Remediation of an acidic mine spoil: Miscanthus biochar and lime amendment affects metal availability, plant growth, and soil enzyme activity

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HIGHLIGHTS

- Blue Wildrye shoot and root growth was improved after lime treatment of spoil.
- Miscanthus biochar had minimal impact on reducing extractable metal concentrations.
- Lime increased mine spoil pH and reduced extractable metal concentrations.
- Water leachates were lower in dissolved metals after lime and biochar additions.
- Microbial enzymatic activity was influenced only at highest biochar and lime application.

ARTICLE INFO

ABSTRACT

Biochar may be a tool for mine spoil remediation; however, its mechanisms for achieving this goal remain unclear. In this study, Miscanthus (Miscanthus giganteus) biochar was evaluated for its ability to reclaim acidic mine spoils (pH < 3) through reducing metal availability, improving soil microbial enzymatic activity, and initial growth of grass seedlings. Biochar was applied at 0, 1, 2.5 and 5% (w/w) along with lime/no lime and fertilizer additions. Blue Wildrye (Elymus glaucus cv. 'Elkton') was planted and later the shoots and roots were collected and metal concentrations determined. Afterwards, each pot was leached with deionized water, and the leachate analyzed for pH, electrical conductivity (EC), dissolved organic carbon (DOC) and soluble metal concentrations. After drying, the spoil was extracted with 0.01 M $CaCl_2$ and Mehlich 3 (M3) to determine extractable Al, Cu, and Zn concentrations. Additionally, microbial activity was measured using a fluorescent β-glucosidase and N-acetyl-β-D-glucosaminidase assay. Spoil treated with lime and biochar had significantly greater pH and EC values. Significantly greater β-glucosidase activity occurred only in the 5% biochar plus lime treatment, while N-acetyl-β-D-glucosaminidase activities were not altered. Metal concentrations in rye shoot and roots were mixed. Lime additions significantly reduced extractable metal concentrations. Increasing biochar rates alone significantly reduced leachate DOC concentrations, and subsequently reduced leachable metal concentrations. Surprisingly, miscanthus biochar, by itself, was limited at mitigation, but when combined with lime, the combination was capable of further reducing extractable metal concentrations and improving β-glucosidase enzyme activity.

1. Introduction

Mine spoils wastes are a waste byproduct from mining activities. Spoils are expensive to reclaim, so they often remain untreated

(Brown et al., 2003). Sulfide ores and unrecoverable heavy metals (e.g., Al, Cu, Zn) in the spoils can cause numerous environmental issues. For example, after exposure to rain and oxygen, sulfide ores undergo oxidation to SO_4^{-2}, which will acidify the spoils (pH values < 2 to 4) and facilitates solubilization and release of heavy metals (Skousen et al., 2000; Kelly et al., 2014). Transport of heavy metals to surface and ground water systems is an environmental (Powlson et al., 2011) and human health concern (Roy and McDonald, 2015), which highlight a central management directive for mine spoil reclamation-to improve soil plant relationships and associate phytostabilization.

Phytostabilization of mine spoil can be a complex process due to several factors. Mine spoils can have unfavorable soil chemical characteristics (e.g., very low pH, phytotoxic metals; Dudka and Adriano, 1997; Paz-Ferreiro et al., 2014), physical limitations (e.g., high bulk density, low soil moisture retention, poor aggregation; Mendez and Maier, 2008), and unsuitable microbial habitat conditions (e.g., low soil organic matter and poor nutrient turnover; Gentcheva-Kostadinova et al., 1994). These aspects limit plant growth. As such, reclamation plans usually involve applying soil amendments to neutralize their low pH, and to raise organic matter levels that favor microbial enzymatic activity for nutrient cycling. Past reclamation studies have employed amendments consisting of lime (Illera et al., 2004; Meiman et al., 2012); composted manures (Norland, 1993; Gudichuttu, 2014); municipal biosolids (Jenness, 2001; Haering et al., 2000; Brown et al., 2003), pulp sludge and fly ash (Li and Daniels, 1997; Gorman et al., 2000), and combinations of these (e.g., Brown et al., 2003). Most of these studies are successful at improving spoil characteristics and better plant growth often occurred, however the duration of the positive effects is uncertain.

An underlying concern in these studies was that a considerable tonnage of amendment was applied per ha (44–220 t/ha in Brown et al., 2003; 45 to 269 t/ha in Gudichuttu, 2014), which could potentially raise N, P, and metals concentrations to be out of balance with plant nutritional requirements (Corker, 2006; Larney et al., 2011). Repeated amendment applications are often necessary because the noted benefits are highly time-dependent, due to carbon mineralization (Larney and Angers, 2012; Gudichuttu, 2014). Additionally, biosolids and composted manures have perceived odor issues and potential release of organic pollutants (Larney et al., 2011).

Biochar has been suggested as an alternate amendment to reclaim mine spoils and mine-impacted soils (Beesley et al., 2010, 2011; Cao et al., 2011). Biochar is a solid product from the pyrolysis of agricultural, forestry and municipal waste byproducts (Laird et al., 2009; Novak et al., 2013). Biochar offers the same benefits as the other amendments; including adding C-based substrates to rebuild soil organic matter levels (Brockhoff et al., 2010; Anawar et al., 2015); promoting heavy metal sequestration (Ehsan et al., 2014; Ippolito et al., 2017a); acting as a liming agent (Yuan and Xu, 2011; Kloss et al., 2012); stimulating microbial organisms involved in nutrient cycling (Ducey et al., 2013); and supplying both plant macro- and micro-nutrients (Novak et al., 2009; Ippolito et al., 2015). Moreover, biochar application rates on mine spoils are typically in the range of 10–40 t/ha (Ehsan et al., 2014; Anawar et al., 2015), although some studies have reported applying up to 74 to 202 t/ha (Reverchon et al., 2015; Phillips et al., 2016). Biochar can offer similar benefits as prior additions, yet as compared to these other amendments, biochar's structural properties favors longer soil residence times (Lehmann et al., 2015; Wang et al., 2016).

