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Response of *Leptospermum scoparium*, *Kunzea robusta* and *Pinus radiata* to contrasting biowastes

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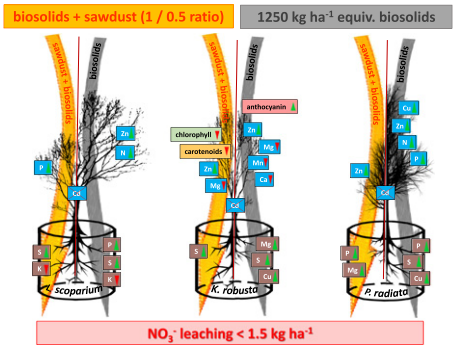
HIGHLIGHTS

- Biosolids increase the growth of mānuka, kānuka and radiata pine.
- Biosolids application did not result in unacceptable heavy metal accumulation.
- Biosolids altered leaf phytochemicals in kānuka but not other species.
- Nitrogen leaching was negligible in all treatments and plant species.
- Blending biosolids with sawdust did not affect nitrate leaching.

GRAPHICAL ABSTRACT



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ABSTRACT

The myrtaceae family has a cosmopolitan distribution and includes the Australasian native species *Leptospermum scoparium* (mānuka) and *Kunzea robusta* (kānuka), which are of economic interest for the production of high-value honey and essential oils. Potentially, these species could be established on low-fertility or degraded soils that have been amended with biowastes, including biosolids and sawdust. We aimed to determine the effect of these biowastes on nitrate leaching and the growth and chemical composition of these plant species compared to *Pinus radiata* (pine), a common forestry species. The addition of biosolids (1250 kg N ha^{-1} equiv.) increased the total dry biomass of mānuka, kānuka, and pine by 117, 90, and 86% respectively. Mixing sawdust with biosolids stimulated growth of mānuka (52%), kānuka (121%) but not pine. Biosolids increased plant uptake of N, P, and trace elements, but not to levels of concern. Nitrate leaching from all treatments was negligible (<2 kg ha^{-1}).

1. Introduction

Monterey pine *(Pinus radiata)* is one of the most common commercially grown tree species, with plantings covering >4.3 million ha (Jeyakumar et al., 2014). *P. radiata* forests are often established on

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poor soils and as a result, nutrient and trace element deficiencies can limit plant growth and biomass production (Jeyakumar et al., 2014; Mosquera-Losada et al., 2010). To alleviate soil nutrient and trace element deficiencies, biosolids from treated municipal or agricultural sewage sources represent an opportunity to improve tree growth in poor soils while enabling safe disposal of a waste stream. Biosolids application to pine forests improves tree growth with minimal environmental impacts (Jeyakumar et al., 2014; Wang et al., 2006).

Plant-soil interactions and nutrient leaching have been thoroughly investigated in combination with moderate and repeated biosolids applications to avoid environmental risks, especially loss of N and P (Kimberley et al., 2004; McLaren et al., 2007; Mosquera-Losada et al., 2010). Various studies have shown positive effects of biosolids fertilization on forest tree species, which can subsequently provide economic returns through increased biomass and soil nutrients, while avoiding accumulation of biosolids derived contaminants above threshold values (Kimberley et al., 2004; Singh and Agrawal, 2008; Wang et al., 2013). However, biosolids application to forest soil can also result in decreased forest productivity because there is a strong dependence on the biosolids composition, soil type and plant species (Cline et al., 2012). Some of the negative effects of biosolids addition to soil can be mitigated by mixing biosolids with wood wastes (Paramashivam et al., 2015) or other biomass products such as biochar (Ammari et al., 2012; Knowles et al., 2011). For example, a beneficial effect of combining biosolids with sawdust on plant growth and soil aggregate stability was shown by Bugbee (1999), Schmidt et al. (2001) and Sandoval et al. (2012). In addition, sawdust potentially can reduce the uptake of heavy metals and contaminants (Fiset et al., 2000), hence improving growth conditions for plants. Sawdust in combination with N-rich material, such as biosolids, undergoes decomposition, hence providing additional functional groups to immobilize metals (Jokova et al., 1997).

Decreases in the price of *P. radiata* logs combined with an increasing interest in restoring degraded lands with native vegetation (Franklin et al., 2015) has focused attention on establishing alternative plant species in low fertility or degraded soils. Candidates include species from Myrtaceae, which comprises some 3100 species with a cosmopolitan distribution (Imatomi et al., 2013). Eucalyptus (subfamily Leptospermoideae) is the most widely studied genus (Imatomi et al., 2013) because of its use for timber, pulp, and paper production. Other members of the Myrtaceae are economically important as cultivated ornamentals (*Callistemon* spp., *Chamelaucium* spp.) and the production of essential oils (*Eucalyptus* spp., *Melaleuca* spp., *Leptospermum scoparium* and *Kunzea robusta*) (Barbosa et al., 2013). Several plant species in this family exhibit high concentrations of terpenes (Keszei et al., 2008) and triketones (Douglas et al., 2004) in their leaves, which may be responsible for allelopathic effects (Fang et al., 2009), antimicrobial properties (Douglas et al., 2004) or the inhibition of nitrification (White, 1988), which could be beneficial traits for improving forestry management.

