Published April 25, 2016

Journal of Environmental Quality

TECHNICAL REPORTS

WASTE MANAGEMENT

Biowaste Mixtures Affecting the Growth and Elemental Composition of Italian Ryegrass (*Lolium multiflorum*)

Jürgen Esperschütz, Obed Lense, Craig Anderson, Simon Bulman, Jacqui Horswell, Nicholas Dickinson, and Brett Robinson*

Abstract

Biosolids (sewage sludge) can be beneficially applied to degraded lands to improve soil quality. Plants grown on biosolids-amended soils have distinct concentrations of macronutrients and trace elements, which can be beneficial or present a risk to humans and ecosystems. Potentially, biosolids could be blended with other biowastes, such as sawdust, to reduce the risks posed by rebuilding soils using biosolids alone. We sought to determine the effect of mixing biosolids and sawdust on the macronutrient and trace element concentration of ryegrass over a 5-mo period. *Lolium multiflorum* was grown in a low fertility soil, typical for marginal farm areas, that was amended with biosolids (1250 kg N ha^{-1}), biosolids + sawdust (0.5:1) and urea (200 kg N ha^{-1}), as well as a control. Biosolids increased the growth of *L. multiflorum* from 2.93 to 4.14 t ha^{-1}. This increase was offset by blending the biosolids with sawdust (3.00 t ha^{-1}). Urea application increased growth to 4.93 t ha^{-1}. The biowaste treatments increased N, P, Cu, Mn, and Zn relative to the control, which may be beneficial for grazing animals. Although biowaste application caused elevated Cd concentrations (0.15–0.24 mg kg^{-1}) five- to eightfold higher than control and urea treatments, these were below levels that are likely to result in unacceptable concentrations in animal tissues. Mixing biosolids with sawdust reduced Cd uptake while still resulting in increased micronutrient concentrations (P, S, Mn, Zn, Cu) in plants. There were significant changes in the elemental uptake during the experiment, which was attributed to the decomposition of the sawdust.

Core Ideas

- Biowastes (biosolids + sawdust) were effective in improving a low-fertility soil.
- The biowaste mixture improved growth and quality of Lolium multiflorum over 5 months.
- The biowaste treatments increased N, P, Cu, Mn, and Zn.
- Mixing biosolids with sawdust reduced Cd uptake.
- Biowaste induced changes in elemental composition increased over the 5-month period.

J. Environ. Qual. 45:1054–1061 (2016)
doi:10.2134/jeq2015.09.0459
Received 10 Sept. 2015.
Accepted 21 Dec. 2015.
*Corresponding author (brett.robinson@lincoln.ac.nz).

Biosolids are a product of municipal wastewater treatment. They are primarily derived from domestic sources, which are combinations of human feces, urine and gray water, as well as small inputs from industry and occasionally stormwater (Lu et al., 2012). Biosolids are produced at an annual rate of 27 kg per person, and their disposal can be expensive (e.g., incineration or in landfills) or environmentally damaging via legal or illegal disposal into waterways (LeBlanc et al., 2009).

Biosolids provide nutrients and organic matter, which can improve soil structure (Antolín et al., 2005; Singh and Agrawal, 2008), enhance plant growth (Miaomiao et al., 2009; Mok et al., 2013), and increase soil microbial activity (Cytryn et al., 2011). The efficacy of biosolids in improving soil quality depends on their provenance and treatment. Biosolids application to soil can also cause negative effects because they can introduce pathogens (Vasseur et al., 1996; Zaleski et al., 2005) and contaminants, including heavy metals (Oliver et al., 1994; Miaomiao et al., 2009; Lomonte et al., 2010; Lopes et al., 2011) that may be hazardous to soil biological processes and to human health. Therefore, the rate of biosolids addition to land is regulated with respect to the levels of heavy metals, organic compounds and pathogens (EEC, 1986; USEPA, 1993; NZWWA, 2003). Excessive biosolids applications to land can result in excessive runoff or leaching of plant nutrients such as nitrate (NO_3^-) and phosphate (PO_4^{3-}) into receiving waters (Agopsowicz et al., 2008; Knowles et al., 2011). Therefore, biosolids are more suitable for rebuilding eroded land, low-fertility with poor soil structure, such as marginal farm areas (Fresquez et al., 1990; Shahid and Al-Shankiti, 2013). Such sites are not directly linked to the human food chain, and the high organic matter contents of biosolids may be more effective than mineral fertilizers for restoring degraded soils.

Some of the negative effects of biosolids addition to soil, namely environmental impacts of heavy metals, can be mitigated

J. Esperschütz, O. Lense, and B. Robinson, Lincoln Univ., Agriculture and Life Sciences, Dep. of Soil and Physical Sciences, PO Box 84, Lincoln 7647, New Zealand; C. Anderson, Plant and Food Research, Soil Sciences Division, Canterbury Agriculture and Science Center, Gerald St., Lincoln 7608, New Zealand; S. Bulman, Plant and Food Research, Microbiology Division, Canterbury Agriculture and Science Center, Gerald St., Lincoln 7608, New Zealand; J. Horswell, Institute of Environmental Science and Research Limited, Kenepuru Science Center, PO Box 50-348, Porirua 5240, New Zealand; N. Dickinson, Lincoln Univ., Agriculture and Life Sciences, Dep. of Ecology, PO Box 84, Lincoln 7647, New Zealand. Assigned to Associate Editor Tracy Punshon.

Abbreviations: PAHs, polycyclic aromatic hydrocarbons; DOM, dissolved organic matter; DOC, dissolved organic carbon; SOM, solid organic matter.

by blending the biosolids with other biowastes including biochar (Knowles et al., 2011), lignite (Simmler et al., 2013) and wood waste (Paramashivam et al., 2015). Bugbee (1999) reported that blending biosolids with sawdust could improve plant growth, while reducing NO_3^- leaching by increasing the C/N ratio. In addition to reducing NO_3^- leaching, wood waste, which can be expensive and environmentally damaging to dispose of, can effectively sorb heavy metals such as Cd, Cr, Cu, Ni, Pb and Zn from industrial effluents (Ajmal et al., 1998; Marchetti et al., 2000; Yu et al., 2000). Bulut and Tez (2007) demonstrated that there was variation in the sorption of individual metals (Pb ≈ Cd > Ni), which was attributed to the affinity of each element to the proteins, carbohydrates, and phenolic compounds in the sawdust.

Sawdust undergoes decomposition when mixed with N-rich material. During decomposition, the cation exchange capacity of the sawdust increases, as more functional groups form on the surface of the sawdust particles (Jokova et al., 1997). Therefore, it is likely that the sorption of metals by sawdust will increase, at least temporarily, as it decomposes. Sawdust may be beneficial in countries with forestry operations that produce large quantities of wood waste, which can be expensive and environmentally damaging to dispose of (Robinson et al., 2007).