Biochar has already been used to remediate mine spoils from the abandoned Formosa Mine (near Riddle, OR; Phillips et al., 2016). In this pot study, two biochars produced from gasified grass seed screenings and mixed conifer wood were applied to spoils at 0 to 202 t/ha, in which wheat (*Triticum aestivum* L.) was planted. Both biochars increased nutrient availability, resulting in significantly more wheat biomass (1.0 g) vs. the un-amended control (0.1 g). However, wheat is not a recommended plant species for revegation and restoration of mine spoils (NRCS, 2005). Moreover, their biochars were selected for soil fertility improvement and not for heavy metal sorption (Phillips et al., 2016).

This research project was designed to further the development of protocols for reclaiming mine spoils at Formosa Mine by evaluating Blue Wildrye growth as an ecologically appropriate grass species (NRCS, 2005; Smith and Hanlon, 2010) and effects on metals (Al, Cu and Zn) availability. In addition to biochar, lime was used as an amendment because of the extreme spoil acidification (pH < 3) and the presence of residual S-bearing materials. Our work is novel because there are few reports that employed lime + biochar as amendments, and we used a grass species recommended for the disturbed ecosystem at the Formosa mine site. We hypothesize that the Miscanthus biochar and lime will increase Blue Wild Rye growth in the mine spoil based on raising pH, sequestering metals, and increasing microbial enzymatic activity. Thus, our experimental objectives were to: i) determine Miscanthus biochars ability after treatment with and without lime addition to sequester metals, raise pH values in spoils and water leachates, reduce rye metal uptake; and ii) ascertain the ability of Miscanthus biochar to improve soil microbial enzymatic activity.

2. Materials and methods

2.1. Formosa Mine site description and spoil characterization

The Formosa mine site (42.851271 N, – 123.38583 W) is located 16 km south of the town of Riddle, OR, USA. The mine site was worked in the early 20th century (1910–1937), then reopened (1990–1993) for silver, gold, and copper extraction from volcanogenic sulfide bearing rock deposits (Phillips et al., 2016). The mine was later abandoned in 1993. During its operation, mine spoils were deposited across the mountain top landscape (Fig. S1). After weathering, the mine spoils became acidified (<3 pH) due to oxidation of residual sulfide-bearing waste rock. Additionally, the spoils contained numerous-sized coarse fragments, lacked aggregates, leading to poor revegetative growth as shown in the foreground of Fig. S1. Further information about the mine site is summarized in Baker-Kircher (2009) and Phillips et al. (2016).

Mine spoils were collected at a depth of 0–10 cm from a location in the south central part of the mine spoil deposits (Fig. S1). Spoils were passed through a 6-mm sieve in the field. The larger coarse fragments were discarded. Back at the laboratory, the particle size distribution was determined by sieving the collected spoils using an Endecott Octagon digital sieve shaker (London, UK). Spoils were passed through a 6.35, 4.0, 2.0, 1.0, and 0.5-mm sieves and the weight distribution was; 0.1, 9.1, 18.5, 18.5, 14.6 and 39.1%, respectively. Total elemental analysis was obtained by digestion of the 6-mm mine spoil size fraction using 4 M HNO_3 and elements were quantified using inductively coupled plasma–optical emission spectrometry (ICP-OES; Bradford et al., 1975). The pH of the mine spoils was measured using 1:2 ratio of spoil to deionized water as outlined by Jones (2001). The %C, N, and S content of the mine spoil was measured using an Elementar VarioMax CNS analyzer (Mt. Laural, NJ, USA). Spoil chemical characterization is presented in Table 1.

2.2. Biochar preparation and characterization

In a preliminary experiment, biochars were produced from

Table 1
Chemical properties of Formosa mine spoil and Miscanthus biochar.

Total elements[a]	Mine spoil mg/kg	Biochar mg/kg
Al	8059	46
Ca	460	5216
Cd	10	0.4
Cu	408	26
Fe	18,881	175
K	1400	10,502
Mg	2568	2501
Mn	285	140
Mo	2	1.3
Na	82	95
Ni	4	10
P	319	1816
Pb	86	7
Zn	339	62
Chemical properties		
pH_{H2O}	<3	10
%ash (w/w)	nd[b]	18.5
%C	0.43	76.7
%H	nd	1.4
%O	nd	3.0
%N	<0.01	0.3
%S	0.96	<0.001
H/C	nd	0.21
O/C	nd	0.03

[a] Dissolution of material using 4 M HNO_3.
[b] nd = not determined.

several feedstocks (i.e., yard debris, hard and softwoods, manures and grasses) and evaluated for their ability to sorb Cu and Zn. Metals sorbed to the biochars were desorbed using 0.01 M $CaCl_2$ and the biochar with greatest Cu and Zn binding was chosen to use in the mine spoil experiment. Among the top three biochars, we chose to evaluate Miscanthus biochar because it removed the most metals, but did not release them with salt extraction. Moreover, Miscanthus biochar possessed a high N_2-BET surface area which is hypothesized to facilitate heavy metal sorption (Janus et al., 2015). Miscanthus feedstock was obtained from the US EPA National Health and Environmental Effects Research Laboratory, Corvallis, OR, USA. The feedstock was oven-dried at 60 °C, and then pulverized into 4-mm flakes using a grinder (Wiley-Mill; Thomas Scientific, Swedesboro, NJ, USA). These raw flakes were then pyrolyzed at 700 °C as outlined by Novak et al. (2014). Miscanthus biochar was placed in plastic sealable plastic bags and stored in a desiccator until later use.

Total elemental contents in the Miscanthus biochar was determined using the acid digestion described in US EPA method 3050B (US EPA, 1996) and were quantified using ICP-OES. The biochar pH was determined in a 1:2 (w/w) biochar to deionized water ratio (Novak et al., 2014). Biochar was characterized by ultimate analysis (ASTM D3176; Hazen Research, Inc.; Golden, CO, USA). The molar H/C and O/C ratios were calculated from these results. The data for the Miscanthus biochar are presented in Table 1.