Mānuka (*Leptospermum scoparium* J.R. Forst & G. Forst) and kānuka (*Kunzea robusta* de Lange & Toelken) are pioneer species in the myrtaceae family, commonly found in degraded environments and low fertility soil in New Zealand and Australia (Bergin et al., 1995; Stephens et al., 2005). *L. scoparium* is of particular interest due to its high value honey (Beitlich et al., 2014; Steinhorn et al., 2011) and the antimicrobial effects of compounds detected in its essential oils (Maddocks-Jennings et al., 2005; Song et al., 2013).

We hypothesise that biosolids will enhance the growth of *P. radiata*, *L. scoparium*, and *K. robusta* in low-fertility soil and lead to increased concentrations of trace elements in the aerial portions. Further, we hypothesise that sawdust will eliminate the initial flush of nitrate leaching associated with biosolids addition, and may offset the growth benefits associated with biosolids addition. We aimed to determine the plant-soil interactions of *P. radiata*, *L. scoparium*, and *K. robusta* with soils amended with biosolids and biosolids combined with sawdust. Specifically, we sought to determine the effect of biowastes on the growth and chemical composition of the plants, as well as determine the effects of the plants on N-losses from the biowaste-amended soils.

2. Material and methods

A low-fertility orthic brown soil, with no history of fertilizer addition, was collected from a marginal farm area near Bideford, New Zealand (40°45′56″S 175°54′42″E). Analyses showed the soil had a mildly acidic pH (pH 6.1), with medium C (6.5%) and N (0.46%) levels, a C/N ratio of 14.3 and a low level of bicarbonate (Olsen) extractable P (11 mg L^{-1}). The Cation Exchange Capacity (CEC) was 21 meq 100 g^{-1}.

The experiment was conducted at the Lincoln University greenhouse facility (43°38′42″S 172°27′41″E). Thirty-six 10 L lysimeters (25 cm in diameter with a height of 29 cm) were filled with 10 kg of fresh soil to a soil bulk density of 0.9 g cm^{-3}. The soil was allowed to settle for eight weeks with regular automated irrigation. *P. radiata* seedlings (GF19) were purchased from Southern Woods Nursery Ltd., Christchurch/New Zealand. To measure NO_3^- leaching, a leachate-sampling device was installed in the bottom of each lysimeter. *L. scoparium* (PH lebsco, 6M) and *Kunzea robusta* (KV kunert, 6M) were purchased from Waiora Nursery Ltd., Christchurch, New Zealand. All plants were planted after the eight-week soil settling period and allowed establish for six weeks before treatments were applied to the soil surface around the plants. The lysimeters were arranged in the glasshouse via a randomized block design.

Two soil treatments (biosolids and biosolids-sawdust) and an unamended control were setup randomly with four replicates for each plant species within the experimental setup. The treatments comprised biosolids (245 g DW) and the same amount of biosolids mixed with sawdust (123 g DW). The biosolids application rate was the equivalent of 50 t ha^{-1} dry weight, providing 1250 kg N ha^{-1}. Control treatments did not receive any fertilization during the experimental period. Biosolids and sawdust were collected from the Kaikoura Wastewater Treatment Plant, New Zealand (42°21′37.40″S, 173°41′27.35″E). Biosolids were homogenized thoroughly after sieving (≤10 mm). The biosolids had a pH of 4.5, a total N concentration of 2.5%, available N of 403.8 mg kg^{-1}, a C/N ratio of 10.6 and a CEC of 17.1 meq 100 g^{-1}. The total C and N in the sawdust were 47.7% and 0.1%, respectively, whereas available N was below detection limit (<5 mg kg^{-1}). Concentrations of further total macro- and micronutrients in the initial substrates used are shown in Table 1a.

An estimation of plant-available elements from biosolids and sawdust was made using a 0.05 M $Ca(NO_3)_2$ extraction following Black et al. (2012), who reported that this extraction was the most effective procedure for determining the plant-availability of metals (Table 1b). Briefly, 5 g of the initial material were weighed into 50 mL centrifuge tubes

Table 1a
Nutrient, trace element and contaminant concentrations in sawdust and biosolids used in the present study [mg kg^{-1}]. Concentrations of trace elements As, Al, Co, Li, Mo and Sr were below the detection limit ($n = 5 \pm$ se).

	Sawdust	Biosolids	Soil
P	42 ± 1	5941 ± 42	544 ± 5
K	455 ± 6	3653 ± 34	1886 ± 46
S	70 ± 1	8681 ± 140	405 ± 2
Ca	838 ± 11	6331 ± 91	4063 ± 67
Mg	212 ± 3	3005 ± 34	1962 ± 22
Na	40 ± 2	202 ± 1	207 ± 5
B	1.9 ± 0.2	26.7 ± 0.1	29.0 ± 0.3
Cu	0.8 ± 0.0	891.0 ± 18.9	4.2 ± 0.0
Fe	116 ± 6	14,534 ± 92	15,461 ± 108
Mn	47 ± 1	185 ± 5	133 ± 3
Zn	8.4 ± 0.4	1073.1 ± 26.8	28.6 ± 0.3
Cd	n.d. ± n.d.	3.97 ± 0.07	0.05 ± 0.00
Ni	0.6 ± 0.5	20.7 ± 0.4	4.1 ± 0.0
Cr	0.2 ± 0.0	47.6 ± 0.8	14.0 ± 0.2
Pb	n.d. ± n.d.	151.3 ± 3.2	8.3 ± 0.1

Table 1b
Plant available ($CaNO_3^-$-extractable) concentrations of nutrients, trace elements and contaminants in sawdust and biosolids used in the present study [mg kg^{-1}]. Concentrations of trace elements As, Al, B, Co, Li, Mo, Pb and Sr were below the detection limit ($n = 5 \pm$ se).