Previous studies have shown that biosolids increases the growth of ryegrass (Crush et al., 2006; Santibanez et al., 2008). Biosolids also increase the uptake of Cd, Cu and Zn into the plant biomass (Ahumada et al., 2009; Bai et al., 2013; Mugica-Alvarez et al., 2015). Therefore, contaminants such as Cd may enter grazing animals and result in concentrations in excess of food safety standards in animal products (Reiser et al., 2014). In contrast, the increase in Cu and Zn in the plant biomass can be beneficial to the health of grazing animals in areas where these elements are deficient, or where high Zn concentrations are needed such as a prophylaxis to facial eczema (Anderson et al., 2012).

We hypothesize that mixing sawdust with biosolids will reduce the solubility and hence the plant uptake of heavy metals from biosolids-amended soil. We aimed to determine whether mixtures of biowastes (biosolids + sawdust) could be used to rebuild a low-fertility soil without resulting in excessive metal concentrations in the aerial portions of ryegrass. Specifically, we sought to elucidate the effect of biosolids, either alone or mixed with sawdust, and urea on the growth and concentration of macronutrients and trace elements in *L. multiflorum*.

Materials and Methods

Experimental Setup

The experiment was conducted at Lincoln University greenhouse facility (43°38′42″ S, 172°27′41″ E). Low-fertility soil, with now history of fertilizer addition, was collected from a marginal farm area near Bideford, New Zealand (40°45′56″ S, 175°54′42″ E). The soil has been classified as an orthic brown soil, and has been chosen as a representative of marginal soils commonly found around farm systems. 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). Tables 1 and 2 show the properties of the soil, biosolids, and sawdust.

Twenty-four 10-L pots (25 cm diam., 29 cm height) were filled with 10 kg of soil to a soil bulk density of 1.3 g cm^{-3}. Pots were incubated at ambient conditions in the greenhouse for 14 wk before treatment application. Three treatments (urea, biosolids, and biosolids + sawdust) and a control were setup randomly with six replicates each within the experimental setup. The treatments comprised urea (2.11 g dry weight), biosolids (245 g dry weight), and the same amount of biosolids mixed with sawdust (123 g dry weight). The applications rates for urea and biosolids were equivalent to 200 and 1250 kg N ha^{-1}, respectively, with the

Table 1. Properties of soil, biosolids and sawdust used in the experiment. Values in parentheses represent standard error of *n* = 5 replicates.

	Soil	Sawdust	Biosolids
pH	6.1	5.7 (0.1)	4.5 (0.0)
Moisture content, % w/w	25.5	230.4 (2.7)	106.2 (4.2)
Dry matter, % w/w	79.7	30.3 (0.2)	48.6 (1.0)
C/N ratio	14.3	908.4 (154.0)	10.6 (0.1)
Total available N, mg kg^{-1}	43.1	n.d. (n.d.)†	403.8 (7.1)
CEC‡, $cmol_c$ kg^{-1}	21.0	8.0 (0.2)	17.1 (0.6)
total base saturation, %BS	55.0	76.2 (0.8)	86.3 (3.0)
C, % w/w	6.5	47.7 (0.1)	27.1 (0.7)
N, % w/w	0.5	0.1 (0.0)	2.5 (0.6)
P, % w/w	0.05 (0.00)	n.d. (n.d.)	0.59 (0.00)
K, % w/w	0.19 (0.00)	0.05 (0.00)	0.37 (0.00)
S, % w/w	0.04 (0.00)	0.01 (0.00)	0.87 (0.01)
Ca, % w/w	0.41 (0.01)	0.08 (0.00)	0.63 (0.01)
Mg, % w/w	0.20 (0.00)	0.02 (0.00)	0.30 (0.00)
B, mg kg^{-1}	29.0 (0.3)	1.9 (0.2)	26.7 (0.1)
Cu, mg kg^{-1}	4.2 (0.0)	0.8 (0.0)	891.0 (18.9)
Zn, mg kg^{-1}	29 (0)	8 (0)	1073 (27)
Mn, mg kg^{-1}	133.5 (2.9)	47.2 (0.8)	184.9 (4.5)
Fe, mg kg^{-1}	15,461 (108)	116 (6)	14,534 (92)
Cd, mg kg^{-1}	0.05 (0.00)	n.d. (n.d.)	3.97 (0.07)

† n.d. = not detected.

‡ CEC = cation exchange capacity.

Table 2. Plant available [$Ca(NO_3)_2$] nutrient and trace element concentrations in biosolids and sawdust at start of the experiment. Values in parentheses represent standard error of n = 5 replicates.

	Sawdust	Biosolids
	mg kg^{-1}	
P	13 (1)	49 (1)
K	295 (6)	170 (5)
S	5 (2)	1193 (64)
Mg	185 (2)	349 (14)
B	n.d. (n.d.)†	n.d. (n.d.)
Cu	0.06 (0.02)	8.90 (0.32)
Zn	6.1 (1.0)	530.7 (12.0)
Mn	33.2 (1.3)	74.0 (2.9)
Fe	0.5 (0.1)	77.6 (1.7)
Cd	0.01 (0.00)	1.32 (0.02)

† n.d. = not detected.

application rate of biosolids equivalent to 50 t ha^{-1} dry weight. The biosolids and biosolids + sawdust mixtures were applied to the surface of the pots before sowing. Urea (50 kg ha^{-1} equivalent) was applied four times during the experimental period. Pots were arranged in a randomized block design.

In September 2013, 2 g of *L. multiflorum* ('Feast II' tetraploid Italian ryegrass) seeds were sown in all pots immediately after treatment application. An automated irrigation system applied a total of 1060 mm of water to each pot over the experimental period of 18 wk to ensure optimal plant growth at conditions near field capacity. The temperature in the greenhouse ranged from 9 to 20°C during the night (10 pm until 6 am) and from 14°C to 28°C during the day. The plant biomass was repeatedly cut back to 2 cm above the soil to simulate grazing. Harvesting occurred fortnightly over the summer (southern-hemisphere) starting from 16 Oct. 2013 to 29 Jan. 2014.

Analyses and Statistical Evaluation

At the end of the experiment, soil and plant samples were dried at 70°C until constant weight was obtained, then ground using a Retch ZM200 grinder. Soil samples were collected and passed through a 5-mm stainless steel sieve prior to chemical analyses. Soil and plant C and N concentrations were measured using an Elementar Vario MAX CN analyzer. Soil pH was determined with pH meter (Mettler Toledo Seven Easy) 24 h after shaking 10 g of soil in 25 mL deionized water. Plant-available elements was estimated with 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 in biosolids-amended soil. In brief, 5 g soil was weighed into 50-mL centrifuge tubes 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 (Whatman 52 filter paper). Extracts were stored in sealed containers until chemical analyses.

