

Production of Biomass Crops Using Biowastes on Low-Fertility Soil: 1. Influence of Biowastes on Plant and Soil Quality

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Abstract

Land application of biosolids to low-fertility soil can improve soil quality by increasing concentrations of macronutrients and trace elements. Mixing biosolids with sawdust could reduce the risks of contaminant accumulation posed by rebuilding soils using biosolids alone. We aimed to determine the effects of biosolids and biosolids-sawdust on the plant quality and chemical composition of sorghum, rapeseed, and ryegrass. Plants were grown in a greenhouse over a 5-mo period in a low-fertility soil amended with biosolids (1250 kg N ha⁻¹), biosolids-sawdust (0.5:1), or urea (200 kg N ha⁻¹). Biosolids application increased the biomass of sorghum, rapeseed, and ryegrass up to 14.0, 11.9, and 4.1 t ha⁻¹ eq, respectively. Mixing sawdust with biosolids resulted in a growth response similar to biosolids treatments in rapeseed but nullified the effect of biosolids in sorghum. Urea fertilization provided insufficient nutrients to promote rapeseed growth and seed production, whereas seed yields after biosolids application were 2.5 t ha⁻¹. Biosolids and biosolids-sawdust application enhanced plant quality by increasing element concentrations, especially Zn, and potentially toxic elements (Cd, Cr, Ni) did not exceed food safety standards. An application of 50 t ha⁻¹ of biosolids, equivalent to 1250 kg N ha⁻¹, did not exceed current soil limits of Cu, Zn, and Cd and hence was effective in rebuilding soil without accumulating contaminants. The effect of mixing sawdust with biosolids varies with plant species but can further enhance plant nutrient quality in biomass and seeds, especially P, Cu, Zn, Mn, Fe, S, and Na.

Core Ideas

- Biosolids application showed potential for Zn enrichment in all plant species.
- Biosolids and sawdust applied to soil enables growth of rapeseed in low-fertility soil.
- Toxic elements like Cd were not increased to levels dangerous for human health.
- Biosolids and sawdust increased seed quality and hence potentially plant products.

GLOBAL INCREASES in population and wealth have resulted in increased production of biowastes, which require sustainable management strategies for their disposal and recycling (Panagos et al., 2013; Río et al., 2011). Biosolids (sewage sludge) are a product of human and industrial effluent. Application of biosolids to agricultural land is widely practiced and can reduce the requirement for mineral fertilizers. Biosolids have also proven suitable to rebuild degraded land (Dere et al., 2012; Oladeji et al., 2013; Speir et al., 2003; Stehouwer et al., 2006), where they can indirectly increase soil C stocks when CO₂-C is fixed by plants whose growth has been promoted by biosolids and their effect on soil quality in terms of physical, chemical, and biological fertility (Torri et al., 2014). Furthermore, in this context biosolids can increase C sequestration by introducing recalcitrant C (Tian et al., 2009), and the formation of metal-organic complexes can limit microbial and enzymatic access, thereby protecting C from rapid mineralization (Keiluweit et al., 2015).

Biosolids can contain elevated concentrations of heavy metals, organic contaminants, and pathogens. This poses potential risks for quality assurance in the human food chain and may negatively affect soil health and function as well as plant growth (Alloway, 2013; Bolan et al., 2014). In addition, organic contaminants and pathogens may pose risks to human health and the environment due to their persistence and potential bioaccumulation in food webs (Clarke and Smith, 2011; Horswell et al., 2010; Sidhu and Toze, 2009). However, treatment technology and processing of biosolids have improved over recent years, and land application as a waste management strategy has become increasingly popular (Park et al., 2011).

Plants grown in biosolids-amended soils can add value to the land through the use of plant parts for industry (e.g., cosmetics and medicine) and bioenergy (e.g., bioethanol, biogas, and biomass burning) purposes. In plants, some of the metals applied with biosolids serve as macro- and micronutrients and

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J. Environ. Qual. 45:1960–1969 (2016)

doi:10.2134/jeq2015.12.0596

Supplemental material is available online for this article.

Received 8 Dec. 2015.

Accepted 25 July 2016.

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Abbreviations: DW, dry weight.

are essential for growth at low concentrations. Over the last century, there has been a reduction in crop quality due to insufficient contents of macro- and micronutrients in soil (Fan et al., 2008; Thomas, 2003); hence, application of micronutrients via biosolids could benefit plant and seed quality (Alloway, 2013). Although in many cases risks of metal accumulation or translocation to grains is limited, plants grown in biosolids-amended soils must be carefully monitored to ensure they do not accumulate metal concentrations in plant tissues above toxicity thresholds (Vamerali et al., 2010).

Some of the negative effects of biosolids addition to soil can also be mitigated by blending the biosolids with wood waste (Ammari et al., 2012). Sawdust can immobilize contaminants (Fiset et al., 2000) and thereby reduce plant uptake. Immobilization and removal of heavy metals, such as Cu, Cr, Ni, Zn, and Cd, by sawdust and other wood waste has been shown in wastewater (Ajmal et al., 1998; Bouziane et al., 2012; Bryant et al., 1992; Bulut and Tez, 2007), where the exchange capacity and general sorption characteristics depend on the materials' contents of cellulose, hemicellulose, pectin, lignin, proteins, and phenolic groups (Bulut and Tez, 2007; Kumar, 2006; Randall et al., 1974).

Plants grown for bioenergy purposes may have a small but significant role in future energy policies (Dickinson et al., 2009). Marginal and degraded land may provide an excellent alternative for growing plants for bioenergy (Field et al., 2008). Combining soil remediation with energy crop production has become increasingly popular because the management of soil contaminants can be combined with waste recycling and profitable output (Dickinson et al., 2009; Evangelou et al., 2012; Gadepalle et al., 2007). Therefore, we chose rapeseed, sorghum, and ryegrass as plant species on the basis that they could add value to the land through fiber or pulp production, through providing materials for food, or through the production of bioenergy through bioethanol, biogas, and biomass burning.