	Sawdust	Biosolids
P	13 ± 1	49 ± 1
K	295 ± 6	170 ± 5
S	5 ± 2	1193 ± 64
Ca	n.d. ± n.d.	n.d. ± n.d.
Mg	185 ± 2	349 ± 14
Na	26 ± 1	54 ± 2
Cu	0.1 ± 0.0	8.9 ± 0.3
Fe	0.5 ± 0.1	77.6 ± 1.7
Mn	33 ± 1	74 ± 3
Zn	6.1 ± 1.0	530.7 ± 12.0
Cd	0.01 ± 0.00	1.32 ± 0.02
Ni	0.03 ± 0.01	3.97 ± 0.06
Cr	n.d. ± n.d.	0.03 ± 0.00

and extracted with 30 mL of 0.05 M $Ca(NO_3)_2$ after 2 h of end-over-end shaking and centrifuging at 3200 rpm for 15 min (pre-leached Whatman 52 filter paper).

After treatments were applied to the lysimeters, the experiment was maintained for 18 weeks in the greenhouse with night-time (10 pm till 6 am) temperatures ranging between 9 °C and 20 °C and day-time temperatures between 14 °C and 28 °C. The lysimeters were weeded fortnightly. An irrigation system allowed the independent watering of each plant species by pressure compensated drippers. Manual irrigation was used to apply additional water to treatments within species. Soil moisture was kept above field capacity to allow drainage. The total irrigation for L. *scoparium*, *K. robusta*, and *P. radiata* was 2400, 1985, and 1240 mm, respectively.

A final destructive harvest of all lysimeters was carried out after 18 weeks. Bulk density measurements were carried out by pushing rings (5 cm in height and 10 cm in diameter) into the soil. The rings with soil were oven-dried and weighed with subsequent determination of the dry bulk density per total ring volume. The total aboveground and belowground plant biomass of all plants was weighed and oven-dried at 70 °C until a constant weight was achieved. Dried plant parts were ground to fine powder using a Retch ZM200 grinder prior to analysis. Soil that was attached to the plant roots (≤1 mm from root surface) was considered rhizosphere soil (Hinsinger, 1998). Rhizosphere soil was sieved ≤5 mm prior to chemical analyses. Total C and N in ground plant and soil material was analysed using a CNS-2000 Element Analyser (LECO Australia Pty Ltd., Australia). Inorganic-N speciation in soil was determined using a KCl extraction from fresh soil (4 °C) within 4 days after harvest according to Blakemore et al. (1987): after adding 40 mL of a 2 M KCl reagent to 4 g of soil, the solution was shaken on an end-over-end shaker for 1 h, centrifuged at 827*g* for 10 min and subsequently filtered through pre-leached Whatman 41 filter paper. Nitrate-N (NO_3^--N) and ammonium-N (NH_4^+-N) was determined using a flow injection analyser (FIA FS3000 twin channel analyser, Alpkem, USA).

Pseudo-total elemental analysis was carried out using microwave digestion 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. Concentrations of P, K, S, Ca, Mg, Na, B, Cu, Fe, Mn, Zn, Cd, Ni, Cr and Pb were determined using inductively coupled plasma optical emission spectrometry (ICP-OES Varian 720 ES - USA). For quality assurance, reference soil and plant material (International Soil analytical Exchange - ISE 921 and International Plant analytical Exchange IPE 100) from Wageningen University, the Netherlands, was analysed with the samples. Recoverable concentrations were 81% - 112% of the certified values.

Significant differences between control soil, biosolids, and biosolids-sawdust treatments were determined by analysis of variance, followed by Duncan post-hoc tests to identify homogenous subsets for $\alpha = 0.05$. The analyses were performed using IBM SPSS v.22 (International Business Machines Corp., New Orchard Road, Armonk, New York 10504 914-499-1900), based on $n = 4$ individual replicates. Results were illustrated in SigmaPlot 11.0 (Systat Software Inc).

3. Results and discussion

3.1. Aerial biomass and root growth

Compared to control treatments in kg ha^{-1} equiv., biosolids application caused an N increase of 165.7 kg ha^{-1} in *P. radiata*, 66.9 kg ha^{-1} in *K. robusta* and 80.3 kg ha^{-1} in L. *scoparium* biomass, respectively, whereas mixing biosolids with sawdust resulted in a N decrease of 117.1 kg ha^{-1}, 13.5 kg ha^{-1} and 22.1 kg ha^{-1} for *P. radiata*, *K. robusta* and L. *scoparium*, respectively, compared to biosolids treatments (data not shown). A reduced biomass N content in biosolids-sawdust compared to biosolids only treatments irrespective plant species could have been related to N competition by microbes involved in the breakdown of the sawdust. The growth response of *P. radiata* to biosolids application was related to both, aboveground (61%) and belowground (25%) biomass compared to controls (Fig. 1). Whereas biosolids had no significant effect on L. *scoparium* and *K. robusta* root growth (Fig. 1b), biosolids stimulated their aboveground plant growth, with increases of 60% and 27%, respectively compared to control treatments (Fig. 1a). Biosolids application to low fertility soil resulted in a positive growth response of *P. radiata*, which is in accordance with a previous study by Kimberley et al. (2004), who reported a positive growth response of *P. radiata* by diameter, volume and height growth. Growth responses associated with biosolids application were comparable to responses to inorganic fertilizer (Prescott and Brown, 1998; Weetman