2.3. Greenhouse experiment

Treatments consisted of 0% (control), 1, 2 and 5% (w w^{-1}) Miscanthus biochar (equivalent to 20, 40 and 100 t/ha) into air-dried 6-mm sieved Formosa mine spoil. 2.2 kg of sieved mine spoil was mixed with 0.8% (w/w, equivalent to 16 t/ha) lime (as CaO) and 0.22 g of NH_4NO_3 (equivalent to 100 kg N/ha) that corresponded to the lime + fertilizer treatments. In preliminary experiments, this quantity of lime was sufficient to raise the pH to between 5.0 and 5.5 (Fig. S2). This is the acceptable soil pH range for Blue Wildrye. The K and P contents from the biochar (10,502 and 1816 mg/kg;

Table 1) were assumed sufficient nutrients for Blue Wildrye growth. Similar treatments without lime (biochar + N fertilizer) were also prepared. For comparison, spoil treated with 0% biochar + no N fertilizer and 0% biochar with N fertilizer served as controls. Deionized water was then mixed into the spoil to give a final moisture content of 15% (w/w) air-dry basis. The treated spoils (n = 4) were then placed into plastic flower pots (15-cm top diameter x 17-cm deep) and gently tapped to a bulk density of 1.5 g/cm^3. Pot drainage holes were covered with nylon mesh to minimize material loss.

Thirty seeds of Blue Wildrye were spread across the spoil surface and were hand raked into the surface to a depth of 5–10-mm. The pots were placed randomly on a greenhouse bench. During the experiment, the greenhouse mean daily temperature and relative humidity was 28.9 °C and 45.7%, respectively. Approximately 1–2 times per week, the pots were re-distributed randomly to minimize growth differences from localized greenhouse light, temperature and humidity fluctuations. During the first 14 days of incubation, pots were re-weighed and deionized water was added to re-adjust the moisture content back to 15% (w/w). Afterwards, deionized water was applied by hand until the spoil appeared to be at field capacity because of pot weight uncertainty from grass growth. On day 28, N as NH_4NO_3 was added to all pots (equivalent to 50 kg/ha) because control plants showed signs of a possible N deficiency. The experiment was terminated after day 50 because the roots grew out the pot bottoms. Stems and roots were harvested from each pot, oven-dried (60 °C), and digested using a combination of conc. HNO_3 and 30% H_2O_2 (Hunag and Schulte, 1985). Aluminum, Cu and Zn in the digestates were quantified using ICP-OES.

2.4. Mine spoil water leaching and characterization

At termination, each pot was placed into a wooden rack and leached with 500 mL of deionized water. Passage of 500 mL of water was equivalent to 1.5 pore volumes and corresponded to a single 5-cm rainfall/infiltration event. The pots drained freely for 24 h or until leaching stopped. The total leachate was collected, weighed, and a 100-mL aliquot was filtered using a 0.45 μm membrane filter. The DOC content along with soluble Al, Cu, and Zn in the leachate were measured using a TOC analyzer (Shimadzu TOC-505A; Shimadzu Scientific Instruments; Kyoto, Japan) and by ICP-OES (Bradford et al., 1975).

The remaining spoil samples from each pot were air-dried (over several days) and then transferred into plastic bags for storage. Five g of this air-dry spoil was weighted into a beaker and the pH and EC were measured as described previously. Aluminum, Cu and Zn metals were the focus of this study because of their phytotoxic impact on plant growth and they represent some of the most abundant heavy metal concentrations in the mine spoil (Table 1). Bioavailable Al, Cu and Zn concentrations were determined by weighing 3.00 g air-dry spoil into a 50-mL centrifuge tube. The spoils were extracted using 30 mL of 0.01 M $CaCl_2$ (salt extractable) or Mehlich 3 reagent (plant available) as outlined by Pueyo et al. (2004). The salt extractable samples were then shaken at 120 rpm for 2 h while the M3 extractable samples were shaken for 5 min. Following the shaking period, both sets of tubes were centrifuged at 1500 rpm (~500 × g) and then 14 mL of liquid was passed through a 0.45 μm membrane filter. One drop of concentrated HNO_3 was added to the $CaCl_2$ extraction solutions. Both the salt and M3 extracts were analyzed for Al, Cu, and Zn via ICP-OES.

2.5. Enzyme analysis

Two enzymes, β-glucosidase (BG; C cycling) and *N*-acetyl-β-ᴅ-glucosamine (NAG; N cycling) were chosen for this study as they are

involved with prominent C (Alef and Nannipieri, 1995) and N (Kim et al., 1998) nutrient cycling pathways. Assays were performed according to Dunn et al. (2014) using a micro titer format as designed and described by Bell et al. (2013). For enzymes analysis, a total of 5 g of field moist spoil was collected from each pot and stored at 5 °C, samples were stored for less than 24 h prior to enzyme analysis. Spoil slurries were prepared by using 1 g of spoil, mixing it with 25 mL of modified universal buffer (MUB) and homogenized by vortexing. Spoil suspensions were then transferred into a 2 mL deep 96 well plate, along with methylumbelliferyl-labeled (MUF) substrates, covered and incubated at 35 °C for 90 min. After the incubation, plates were centrifuged for 3 min at $2900 \times g$, and 250 μL of supernatant was transferred to a black micro titer plate. Fluorescence at 450 nm was measured (BioTek FLx800 fluorometer) with an excitation at 330 nm. Autohydrolysis of the substrate was evaluated and subtracted from spoil sample emission values. A second portion of 1 g field moist spoil was weighed, oven dried for 24 h at 70 °C, and used to calculate enzyme activities on a per gram dry weight spoil basis.