Pseudo-total elemental analysis was performed using microwave digestion in 8 mL of nitric acid (Aristar; ±69%), filtered through Whatman 52 filter paper, and diluted with filtered (MilliQ) water to a volume of 25 mL. Concentrations of B, Ca, Cd, Cu, Fe, K, Mg, Mn, P, S, and Zn were determined using inductively coupled plasma optical emission spectrometry (ICP–OES Varian 720 ES). For quality assurance, reference soil and plant material from Wageningen University, the Netherlands (International Soil analytical Exchange 921 and International Plant analytical Exchange 100) was analyzed with the samples. Recoverable concentrations were 81–112% of the certified values.

Significant differences (α = 0.05) between control soil, urea, biosolids, and biosolids + sawdust treatments were determined by analysis of variance (Trillas et al., 2006), followed by Duncan post-hoc tests at $p \leq 0.05$. The analyses were performed using SPSS v.22 (IBM, 2013). Correlation analyses between dry biomass production and element concentrations were performed in Microsoft Excel 2013 (Microsoft Office, 2013).

Results

Figure 1 shows the cumulative pasture biomass production over the 18 wk experimental period. Control treatments showed a total average of 10.56 g biomass dry weight per pot, equivalent to 2.15 t ha^{-1}. Urea fertilization increased the cumulative biomass to 24.19 g, equivalent to 4.93 t ha^{-1}. Biosolids application also resulted in a significant biomass response (20.32 g, equivalent to 4.14 t ha^{-1}), while mixing sawdust with biosolids lowered the biomass growth of *L. multiflorum* compared to biosolids alone (14.72 g, equivalent to 3.00 t ha^{-1}). Six weeks after sowing, significant differences were detected in the growth response of *L. multiflorum* as a result of different treatments ranking in order of urea > biosolids > biosolids + sawdust > control, which remained unchanged throughout the duration of the experiment.

Table 3 shows that there were significant differences macronutrient uptake between untreated control and the treatments. Biosolids addition significantly increased the concentrations of P and S, but surprisingly not N, relative to the control. Biosolids decreased plant K concentration. Biosolids + sawdust increased both N and P. Urea application only caused a significant increase in N and caused significant decreases in P, K and S.

Within treatments, there were significant differences in the uptake of K, P, and S over the experimental period (Fig. 2). Whereas K concentration in the plant biomass showed a decreasing trend in all treatments (Fig. 2b), the highest concentrations of P and S were detected in plant biomass harvested 10 and 12 wk after sowing, as well as at the end of the experiment (Fig. 2c and 2d). For the control, biosolids, and biosolids + sawdust treatments, the N concentration varied between 2 and 3% throughout the experimental period. Concentrations of N were between 3 and 5% during the experiment in urea treatments, with distinct peaks in the initial harvest and at the 10-wk harvest, whereas N concentrations in control, biosolids and biosolids + sawdust treatments ranged between 2 and 3% (Fig. 2a). Plant P at individual harvest time points was negatively correlated with the corresponding biomass in the biosolids and biosolids + sawdust treatments ($r = -0.97$, $p \leq 0.001$; $r = -0.89$, $p \leq 0.01$), as well as plant S ($r = -0.88$, $p \leq 0.01$; $r = -0.78$, $p \leq 0.05$, data not shown).

The application of biosolids and biosolids + sawdust increased the average plant Zn concentrations up to nine- and sixfold, respectively. Foliar Cu concentrations were increased by up to 50% in the biosolids, and 70% in the biosolids + sawdust treatments (Table 4). Average concentrations of Mn were increased by approximately 50% after biosolids application compared to control treatments. Plant Cd concentrations in the biosolids treatment were approximately eightfold higher than the control

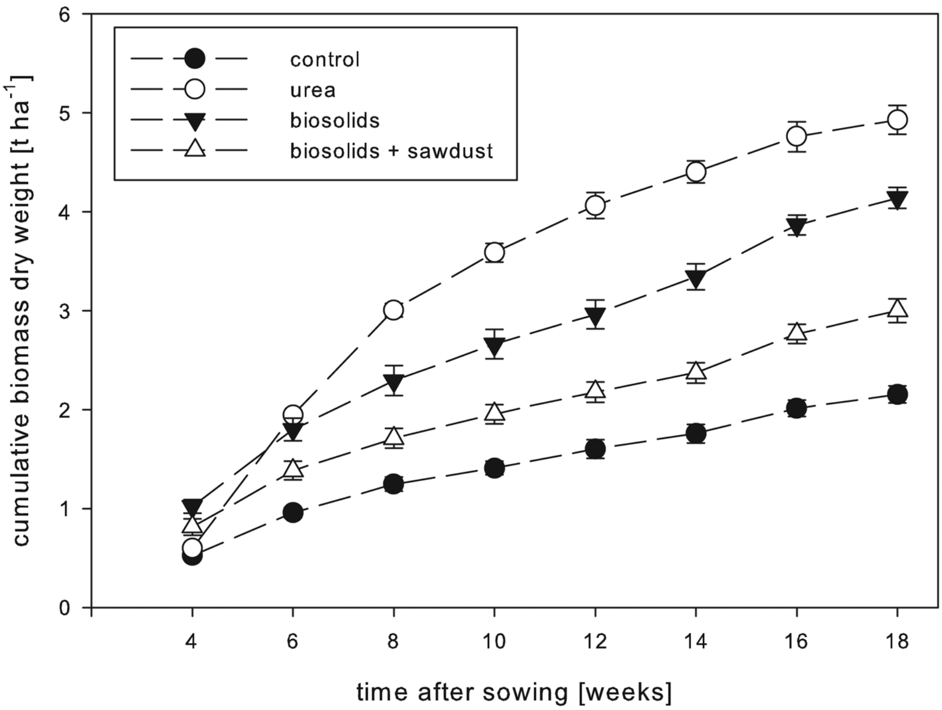

Fig. 1. Cumulative biomass (dry weight) in t ha^{-1} equivalent during the 18-wk experimental period. Each point is the average of six replicates with bars representing the standard error of the mean. Non-overlapping bars indicate significant differences (*p* ≤ 0.05).

and urea treatments. This increase was only a fivefold increase in the biosolids + sawdust treatment. Urea application did not cause significant differences in average foliar concentration of any trace element.