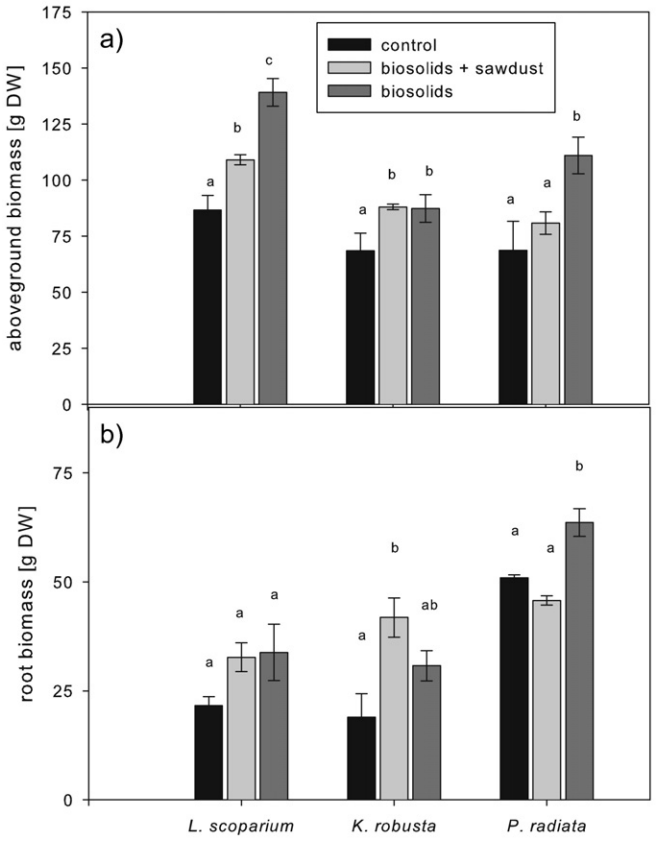

Fig. 1. Aboveground (a) and belowground (b) plant biomass [g DW] after a growing period of 18 weeks in control, biosolids-sawdust and biosolids treatments ($n = 4 \pm$ se). Significant differences between treatments at $p \leq 0.05$ are indicated by letters (a, b, c) within plant species.

et al., 1993). An increased root growth of *P. radiata* suggests a stimulation of roots by nutrients associated with biosolids. Our results suggest that biosolids may be an effective tool for establishing these species on low-fertility or degraded soils.

Biosolids-sawdust stimulated aboveground as well as belowground plant growth of L. *scoparium* and *K. robusta,* whereas no increase was observed in *P. radiata*, neither aboveground nor belowground. In *K. robusta*, mixing sawdust with biosolids resulted in similar aboveground biomass gain for *K. robusta* compared to biosolids only, whereas the biomass gain was lower for L. *scoparium*. While small increases/decreases in L. *scoparium* and *P. radiata* root biomass were not significant, *K. robusta* unlike the other species, had a higher root biomass in the biosolids-sawdust treatment (Fig. 1b). Aboveground and belowground biomass increases with biosolids-sawdust for *K. robusta* was 62% and 121%, whereas that of L. *scoparium* was 57% and 52%, respectively. Since we observed negligible biomass increase of *P. radiata* after biosolids-sawdust application, these results indicate superior growth of the myrtaceae species (*L. scoparium* and *K. robusta*) in combination with biosolids-sawdust when compared with *P. radiata*, although L. *scoparium* and *K. robusta* species are naturally adapted to low fertility soil. Therefore, recycling fresh sawdust for land application via mixing with biosolids provides a suitable alternative to biosolids alone in combination with these myrtaceae species.

A positive root growth in *K. robusta* treatments was only observed when mixing biosolids with sawdust (Fig. 1b). The lack of growth response in *K. robusta* biosolids compared to biosolids-sawdust treatments may have been due to either the physical properties of the soil and/or the mycorrhizal state of the different plants. Mixing sawdust with biosolids may have resulted in higher porosity of the growth media (favourable physical properties) thus enabling better growth of *K. robusta* roots which are smaller in diameter and thus more fragile when compared to L. *scoparium* and *P. radiata* roots (Haynes and Goh, 1987; Watson and Marden, 2004). This was confirmed by bulk soil density measurements at the end of the experiment, where biosolids-sawdust treatments showed significantly lower densities (0.57–0.64 g cm^{-3}) when compared to control soil (0.74–0.81 g cm^{-3}), irrespective plant species (Table 2). Considering the total biomass growth in L. *scoparium* and *K. robusta* in biosolids-sawdust treatments, the increase in L. *scoparium* was mainly reason of aboveground growth, whereas *K. robusta* invested belowground, suggesting different growth strategies of both plant species.