2.6. Statistics

Biochar and lime were the fixed factors, whereas the mean shoot, root, final spoil pH and EC values were the dependent variables in the statistical analysis using a 2-way ANOVA with significance determined at a $P < 0.05$. A value of 0 was used for the mean rye shoot and root values in the spoil + % biochar without lime treatment due to no plant growth (Table 2). Additionally, only metal concentrations in shoot and roots were quantified in the

spoil + lime + %biochar samples because they were the only treatments where plant material was recovered for digestion. A 1-way ANOVA was used to determine significance among these means (Table 3).

Additionally, mean values were calculated from the water, salt extractable (i.e., exchangeable) and plant available metals concentrations ($n = 4$) and were tested using a separate 2-way ANOVAs to determine if biochar, lime, and the biochar × lime interaction were significant. The leachates pH, Al, Cu and Zn concentrations were also tested using a 2-way ANOVA to determine the impact of lime and biochar additions. The DOC, Al, Cu, and Zn concentrations in the leachates were evaluated using linear regression to determine if a relationship among between soluble metals and DOC existed. All statistics were completed using Sigma Stat v. 3.5 software (SSPS Corp., Chicago, IL, USA).

3. Results and discussion

3.1. Characterization of mine spoils and biochar

The mine spoil has a low pH, which was attributable to it containing 0.96% (w/w) S-bearing mineral (Table 1). Inorganic S can be oxidized by *Thiobacillus* organisms to SO_4^{-2} and releases H^+, which reduces the pH values (Alexander, 1977). The spoil also contained relatively high concentrations of total Al, Fe, Cu, and Zn, and lesser amounts of Cd, Ni and Pb (<90 mg/kg). In comparison, Miscanthus biochar had lesser amounts of the same heavy metals, but relatively moderate concentrations of plant macro nutrients (e.g., Ca, K, Mg, P) and micronutrients (e.g., Cu, Zn, etc.). This biochar has an alkaline pH, a low ash content (18.5%; Table 1) suggesting it has limited acid neutralizing capacity (Yuan and Xu, 2012; Xiao et al., 2016). Generally, the higher the biochar % ash content, the greater the acid neutralizing capacity (Yuan and Xu, 2012). The Miscanthus biochar had H/C (0.21) and O/C (0.03) molar ratios that are typical for grass feedstocks pyrolyzed at 500 to 700 °C (Spokas, 2010). The H/C ratio of <0.3 and O/C ratio of <0.1 suggests loss of hydroxyl and carboxylic functional groups losses during pyrolysis (Antal and Grønli, 2003).

3.2. Blue Wildrye growth and metal uptake

Regardless of the presence of the Miscanthus biochar, there was poor Blue Wildrye growth in spoils without lime addition (Table 2). In fact, there was insufficient shoot and root masses recovered from this group of treatments for metal concentration analysis. After lime was added to the mine spoil, in contrast, there was a significant increase in plant growth. The incorporation of Miscanthus biochar into the spoils treated with lime had minimal impact on Blue Wildrye shoot and root masses, with the only significant influence occurring in mean root mass without biochar (Table 2). The impact of lime on both mean shoot and root masses when compared to the Miscanthus biochar results is evident by the very significant P value (<0.001; Table).

Total Al, Cu and Zn concentrations in shoots and roots of Blue Wildrye in spoil treated with lime and biochars is shown in Table 3. Only in this set of treatments was rye growth sufficient to recover shoot and roots masses for metal determination. Aluminum and Cu concentrations in shoots were significantly reduced by the addition of Miscanthus biochars, however, there was no significant differences between means with increasing biochar addition (Table 3). Zinc concentrations measured in rye shoots were not impacted by lime nor biochar addition. Aluminum, Cu and Zn concentrations uptake by the rye roots showed mixed results probably due to the high standard deviation about the means. It appears that the Miscanthus biochar can reduce Al, Cu and Zn plant concentrations

Table 2
Mean shoot and root weights of Blue Wildrye at the of study ($n = 4$, oven dry weights).

Spoil + % biochar	Shoot (g)[a]		Root (g)	
(w/w)	No lime	Lime	No lime	Lime
0	0.0 a, A	0.245 b, A	0.0 a, A	0.199 a, A
1	0.001 a, A	0.384 b, A	0.001 a, A	0.371 b, B
2.5	0.004 a, a	0.317 b, A	0.004 a, A	0.272 b, A
5.0	0.007 a, A	0.342 b, A	0.019 a, A	0.285 b, B
Mean	0.003 a	0.322 b	0.006 a	0.282 b
Factor	P		P	
Biochar	0.456		0.196	
Lime	<0.001		<0.001	
Biochar X Lime	0.480		0.209	

[a] Lower case letter indicates significant differences among means between columns, while capital letter indicates significant difference among means within a column using a 2-way ANOVA at a $P < 0.05$ level of significance.

Table 3
Total metal concentrations in Blue Wildrye shoots and roots ($n = 4$; standard deviation in brackets).

Spoil + lime + % biochar (w/w)	Al[a] mg/kg	Cu mg/kg	Zn mg/kg
Shoot			
0	618 (346) a	98 (29) a	490 (170) a
1	94 (31) b	44 (15) b	246 (77) a
2.5	143 (93) b	66 (41) b	453 (385) a
5	53 (10) b	34 (3) b	196 (25) a
Root			
0	895 (173) ab	431 (58) a	436 (133) a
1	1082 (238) a	332 (15) bc	244 (22) bc
2.5	1143 (112) a	370 (48) ac	283 (66) ac
5	743 (42) b	285 (30) bc	237 (23) bc

[a] Means within a column were tested for significant differences using a 1-way ANOVA at a $P < 0.05$ level of significance.

more at the 1 and 5% application rate compared to the control (0% biochar; Table 3).

