoot: root ratio.

3.2. Root distribution

Fig. 3 shows that there was a similar proportion of roots (20–25%) in the top 3 cm in both control soils, even though the root biomass in S1 was significantly lower than in S2 (Fig. 2). When biosolids were incorporated (S1+M treatment), there was an increased concentration of roots in the surface layer. This effect was not observed in S2.

In the treatment where biosolids were applied to the surface (+T), there was an obvious concentration of roots in the top of the pot compared with control and mixed application (+M) (Fig. 3). This effect was more pronounced in S1+T, where half of the root biomass was concentrated in the top 3 cm of the pot, which comprised only 20% of the total soil volume.

The response of the root system to heterogeneous or homogeneous distribution of biosolids in the rhizobox experiment (Figs. 4 and 5) demonstrated a visible proliferation of the roots in the biosolids vertical band treatment (S1+V). Similar to the results in pot experiment, the rhizobox experiment demonstrated a higher root biomass in the right side of the rhizobox where biosolids are concentrated in the case of vertical application, compared with the control and mixed application (S1 and S1+M) (Fig. 4).

Fig. 5 shows both the proliferation of *L. scoparium* roots into the vertical band with biosolids by way of creating new lateral roots, and also the main roots growing towards the patch of biosolids.

3.3. Uptake of nutrients and trace elements

Table 2 shows the concentration of nutrients and trace elements in *L. scoparium* leaves in both the pot experiment and rhizobox experiment. The control plants in the rhizobox experiment developed insufficient biomass for all chemical analysis. Nitrogen, S and P concentration in *L. scoparium* leaves increased in the biosolids treatments in both experiments; this effect was more pronounced in S1 than in S2 (Table 1).

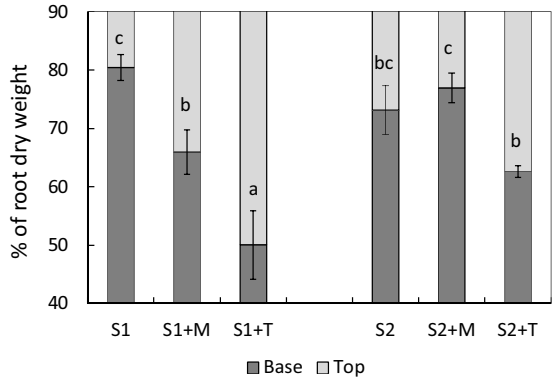

Fig. 3. Distribution of root dry weight in the pots for each treatment, where "top" is the upper 3 cm of the soil in the pots and "base" is the remaining soil underneath. S1 and S2 represent the soil 1 and soil 2 controls respectively; +M = biosolids homogeneously incorporated into soil; +T = top application of biosolids. Error bars show standard error. Different letters indicate significant differences (95%) between soils and treatments (n = 3).

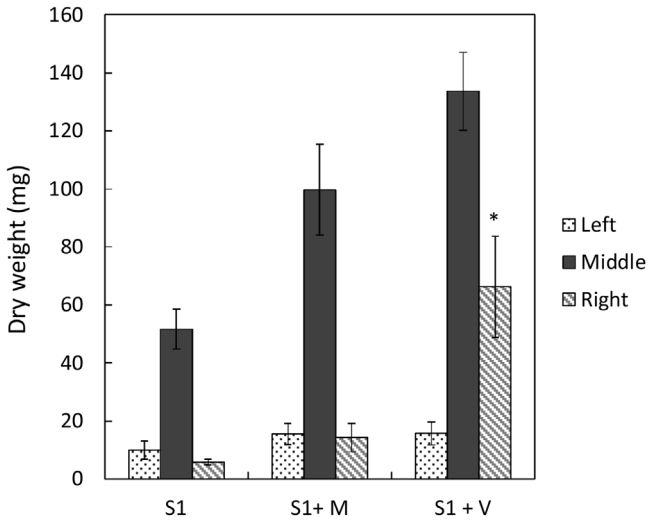

Fig. 4. Root biomass per vertical segment in the different treatments of the rhizobox trial. Where S1 = soil 1 control; S1 + M = soil 1 + biosolids homogeneously incorporated into soil; S1 + V = soil 1 + vertical application of biosolids. Left, middle and right vertical segment of rhizobox labelled.* indicates significant difference (95%) between dry weight in the two lateral segments.

The concentrations of K, Mg, Ca and Na were lower in plants growing in the biosolids treatments. However, the concentration of these elements in the soil was unaffected by biosolids addition (Table 1), and the higher biomass in the biosolids treatments may have reduced concentrations through dilution.