As with the macronutrients, there was significant variation in the uptake of trace elements over the 18-wk experimental period (Fig. 3). In the biosolids and biosolids + sawdust treatments, the concentrations of Zn, Cd and Cu increased throughout the experimental period (Fig. 3b, 3c, and 3e). In contrast, there was little difference in the Cu concentration between biosolids and biosolids + sawdust treatments (Fig. 3e). For Cd and Zn, the difference between the biosolids and the biosolids + sawdust treatment increased over time (Fig. 3b and 3c). Between the 6- and 8-wk harvests, the results showed a pronounced increase in the elemental concentrations of Fe, Cd, Mn and Cu, especially in biosolids treatments.

Discussion

The total biomass production (2–5 t ha^{-1} equivalent) of *L. multiflorum* over 18 spring and summer weeks under biosolids and biosolids + sawdust treatments is comparable to the average biomass production of 2.2 and 8.7 t ha^{-1}, depending on the growth period, reported for 'Feast II' (Hanson et al., 2006; Moir et al., 2013). Smith and Tibbett (2004) reported annual biomass production of 1.7, 2.0, and 2.4 t ha^{-1} in pastures receiving 4, 8, and 16 t ha^{-1} of dried biosolids, which is somewhat lower than our study equating to approximately 50 t ha^{-1} of dried biosolids. Biosolids and biosolids + sawdust hence were effective in increasing plant growth on a low-fertility soil. The results indicate that mixing sawdust with biosolids significantly reduced the growth increase compared to biosolids alone and that neither biosolids nor biosolids + sawdust was as effective as urea in increasing biomass. The lower biomass production of the biosolids + sawdust treatment compared to the biosolids-alone treatment is consistent with sawdust immobilizing N. While the average N concentration in the biosolids + sawdust was not significantly lower than the biosolids-alone treatment (Table 3), the mass that was extracted (biomass × N concentration) was significantly higher for the biosolids alone treatment.

In the control treatment, the N concentration in our study was in a similar range of the value reported for annual ryegrass (*L. multiflorum*) in a study comparing different grass species under different rates of N loading (Moir et al., 2013). That the urea treatment (N) resulted in a greater increase in biomass than either of the biosolids treatments (N plus a suite of other plant nutrients) indicates that other components in the biosolids, such as heavy metals, reduced the effectiveness of the added N. In the biosolids and biosolids + sawdust treatments, only a limited amount of the total N applied with biosolids (1250 kg ha^{-1}) was immediately plant available. Most of the N in biosolids is locked up in organic compounds which need to undergo (microbial) transformation processes to become available (Sommers, 1977).

With the exception of N, the concentrations of macronutrients in our study were similar to those reported for perennial ryegrass (Harrington et al., 2006). Even though urea significantly increased the biomass, the concentrations of other essential

Table 3. Average concentration of trace elements in *L. multiflorum* over the experimental period. Values in parentheses represent the standard error of the average concentration per pot (*n* = 6) throughout the experiment (*n* = 8).

	Control	Urea	Biosolids	Biosolids + sawdust
	% w/w			
N	2.39 (0.04) a†	3.35 (0.09) c	2.56 (0.05) ab	2.63 (0.12) b
P	0.30 (0.01) b	0.17 (0.00) a	0.43 (0.02) d	0.35 (0.02) c
K	3.21 (0.03) c	1.93 (0.02) a	2.73 (0.06) b	3.00 (0.12) c
S	0.38 (0.01) bc	0.26 (0.00) a	0.40 (0.01) c	0.35 (0.02) b
Ca	0.80 (0.01) c	0.77 (0.02) bc	0.73 (0.01) b	0.66 (0.02) a
Mg	0.23 (0.00) a	0.24 (0.01) bc	0.23 (0.00) b	0.21 (0.01) a

† Different lowercase letters indicate significant differences between treatments at *p* ≤ 0.05.

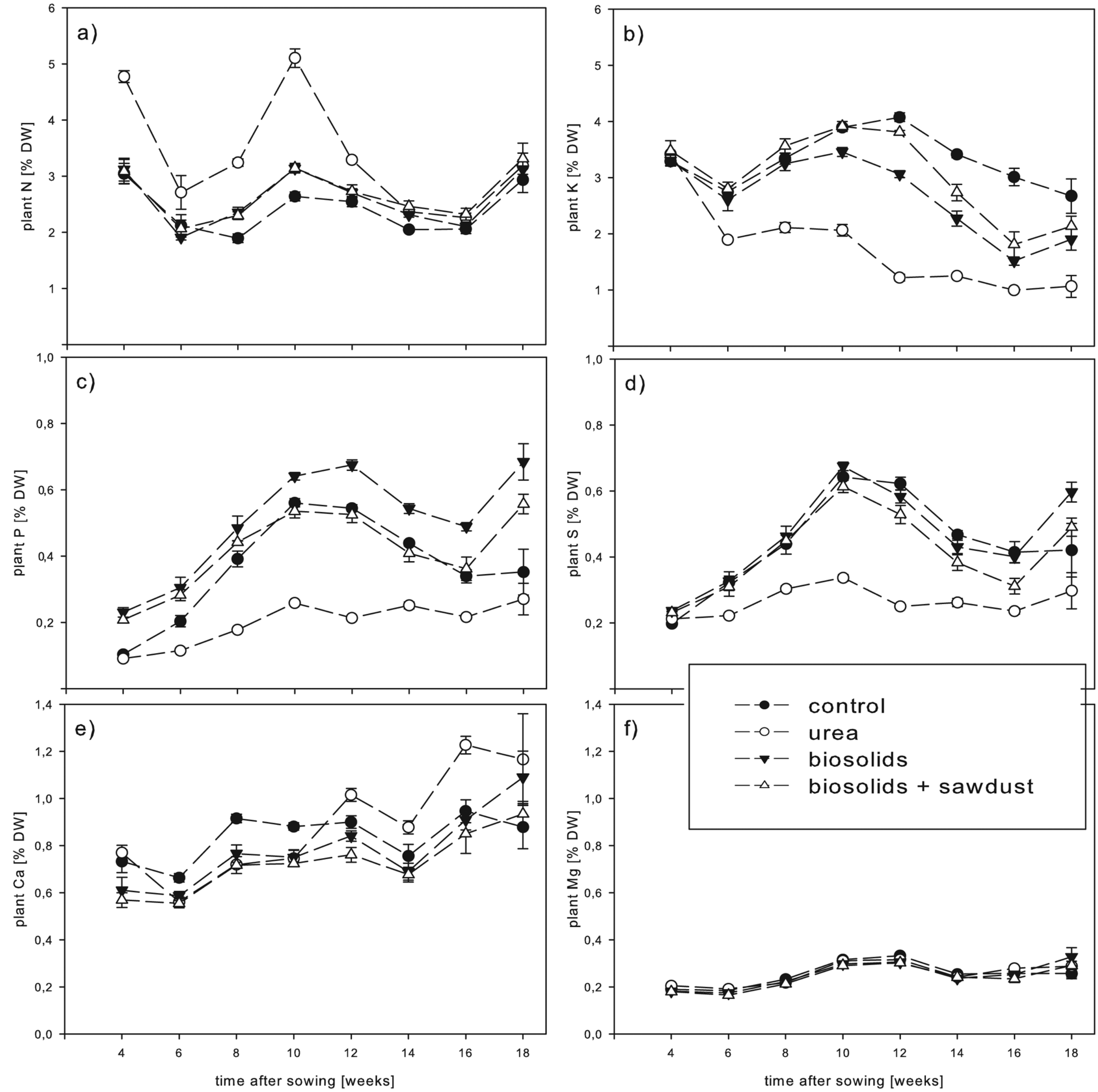

Fig. 2. Average concentrations of macronutrients over the experimental period (*n* = 6). Error bars represent the standard error of the mean. Non-overlapping error bars indicate significant difference between means ($p \leq 0.05$).