We aimed to determine the plant growth and element concentrations in a low-fertility soil amended with biosolids and a mixture of biosolids-sawdust. Specifically, we sought to identify the effect of nutrients, trace elements, and heavy metals on plant and seed quality. The effects of organic contaminants and pathogens were beyond the scope of the study. We hypothesize that biosolids increase plant quality and that contaminants do not accumulate in plant tissues at concentrations above toxicity thresholds. Because sawdust can be used to immobilize elements, a sawdust application in combination with biosolids can prevent plant uptake of elements that are not essential for plant growth and development. The focus in this manuscript is on plant and soil nutrients and trace elements; N transformation processes are discussed in Part 2 of this study (Esperschütz et al., 2016a).

Materials and Methods

Experimental Setup

In April 2013, 10-L lysimeters were constructed and installed at the Lincoln University plant growth facility (43°38'42" S, 172°27'41" E). Low-fertility soil, as defined according to its low Olsen P of 11 mg L⁻¹, was collected from the North Island of New Zealand near Bideford, mainly classified as orthic brown soil with a clay-loam texture (40°45'56" S, 175°54'42" E). Soil analyses showed the soil at a medium

pH range (pH 6.1), with medium carbon (6.5%) and nitrogen (0.46%) levels and a C/N ratio of 14.3. Cation exchange capacity was determined at 21 me 100 g⁻¹. Potassium, Mg, and Na occurred at concentrations of 0.30, 0.63, and 0.14 me 100 g⁻¹, respectively. The soil was homogenized before it was placed into lysimeters (25 cm in diameter; 29 cm in height). To measure NO₃⁻ leaching, a leachate-sampling device was installed in the bottom of each lysimeter. The device was covered by fleece sheets and a gravel drainage layer to avoid stagnant moisture. Each lysimeter was filled with 10 L of soil at an average soil bulk density of 1.3 g cm⁻³. Soil was packed in three layers to avoid gradients. Lysimeters were incubated at near field capacity conditions and ambient conditions in the greenhouse for 14 wk before treatment application.

The experiment was set up in four soil treatments (control, biosolids, biosolids-sawdust, urea) and arranged in a randomized block design. Biosolids (untreated pond sludge, characterized as Grade "Bb" according to) (NZWWA, 2003) were collected from settlement ponds of the Kaikoura Sewage Treatment Plant; sawdust (*Pinus radiata* D. Don, untreated) was obtained from an adjacent wood-waste disposal area (Kaikoura, New Zealand, 42°21'37.40" S, 173°41'27.35" E). Biosolids were homogenized thoroughly after sieving (≤10 mm). Table 1 provides a detailed description of sawdust and biosolids. The treatments comprised urea (2.11 g dry weight [DW]), biosolids (245 g DW), and the same amount of biosolids mixed with sawdust (123 g DW). The application rates for urea and biosolids were equivalent to 200 and 1250 kg N ha⁻¹, respectively; the biosolids application rate was equivalent to 50 t ha⁻¹ dry weight. For biosolids-sawdust treatments, the sawdust was mixed with the biosolids before application at a ratio of 1:0.5 (biosolids/sawdust). The biosolids and biosolids-sawdust mixtures were applied to the surface of the pots before sowing. Urea (50 kg N ha⁻¹ equivalent) was applied four times over the experimental period. Concentrations of total and Ca(NO₃)₂-extractable, plant-available elements in the initial sawdust and biosolids material are shown in Table 1.

Seeds of ryegrass (*Lolium multiflorum* LAM. Feast II tetraploid Italian ryegrass; 2 g), sorghum [*Sorghum bicolor* (L.) Moench 'Sudanese'], and rapeseed (*Brassica napus* L. 'MAKRO') were sown directly into the lysimeters after treatment application. After germination, sorghum and rapeseed were thinned to three and five plants per lysimeter, respectively. The experiment was maintained for 18 wk in the greenhouse with temperatures ranging between 9 and 20°C during the nighttime (10 PM until 6 AM) and between 14 and 28°C during the daytime. 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 rapeseed, sorghum, and ryegrass was 2160, 1190, and 1060 mm, respectively. The amount of leachate was sampled and recorded weekly throughout the experimental period. Aliquots were stored at -20°C until further analyses (see Esperschütz et al., 2016a). Ryegrass was repeatedly cut back to 2 cm above the soil to simulate grazing. Individual harvests were analyzed separately as reported in Esperschütz et al. (2016b).

Table 1. Initial conditions of the sawdust and biosolids used for the lysimeter experiment at the Lincoln University Plant Growth Unit ($n = 5$; $SE < 10\%$ if not indicated otherwise).