In combination with such physical properties, plant growth could have been influenced via the composition of mycorrhiza. Biosolids may have stimulated ectomycorrhiza, especially in context with providing additional P (Smith et al., 2011). Ectomycorrhiza has been identified with L. *scoparium* and *K. robusta* (Moyersoen and Fitter, 1999; Weijtmans et al., 2007) as well as *P. radiata* (Walbert et al., 2010). An influence of biosolids on the growth of ectomycorrhizal symbionts would explain results from our study, showing an increased total plant biomass (aboveground + belowground), somewhat in a similar range in L. *scoparium*, *K. robusta* and *P. radiata* (117%, 90% and 86%, respectively compared to control treatments). When sawdust was mixed with biosolids, additional C was provided to the plant, whereas biosolids provided organic-N, potentially promoting the growth of arbuscular mycorrhiza (Smith et al., 2011; Whiteside et al., 2012). Both plant species, *K. robusta* and L. *scoparium* have been identified with dual colonisation, ectomycorrhiza as well as arbuscular mycorrhiza (Moyersoen and Fitter, 1999; Weijtmans et al., 2007), whereas *P. radiata* only supports ectomycorrhiza (Walbert et al., 2010). We therefore suggest that an increased mycorrhization in *K. robusta* and L. *scoparium* due to favourable conditions caused by sawdust mixed within biosolids resulted in a higher growth increase (149% and 77% for *K. robusta* and L. *scoparium*, respectively) compared to *P. radiata* (8%).

Table 2
Soil bulk density (0–5 cm) at the end of the experiment ($n = 4 \pm$ se). Letters (a, b) indicate significant differences ($p \leq 0.05$) within plant species between treatments.

	Control	*Sawdust + biosolids*	*Biosolids*
L. scoparium	0.74 ± 0.04^{a}	0.60 ± 0.02^{b}	0.67 ± 0.02^{ab}
K. robusta	0.81 ± 0.04^{a}	0.57 ± 0.08^{b}	0.66 ± 0.04^{ab}
P. radiata	0.75 ± 0.03^{a}	0.64 ± 0.04^{b}	0.73 ± 0.01^{a}

3.2. Plant element concentration

Biosolids as well as biosolids-sawdust application significantly affected the elemental concentrations in the leaves. Whereas biosolids application to *P. radiata* significantly increased plant N, P, Zn and Cu concentrations, only N and Zn were increased in L. *scoparium*. In combination with *K. robusta*, biosolids application resulted in an increase of plant Zn, but resulted in a decrease of Ca, Mg, and Mn (Fig. 2). As discussed by Koo et al. (2013), biosolids application could have stimulated root exudation, including organic acids, which in turn are responsible for solubilisation and mobilization of nutrients (Bertin et al., 2003). Such compounds can increase the availability of P and Zn (Hinsinger, 2001; Keller and Römer, 2001). Since exudate composition strongly varies with plant species (Walker et al., 2003), this can lead to contrasting plant responses in terms of nutrient and contaminant uptake, and may explain the differences in nutrient concentration increases observed between plant species in our study. A different exudate composition of myrtaceae species may have mobilized only Zn in *K. robusta* and Zn and N in L. *scoparium* treatments, whereas P and Cu uptake were increased in addition to Zn and N due to specific compounds in *P. radiata* exudates. Foliar Zn concentrations in *K. robusta* in biosolids treatments increased by 118% compared to control treatments, whereas in *P. radiata* and L. *scoparium* leaves, Zn increased by 27% and 32%, respectively (Fig. 2d). However, determination of the root exudate composition was outside the scope of this study. Interestingly, biosolids application reduced Ca and Mg concentrations in *K. robusta* leaves. Calcium is an essential plant nutrient, and is required for many structural roles in cell walls and membranes, as well as inter- and intracellular functions (Marschner, 1995). Uptake of Ca into the plants is primarily occurring through root tips (White and Broadley, 2003), hence biosolids application could have altered root growth or chemical composition of available nutrients in the rhizosphere less favourable for uptake into aboveground plant parts, although Ca was additionally applied with biosolids (Table 1b).

Mixing biosolids with sawdust did not result in significantly different element concentrations compared to biosolids. However, Zn was found the only element, which concentrations were significantly further increased in *P. radiata* in biosolids-sawdust compared to biosolids only treatments. Bowen et al. (1974) discussed the influence of ectomycorrhizal fungi on the uptake and translocation of Zn into *P. radiata.* An increased Zn uptake by *P. radiata* in biosolids-sawdust treatments compared to biosolids may hence indicate an increased mycorrhization of pine roots in our study. However, this is contradictory with results obtained from biomass results, where neither root nor shoot biomass was increased in *P. radiata* in biosolids-sawdust treatments compared to controls.

As discussed previously, in contrast to *P. radiata*, *K. robusta* and L. *scoparium* had a positive growth response after biosolids-sawdust application, although in L. *scoparium* this was lower compared to biosolids. Sawdust may have immobilized plant available N (Schmidt et al., 2001), thus decreasing the growth response compared to biosolids in L. *scoparium* and *P. radiata* treatments. The high C/N ratio of sawdust would have resulted in the immobilization of available N in the biosolids (Haynes and Goh, 1987). *K. robusta* and L. *scoparium* are adapted to low N environments, therefore N immobilization by sawdust did not affect plant growth as compared to *P. radiata.* Assuming that sawdust immobilizes N (Schmidt et al., 2001), our results indicate that *K. robusta* is more adapted to low N environments than L. *scoparium*, since mixing sawdust