Table 4. Average concentration of trace elements in *L. multiflorum* over the experimental period. Values in parentheses represent the standard error of the average concentration per pot (*n* = 6) throughout the experiment (*n* = 8).

	Control	Urea	Biosolids	Biosolids + sawdust
	mg kg^{-1} dry wt.			
B	11.4 (1.0) b†	8.9 (0.3) a	10.5 (0.3) ab	9.9 (0.8) ab
Cu	5.9 (0.1) a	6.0 (0.2) a	10.3 (0.6) c	8.7 (0.4) b
Zn	21.6 (2.3) a	19.8 (0.7) a	150.4 (8.3) c	91.7 (3.7) b
Mn	37.4 (1.0) a	35.2 (0.8) a	60.2 (1.7) c	51.0 (2.4) b
Fe	96.0 (3.9) a	105.8 (13.6) a	118.7 (14.4) a	105.5 (7.1) a
Cd	0.03 (0.01) ab	0.02 (0.00) a	0.26 (0.06) c	0.13 (0.00) b

† Different lowercase letters indicate significant differences between treatments at $p \leq 0.05$.

macronutrients, namely P, S and K were significantly lower in the urea treatment, indicating that these elements were not limiting in the control soil. These elements dropped to near-deficient concentrations (McNaught, 1970) in the urea treatment, possible due to a dilution-by-growth effect. The biosolids + sawdust treatment (Table 3) showed that the concentrations of K, P, and S were higher than the critical deficiency threshold concentrations (28, 2.1, and 1.8 g kg^{-1}, respectively) reported for perennial ryegrass (*L. perenne*) (McNaught, 1970; Smith et al., 1985). This is consistent with using biosolids and biosolids + sawdust not only to improve plant growth, but also to enhance plant nutrient uptake in a low fertility environment. The concentration of plant K decreased throughout the experimental period, which could

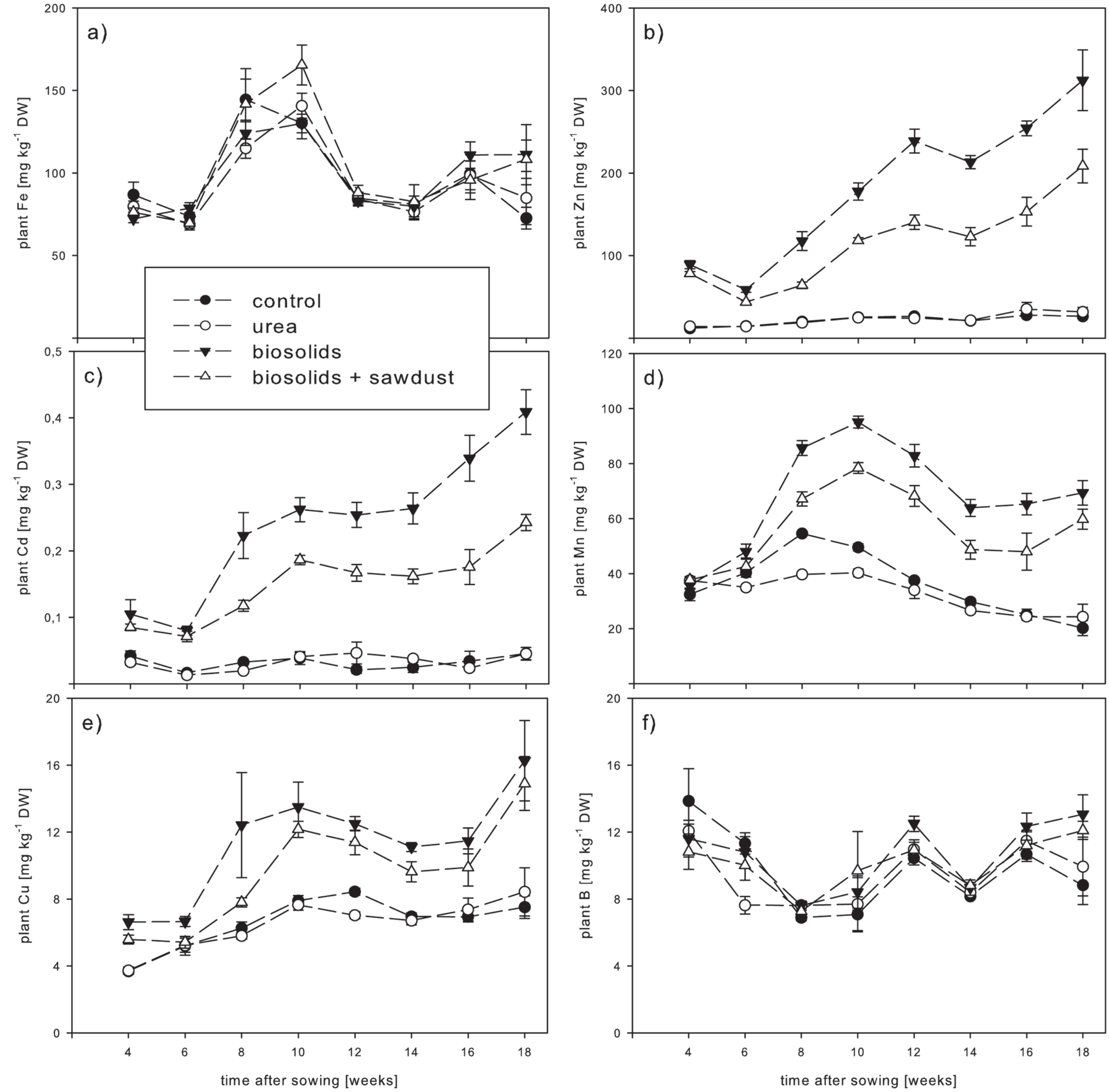

Fig. 3. Average (*n* = 6) concentrations of trace elements over the experimental period. Error bars represent the standard error of the mean. Non-overlapping error bars indicate significant difference between means ($p \leq 0.05$).

be attributed to a limited supply of K from the initial soil, as well as biosolids and biosolids + sawdust treatments (Table 2).