	Sawdust		Biosolids	
	Total	Extractable†	Total	Extractable†
pH	5.7 ± 0.1	n.d.‡	4.5 ± 0.0	n.d.
Dry matter, %	30.3 ± 0.2	n.d.	48.6 ± 1.0	n.d.
Total C, %	47.7 ± 0.1	n.d.	27.1 ± 0.7	n.d.
C/N ratio	908 ± 154	n.d.	10.6 ± 0.1	n.d.
CEC,§ me 100 g ⁻¹	8.0 ± 0.2	n.d.	17.1 ± 0.6	n.d.
Total base saturation, %BS	76.2 ± 0.8	n.d.	86.3 ± 3.0	n.d.
N, %	0.1 ± 0.0	b.d. ± b.d.¶	2.5 ± 0.6	403.8 ± 7.1
P, mg kg ⁻¹	42 ± 1	13 ± 1	5941 ± 42	49 ± 1
K, mg kg ⁻¹	455 ± 6	295 ± 6	3653 ± 34	170 ± 5
S, mg kg ⁻¹	70 ± 1	5 ± 2	8681 ± 140	1193 ± 64
Ca, mg kg ⁻¹	838 ± 11	n.d. ± n.d.	6331 ± 91	n.d. ± n.d.
Mg, mg kg ⁻¹	212 ± 3	185 ± 2	3005 ± 34	349 ± 14
Na, mg kg ⁻¹	40 ± 2	26 ± 1	202 ± 1	54 ± 2
B, mg kg ⁻¹	1.9 ± 0.2	n.d. ± n.d.	26.7 ± 0.1	n.d. ± n.d.
Cu, mg kg ⁻¹	0.8 ± 0.0	0.1 ± 0.0	891.0 ± 18.9	8.9 ± 0.3
Fe, mg kg ⁻¹	116 ± 6	0.5 ± 0.1	14,534 ± 92	77.6 ± 1.7
Mn, mg kg ⁻¹	47 ± 1	33 ± 1	185 ± 5	74 ± 3
Zn, mg kg ⁻¹	8.4 ± 0.4	6.1 ± 1.0	1073.1 ± 26.8	530.7 ± 12.0
Cd, mg kg ⁻¹	n.d. ± n.d.	0.01 ± 0.00	3.97 ± 0.07	1.32 ± 0.02
Ni, mg kg ⁻¹	0.6 ± 0.5	0.03 ± 0.01	20.7 ± 0.4	3.97 ± 0.06
Cr, mg kg ⁻¹	0.2 ± 0.0	n.d. ± n.d.	47.6 ± 0.8	0.03 ± 0.00
Pb, mg kg ⁻¹	n.d. ± n.d.	n.d. ± n.d.	151.3 ± 3.2	n.d. ± n.d.

† Estimation of plant-available elements using 0.05 mol L⁻¹ Ca(NO₃)₂ extraction.

‡ Not determined.

§ Cation exchange capacity.

¶ Below detection.

Analyses and Measurements

A final destructive harvest of all lysimeters was performed after 18 wk. The total plant biomass was weighed to investigate the growth responses of each plant species to soil amendments after oven-drying at 70°C until constant weight. Dried plant parts were further separated into roots, stems, and leaves. Soil attached to roots ≤2 mm was considered as rhizosphere soil. Rhizosphere soil was harvested, subsampled, and stored for further analyses after sieving (≤5 mm). Plant and soil samples were ground using a Retch ZM200 grinder. Plant C and N concentrations were measured using a Vario MAX CN analyzer (Elementar). Pseudo-total elemental analysis was performed using microwave digestion in 8 mL of Aristar nitric acid (±69%), filtered using Whatman 52 filter paper, and diluted with milliQ water to a volume of 25 mL. Sample digestion and analyses was performed using an inductively coupled plasma optical emission spectrometer (720-ES ICP-OES, Varian) as described by Esperschütz et al. (2016b). Concentrations of Al, Co, Sr, and Pb were below concentrations toxic to plants and were unlikely to be significant for human health or ecosystem functioning (Aral and Vecchio-Sadus, 2008; Wuana and Okieimen, 2011) and hence are not further discussed in the present study.

An estimation of the plant-available elements was made using a 0.05 mol L⁻¹ Ca(NO₃)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 were weighed into

50-mL centrifuge tubes and extracted with 30 mL of 0.05 mol L⁻¹ Ca(NO₃)₂ after 2 h of end-over-end shaking and centrifuging at 3200 rpm for 15 min (Whatman 52 filter paper).

Statistical analyses were based on four individual replicates for rapeseed and sorghum and six replicates for ryegrass, respectively. Using SPSS 22 (IBM SPSS statistics), ANOVAs were performed followed by Duncan's post hoc tests to identify homogenous subsets for $\alpha = 0.05$. Graphs were prepared using SigmaPlot 11.0 (Systat Software Inc.).

Results

Biomass and Seed Yield

Biosolids application resulted in a positive growth response in all plant species compared with control treatments during the 18-wk experimental period (Fig. 1a). Mixing sawdust with biosolids resulted in a growth response similar to biosolids treatments in rapeseed but nullified the biosolids effect in sorghum. Urea application increased sorghum and ryegrass biomass but showed no effect on rapeseed biomass compared with control treatments.

When grown on unamended soil, rapeseed produced negligible biomass and did not respond to urea application; hence, no seeds were obtained from control and urea treatments at the end of the experiment. No significant differences were observed between rapeseed seed biomass in biosolids and biosolids-sawdust treatments (Fig. 1b). Soil amendments significantly increased the seed biomass of sorghum, with highest yields in biosolids and urea treatments.

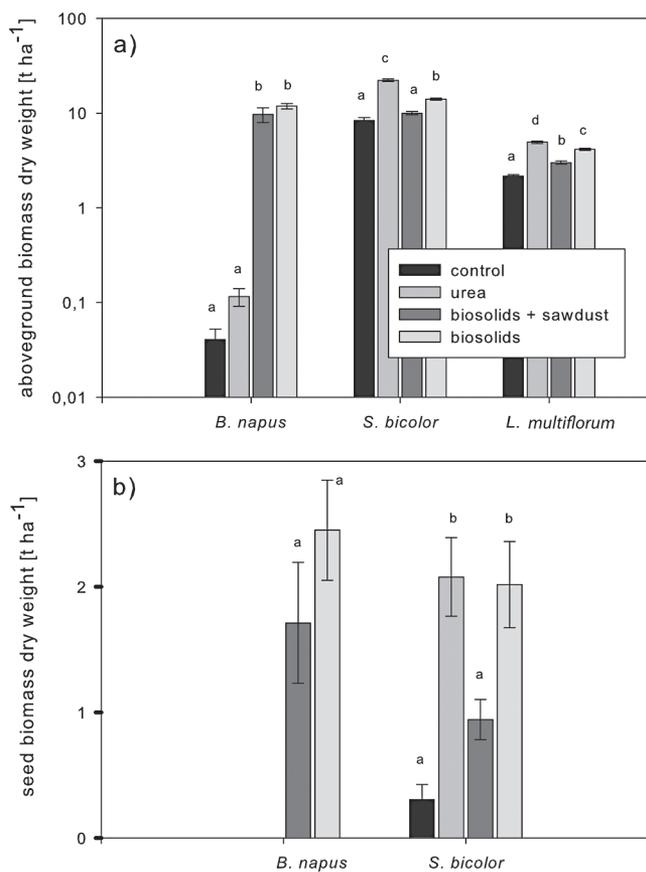


Fig. 1. Total aboveground plant biomass (a) and total seed biomass (b) of sorghum (*S. bicolor*), rapeseed (*B. napus*), and ryegrass (*L. multiflorum*) at the end of the experiment. Significant differences ($p \leq 0.05$) are represented by lowercase letters.