The Al concentration in biosolids-amended soils was not different from that in S1 & S2. Even with the low pH in S2 amended with biosolids (Table 1), the exchangeable Al concentration was not different from the control. The Al concentration in leaves was the same in S2, S2+M and S2+T. In S1 the Al concentration was higher in S1 and S1+M than in S1+T.

The addition of biosolids increased the concentration of extractable Mn, and the concentration of total and extractable Zn and Cd in both soil types (Table 1). This was reflected in *L. scoparium* leaves. Mn concentration in leaves was higher in S2+M and S2+T compared with S2, but not in S1. Leaf Zn and Cd concentrations were higher in the treatments than the controls. Leaf Zn and Cd concentrations were affected by the distribution of the biosolids, being higher in the incorporated treatments (+M), except for Zn in S1. Although biosolids significantly increased the soil Cu concentration (Table 1), this was not reflected in the leaf Cu concentration.

Fig. 6A shows the results of a Principal Component Analysis (PCA) that incorporated the shoot dry weight, and leaf concentrations of N, S, P, Ca, Na, Mg, K, Al, Mn, Zn, Cu, Cd. The first component separated the non-limiting nutrients in the positive part of the axis and dry weight and Mn in the negative part of the axis. The second component was more related to the limiting nutrients (N, P and S) and Zn, but was unrelated to dry weight. Cadmium had a low weighting in both components. Fig. 6B shows the distribution of the results in the two components. The plant response to the treatments was three times greater in S1 compared to S2. The most interesting result of the PCA was that the type of biosolids application affects the results in S2, but not in S1. In S2, results were grouped along axis 2, indicating a concentration gradient of limiting nutrients, where the incorporated application had the highest concentration of limiting nutrients. In S1, there was a large difference between the control and the biosolids application in the two axis, but the distribution of biosolids did not affect the response. While there were two distinct groupings (one for the rhizobox experiment and one for the pot experiment), there was no

Fig. 5. Pictures of one rhizobox per treatment at the end of the experiment. These three rhizoboxes are representative of the response of the four replicates per treatment. In the vertical band treatment, the area to the right of the red line denotes vertical segment of biosolids application (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

Table 2
Mean and standard error of element concentration in plant leaves in each treatment and experiments. Different letters indicate significant difference (95%) of element concentration between treatments in pot experiment. * and ** indicates significant differences (95% or 99%) of element concentration between the two treatments in rhizobox experiment.

Element	Pot experiment (n = 4)												Rhizobox experiment (n = 3)		
	S1		S1 + M		S1 + T		S2		S2 + M		S2 + T		S1 + M	S1 + V	
N (%)	1.21 ± 0.22	a	2.61 ± 0.16	c	2.61 ± 0.18	c	1.59 ± 0.11	a	2.03 ± 0.14	b	2.08 ± 0.07	b	2.35 ± 0.1	2.32 ± 0.1	
P (g kg^{-1})	1.5 ± 0.04	bc	2.0 ± 0.33	cd	2.1 ± 0.12	d	0.89 ± 0.06	a	1.5 ± 0.13	c	0.95 ± 0.12	ab	0.810 ± 0.110	0.740 ± 0.08	
S (g kg^{-1})	2.2 ± 0.17	ab	3.9 ± 0.86	c	3.6 ± 0.47	bc	1.3 ± 0.16	a	2.0 ± 0.09	a	1.9 ± 0.09	a	4.500 ± 0.740	2.400 ± 0.25	*
K (%)	1.35 ± 0.05	b	0.89 ± 0.10	a	0.78 ± 0.02	a	0.82 ± 0.02	a	0.85 ± 0.02	a	0.79 ± 0.02	a	0.79 ± 0.07	0.77 ± 0.04	
Ca (%)	2.6 ± 0.12	d	1.24 ± 0.08	c	1.37 ± 0.07	c	0.59 ± 0.02	a	0.84 ± 0.03	b	0.77 ± 0.06	ab	1.4 ± 0.06	1.54 ± 0.08	
Mg (g kg^{-1})	5.6 ± 0.14	c	2.2 ± 0.16	b	2.0 ± 0.05	ab	1.9 ± 0.05	ab	1.9 ± 0.10	ab	1.7 ± 0.15	a	3.8 ± 0.14	2.9 ± 0.17	**
Na (g kg^{-1})	2.4 ± 0.11	d	1.2 ± 0.16	bc	1.6 ± 0.15	c	0.93 ± 0.06	ab	0.70 ± 0.08	a	0.88 ± 0.16	ab	1.8 ± 0.21	1.4 ± 0.06	
Mn (mg kg^{-1})	186 ± 9.5	b	110 ± 14	a	230 ± 23	b	251 ± 16	b	596 ± 47	c	633 ± 171	c	331 ± 31	191 ± 20	**
Al (mg kg^{-1})	167 ± 64	c	154 ± 63	bc	29 ± 1.8	a	76 ± 11	abc	79 ± 8.5	abc	56 ± 8.5	ab	98 ± 24	48 ± 12	
Cu (mg kg^{-1})	10.6 ± 0.7	b	10.5 ± 2.2	b	7.0 ± 0.4	a	3.8 ± 0.2	a	6.4 ± 0.3	a	5.3 ± 0.5	a	7.3 ± 1.2	5.4 ± 0.5	
Zn (mg kg^{-1})	42 ± 1.0	a	135 ± 12	bc	211 ± 8.0	d	20 ± 1.2	a	153 ± 13	c	107 ± 12	b	145 ± 12	146 ± 19	
Cd (mg kg^{-1})	0.0 ± 0.0	a	0.26 ± 0.15	a	0.19 ± 0.04	a	0.06 ± 0.03	a	0.57 ± 0.12	b	0.19 ± 0.04	a	1.55 ± 0.66	0.52 ± 0.09	