Lolium multiflorum growing in the biosolids and biosolids + sawdust treatments had significantly higher concentrations of Cd, Cu and Zn compared to control and urea treatments. Mixing sawdust with biosolids significantly decreased the Cd and Zn concentrations compared to the biosolids treatment. Clearly, this is beneficial in the case of Cd; sawdust addition can reduce the entry of this toxic element into fodder and food plants. In our study, Cd concentrations were within the range of acceptable daily intake of Cd concentration based on both food standards of New Zealand ($\leq$1.25 mg kg^{-1} for kidney and $\leq$2.5 mg kg^{-1} for liver) and the European Union ($\leq$1.0 mg kg^{-1} for kidney and $\leq$0.5 mg kg^{-1} for liver) (Reiser et al., 2014). The average Cd concentrations in our study (Table 3) were lower compared to others studies where biosolids had been used as a soil conditioner at similar rates (Antoniadis and Alloway, 2001; Black et al., 2012).

Some of the negative effects of elevated Cd may be offset by the elevated Zn concentrations (Oliver et al., 1994; Khoshgoftar et al., 2004; Reiser et al., 2014). Since Cd is absorbed by the root Zn transporter, a low supply of plant available Zn promotes Cd accumulation by plants (Khoshgoftar et al., 2004). Applying Zn fertilizer inhibits Cd uptake and translocation, especially in soils with low plant available Zn (Oliver et al., 1994). Khoshgoftar et al. (2004) reported that when Zn fertilizer was applied in a greenhouse experiment, Zn concentration in wheat shoot increased from 26 to 56 mg kg^{-1}, and Cd concentration was reduced from

0.90 to 0.09 mg kg^{-1}. In our study, foliar Zn concentrations were similar to the 129 to 390 mg kg^{-1} range reported by Santibanez et al. (2008) and Torri and Lavado (2009), who used higher rates of biosolids addition (150–400 t ha^{-1}) for perennial ryegrass. Biomass Zn concentrations in *L. multiflorum* in this study were higher than those reported for similar or lower rates of biosolids addition in combination with *L. perenne* (Antoniadis and Alloway, 2001; Ahumada et al., 2009; Black et al., 2012), hence they could have offset Cd uptake into plant biomass. The high Zn concentrations in our study can be explained by the relatively high Zn content in the used biosolids, as well as the mildly to moderately acidic nature of the soil and biosolids respectively (Table 1). The Zn concentrations in *L. multiflorum* in the biosolids treatment were within the range that Anderson et al. (2012) reported to cause a beneficial increase in blood Zn concentrations in sheep.

Although Cd and Zn were significantly higher in biosolids compared to biosolids + sawdust, plant Cu concentrations in plant biomass increased after mixing biosolids with sawdust compared to pure biosolids application. Copper deficiency is a widespread problem in all agricultural systems (Sinclair and Edwards, 2008; White and Broadley, 2009); thus increasing Cu uptake by plants by mixing biosolids with sawdust can provide agricultural benefits. However, the Cu concentration in our study were generally lower than those reported for *L. perenne* (Antoniadis and Alloway, 2001; Ahumada et al., 2009; Black et al., 2012). Urea application caused significant differences in uptake of B, Cu, and Zn, however, the differences were small and unlikely to be of agricultural significance.

During the experimental period, an accumulation of Zn, Cd and Cu was observed in *L. multiflorum* biomass. An increase of these elements at the end of the growing season may be related to decreased metabolic processes and smaller changes in the plant biomass, as suggested in studies investigating seasonal variations in trace metal uptake by *Phragmites australis* (Kastratović et al., 2013; Eid and Shaltout, 2014). This is consistent with the results obtained from total biomass harvests (Fig. 1), which show only a small growth increase toward end of the experiment. In the biosolids + sawdust treatment, Cd and Zn concentrations increased at a lower rate compared to the biosolids treatments, indicating that sawdust reduced the mobility of these elements. It was likely that the sawdust started to decompose during the experiment, resulting in increased metal sorption. Kostov et al. (1991) showed that the C/N ratio of *Picea excelsa* sawdust decreased from 251 to 62 only 6 mo after treatment with nutrient solution. Sawdust decomposition could explain the greater difference between the biosolids + sawdust treatment and biosolids treatments at the end of the experiment compared to the harvests before 6 wk, where difference were minimal.

The application of biosolids and biosolids mixed with sawdust improved the growth of *L. multiflorum* on a low-fertility soil, while the biowaste mixture (biosolids + sawdust) was less effective in restoring fertility compared to biosolids alone. Although less growth promoting, the advantage of using sawdust was seen in a reduction of the Cd uptake by the plants. There were significant changes in the elemental composition of the pasture over time, with the differences between the biosolids and biosolids + sawdust treatments increasing over time to favorable agronomic levels. Our results indicate that a single harvest of pasture can be insufficient to determine the effect of a soil treatment on element uptake, since results highly vary with environmental conditions, plant growth, and metabolism. Future work could involve a field study to reveal the effect of sawdust decomposition on the long-term fertility of soils amended with a mixture of biowastes.

Acknowledgments

The study was financially supported by the Deutsche Forschungsgemeinschaft (DFG, ES 416/1-1), the Center for Integrated Biowaste Research (CIBR) as well as Plant and Food Research (Blueskies). We highly appreciate H. Lowe (Lowe Environmental Impact) for providing and assisting with soil and soil collection, and acknowledge Lincoln University, especially L. Clucas, B. Richards, D. Paramashivan, S. Mammun, Y. Kim, T. Zhongtao, N. Stief, Y. Yang and H. Franklin for their support in sample analyses and maintaining the experiment.

References

Agopsowicz, M., A. Bialowiec, and P. Pijarczyk. 2008. Sewage sludge land disposal effects on groundwater. Archives of Environmental Protection 34:73–82.

