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RESEARCH ARTICLE

Plant response to biochar, compost, and mycorrhizal fungal amendments in post-mine sandpits

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Extreme growing conditions inhibit restoration in sandpit mines. Co-amendment of soil conditioners such as biochar, compost, and arbuscular mycorrhizal fungi (AMF) may alleviate these stresses and lead to a more successful restoration. We conducted a multiyear restoration experiment in a sandpit in Southern Ontario, Canada, following industrial-scale grassland restoration protocols. The sandpit substrate was sand with low carbon (C) and nutrients. We tested the effect of biochar, compost, and AMF inoculum in two experiments (plant plugs vs. seed application). In the plant plug trial, we investigated the treatment effects on the growth of eight grassland plant species and colonization of plant roots by AMF over two growing seasons. We found that co-amending soils with compost plus biochar (20 T/ha + 10 T/ha) was more beneficial than other amendment combinations. Amendments including AMF were not more beneficial to plant growth than those without AMF. In the seed application trial, direct inoculation of AMF in the field combined with high compost addition (20 T/ha or 40 T/ha) resulted in the highest plant cover compared to other treatment combinations. Our results indicate that co-amending sandpit substrates with biochar, compost, and AMF are practical restoration tools that enhance grassland restoration.

Key words: arbuscular mycorrhizal fungi, biochar, compost, grassland, microbial, sandpit

Implications for Practice

- Sandpit restoration is inhibited by low organic matter and low nutrients in the soil substrate.
- Organic amendments can help accelerate plant growth in former sandpits.
- Biochar and compost co-amendments are more effective than single amendments.
- Direct arbuscular mycorrhizal fungi inoculation in the field may be important for seed-based restoration, but not when using plant plugs.

Introduction

Aggregate mining is an important industry worldwide, with an estimated 50 billion tonnes of aggregates being removed from riverbeds, coastal dunes, and marine sediments every year (Steinberger et al. 2010). Due to the exponential rate at which these materials are being used, particularly in developing nations (Peduzzi 2014), the restoration of these landscapes is a pressing environmental issue.

In Ontario, Canada, sand plain prairie ecosystems have diminished due to sand excavation, agriculture, fire suppression, and urbanization (Gartshore et al. 1987). Excavated sandpits in Ontario are candidate areas to restore prairie plant species but edaphic conditions limit the development of high diversity plant communities (Wali 1999; Prach & Hobbs 2008). These soils are characterized by a lack of organic material and consist largely of subsoil and rock material (Bradshaw 2000). These conditions can directly affect root growth (Szota et al. 2007) and inhibit soil microbial activity (Larney & Angers 2012). If restoration efforts fail to restore soil biodiversity and functioning at these sites, then plant communities may take decades to establish, or fail altogether (Bradshaw 1997).

Soil amendments are important components of restoration events, particularly in soils with low organic material (Ohsowski et al. 2012). These amendments may improve the physiochemical properties of the soil to increase soil biodiversity (Harris 2003). However, the literature is varied in reports of their utility in promoting plant growth.

Compost

As a solitary soil amendment, compost has ameliorative effects on soils in agricultural and mine restoration settings (Shiralipour et al. 1992; Ouédraogo et al. 2001; Novo et al. 2013). Compost amendment increases soil organic matter content, water holding capacity, and nutrients (Termorshuizen et al. 2004). In addition,

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compost addition stimulates soil microbial communities (Carlson et al. 2015; Cozzolino et al. 2016). Compost application to severely degraded landscapes has been shown to increase grassland plant survivorship and primary production (Noyd et al. 1996; Kohler et al. 2014; Gil-Loaiza et al. 2016).

Biochar

Biochar is the carbon-rich residue from partially charred organic material (Schmidt & Noack 2000) widely used as a soil additive to improve soil nutrient status (Spokas et al. 2012). It can alter physicochemical soil properties by directly releasing nutrients or indirectly altering plant available nutrient concentrations (Chan & Xu 2009). Meta-analysis shows that biochar significantly translates to increased crop biomass and plant macronutrients across all soil types and climates (Biederman & Harpole 2012), but the largest positive influences on plant production have been shown in acidic, nutrient poor soils (Jeffery et al. 2011). In mine substrates, biochar has shown positive effects on native plant growth (Anawar et al. 2015; Roberts et al. 2015), but inconsistent effects on plant biomass (Jones et al. 2012; Adams et al. 2013) Thus, the utility of biochar in restoring plant communities is not clear.

Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhizal fungi (AMF) are obligate root symbionts that have been used in the restoration of mine areas for more than 30 years due to their ability to enhance plant establishment and survival (Khan 1981; Johnson 1998; Enkhtuya et al. 2005; Rydlová et al. 2008). Mine landscapes typically have low AMF diversity and abundance in addition to low nutrients and organic matter (Stahl et al. 1988; Ganesan et al. 1991; Diaz & Honrubia 1994). This means that plants may be unable to establish and persist, even if physical properties of the soil improve (Rivera et al. 2012).

Synergism Among Amendments. Co-amending soils with multiple soil conditioners may lead to larger responses in plants, as shortfalls in one amendment may be compensated for by the activity of another (Fischer & Glaser 2012). For example, the nutrient-sequestering capacity of biochar may be beneficial to plants growing in soils at risk of leaching—particularly when combined with a compost amendment (Schulz & Glaser 2012; Schulz et al. 2013; Agegnehu et al. 2015). Conversely, biochar in a nutrient poor, highly leached soil may not provide benefits to plants.

Combining AMF inoculum with compost should promote plant growth compared to either amendment alone, because AMF may exploit nutrients released by mineralization of organic matter (Hodge & Fitter 2010). Subsequently, organic amendments have been shown to have a positive effect on the proliferation of AMF naturally occurring in agricultural systems (Harinikumar et al. 1990) and produce larger plants (Caravaca et al. 2003; Püschel et al. 2011). The combined use of biochar and AMF inoculation, however, may not be beneficial. If nutrients are limiting, the addition of AMF and biochar without compost may have no measurable benefit for plant growth. Although this co-amendment has not been rigorously tested, results show both positive (Warnock et al. 2007) and negative (Birk et al. 2009; Warnock et al. 2010) effects on plant response.

The aim of this study is to understand the effects of soil conditioners and a microbial inoculant on grassland plant response in post-mine sandpits. We performed two long-term field experiments in a recently excavated sandpit, examining the effects of compost, biochar, and AMF inoculum alone or in combination on: (1) plant plug establishment and growth and (2) seed germination and growth. We hypothesized that AMF inoculation, compost, and biochar addition would individually increase plant dry mass and cover compared to nonamended control treatments. We further hypothesized that synergistic effects among amendments and AMF would yield the greatest plant benefits.

Methods

Research Site

Our research was conducted on a recently active sand extraction site (0.5 hectares [ha]) near Port Rowan, Ontario, Canada (42°40′17″N, 80°28′45″W, elevation 211 m). At the start of this project (May 2010), the post-mine area was graded flat by an earthmover. At the time of planting, the mine area substrate was poorly developed and composed of unconsolidated mineral substrate with no evidence of coarse soil organic material. Average annual temperature is 7.7°C, and annual rainfall averages 942 mm (Environment Canada).

Experimental Design

We tested the effects of three soil amendments (biochar, compost, and AMF) on the establishment and growth of plants in a post-mining sandpit using two planting approaches: plant plugs (plants were established in a greenhouse and planted as plugs) and seed application. Biotic and soil nutrient responses were collected in both experiments. In both trials, biochar was supplied by New Earth Renewable Energy Inc. (Quebec, Canada), and was created from wood pellet feed stock that was pyrolyzed at 500°C in an industrial scale nonoxygenated vacuum reactor. We tested two industrially feasible biochar rates (5 tonnes [T]/ha and 10 T/ha) in the plant plug experiment. Six rates of biochar were tested in the seed application trial ranging from 0 to 40 T/ha (Table 1). Compost (Try Recycling, London, Ontario, Canada) was derived from municipal lawn and leaf urban waste streams (pH = 6.3; C:N ratio = 24:1) (chemical composition is given in Table S1, Supporting Information). We tested one industrially feasible compost rate (20 T/ha) in the plant plug trial, and six rates of compost ranging from 0 to 40 T/ha (Table 1) in the seed application trial. AMF inoculum was a commercial isolate, *Rhizophagus irregularis* (Blaszk., Wubet, Renker & Buscot; Schüßler & Walker 2010), supplied by Mikro-Tek (Timmins, Ontario, Canada).

Eight grassland plants (two C_4 grasses; two C_3 grasses; two N-fixing forbs; and two composite forbs) were selected for

Table 1. Experimental treatments for the seed application experiment. All treatment levels are fully factorial. Each treatment combination was applied to one plot only. Total number of plots was 72.