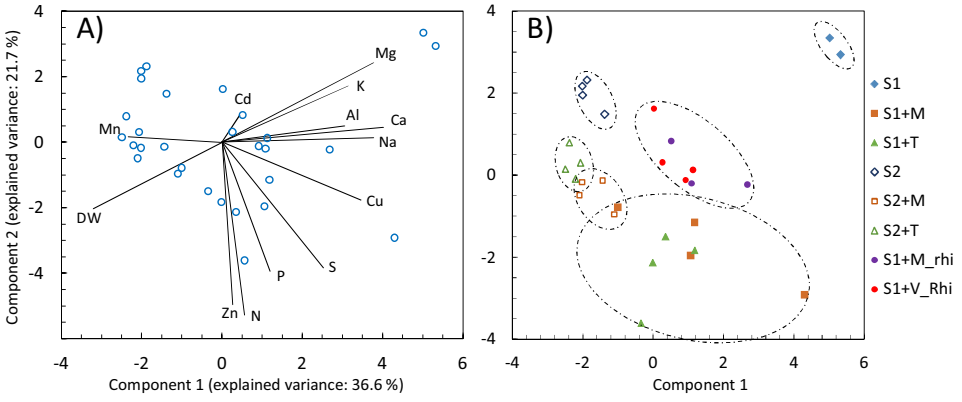

Fig. 6. Principal Component Analysis of the response of *L. scoparium* to different biosolids applications in both the pot experiment and the rhizobox experiment. A) Represents the weight of the variables for each of the components and the scatterplot of the results; B) shows the distribution of the results between the two components depending on each treatment.

difference between the mixed, vertical or surface application treatments (Fig. 6B).

4. Discussion

4.1. The better growth and nutrient status of L. scoparium in biosolids-amended soils

Biosolids addition to the two low fertility soils improved the growth and the nutrient status of *L. scoparium*, regardless the type of biosolids application. The improvement was more pronounced, though, in S1 than in S2. The low levels of nutrients and high pH in S1, compared with S2, may account for the better response of *L. scoparium* when biosolids are added. The greater amelioration of soil conditions when biosolids were applied to sand (such as S1), compared with soil substrates (such as S2) was reported by Sarooshi et al. (2002). This effect was not only evident by the dry weight, but also by the distribution of biomass between shoots and roots. Only in S1 treatment did the shoot: root ratio change. Our results are consistent with Mata-González et al. (2002), who found a larger shoot: root ratio in two perennial grasses with increased biosolids application rate. This can be explained by the severe macronutrient deficiency in S1, which induces a higher investment in root than in shoot growth (Marschner, 2012). We can identify N, S and P as the limiting macronutrients in both soils, since they were the ones with the greatest increase in *L. scoparium* leaves when biosolids were added, above all in the case of S1. Only in biosolids-amended soils were the levels of nutrients in leaves comparable to New Zealand native vegetation in natural areas (Hahner et al., 2014).

4.2. The foraging behaviour of L. scoparium roots in biosolids

The results demonstrate morphological foraging of *L. scoparium* roots into or near biosolids (when they are surface applied or in a vertical band). This proliferation was more pronounced in S1, due to the large difference in nutrient status between this soil and biosolids, a phenomenon explained by Hodge, (2004). This positive growth effect could be due to sensing a chemical gradient or even the presence of a signalling mechanism in this species (Ruffel et al., 2011). However, further research is needed to confirm a chemical gradient that might be detected by the plant roots.