Ahumada, I., O. Gudenschwager, M.A. Carrasco, G. Castillo, L. Ascar, and P. Richter. 2009. Copper and zinc bioavailabilities to ryegrass (*Lolium perenne L.*) and subterranean clover (*Trifolium subterraneum* L.) grown in biosolid treated Chilean soils. J. Environ. Manage. 90:2665–2671. doi:10.1016/j.jenvman.2009.02.004

Ajmal, M., K.A. Hussain, S. Ahmad, and A. Ahmad. 1998. Role of sawdust in the removal of copper(II) from industrial wastes. Water Res. 32:3085–3091. doi:10.1016/S0043-1354(98)00067-0

Anderson, C.W.N., B.H. Robinson, D.M. West, L. Clucas, and D. Portmann. 2012. Zinc-enriched and zinc-biofortified feed as a possible animal remedy in pastoral agriculture: Animal health and environmental benefits. J. Geochem. Explor. 121:30–35. doi:10.1016/j.gexplo.2012.01.009

Antolín, M.C., I. Pascual, C. García, A. Polo, and M. Sánchez-Díaz. 2005. Growth, yield and solute content of barley in soils treated with sewage sludge under semiarid Mediterranean conditions. Field Crops Res. 94:224–237. doi:10.1016/j.fcr.2005.01.009

Antoniadis, V., and B.J. Alloway. 2001. Availability of Cd, Ni and Zn to Ryegrass in Sewage Sludge-Treated Soils at Different Temperatures. Water Air Soil Pollut. 132:201–214. doi:10.1023/A:1013202104550

Bai, Y., C. Gu, T. Tao, L. Wang, K. Feng, and Y. Shan. 2013. Growth characteristics, nutrient uptake, and metal accumulation of ryegrass (*Lolium perenne L.*) in sludge-amended mudflats. Acta Agriculturae Scandinavica Section B, Soil and Plant Science 63:352–359. doi:10.1080/09064710.2013.782424

Black, A., R.G. McLaren, S.M. Reichman, T.W. Speir, L.M. Condron, and G. Houliston. 2012. Metal bioavailability dynamics during a two-year trial using ryegrass (*Lolium perenne L.*) grown in soils treated with biosolids and metal salts. Soil Res. 50:304–311. doi:10.1071/SR11315

Bugbee, G.J. 1999. Effects of hardwood sawdust in potting media containing biosolids compost on plant growth, fertilizer needs, and nitrogen leaching. Commun. Soil Sci. Plant Anal. 30:689–698. doi:10.1080/00103629909370238

Bulut, Y., and Z. Tez. 2007. Removal of heavy metals from aqueous solution by sawdust adsorption. J. Environ. Sci. (China) 19:160–166. doi:10.1016/S1001-0742(07)60026-6

Crush, J.R., U. Sarathchandra, and A. Donnison. 2006. Effect of plant growth on dehydration rates and microbial populations in sewage biosolids. Bioresour. Technol. 97:2447–2452. doi:10.1016/j.biortech.2005.04.019

Cytryn, E., L. Kautsky, M. Ofek, R.T. Mandelbaum, and D. Minz. 2011. Short-term structure and functional changes in bacterial community composition following amendment with biosolids compost. Appl. Soil Ecol. 48:160–167. doi:10.1016/j.apsoil.2011.03.010

EEC (European Economic Community). 1986. Using sewage sludge in farming. Directive 86/278, soil protection when sewage sludge is used in agriculture. 12 June 1986. European Economic Community, Brussels, Belgium.

Eid, E.M., and K.H. Shaltout. 2014. Monthly variations of trace elements accumulation and distribution in above- and below-ground biomass of *Phragmites australis* (Cav.) Trin. ex Steudel in Lake Burullus (Egypt): A biomonitoring application. Ecol. Eng. 73:17–25. doi:10.1016/j.ecoleng.2014.09.006

Fresquez, P., R. Francis, and G. Dennis. 1990. Sewage sludge effects on soil and plant quality in a degraded, semiarid grassland. J. Environ. Qual. 19:324–329. doi:10.2134/jeq1990.00472425001900020020x

Hanson, R., K. Armstrong, J. de Ruiter, A. Hay, and G. Milne. 2006. Cereal forage breeding for New Zealand agriculture. In: C.F. Mercer, editor, Breeding for success: Diversity in action. 13th Australasian Plant Breeding Conference. Christchurch, New Zealand. 18–21 April 2006. p. 84–92.

Harrington, K.C., A. Thatcher, and P.D. Kemp. 2006. Mineral composition and nutritive value of some common pasture weeds. New Zealand Plant Protection 59:261–265.

IBM (International Business Machines Corp.). 2013. SPSS Statistics V22.0. Armonk, NY.

Jokova, M., O. Kostov, and O. Van Cleemput. 1997. Cation exchange and reducing capacities as criteria for compost quality. Biol. Agric. Hortic. 14:187–197. doi:10.1080/01448765.1997.9754809

Kastratović, V., S. Krivokapić, D. Đurović, and N. Blagojević. 2013. Seasonal changes in metal accumulation and distribution in the organs of *Phragmites australis* (common reed) from Lake Skadar, Montenegro. Journal of the Serbian Chemical Society 78:1241–1258. doi:10.2298/JSC121026153K

Khoshgoftar, A., H. Shariatmadari, N. Karimian, M. Kalbasi, S. Van der Zee, and D. Parker. 2004. Salinity and zinc application effects on phytoavailability of cadmium and zinc. Soil Sci. Soc. Am. J. 68:1885–1889. doi:10.2136/sssaj2004.1885

Knowles, O.A., B.H. Robinson, A. Contangelo, and L. Clucas. 2011. Biochar for the mitigation of nitrate leaching from soil amended with biosolids. Sci. Total Environ. 409:3206–3210. doi:10.1016/j.scitotenv.2011.05.011

Kostov, O., V. Rankov, G. Atanacova, and J.M. Lynch. 1991. Decomposition of sawdust and bark treated with cellulose-decomposing microorganisms. Biol. Fertil. Soils 11:105–110. doi:10.1007/BF00336373

LeBlanc, R.J., P. Matthews, and R.P. Richard, editors. 2009. Global atlas of excreta, wastewater sludge, and biosolids management: Moving forward the sustainable and welcome uses of a global resource. United Nations Human Settlements Program, Nairobi, Kenya.

Lomonte, C., A.I. Doronila, D. Gregory, A.J.M. Baker, and S.D. Kolev. 2010. Phytotoxicity of biosolids and screening of selected plant species with potential for mercury phytoextraction. J. Hazard. Mater. 173:494–501. doi:10.1016/j.jhazmat.2009.08.112

Lopes, C., M. Herva, A. Franco-Uría, and E. Roca. 2011. Inventory of heavy metal content in organic waste applied as fertilizer in agriculture: Evaluating the risk of transfer into the food chain. Environ. Sci. Pollut. Res. Int. 18:918–939. doi:10.1007/s11356-011-0444-1

Lu, Q., Z.L. He, and P.J. Stoffella. 2012. Land application of biosolids in the USA: A review. Appl. Environ. Soil Sci. 11.