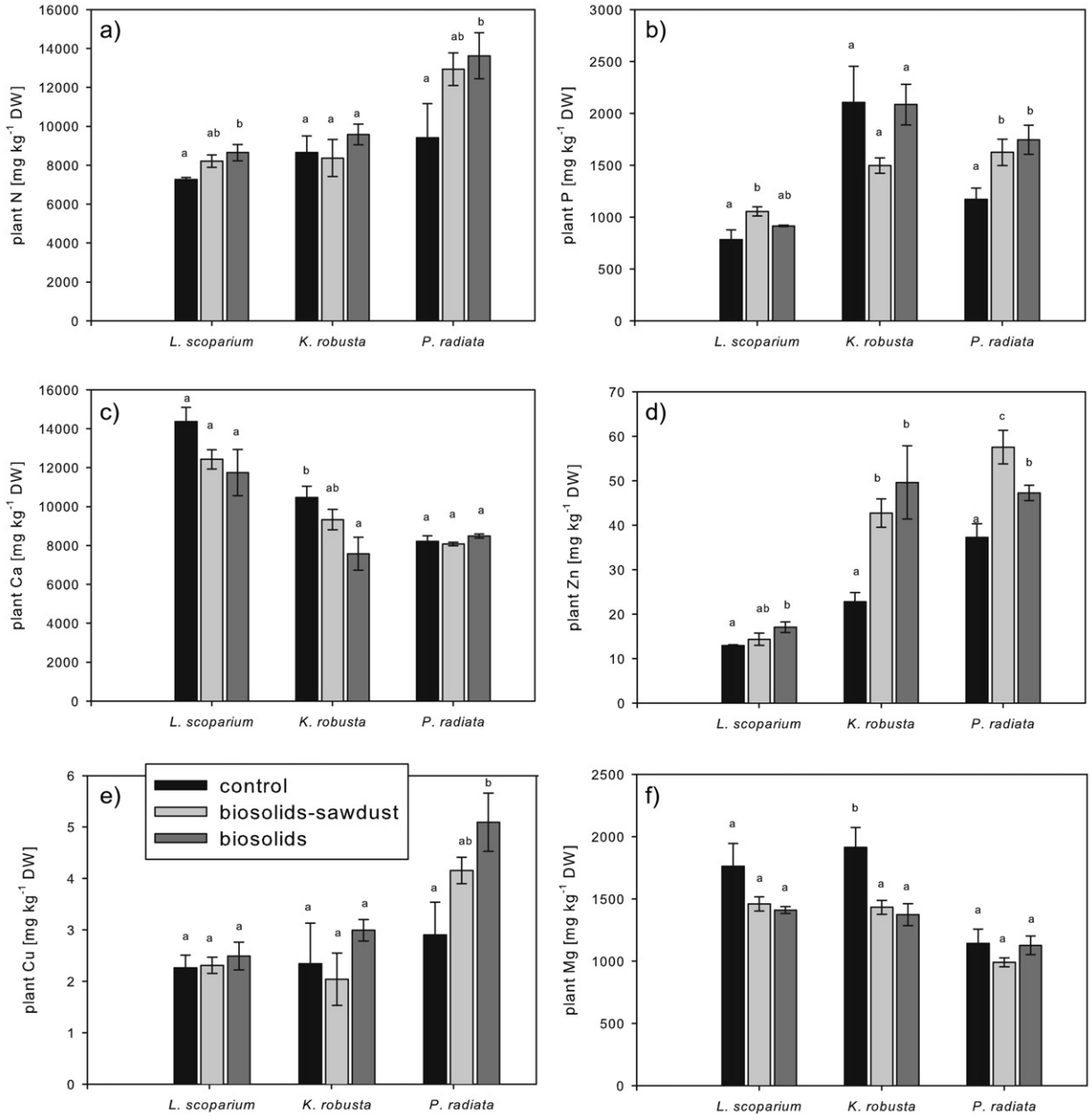

Fig. 2. Concentration of selected macro- and micronutrients in plant leaves [mg kg^{-1} DW] after a growing period of 18 weeks in control, biosolids-sawdust and biosolids amended treatments ($n = 4 \pm$ se). Significant differences between treatments at $p \leq 0.05$ are indicated by letters (a, b, c) within plant species.

with biosolids lowered the growth response of L. *scoparium* compared to biosolids, whereas similar growth compared to biosolids was observed for *K. robusta*. Plants benefit from other macro- and micronutrients aside from N, which are applied with biosolids (Anderson et al., 2012; Antoniadis et al., 2015).

Other elements (K, B, S, Mn, Fe, Mo, Al and Na) were analysed, but the effects of the treatments on plant leaf concentrations were negligible, or not of environmental significance (Table S1 – supplemental material). Concentrations of S, Mo, Al and Na, did not differ between treatments for any plant species. Potential contaminants, such as Cr, Ni, Cd and As, were detected in plant leaves only in low concentrations, and were not significantly increased by either biosolids or biosolids-sawdust application compared to control treatments. There was no significant difference in foliar Cd concentrations, and in many cases, Cd was below detection limit (Table S1 – Supplemental material).

3.3. Phytochemical analysis

None of the treatments affected any of the measured plant pigment concentrations in *P. radiata* and L. *scoparium* (Fig. 3). Biosolids significantly increased anthocyanin in *K. robusta.* (Fig. 3b). The biosolids and sawdust treatment significantly increased chlorophyll and carotenoid concentration *K. robusta* (Fig. 3a, c).