3.3. Biochar and lime impact on mine spoil chemical properties

Lime was more effective at increasing mine spoils pH and EC than the Miscanthus biochar, even at the highest biochar application rate (5% w/w; Table 4). Only biochar applied at 5% however, was able to significantly raise the pH compared to the other treatments (0, 1 and 2.5% biochar). Overall, application of 0.8% (w/w) lime was sufficient to adjust the spoil to a pH range acceptable for Blue Wildrye growth (NRCS, 2013).

Table 4
Mean pH and electrical conductivity (EC) values of Formosa mine spoil at end of study (n = 4).

Spoil + % biochar (w/w)	pH$_{H2O}$		EC (mS/cm)	
	No lime[a]	Lime	No lime	Lime
0	2.81 a, A	6.35 b, A	1.738 a, A	2.619 b, A
1	2.88 a, A	6.46 b, AB	1.776 a, A	2.750 b, A
2.5	3.00 a, AB	6.50 b, AB	1.432 a, A	2.884 b, A
5	3.10 a, B	6.65 b, B	1.507 a, A	2.857 b, A
Mean	2.95 a	6.50 b	1.613 a	2.777 b
Factor	P		P	
Biochar	<0.001		0.786	
Lime	<0.001		<0.001	
Biochar X Lime	0.855		0.040	

[a] Lower case letter indicates significant differences among means between columns, while capital letter indicates significant difference among means within a column using a 2-way ANOVA at a P < 0.05 level of significance.

In the current study, liming should be a high priority reclamation goal to reduce phytotoxic Cu, Zn, and Al concentrations since acidic mine spoils will have increased heavy metal solubility, which facilitates metal movement into ground and surface waters. Additionally, as heavy metal concentrations increase in pore water, the probability of plant phytotoxicity also increases (Marschner, 1998; Kabata-Pendias, 2001). The relative degree of toxicity depends on the form of the metal, solution acidity, and presence of organic compounds (Kabata-Pendias, 2001). However, sulfidic spoils often have additional acidity production potential that needs to be quantified and considered as the lime addition may have a short-lived effect if there is sufficient reserve acidity capacity.

Aluminum concentrations were mostly influenced by lime additions, but increasing biochar application amounts also reduced Al concentrations (Table 5). Decreasing Al concentrations were probably obtained through a combination of lime reducing the solubility of Al forms and to complexation/chelation reactions by the biochar (McLean, 1976; Stevenson, 1994). Additionally, lime and biochar additions impacted salt and M3 extractable Al forms, as evident by the significant interaction between these variables.

Lime addition significantly (P < 0.001) reduced extractable Cu forms. When biochar alone was added to Formosa spoil, there was no significant impact on extractable forms of Cu, even at 5% biochar application (equivalent to 100 t/ha). Similar results were observed for extractable Zn forms (Table 5). There is a significant interaction between lime and % biochar for M3 extractable Cu and Zn concentrations, probably due to the strong lime influence.

Raising the mine spoil pH was sufficient to reduce extractable metal concentrations probably through oxyhydroxide, hydroxide, and carbonate formation (Bohn et al., 1979; Ippolito et al., 2017a).

Table 5
Mean Total, CaCl$_2$ and Mehlich 3 (M3) extractable metals from Formosa spoils (n = 4) at end of incubation.[a]

Spoil + % biochar (w w^{-1})	Total (mg/kg)		CaCl$_2$ extractable (mg/kg)		M3 extractable (mg/kg)	
	No lime	lime	No lime	lime	No lime	lime
Aluminum						
0	8069 a, A	7216 a, A	75 a, A	0 b, A	409 a, A	268 b, A
1	7848 a, A	7113 a, A	75 a, A	0 b, A	475 a, B	202 b, A
2.5	7346 a, A	7150 a, A	46 a, B	0 b, A	411 a, A	244 b, A
5	7119 a, A	6531 a, A	45 a, B	0 b, A	358 a, A	186 b, B
mean	7596 a	7007 b	60 a	0 b	413 a	225 b
Factor		P		P		P
Biochar		0.065		0.003		0.005
Lime		0.012		<0.001		<0.001
Biochar * Lime		0.732		0.003		0.01
Copper						
0	408 a, A	426 a, A	22.6 a, A	0 b, A	30.0 a, A	18.4 b, A
1	354 a, A	424 a, A	24.9 a, A	0 b, A	36.6 a, A	13.2 b, A
2.5	366 a, A	359 a, A	17.7 a, A	0 b, A	28 a, A	17.6 b, A
5	327 a, A	357 a, A	19.1 a, A	0 b, A	28.7 a, A	13.5 b, B
mean	364 a	391 a	21.1 a	0 b	30.8 a	15.6 b
Factor		P		P		P
Biochar		0.332		0.200		0.363
Lime		0.359		<0.001		<0.001
Biochar * Lime		0.833		0.200		0.037
Zinc						
0	391 a, A	385 a, A	29.7 a, A	0.7 b, A	35.3 a, A	37.3 a, A
1	267 a, BC	259 a, B	35.4 a, A	0.06 b, A	54.1 a, A	21.0 b, A
2.5	277 a, B	272 a, B	30.5 a, A	0.5 b, A	42.8 a, A	40.0 a, A
5	233 a, BC	304 a, B	33.6 a, A	0.17 b, A	42 a, A	34.4 a, A
mean	292 a	305 a	32.3 a	0.36 b	43.6 a	33.7 a
Factor		P		P		P
Biochar		<0.001		0.79		0.87
Lime		0.483		<0.001		<0.027
Biochar * Lime		0.389		0.65		0.043

[a] Lower case letter indicates significant differences among means between columns, while capital letter indicates significant differences among means within a column using a 2-way ANOVA at a P < 0.05 level of significance.