Nutrient and Trace Element Concentration in Plants and Seeds

Biosolids and biosolids-sawdust application to rapeseed showed lower N, S, Ca, and Mg concentrations compared with controls. There was no effect on P and K accumulation (Table 2). An increased accumulation of S and Mg was detected in sorghum biosolids and biosolids-sawdust treatments. In combination with sorghum, urea application showed increased N concentration, whereas decreased concentrations of P and K were detected compared with control treatments. In ryegrass treatments, biosolids and biosolids-sawdust caused an increase in plant P but resulted in lower concentrations of K and Ca compared with controls. Compared with rapeseed and sorghum, the highest concentrations of P and K were detected in ryegrass, irrespective of soil treatments. A detailed discussion of N uptake and leaching was performed by (Esperschütz et al., 2016a).

In rapeseed, biosolids and biosolids-sawdust treatments resulted in lower B and Fe but showed up to 10-fold higher concentrations of Zn compared with control treatments (Table 3). Biosolids and biosolids-sawdust caused fivefold and eightfold higher Zn concentrations in sorghum, respectively. Biosolids-sawdust application resulted in Cu and Mn accumulations that were up to 30 and 40% higher than biosolids treatments. In ryegrass, biosolids treatments showed increased Cu, Mn, and Zn concentrations up to 65, 85, and 540%, respectively, and levels in biosolids-sawdust treatments were increased by 65, 110, and

Table 2. Total macronutrients in plant biomass in response to different soil amendments.†

	Control	Urea	Biosolids-sawdust	Biosolids
	%			
Rapeseed				
N	4.50 ± 0.09b‡	4.55 ± 0.36b	0.58 ± 0.11a	0.47 ± 0.04a
P	0.07 ± 0.02a	0.09 ± 0.01a	0.08 ± 0.01a	0.07 ± 0.01a
K	1.29 ± 0.56ab	1.48 ± 0.49b	0.53 ± 0.13ab	0.39 ± 0.04a
S	1.49 ± 0.09c	0.99 ± 0.04b	0.24 ± 0.03a	0.21 ± 0.02a
Ca	4.58 ± 0.29b	5.00 ± 0.51b	1.29 ± 0.18a	1.19 ± 0.05a
Mg	0.30 ± 0.02b	0.33 ± 0.00b	0.13 ± 0.02a	0.11 ± 0.01a
Sorghum				
N	0.21 ± 0.05a	1.51 ± 0.16b	0.29 ± 0.01a	0.19 ± 0.03a
P	0.10 ± 0.01bc	0.04 ± 0.00a	0.17 ± 0.00c	0.10 ± 0.01b
K	0.81 ± 0.03bc	0.31 ± 0.00a	0.92 ± 0.07c	0.61 ± 0.11b
S	0.07 ± 0.00a	0.06 ± 0.00a	0.09 ± 0.00b	0.09 ± 0.01b
Ca	0.40 ± 0.02a	0.49 ± 0.03b	0.50 ± 0.02b	0.52 ± 0.03b
Mg	0.11 ± 0.00a	0.16 ± 0.01c	0.12 ± 0.01ab	0.14 ± 0.01b
Ryegrass				
N	2.39 ± 0.04a	3.35 ± 0.09c	2.63 ± 0.12b	2.56 ± 0.05ab
P	0.30 ± 0.01b	0.17 ± 0.00a	0.35 ± 0.02c	0.43 ± 0.02d
K	3.21 ± 0.03d	1.93 ± 0.02a	3.00 ± 0.12c	2.73 ± 0.06b
S	0.38 ± 0.01bc	0.26 ± 0.00a	0.35 ± 0.02b	0.40 ± 0.01c
Ca	0.80 ± 0.01c	0.77 ± 0.02bc	0.66 ± 0.02a	0.73 ± 0.01b
Mg	0.23 ± 0.00b	0.24 ± 0.01bc	0.21 ± 0.01a	0.23 ± 0.00b

† A detailed investigation of macronutrients in individual ryegrass biomass throughout the experiment is reported in Esperschütz et al. (2016b).

‡ The average macronutrient concentrations are based on a weighted average across individual harvests. Lowercase letters indicate significant differences between treatments at $p \leq 0.05$.

400%, respectively, compared with controls. Whereas no differences between treatments were detected in Mo concentration in rapeseed, higher Mo concentrations were measured in sorghum and ryegrass biosolids and biosolids-sawdust treatments compared with controls. In ryegrass and in sorghum, a decrease in Mo concentration was detected after urea application.