Photosynthetic activity has been related to nutrient supply and uptake, including P (Singh et al., 1939), whereas other elements, such as heavy metals can have a negative impact on photosynthetic pigments (Sai Kachout et al., 2015). In our study, chlorophyll and carotenoids decreased in *K. robusta* plants when amended with biosolids-sawdust, while an increase in anthocyanin was achieved with biosolids application alone (Fig. 3b). Sawdust mixed with biosolids did not reduce plant available P (Table S2, supplemental material), nor plant P (Fig.

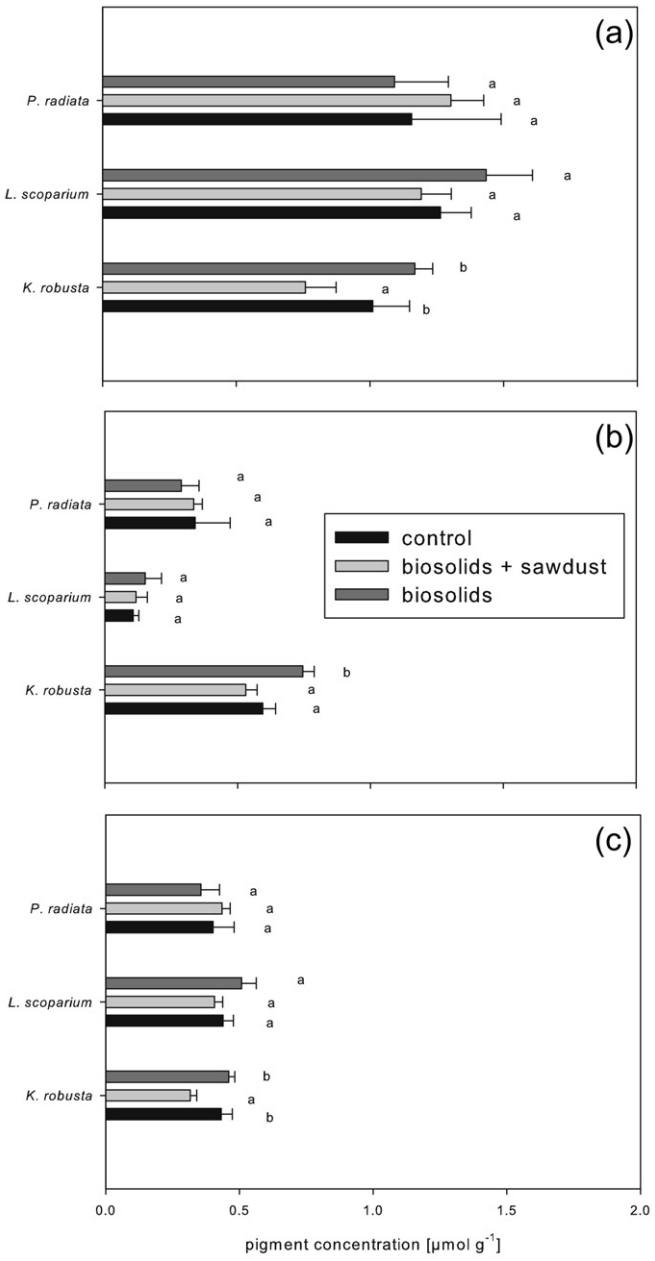

Fig. 3. Concentration of chlorophyll (a), anthocyanin (b) and carotenoids (c) in plant leaves [µmol g^{-1}] after a growing period of 18 weeks in control, biosolids-sawdust and biosolids amended treatments ($n = 4 \pm$ se). Significant differences between treatments at $p \leq 0.05$ are indicated by letters (a, b, c) within plant species.

2) in *K. robusta*. Therefore our results did not support a decrease in photosynthetic activity due to an immobilization of P. Higher anthocyanin concentrations in *K. robusta* biosolids treatment could be a result of an increased Zn uptake due to biosolids application, however, results were inconsistent and higher Zn in *P. radiata* did not result in increased anthocyanin concentrations in biosolids amended soil. Similarly, plant available concentrations of S, Mg and Cu could have stimulated anthocyanin synthesis in *K. robusta*, but at the same time, increased concentrations of these elements in the rhizosphere of *P. radiata* did not result in increased anthocyanin contents. The synthesis of anthocyanins is induced by several factors, including nutrient deficiency (Pinto et al., 1999) and heavy metal stress (Dai et al., 2006). It is possible that decreased concentrations of Ca and Mg in *K. robusta* plant leaves hence could have been responsible for an increased anthocyanin production after biosolids application.

3.4. Rhizosphere soil elements and N-flux

Biosolids and biosolids-sawdust treatments had increased element concentrations in the rhizosphere soil at the end of the experiment, depending on the plant species. In *P. radiata*, rhizosphere, P was increased in biosolids and biosolids-sawdust, whereas S was only increased in the biosolids treatment, and Mg only in biosolids-sawdust treatments compered to controls (Table S2 – Supplemental material). In combination with *K. robusta*, biosolids application increased S, Mg and Cu concentrations, and S concentrations in biosolids-sawdust treatments. Biosolids significantly increased concentrations of P and S in the rhizosphere of L. *scoparium* and increased S in biosolids-sawdust. The same pattern occurred in the *K. robusta* treatments. There was a decrease in K in the L. *scoparium* rhizosphere after biosolids and biosolids-sawdust application compared to control treatments.