Marchetti, V., A. Clément, P. Gérardin, and B. Loubinoux. 2000. Synthesis and use of esterified sawdusts bearing carboxyl group for removal of cadmium(II) from water. Wood Sci. Technol. 34:167–173. doi:10.1007/s002260000040

McNaught, K. 1970. Diagnosis of mineral deficiencies in grass-legume pastures by plant analysis. In: Proceedings of the 11th International Grassland Congress, Surfers Paradise, QLD, Australia. 12–23 Apr. 1970. Univ. Queensland Press, Saint Lucia, Queensland. p. 334–338.

Miaomiao, H., L. Wenhong, L. Xinqiang, W. Donglei, and T. Guangming. 2009. Effect of composting process on phytotoxicity and speciation of copper, zinc and lead in sewage sludge and swine manure. Waste Manag. 29:590–597. doi:10.1016/j.wasman.2008.07.005

Microsoft Office. 2013. Microsoft Excel 2013. Microsoft Corporation, Redmond, WA.

Moir, J.L., G.R. Edwards, and L.N. Berry. 2013. Nitrogen uptake and leaching loss of thirteen temperate grass species under high N loading. Grass Forage Sci. 68:313–325. doi:10.1111/j.1365-2494.2012.00905.x

Mok, H.F., R. Majumder, W.S. Laidlaw, D. Gregory, A.J.M. Baker, and S.K. Arndt. 2013. Native Australian species are effective in extracting multiple heavy metals from biosolids. Int. J. Phytoremediation 15:615–632. doi:10.1080/15226514.2012.723063

Mugica-Alvarez, V., V. Cortés-Jiménez, M. Vaca-Mier, and V. Domínguez-Soria. 2015. Phytoremediation of mine tailings using *Lolium multiflorum*. Intl. J. Environ. Sci. Dev. 6:246–251. doi:10.7763/IJESD.2015.V6.599

NZWWA (New Zealand Water and Wastewater Association). 2003. Guidelines for the safe application of biosolids to land in New Zealand. Ministry for the Environment, New Zealand Water and Wastewater Association, Wellington, New Zealand.

Oliver, D.P., R. Hannam, K. Tiller, N. Wilhelm, R.H. Merry, and G. Cozens. 1994. The effects of zinc fertilization on cadmium concentration in wheat grain. J. Environ. Qual. 23:705–711. doi:10.2134/jeq1994.00472425002300040013x

Paramashivam, D., T. Clough, N. Dickinson, J. Horswell, O. Lense, L. Clucas, and B. Robinson. 2015. The effect of pine waste and pine-biochar on nitrogen mobility in biosolids. J. Environ. Qual.

Reiser, R., M. Simmler, D. Portmann, L. Clucas, R. Schulin, and B. Robinson. 2014. Cadmium concentrations in New Zealand pastures: Relationships to soil and climate variables. J. Environ. Qual. 43:917–925. doi:10.2134/jeq2013.09.0367

Robinson, B.H., S.R. Green, B. Chancerel, T.M. Mills, and B.E. Clothier. 2007. Poplar for the phytomanagement of boron contaminated sites. Environ. Pollut. 150:225–233. doi:10.1016/j.envpol.2007.01.017

Santibanez, C., C. Verdugo, and R. Ginocchio. 2008. Phytostabilization of copper mine tailings with biosolids: Implications for metal uptake and productivity of *Lolium perenne*. Sci. Total Environ. 395:1–10. doi:10.1016/j.scitotenv.2007.12.033

Shahid, S.A., and A. Al-Shankiti. 2013. Sustainable food production in marginal lands—Case of GDLA member countries. Intl. Soil Water Conserv. Res. 1:24–38. doi:10.1016/S2095-6339(15)30047-2

Simmler, M., L. Ciadamidaro, R. Schulin, P. Madejoìn, R. Reiser, L. Clucas, P. Weber, and B. Robinson. 2013. Lignite reduces the solubility and plant uptake of cadmium in pasturelands. Environ. Sci. Technol. 47:4497–4504. doi:10.1021/es303118a

Sinclair, A., and A. Edwards. 2008. Micronutrient deficiency problems in agricultural crops in Europe. In: B. Alloway, editor, Micronutrient deficiencies in global crop production. Springer, the Netherlands. p. 225–244.

Singh, R.P., and M. Agrawal. 2008. Potential benefits and risks of land application of sewage sludge. Waste Manag. 28:347–358. doi:10.1016/j.wasman.2006.12.010

Smith, G.S., I.S. Cornforth, and H.V. Henderson. 1985. Critical leaf concentrations for deficiencies of nitrogen, potassium, phosphorus, sulphur, and magnesium in perennial ryegrass. New Phytol. 101:393–409. doi:10.1111/j.1469-8137.1985.tb02846.x

Smith, M.T.E., and M. Tibbett. 2004. Nitrogen dynamics under *Lolium perenne* after a single application of three different sewage sludge types from the same treatment stream. Bioresour. Technol. 91:233–241. doi:10.1016/S0960-8524(03)00205-0

Sommers, L. 1977. Chemical composition of sewage sludges and analysis of their potential use as fertilizers. J. Environ. Qual. 6:225–232. doi:10.2134/jeq1977.00472425000600020026x

Torri, S., and R. Lavado. 2009. Plant absorption of trace elements in sludge amended soils and correlation with soil chemical speciation.

Trillas, M.I., E. Casanova, L. Cotxarrera, J. Ordovás, C. Borrero, and M. Avilés. 2006. Composts from agricultural waste and the *Trichoderma asperellum* strain T-34 suppress *Rhizoctonia solani* in cucumber seedlings. Biol. Control 39:32–38. doi:10.1016/j.biocontrol.2006.05.007

USEPA. 1993. The standards for the use or disposal of sewage sludge, Title 40 of the Federal Regulations Part 503. USEPA, Washington, DC.

Vasseur, L., C. Cloutier, A. Labelle, J.N. Duff, C. Beaulieu, and C. Ansseau. 1996. Responses of indicator bacteria to forest soil amended with municipal sewage sludge from aerated and non-aerated ponds. Environ. Pollut. 92:67–72. doi:10.1016/0269-7491(95)00079-8

White, P.J., and M.R. Broadley. 2009. Biofortification of crops with seven mineral elements often lacking in human diets–iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytol. 182:49–84. doi:10.1111/j.1469-8137.2008.02738.x

Yu, B., Y. Zhang, A. Shukla, S.S. Shukla, and K.L. Dorris. 2000. The removal of heavy metal from aqueous solutions by sawdust adsorption: removal of copper. J. Hazard. Mater. 80:33–42. doi:10.1016/S0304-3894(00)00278-8

Zaleski, K.J., K.L. Josephson, C.P. Gerba, and I.L. Pepper. 2005. Potential regrowth and recolonization of salmonellae and indicators in biosolids and biosolid-amended soil. Appl. Environ. Microbiol. 71:3701–3708. doi:10.1128/AEM.71.7.3701-3708.2005