Macronutrient concentration (Supplemental Table S1) in rapeseed seeds was not significantly different between biosolids and biosolids-sawdust. Concentrations of N, P, and S in sorghum treatments were up to 60, 108, and 57% higher after biosolids application compared with controls. Among micronutrients (Supplemental Table S2), Cu, Fe, and Zn were increased in sorghum seeds after biosolids-sawdust application up to 25, 305,

Table 3. Total micronutrients in plant biomass in response to different soil amendments.†

	Control	Urea	Biosolids-sawdust	Biosolids
	mg kg ⁻¹			
Rapeseed				
B	41.6 ± 3.6b‡	64.7 ± 13.2a	19.2 ± 2.2c	16.5 ± 0.6c
Cu	2.1 ± 0.4a	2.5 ± 0.1a	2.7 ± 0.3a	2.3 ± 0.2a
Fe	46.5 ± 6.3b	53.0 ± 3.0b	20.5 ± 3.4a	15.8 ± 1.7a
Mn	21.4 ± 0.2a	41.2 ± 6.8b	25.3 ± 3.6a	15.7 ± 1.7a
Zn	21.7 ± 6.8a	31.4 ± 6.9a	249.7 ± 16.9b	232.0 ± 18.4b
Mo	3.11 ± 1.66a	0.76 ± 0.08a	2.03 ± 0.58a	3.05 ± 0.54a
Sorghum				
B	2.7 ± 0.3a	2.9 ± 0.3a	2.7 ± 0.3a	2.8 ± 0.3a
Cu	2.0 ± 0.1a	2.2 ± 0.3ab	3.7 ± 0.1c	2.8 ± 0.2b
Fe	22.8 ± 3.1a	35.2 ± 6.7a	22.9 ± 0.6a	26.2 ± 4.5a
Mn	11.9 ± 0.4a	10.9 ± 1.6a	19.6 ± 2.1b	13.9 ± 0.9a
Zn	9.4 ± 0.3a	6.5 ± 1.0a	81.6 ± 6.6c	54.8 ± 2.1b
Mo	0.46 ± 0.16b	0.33 ± 0.02a	0.93 ± 0.08c	1.18 ± 0.12c
Ryegrass				
B	11.4 ± 1.0b	8.9 ± 0.3a	9.9 ± 0.8ab	10.5 ± 0.3ab
Cu	5.9 ± 0.1a	6.0 ± 0.2a	8.7 ± 0.4b	10.3 ± 0.6c
Fe	96.0 ± 3.9a	105.8 ± 13.6a	105.5 ± 7.1a	118.7 ± 14.4a
Mn	37.4 ± 1.0a	35.2 ± 0.8a	51.0 ± 2.4b	60.2 ± 1.7c
Zn	21.6 ± 2.3a	19.8 ± 0.7a	97.1 ± 3.7b	150.4 ± 8.3c
Mo	1.11 ± 0.08b	0.68 ± 0.02a	2.33 ± 0.48c	4.24 ± 0.29d

† A detailed investigation of macronutrients in individual ryegrass biomass throughout the experiment is reported in Esperschütz et al. (2016b).

‡ The average macronutrient concentrations are based on a weighted average across individual harvests. Lowercase letters indicate significant differences between treatments at $p \leq 0.05$.

and 51%, respectively. Concentrations of Cu, Fe, Zn, and Mn were higher compared with biosolids treatments. Rapeseed seeds showed higher concentrations of Mn in biosolids-sawdust treatments compared with biosolids.

Contaminant Uptake into Plants

Compared with controls, a higher concentration of Ni was detected in rapeseed and ryegrass after biosolids and biosolids-sawdust addition (Table 4). Ryegrass showed Ni concentrations between 2.5-fold and 5-fold higher compared with sorghum and rapeseed, irrespective of soil treatment. The accumulation of Cd in rapeseed was not affected by any treatment. Biosolids and biosolids-sawdust treatments showed increased Cd concentrations in ryegrass, to a similar level as in rapeseed. In sorghum biomass, Cd concentrations were low (≤ 0.1 mg kg⁻¹) and close to detection limit (0.01 mg kg⁻¹). Cadmium concentrations in rapeseed seeds were not affected by mixing sawdust with biosolids compared with pure biosolids (Supplemental Table S3). In sorghum, similar to rapeseed, no differences were observed regarding Cd accumulation. Biosolids and biosolids-sawdust resulted in an increase in Li and As uptake in ryegrass and an increased Li accumulation in rapeseed, respectively (Table 4).

Extractable Elements in Rhizosphere Soil

Limited amounts of rhizosphere soil was harvested from rapeseed; hence, analyses of macronutrients and trace elements in rhizosphere soil were only performed for sorghum and ryegrass treatments. A plant-available fraction of macro- and micronutrients was estimated after Ca(NO₃)₂ extraction. Available N (NO₃⁻, NO₂⁻, and NH₄⁺) was determined after KCl extraction and has been discussed in detail by (Esperschütz et al., 2016a).

Sorghum rhizosphere soil showed increased plant-available P in response to biosolids application and an increase in S after biosolids and biosolids-sawdust application (Table 5). In ryegrass treatments, Mg and Zn increased in response to biosolids application, whereas P, S, and Mn showed higher concentrations in biosolids- and biosolids-sawdust-amended soil. The potential contaminants Cd, Cr, Ni, and Pb were below detection limit in all treatments (<0.01, <0.02, <0.08, and <0.13 mg kg⁻¹, respectively; data not shown).

Plant species had a significant effect on extractable elements in the rhizospheres of plants in control, urea, and biosolids treatments but not in the biosolids-sawdust treatment. Control, urea, and biosolids treatments with sorghum reduced available K compared with ryegrass (Table 5). In urea treatments, sorghum growth lowered concentrations of K, Mg, Mn, and Zn compared with ryegrass. After biosolids application, available K, S, Mg, Cu, and Zn concentrations were reduced compared with ryegrass. Mixing biosolids with sawdust negated any plant species effect on the availability of nutrients and trace elements in soil.