Table 6
Mean pH, DOC, Al, Cu and Zn measured in deionized water leachates (n = 4) collected at end of study.[a]

Spoil + % biochar (w/w)	pH		DOC		Al		Cu		Zn	
	No lime mg/L	lime mg/L	No lime mg/L	lime mg/L	No lime mg/L	lime mg/L	No lime mg/L	lime mg/L	No lime mg/L	lime mg/L
0	2.95 a, A	4.69 b, A	31.49 a, A	7.34 a, A	212 a, A	0.318 b, A	4.61 a, A	0.028 b, A	5.87 a, A	0.308 b, A
1	3.04 a, A	5.16 b, B	18.26 a, B	8.26 a, A	125 a, B	0.04 b, A	2.58 a, B	0.002 b, B	3.98 a, B	0.059 b, A
2.5	3.10 a, A	5.05 b, C	18.04 a, B	8.92 a, A	86 a, C	0.089 b, A	1.50 a, C	0.007 b, B	3.33 a, C	0.096 b, A
5	3.11 a, A	5.36 b, D	11.94 a, B	8.85 b, A	48 a, C	0.031 b, A	1.07 a, D	0.001 b, B	2.13 a, D	0.042 b, A
mean	3.05 a	5.06 b	19.94 a	8.34 b	117 a	0.12 b	2.44 a	0.009 b	3.83 a	0.126 b
Factor	P		P		P		P		P	
biochar	<0.001		0.007		<0.001		<0.001		0.002	
lime	<0.001		<0.001		<0.001		<0.001		<0.001	
biochar x lime	0.009		0.001		<0.001		<0.001		0.007	

[a] Lower case letter indicates significant differences among means between columns, while capital letter indicates significant difference among means within a column using a 2-way ANOVA at a $P < 0.05$ level of significance.

On the other hand, the Miscanthus biochar had no significant influence on reducing extractable Cu and Zn concentrations, and a limited impact on Al concentration reductions. These results are contrary to previous biochar treated mine spoil investigations that reported reduced extractable Zn (Beesley et al., 2010; Puga et al., 2015), Cu (Brennan et al., 2014; Rodríguez-Vila et al., 2015), and Al (Phillips et al., 2016) concentrations. Our contrary results could be due to the experimental design of the greenhouse pots, which possessed limited homogenization of the material with the water solution compared to the batch equilibrium tests. Additionally, it is possible that lack of metal sorption in the pot experiment was due to the biochar's inability to chemically/electrostatically attract available heavy metals to form solid phases by: (*i*) possessing an ash that contained material not capable of precipitating metals (i.e., low $CaCO_3$ equivalence); (*ii*) minimal surface functional groups that limited electrostatic interactions with the metal cations; (*iii*) having a poor ionic exchange between ionizable protons on acidic carbons and metal cations; and (*iv*) lacking delocalized π electrons on carbon moieties that limits their sorption potential (Sohi et al., 2010; Ahmad et al., 2014; Ippolito et al., 2017a). Overall, this Miscanthus biochar (by itself) appears to have a limited capability as a reclamation agent to reduce extractable metals.

3.4. Water leachate characteristics

Lime addition to the spoil significantly raised the grouped leachate mean pH value from 2.95 to 4.69 (Table 6). Miscanthus biochar by itself did not significantly increase leachate pH, regardless of application amount. However, when the biochar is applied with lime, the leachate pH did significantly increase with greater biochar applications (4.69–5.36; Table 6). This is hypothesized to result from solublized Ca^{+2} from lime, and the ability of Ca^{+2} to outcompete other cations, as positioned on the lyotropic scale (Bohn et al., 1979). Others have observed that aromatic carbon nanoparticles attract cations through static surface charges based on the Hofmeister effect (Xia et al., 2017), which advocates that both ion size and charge density are important factors. Moreover, Li et al. (2013) reported small increases in solution pH after Ca^{+2} doping of an acidic humic acid solution. This scenario could also explain the significant lime × biochar interaction ($P = 0.009$; Table 6).

Leachate DOC concentrations were also measured because dissolved organic matter can impact adsorption/desorption equilibrium between dissolved phases of soil material, aqueous metals, and interaction with solid phases (Smernik, 2009; Lin et al., 2012; Smebye et al., 2016). Functional groups (e.g., COOH, OH, etc.) associated with DOC structures has been reported (Stevenson,

1994) to influence heavy metal availability for plant uptake and their potential movement with water through complexation reactions with DOC that is mobilized by biochar additions (Beesley et al., 2010; Beesley and Marmiroli, 2011; Park et al., 2011; Tan et al., 2017). Conversely, DOC releases from biochars can bind to DOC that is solubilized from indigenous soil organic matter pool and precipitates out of solution resulting in a net DOC concentration reduction (Joseph et al., 2010; Lin et al., 2012; Mukherjee and Zimmerman, 2013).

The highest DOC concentration was measured in the control without lime treatment (Table 6). When biochar was added to the mine spoils, there was a significant DOC concentration reduction when compared to the control. In fact, the mean DOC concentration reduction between the control and biochar treatments (with no lime) was almost 40%. The reduction in leachate DOC concentrations is consistent with other reports (Joseph et al., 2010; Lin et al., 2012; Mukherjee and Zimmerman, 2013). It is interesting; however, that the mean DOC concentrations among the biochar treatments were statistically similar implying that there was no corresponding increase in DOC with additional biochar. Finally, when combined with lime application, all mean DOC concentrations declined to <9 mg/l and the differences between these treatments were not significant. Since similar amounts of lime were added to the treatments (except controls), we speculate that Ca^{+2} solublized from CaO could uniformly reduce DOC concentrations probably through Ca^{+2} bridging with organic ligands leading to flocculation (McLean, 1976; Stevenson, 1994).

Heavy metal concentrations in the leachates were impacted both by the addition of lime and Miscanthus biochar (Table 6). Lime addition significantly reduced Al, Cu and Zn concentrations in the leachates compared to treatments with just the Miscanthus biochar alone ($P < 0.001$). This can be explained by pH dependency in metal solubility (Bohn et al., 1979). These observations stress the important role of lime in reducing metal solubility.