Root foraging by *L. scoparium* is contrary to the general assumption that species adapted to low fertility environments would not show morphological plasticity for the acquisition of nutrients (Hutchings and De Kroon, 1994). Watson and O'Loughlin (1985) showed that more than 90% of root length of *L. scoparium* roots were in the diameter class between 2 and 20 mm, being only 20% of the root biomass. According to Hodge (2004) plants with higher specific root length (root length per unit mass) are more prone to rapid root proliferation in high nutrient patches.

In the treatments with heterogeneous application of biosolids (top application in pot experiment or vertical band application in rhizobox one), only part of the root biomass was in direct contact with biosolids, compared with the mixed application treatments, where the whole root system was in contact with the biosolids. Even so, the total nutrient extraction (Reis, 2015) and the plant growth were unaffected by the type of application.

4.3. Trace elements and toxicity of biosolids

As reported in other studies (Knowles et al., 2011; Mosquera-Losada et al., 2010), Zn is the one of the most abundant trace element in the biosolids used in our experiments (1239 mg kg^{-1}). However, the increased Zn concentration in leaves is unlikely to cause toxicity, either to the plant (where tolerable levels are <100–300 mg kg^{-1}, Marschner, 2012) or to animals (tolerable levels are <300–500 mg kg^{-1}, Chaney, 1989). The Cd concentrations were within the range found in different vegetables (Bešter et al., 2013) and are unlikely to pose a risk to plant health (where tolerable levels are <5 mg kg^{-1}, Marschner, 2012). However, in the treatments where the biosolids were incorporated into the soil (+M), the Cd concentration was close to the tolerable levels for livestock (<0.5 mg kg^{-1}, Chaney, 1989). Potentially, elevated Mn may be phytotoxic in S2 with treatments, since its concentration was higher than 400 mg kg^{-1} (Chaney, 1989).

Even though the pH in S2 was low (4.3) in the treatment, there was no evidence of Al toxicity caused by biosolids, since P, Ca and Mg concentrations in leaves (nutrients that are affected by Al toxicity in acidic soils, (Marschner, 2012)) were not lower than in control. The significant difference in leaf Ca and Mg concentration in all treatments in S2 compared with S1 could be attributed to the different concentration of these nutrients in soils, rather than pH difference (Table 1).

4.4. Implications for biosolids application method

The application of biosolids in low fertility soils could accelerate the growth of *L. scoparium,* especially if this species were to be planted soils with low fertility and high pH (such as S1). Due to the foraging behaviour of *L. scoparium* roots into or near the biosolids, the nature of the biosolids application would not greatly affect growth and nutrient status. Although *L. scoparium* root system is characterized by shallow roots with a strong tendency for lateral roots in the top 20 cm of soils (Watson and O'Loughlin, 1985), a surface application may induce an inordinately high proliferation of roots at the soil surface (up to 50% of total root biomass, Fig. 3). According to Roychoudhry and Kepinski (2015), surface root proliferation may result in the physical instability of the plant and reduce access to water deep in the soil profile during times of drought. Alternatively, the mixed or the vertical band application (to replicate strip tilling incorporation), could induce the development of a bigger and deeper root system. In any case, the uptake of trace elements should not pose a problem.

If the nutrient deficiency is less severe (such as in S2), the surface application of biosolids would not induce such a high location of roots in the soil surface. In this case, surface application might be a better option for economic reasons, and also for reducing the risk of trace element accumulation in aerial parts.

Further research is necessary to better understand the response of *L. scoparium* to different doses of biosolids application in more realistic scenarios at the field scale. Moreover, the leachate and distribution of nutrients and trace elements in soil are also an important factors to take into account when soil amendment programs are designed (Knowles et al., 2011).

5. Conclusions

L. scoparium responded positively to biosolids application by increasing dry weight and improving its nutrient status. The foraging behaviour of its roots allowed *L. scoparium* to adapt to a heterogeneous distribution of biosolids. As a result, the growth and nutrient status of *L. scoparium* was similar regardless the type of biosolids application (top, mixed or concentrated in a vertical band). However, there are some concerns about biosolids management that should be taken into account at the field scale. If the soil to be amended is extremely low in nutrients or organic matter, as is the case in S1, surface application of biosolids could induce a large concentration of roots in the surface. However, if the soil conditions are not too extreme (such as soil S2), a surface application could be more desirable, as it is more economic and less likely to increase the concentration of potentially toxic

elements. A heterogeneous incorporation of biosolids, like strip tilling, might be an intermediate solution, forcing roots to grow deeper and decreasing the amount of roots in direct contact with biosolids and so with trace elements. Further experiments with more realistic scenarios at the field scale are needed to better understand the response of *L. scoparium* to biosolids application and to study the movement and leachate of nutrients and trace elements depending on type of application.

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