The total cumulative mass of NO_3^- recovered in drainage during the experimental period was <1.5 kg N ha^{-1} equiv. for all plant species (Fig. 4a). There were no differences between the biosolids and biosolids-sawdust treatments, irrespective plant species. The total amount of N in soil at the end of the experiment increased with biosolids and biosolids-sawdust application (Fig. 4b). Specifically, soil planted with *P. radiata* showed N increases of 686 kg ha^{-1}, soil planted with L. *scoparium* had increases of 1602 kg ha^{-1} and soil planted with *K. robusta* had increases of 1449 kg ha^{-1}.

Blending biosolids with sawdust was shown previously to improve plant growth while reducing (NO_3^-) leaching by increasing the C:N (Bugbee, 1999; Paramashivam et al., 2015). In our study, mixing sawdust with biosolids did not result in differences in N leaching, irrespective plant species (Fig. 4a). However, total NO_3^- recovered in leachate ranged below 2 kg ha^{-1} in all treatments and plant species, hence was considered negligible in our experiment. Biosolids was applied at

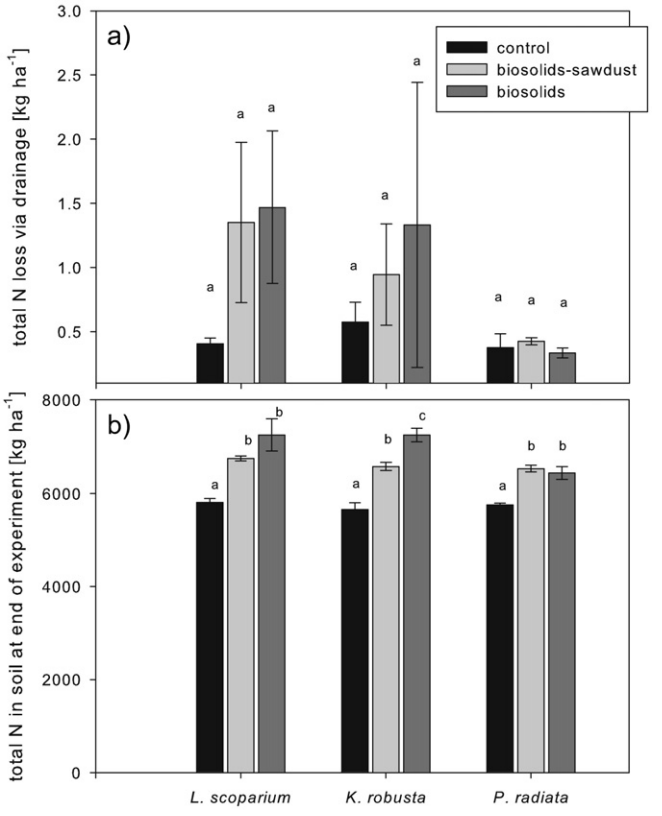

Fig. 4. Total N loss via NO^{3-} leaching (a) and total N in soil (b) at end of the experiment [kg ha^{-1}] after a growing period of 18 weeks in control, biosolids-sawdust and biosolids amended treatments ($n = 4 \pm$ se). Significant differences between treatments at $p \leq 0.05$ are indicated by letters (a, b, c) within plant species.

a rate of 1250 kg ha^{-1}, increasing both plant N and total N in soil at the end of the experiment in combination with all plant species.

Whereas similar total N contents were detected in control soils irrespective plant species (5650–5809 kg ha^{-1}), an increase in total N up to 7260 kg ha^{-1} was measured in soil planted with *K. robusta* and L. *scoparium* treatments after biosolids application, with smaller increases (6440 kg ha^{-1}) detected in *P. radiata* biosolids lysimeters. This difference of 820 kg ha^{-1} could not be completely explained by a larger N use for biomass production of *P. radiata* compared to L. *scoparium* and *K. robusta*. The growth response of *P. radiata* resulted in an additional 222 kg ha^{-1} of N compared to *K. robusta*, and 183 kgha^{-1} N compared to L. *scoparium* growth leaving up to ~640 kg ha^{-1} N retained in the soil associated with myrtacece species. These calculations show that both, *L. scoparium* and *K. robusta* planted on degraded soil amended with biosolids showed evidence of interference with the N cycle, and could potentially reduce gaseous N losses compared to *P. radiata* trees. However, given the low levels of N-leaching in this experiment, further studies need to verify N emissions and overall N balances from *K. robusta* and L. *scoparium* compared to *P. radiata*.

4. Conclusions

Biosolids application to low-fertility soil can enhance the growth of L. *scoparium, K. robusta* and *P. radiata*, increase N uptake, and not result in unacceptable concentrations of trace element contaminants such as Cd. Mixing sawdust with biosolids had no effect on N leaching, but reduced benefits from biosolids application in combination with *P. radiata* and L. *scoparium*. Biosolids-sawdust application to L. *scoparium* and *K. robusta* could provide a suitable way of recycling wood-waste while increasing plant growth. Differences in biomass increase between tree species in biosolids compared to biosolids-sawdust treatments might result from a stimulation of different mycorrhiza types, associated with the respective tree species, which will be an interesting area for future research. Biosolids may influence plant rhizodeposition, hence in future studies there is a strong need to include investigations of plant-root-microbe interactions in terms of N dynamics and plant element uptake.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.02.134.

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