In spoil treated with only Miscanthus biochar, there was a significant decline in leachate Al, Cu and Zn concentrations (Table 6). The metal concentration decrease was most noticeable with Al (4.4-fold), followed by Cu (4.3-fold) and Zn (2.8-fold) when treatment with 0% biochar (control) was compared to biochar treatments. This finding is similar to reports of reduced heavy metal (e.g., Cd, Pb, and Zn) concentrations in pore water from biochar treated mine spoils (Beesley et al., 2014; Puga et al., 2015). We speculated that there was a relationship between leachate DOC and Al, Cu and Zn concentrations since there was a significant metal concentration decline in the no lime treatments as the % biochar application rate increased. Thus, linear regression was used to determine relationships between leachate mean DOC concentrations and Cu, Zn and
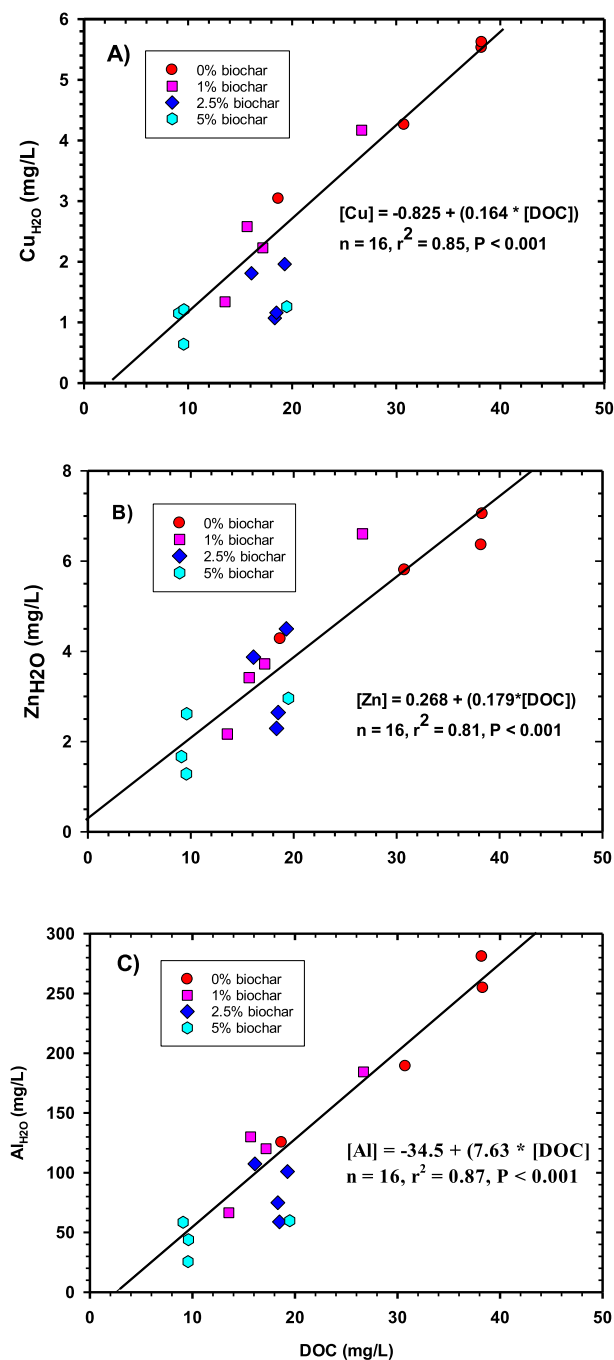
Fig. 1. Regression results between leachate dissolved organic carbon (DOC) concentrations and Cu (A), Zn (B), and Al (C) concentrations collected from un-limed treatments (n = 16).
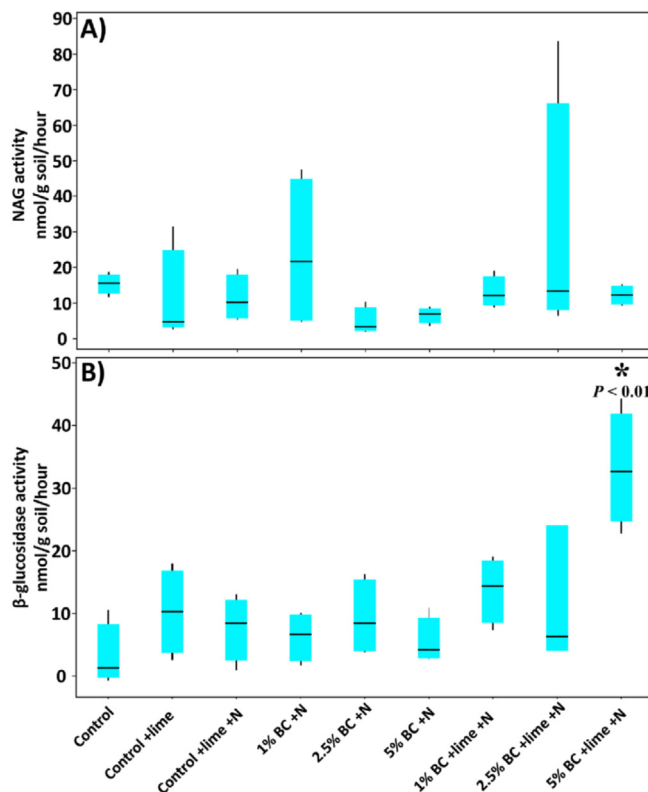

Fig. 2. *N*-acetyl-β-D-glucosaminidase (A; NAG) and β-glucosidase (B) enzyme activity in treated and untreated Formosa mine spoil.

metal binding to functional groups through electrostatic attraction. In other words, H^+ is outcompeting Al^{+3}, Cu^{+2}, and Zn^{+2} for exchange sites. Thus, we speculate that the metal and DOC concentration reductions were a result of complexation reactions, whereby a proton is dislodged from functional group resulting in metal binding (Li et al., 2017) and bridging between DOC and biochar (Bohn et al., 1979). Others have suggested that DOC bound metals, especially As, Cd and Cu, can pose a water quality risk if their movement is expedited by soluble complex formation (Kim et al., 2014; Wagner and Kaupenjohann, 2014; Anawar et al., 2015). Thus, the presence of DOC from organic sources (e.g., biosolids, manures), in conjunction with biochar, could be an important mechanism for reducing soluble metal concentrations in acidic pore water. Furthermore, increasing biochar application alone appeared to lower metal concentrations to be equal to or below US EPA secondary drinking water standards (US EPA, 2013). For example, soluble Cu and Zn concentration in water leachates were reduced to <1.5 and 3 mg/L (Fig. 1), respectively, which is close to the secondary drinking water standards of 1.3 and 5.0 mg/L (US EPA, 2013). On the other hand, biochar did not reduce soluble Al concentrations (<50 mg/L; Fig. 1) to match the 0.05−0.2 mg/L, which exceeds the secondary drinking water standard for Al.