Biochar Level	*Compost Level*	*AM Level*
0.0 T/ha	0.0 T/ha	No inoculum
2.5 T/ha	2.5 T/ha	*Rhizophagus irregularis*
5.0 T/ha	5.0 T/ha	
10.0 T/ha	10.0 T/ha	
20.0 T/ha	20.0 T/ha	
40.0 T/ha	40.0 T/ha	

Factorial = biochar level × compost level × AM fungal inoculum level

this project using the following criteria: common in Ontario's sand plain prairies, tolerant of sandy soils and dry conditions, endemic to the study area, and known to associate with AMF. Details about these plants are given in Table S2.

In 2011, the topography of the experimental site was mapped with land surveying equipment to account for any potential influence of microclimate differences. The relative height of each experimental plot was included as a factor in the statistical models for both trials.

Plant Plug Trial

Plants were grown in a commercial greenhouse by Pterophylla/St. Williams Nursery & Ecology Centre (St. Williams, Ontario, Canada) from 1 April to 24 June 2010 in 72 cell Landmark plug trays each filled with 57 cm^3 of a proprietary growing medium (containing pine bark, sphagnum peat, leaf and yard waste compost, and perlite). To inoculate plant plugs, 20 AMF spores in a proprietary powder medium were added just below the soil surface at the time of seed sowing. The growth medium was not sterilized to mimic industrial growing conditions. Before sowing plugs in the field, 10 noninoculated and 10 inoculated plugs from each plant species were randomly selected to assess root colonization.

Plots (10.2 m^2) were established in May 2010 using a fully factorial randomized design. The three factors were: biochar (no amendment, 5 T/ha biochar, 10 T/ha biochar), compost (no amendment, 20 T/ha compost), and AMF inoculation (±). Each of the 12 factorial combinations was replicated 10 times totaling 120 plots. The locations of 72 plant plugs per plot (total 8,640 plant plug positions) were mapped to have identical positions across all field plots (plug spacing = 33 cm) (Fig. S1). A hexagonal plug arrangement was chosen to minimize spatial variability between plugs. Compost and biochar were hand raked into the upper 6 cm of substrate. Control plots were not amended and were planted with noninoculated plant plugs. A buffer zone (1 m) separated each plot.

Responses Measured. *AMF quantification:* In the field plots, 16 soil cores per plot were collected near designated plug locations in September 2011/September 2012 and pooled to minimize spatial variability. In all cases, washed roots were cut into 1 cm pieces, and preserved in 50% ethanol until processing. Roots were stained with Chlorazol Black E (Brundrett et al. 1984) and fungal structures were counted under a microscope using the gridline intersect method (McGonigle et al. 1990).

Soil nutrients: The 2012 soils that were collected from the plug experiment for AMF quantification (see above) were also used to measure soil chemical variables (pH, C, N, P, Ca, Mg, and K). Although there were two biochar treatments, we only analyzed soil from the higher biochar rate treatment (10 T/ha). The pH was determined using a 2:1 mixture of dried soil and 0.01 M $CaCl_2$ (Hendershot et al. 1993). Total soil carbon was determined following dry combustion using a LECO CR-12 analyzer (Leco Corporation, St. Joseph, MI, U.S.A.) (Wang & Anderson 1998). Total soil nitrogen was determined following dry combustion using a LECO FP-228 analyzer (Leco Corporation, St. Joseph, MI, U.S.A.), using manufacturer's protocol. Total P was determined colorometrically, following digestion with nitric acid (Olsen & Sommers 1982). Soil Ca, Mg, and K were determined using the Mehlich III-exchangeable method (Mehlich 1984).

Plant biomass estimation: To avoid destructively harvesting the plants, we used partial least squares (PLS) regression to predict plant biomass (Ohsowski et al. 2016). To estimate biomass, we measured plant characters related to height, diameter, and stem counts when appropriate for each plant species separately for the fall 2011 and fall 2012 growing seasons, but these data were later pooled to give an estimate of total plant biomass (Table S3). To reduce edge effects, only plants in the center of the plots were measured (i.e. the "core area"; Fig. S1). Thirty-three plant plugs in the core area were measured, totaling 3,960 plugs per growing season. Relevant predictor variables were selected via Bayesian information criterion (Bic) model selection and PLS regression was used to predict the plant mass of the plugs in the fall of each field season (Table S4). Statistical details of measurement accuracy for each species are given in Table S5.