Discussion

Plant and Seed Yield in Response to Biowaste Application

According to the characterization of the initial soil used for the experiment, all plants grown in the control treatment encountered a low-fertility environment, particularly N and P. Urea application increased sorghum and ryegrass biomass significantly compared with controls, biosolids, and biosolids-sawdust treatments. In urea treatments, 200 kg ha⁻¹ of plant-available N was applied. Because urea rapidly hydrolyses to form NH₄⁺ (Robertson and Groffman, 2015), the urea treatments may have

provided more available N than the biowaste treatments, which contain >95% organic N (Gilmour et al., 2003). The lack of rapeseed growth in the urea treatment indicates that nutrients other than N were limiting growth. Rapeseed has shown higher requirements for S and P compared with other species, such as wheat or maize (Abdallah et al., 2010; Ahmad et al., 2007; Chen et al., 2015; Jackson, 2000). High N levels in rapeseed control and urea treatments were most likely the effect of a N concentration effect due to scarce biomass growth in these treatments

compared with biosolids and biosolids-sawdust. In the control soil, Olsen P was only 11 mg L⁻¹, and the soil total S content in the initial soil was measured at 405 mg kg⁻¹, which was characterized as S-deficient according to Rajendram et al. (2008). The application of the equivalent of 375 kg ha⁻¹ of total S and 250 kg ha⁻¹ of total P with biosolids and biosolids-sawdust, of which 41.5 and 2.5 kg ha⁻¹, respectively, were available to plants, provided sufficient S and P for rapeseed growth. By applying nutrients and trace elements with biosolids and biosolids-sawdust,

Table 4. Contaminant accumulation in plant biomass in response to different soil amendments.†

	Control	Urea	Biosolids-sawdust	Biosolids
	mg kg ⁻¹			
Rapeseed				
Cd	0.24 ± 0.03a‡	0.27 ± 0.04a	0.30 ± 0.02a	0.26 ± 0.03a
Ni	0.43 ± 0.26a	0.48 ± 0.24a	1.41 ± 0.12b	1.25 ± 0.15b
Cr	0.87 ± 0.71a	0.48 ± 0.16a	1.00 ± 0.26a	0.77 ± 0.23a
As	<0.50	0.22 ± 0.02	<0.18	<0.02
Li	<0.07	0.07 ± 0.00a	0.28 ± 0.02b	0.23 ± 0.03b
Sorghum				
Cd	n.d. ± n.d.§	0.01 ± 0.01a	0.11 ± 0.02b	0.09 ± 0.02b
Ni	1.02 ± 0.22bc	0.52 ± 0.09a	0.73 ± 0.02ab	0.97 ± 0.08b
Cr	2.21 ± 0.53c	0.97 ± 0.10a	1.50 ± 0.08ab	1.71 ± 0.07bc
As	<0.38	0.35 ± 0.11a	<0.18	0.50 ± 0.23a
Li	<0.01	0.02 ± 0.02a	0.01 ± 0.00a	<0.03a
Ryegrass				
Cd	0.03 ± 0.01ab	0.02 ± 0.00a	0.13 ± 0.00b	0.26 ± 0.06c
Ni	2.57 ± 0.22a	2.52 ± 0.32a	3.79 ± 0.14b	4.47 ± 0.21b
Cr	0.02 ± 0.00a	0.03 ± 0.00a	0.03 ± 0.00a	0.03 ± 0.00a
As	0.36 ± 0.07a	0.15 ± 0.04a	1.03 ± 0.08b	0.71 ± 0.04b
Li	0.17 ± 0.03a	0.09 ± 0.01a	0.92 ± 0.08b	0.58 ± 0.02b

† A detailed investigation of macronutrients in individual ryegrass biomass throughout the experiment is reported in Esperschütz et al. (2016b).

‡ The average macronutrient concentrations are based on a weighted average across individual harvests. Lowercase letters indicate significant differences between treatments at $p \leq 0.05$.

§ Not determined.

Table 5. Extractable (CaNO₃) nutrient and trace element concentrations in soil detected in combination with different plant species and soil amendments.

	Control	Urea	Biosolids-sawdust	Biosolids
	mg kg ⁻¹			
Sorghum				
P	0.65 ± 0.06a† ↑	0.60 ± 0.00a	0.79 ± 0.13b	0.60 ± 0.01a
K	17.1 ± 0.66ab↓	14.4 ± 0.55a↓	18.9 ± 2.61b	16.0 ± 1.03ab↓
S	4.39 ± 0.24a	3.53 ± 0.35a	10.19 ± 2.26b	7.88 ± 1.33b↓
Mg	90.0 ± 3.43b	70.5 ± 1.40a↓	90.6 ± 4.01b	84.9 ± 2.57b↓
Cu	0.01 ± 0.00a	0.01 ± 0.00a	0.02 ± 0.01a	0.01 ± 0.00a↓
Fe	1.10 ± 0.09a	2.15 ± 0.32b	1.01 ± 0.05a	1.29 ± 0.06a↑
Mn	7.29 ± 1.00b ↑	3.10 ± 0.25a↓	3.05 ± 0.76a	4.85 ± 1.67ab
Zn	0.05 ± 0.03a	0.02 ± 0.02a↓	0.33 ± 0.14a	0.36 ± 0.18a↓
Ryegrass				
P	0.48 ± 0.02a↓	0.57 ± 0.05ab	0.62 ± 0.03b	0.62 ± 0.01b
K	23.1 ± 1.86ab↑	26.4 ± 1.92b↑	22.7 ± 1.44ab	19.8 ± 0.69a↑
S	5.19 ± 0.35a	3.34 ± 0.15a	11.85 ± 1.59b	14.54 ± 0.93c↑
Mg	94.1 ± 3.20a	88.8 ± 3.41a↑	98.7 ± 4.09ab	106.8 ± 4.65b↑
Cu	0.02 ± 0.01a	0.02 ± 0.01a	0.05 ± 0.02a	0.03 ± 0.00a↑
Fe	0.88 ± 0.07a	4.64 ± 0.27b	0.97 ± 0.04a	0.96 ± 0.04a↓
Mn	2.07 ± 0.05a↓	6.57 ± 0.46c↑	2.88 ± 0.11b	2.98 ± 0.16b
Zn	0.13 ± 0.06a	0.11 ± 0.01a↑	0.84 ± 0.20a	1.74 ± 0.46b↑

† Lowercase letters indicate significant differences between treatments at $p \leq 0.05$. Arrows indicate significantly higher (↑) or lower (↓) concentrations ($p \leq 0.05$) between plant species grown under the same treatment.

rapeseed biomass was recorded in a similar range compared with mineral fertilization with 150 kg N ha⁻¹ (Muchechei et al., 2011). Although an equivalent of only 20 kg ha⁻¹ available N was applied with biosolids, biosolids application improved soil fertility and performance of rapeseed in the low-fertility soil compared with urea-only treatment.