3.5. Relationship of microbial soil enzyme activity to biochar

The response of microbial soil C and N cycling enzymes to biochar amendment is shown in Fig. 2. As a predictor of N cycling, NAG activity ranged from 4.8 to 29.2 nmol/g soil/h (Fig. 2 A). Control samples (no amendment) had NAG activity of 15.4 nmol/g soil/h, with all treatments showing no statistical differences. Regarding C cycling, β-glucosidase activity ranged from 3.1 to 33.1 nmol/g soil/h (Fig. 2B). Control samples had a β-glucosidase activity of 3.1 nmol/g

Al concentrations (Fig. 1). The regression function indicates a dissolved metal concentration reduction with increasing biochar application rate. The linear relationship is significant (P < 0.001) in all three cases. The fitted regression functions calculated for all three metals explain between 81 and 87% of the variation in the relationship between metal vs. DOC concentrations (Fig. 1).

The leachates from mine spoil treated only with the Miscanthus biochar were highly acidic, so it would be expected that functional groups associated with DOC are protonated. This would minimize

soil/h, the lowest activity reported out of all the treatment groups. With the exception of 5% biochar with lime (mean of 33.1 nmol/g soil h^{-1}), however, all other treatments were statistically similar to the control samples.

The literature has reported mixed soil enzyme activity on exposure to biochars with results ranging from highly variable (Bailey et al., 2011) to having a significant positive impact (Lu et al., 2015; Jain et al., 2016). Furthermore, when compared to farmland soils under both tillage and no-tillage management practices (Hou et al., 2016), or farmland soil under various irrigation practices (Ippolito et al., 2017b), the β-glucosidase activity from these mine spoil treatments were reduced by at least an order of magnitude. Likewise, NAG activity in the mine spoil soils were on average approximately 50% less than reported in the same study by Hou et al. (2016).

These results seem to agree with Lee et al. (2002), which demonstrated that microbial activity in soils contaminated with heavy metals (Cd, Zn, Cu, and Pb) was significantly reduced when compared to adjacent healthy soils; they hypothesized that microbial activity was inhibited by the presence of heavy metals. While inhibition may be one possibility for the low activity reported in this study, the poor response to almost all treatments - for both β-glucosidase and NAG — is potentially indicative of a lack of sufficient bacterial and/or fungal communities, both in number and diversity. A study by Ajwa et al. (1999) reported a correlation between β-glucosidase and both microbial biomass-C and microbial biomass-N. Additionally, NAG (chitinase) has been demonstrated to be elevated in ecosystems with increased fungal biomass (Miller et al., 1998). The low activity rates of NAG in this study, coupled with a lack of response to any of the treatments, is more than likely indicative of low fungal populations in the spoil utilized in this study as determined by PFLA results (Fig. 2). T.

The statistically significant increase in β-glucosidase activity in the spoil amended with 5% biochar + lime (Fig. 2B), may be the result of heterotrophic activity response to biochar's labile C pool. These results are similar to Ducey et al. (2013) and Kolb et al. (2009) reports that demonstrated a positive correlation between microbial communities and biochar amendment rates. It is possible that the microbial communities in the Formosa mine spoil are significantly degraded, to a point where only a combination of the highest biochar amendment rate - with a pH adjustment to more neutral conditions - was able to elicit a significant β-glucosidase activity response.

4. Conclusions

This study tested the ability of a Miscanthus biochar to sequester heavy metals and improve microbial enzymatic activity in an acidic mine spoil. The Miscanthus biochar alone performed poorly by failing to eliminate the acidic spoil conditions and consequently had a minimal impact on reducing extractable metal concentrations. There was very poor Blue Wildrye growth in spoils without lime addition, regardless of the presence of the Miscanthus biochar. Total Al and Cu concentrations in shoots were significantly reduced by the addition of Miscanthus biochars, but, additional biochar did not correspondingly reduce their concentrations. Aluminum, Cu and Zn concentrations by the rye roots showed mixed results.

Salt extractable Al concentrations were reduced by this Miscanthus biochar. Biochar additions did reduce the DOC leaching from the biochar treated spoils, which suggests a benefit of reducing indigenous DOC loss. The Miscanthus biochar at the highest application rate and with lime was found to significantly improve β-glucosidase activity. Lime alone, however, was more effective at reducing Mehlich 3 extractable metal concentrations. We recognize that the Miscanthus biochar was tested in only one

mine spoil material (Formosa), and our conclusions cannot be extended to other mine spoil materials. However, this study does suggest caution in extending biochars mitigation potential, given that the removal of metals from laboratory experiments might not correlate with realistic soil conditions (i.e., short-term static pot experiments). Nevertheless, we conclude that when teamed with lime (as CaO), this Miscanthous biochar reduced extractable metal concentrations and bolstered enzymatic properties in the Formosa mine spoil material. Perhaps using a strategy of amending metal contaminated soils with biochar, lime and nutrients and allowing them to react with the spoil for a much longer period than the length of this study (i.e., 50 d) may promote a more complete soil reaction, thereby, optimizing amendment conditions.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.chemosphere.2018.04.107.

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