In sorghum treatments, a high biomass response was observed with mineral N (urea) fertilization, which was in accordance with Fellet and Marchiol (2011) and Marchiol et al. (2007), who found greater biomass production in mineral-fertilized sites (22.1 t ha⁻¹) compared with organically fertilized sites (16.9 t ha⁻¹). A higher biomass yield due to mineral fertilization has been explained by mineral fertilization causing a longer vegetative period, thereby delaying the senescence of the canopy (Fellet and Marchiol, 2011).

Total seed yields obtained from rapeseed biosolids and biosolids-sawdust treatments were comparable to the study by Ahmadi (2010), where plants received biosolids and biosolids-sawdust fertilization at rates between 50 and 150 kg N ha⁻¹, respectively. Jackson (2000) identified a rapeseed seed yield of 2.65 t ha⁻¹ at an optimum of 200 kg N ha⁻¹. Seed yields in our study were 2.5 t ha⁻¹ in biosolids treatments, achieved with an application of only 20 kg ha⁻¹ of plant-available N applied with biosolids. Biosolids application to degraded soil would therefore provide a viable alternative to urea fertilization to grow rapeseed while simultaneously achieving an optimum seed yield.

Sorghum produced seeds in all treatments. Both urea and biosolids resulted in a seed biomass range of 2 t ha⁻¹, which is in the lower range of recorded grain yield of sweet sorghum in North China (Zhao et al., 2009). Biosolids application increased nutrient use efficiency, offering a low-cost strategy to achieve similar grain yield with only 20 kg ha⁻¹ equivalent available N, compared with 200 kg ha⁻¹ available N for urea. An increased seed yield in biosolids treatments can be further linked to Zn because increasing levels of Zn have been shown to improve conditions for pod formation and the number of seeds per pod, thereby enhancing seed yield (Olama et al., 2014). Biosolids-sawdust did not increase biomass or seed yield when compared with control treatments. This could be related to C increase as a result of the sawdust component, stimulating heterotrophic bacteria that immobilize N while they degrade the C substrate (Schmidt et al., 2001).

Nutrient and Trace Elements in Vegetative Plant Biomass

In sorghum and ryegrass plants, we generally detected higher or similar element concentrations in biosolids treatments compared with urea. Higher concentrations of several macro- and micronutrients were found in rapeseed urea compared with biosolids treatments. This was most likely a concentration effect due to scarce biomass growth in the urea treatment. Urea application caused a decrease of P and K in sorghum biomass, presumably a dilution effect, related to a high biomass increase that diluted plant P and K, in combination with a lack of P and K uptake, because only small amounts of P and K were available in soil (Jarrell and Beverly, 1981). These results are in accordance with Riedell (2010), who describes lower shoot P and K concentrations in maize after high-N application. Biosolids application increased Ca, Mg, S, and Cu concentrations in vegetative plant parts of sorghum compared with controls. Although these

elements were increased, the levels in all treatments were in the reported range of 1000 to 50,000, 1500 to 3500, 1000 to 5000, and 1 to 10 mg kg⁻¹, which are levels typically found in food crops (Alloway, 2013).

Irrespective of plant species, biosolids and biosolids-sawdust treatments increased plant Zn in the order of rapeseed > ryegrass > sorghum, above the typical range found in crop species (Alloway, 2013). Zinc concentrations in sorghum were below 15 to 20 mg kg⁻¹ in control and urea treatments, a level that has been reported as a typical range for adequate growth in most crop species (Marschner, 1995). In rapeseed, Zn concentrations in biosolids and biosolids-sawdust treatments were 150% higher compared with sorghum and ryegrass. Generally, Zn is important in many biological functions, but recent studies increasingly show free ionic Zn (Zn²⁺) as more biologically toxic than traditionally presumed (Plum et al., 2010). Visible Zn toxicity in plants has been described above 300 mg kg⁻¹ (Broadley et al., 2007). Our results indicate that biosolids as well as biosolids mixed with sawdust could potentially overcome Zn deficiency in crop species without reaching toxic thresholds.

Contaminant Uptake into Plants

Nickel concentrations increased with biosolids and biosolids-sawdust application in all plant species but were detected in a range typical for food crops (Alloway, 2013). Concentrations of Cd in rapeseed were measured at 0.1 to 0.3 mg kg⁻¹, which has been reported as a normal range in plants (Alloway, 2013; Chaney, 1989). In sorghum and ryegrass biomass, Cd was significantly increased due to biosolids and biosolids-sawdust applications compared with controls but in a range not toxic to human or animal health (Alloway, 2013; Chaney, 1989). This indicates that biosolids and biosolids-sawdust can enhance uptake of essential trace elements in plant parts while not increasing toxic elements like Cd to levels dangerous for animal and human health. Plant species like rapeseed did not accumulate contaminants such as Cd from biosolids and biosolids-sawdust treatments; hence, potentially higher rates of biosolids could be applied without reaching threshold levels for food products. Although there were higher Li concentrations in ryegrass and rapeseed biosolids and biosolids-sawdust treatments, as well as higher As concentrations in biosolids and biosolids-sawdust ryegrass treatments, these levels were below concentrations shown to be toxic to plants and were unlikely to be significant for human health or ecosystem functioning (Aral and Vecchio-Sadus, 2008; Wuana and Okieimen, 2011).

Influence of Biosolids and Sawdust on Seed Quality

In rapeseed treatments, an increase in seed quality compared with control treatments could not be verified because no seed production was obtained in control and urea treatments. Macro- and micronutrients in seeds in biosolids and biosolids-sawdust treatments (Supplemental Tables S1 and S2) were in a range as expected under normal P fertilization or higher in cases of Ca, Zn, Cu, or Fe (Ding et al., 2010). Copper concentrations were higher than the critical content of 2.2 mg kg⁻¹ for Cu deficiency as stated by Khurana et al. (2006); hence, seed Cu concentrations are not correlated to the Cu deficiency of vegetative parts as discussed above. This indicates that biosolids application can increase the quality of rapeseed seeds and therefore potentially

plant oil. Increased mineral concentrations in seeds, especially P and Zn, could be beneficial for seedlings and result in a faster seedling establishment and higher yields (Broadley et al., 2007; Zhu and Smith, 2001).

Sorghum seeds showed higher N, P, and S concentrations in biosolids treatments compared with controls, and biosolids-sawdust further increased Cu, Fe, and Zn compared with control treatments. Using biosolids and sawdust as soil amendments could potentially overcome nutrient deficiency in plant foods, which is becoming an increasingly important global problem (Musa et al., 2012). Concentrations of Cd and Ni in seeds in biosolids and biosolids-sawdust treatments remained low (0.05 and 1.5 mg kg⁻¹, respectively) and did not endanger food safety according to current thresholds (Codex, Alimentarius Commission 2001).

Nutrient Availability in Soil

Biosolids and biosolids-sawdust application influenced P and S concentrations in the rhizosphere; however, different effects were observed in biosolids-sawdust depending on the plant species. This could be related to different nutrient mobilization via different compounds exuded by plant species (Carvalho et al., 2011; Nardi et al., 2000; Ström et al., 2002). However, plants might have influenced nutrient availability via uptake, evapotranspiration, or preferential flow down through root channels (Allaire et al., 2011; Mitchell et al., 1995; Vasquez, 2008). In addition, sawdust could have altered nutrient availability by exerting influence on microbial communities through compounds leached into the soil from sawdust. It has been previously reported that organic compounds such as phenols, tannins, lignin, and terpenes can be leached from sawdust, which could potentially alter microbial processes in toxic concentrations. (Hedmark and Scholz, 2008; Keeling and Bohlmann, 2006; Rupar and Sanati, 2005; Tao et al., 2005).

An increase of Mg, Mn, and Zn was seen in ryegrass rhizosphere in response to biosolids. This could be due to a lower demand for plant nutrients due to lower biomass production compared with sorghum and hence higher concentrations of available elements remaining in the soil. Cadmium, Cr, and Ni were not detected in plant-available concentrations, which is in accordance with the biomass results, suggesting these contaminants do not accumulate in plants after a biosolids application rate of 50 t ha⁻¹.

Sorghum and ryegrass exerted a diverse influence on the availability of nutrients and trace elements in soil, presumably due to root growth and exudation (Nascimento and Xing, 2006). Potassium, S, Mg, Mn, Cu, and Zn had lower availability in sorghum treatments compared with ryegrass. Root exudates, including organic anions, are responsible for metal complexation and uptake into plants or immobilization in soil (Bais et al., 2006). Our results suggest a higher influence of sorghum exudates on the nutrient availability in soil compared with ryegrass. However, biosolids-sawdust application was the only treatment where no differences occurred between sorghum and ryegrass treatments with regard to nutrient availability. Sawdust may well provide higher amounts of available C, which likely attracted heterotrophic bacteria, consuming root exudates and available nutrient sources in soil (Cébron et al., 2015). Therefore, sawdust could have overcome the difference in plant exudation (in

volume as well as composition) between sorghum and ryegrass by stimulating the rhizosphere microbial biomass.

The leaching of heavy metals from biosolids-amended soils has been discussed in several studies with different types of biosolids and soil types (Brown et al., 1983; Esteller et al., 2009; Gove et al., 2001). Heavy metal leaching in these studies was reported in very low or negligible concentrations and hence was not focus of our experiment. However, because an increasing amount and repeated biosolids application to land in the near future could potentially pose risks in terms of heavy metal leaching into groundwater, the leaching composition of heavy metals is an interesting topic for further research.

Conclusions

High rates of biosolids application to low-fertility land in combination with rapeseed, sorghum, and ryegrass provided a complete fertilization with macro- and micronutrients without significantly increasing contaminants such as Cr, Ni, or Cd in the plant biomass. Biosolids application increased plant and seed biomass as well as seed and crop quality by enhancing the concentration of important elements in the vegetative biomass and seeds, especially Zn and P. Results from blending biosolids with fresh sawdust varied strongly depending on plant species, and therefore the use of sawdust in these scenarios must be decided on a case-by-case basis depending on the required outcome. The use of sawdust can immobilize and reduce available N for plant growth; however, sawdust can also enhance plant quality with respect to individual nutrients in biomass and seeds, including P, Cu, Zn, Mn, Fe, S, or Na. Further investigation of biosolids application rates in combination with sawdust and different plant species could maximize the plant quality benefits derived from sawdust, thereby improving its value for recycling in combination with biosolids on land instead of increasing landfill deposition. In addition to metal contaminants and N, analyses of organic contaminants and pathogens have to be included into future studies.

Acknowledgments

The study was supported by the Deutsche Forschungsgemeinschaft (DFG, ES 416/1-1), the Center of Integrated Biowastes (CIBR), and Plant & Food Research (Blueskies). The authors thank H. Lowe (Lowe Environmental Impact) for assisting with soil collection; Lincoln University for providing facilities and laboratories; and L. Clucas, B. Richards, D. Paramashivan, S. Mammun, Y. Kim, T. Zhongtao, and H. Franklin for technical and physical assistance.

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