Seed Application Trial

Plots were amended with compost and biochar following the same procedure as the plant plug trial protocol in August 2010. Factor levels for biochar and compost were replicated once for a total of 72 plots (see Table 1). To minimize overwinter seed mortality and undesired seed movement via wind scour, seeding and inoculation were performed in spring 2011. Prior to planting, seeds were cold-moist stratified by mixing with moist vermiculite, and stored at 4°C for 1 month in a refrigerator. In May 2011, seeds were lightly mixed into plots with a rake, and pressed into soil with a seed roller. AMF inoculum was added to half of the plots via a liquid medium containing spores (2 L of liquid medium per plot) following seed compaction. Spores were applied at Mikro-Tek's recommended rate of 1,000 spores/m^2. Seeds were applied at double the standard rate recommended for a grassland restoration project to ensure measurable plant establishment rates in the severely degraded sandpit substrate (see Table S6 for information on species and seeding rate). We did not collect data on individual plant species, AMF root colonization, or soil nutrients for this trial.

Responses Measured. *Plant cover estimation:* A repeated measure photographic time-series technique was used to nondestructively estimate the percent plant cover in the seed application trial for 3 years. An angle camera monopod was constructed to take overhead pictures in each plot (Fig. S2). Photos were cropped to represent a 2.6 m^2 area in the center of the plot. Plant cover was measured via a 100-point overlaying grid to classify pixels representing grasses, composite forbs, N-fixing forbs, soil, ruderal recruits, or plant litter using the software SamplePoint (Booth & Cox 2008). Plant cover was estimated for green photosynthetic plant tissues in each photo. To estimate total plant cover, grass, forb, and N-fixing forb pixels were summed and subsequently divided by total pixels estimated. Photographs of each plot were taken in September for three growing seasons (2011–2013).

Statistical Analyses

Linear mixed effect models were used to test the effect of biochar, compost, and AMF inoculation on plant biomass responses and AMF colonization of roots in the plant plug trial and plant cover in the seed application trial. Relative plot height was included as a covariate in all linear mixed effect models. Linear mixed effect model selection procedures iteratively removed nonsignificant treatment variables using chi-squared model comparison estimations. This resulted in the most parsimonious models to analyze statistical significance for each response variable. Thus, not all treatment variables are included in the most parsimonious model in each statistical analysis. Linear mixed effect models were analyzed using the lme4 package in R (R Core Team 2015; Bates et al. 2014). Significance levels (p-values ≤ 0.05) derived from Markov Chain Monte Carlo methods, % explained deviance (an R-squared proxy, abbreviated: % expl. dev.), and main level post hoc comparisons were calculated using the R package LMERConvenienceFunctions by Tremblay and Ransijn (2013). Data transformations were used when necessary to approximate a normal distribution of model residuals.

Results

Plant Plug Trial

AMF Establishment in Greenhouse Plug Roots. All plant species were colonized by AMF in the greenhouse (Fig. S2). As expected, low levels of AMF colonization of roots were detected in noninoculated plant plugs across all plant species (<5.0% colonization of roots, across all taxa). AMF inoculation resulted in significant increases in percent colonization in all species compared to noninoculated plants ($p = 0.001$), with root colonization ranging from 16.9 (*Elymus canadensis*) to 30.1% (*Andropogon gerardi*).

AMF Establishment in Field Roots. Colonization of roots was significantly higher in inoculated plots compared to noninoculated plots ($p = 0.001$) (Fig. 1). Mean % colonization in the inoculated plots nearly doubled between September 2011 (22.4%) and September 2012 (45.8%). Mean % colonization of roots in the noninoculated plots tripled from 5.5 (2011) to 15.8% (2012). Although roots in inoculated plots had higher overall colonization, noninoculated plants displayed a larger relative increase in % colonization between September 2011 and September 2012 compared to inoculated treatments ($p = 0.001$) (Table S7).

Plant Biomass Responses in the Plant Plug Trial. Although estimated plant biomass did not differ significantly among amended and nonamended plots ($p = 0.056$), there was an overall trend of increased biomass in amended treatments compared to nonamended control plots. Compared to biochar alone, compost and compost + biochar amendments increased plant biomass significantly in most instances ($p < 0.05$; Table 2). Estimated biomass was significantly larger in fall 2012 compared to fall 2011 ($p = 0.001$). Surveyed experimental plots relatively lower on the landscape resulted in a trend of increased plant biomass compared to plots higher on the landscape ($p = 0.068$). No significant interactions among the model terms were detected. Compared to nonamended control plots, 5 T/ha of biochar ($p = 0.642$) and 10 T/ha of biochar ($p = 0.798$) did not influence total plant biomass (Fig. 2). When analyzed individually, only *A. gerardi* responded negatively to the addition of biochar compared to nonamended control plots (Fig. S4A). All other measured plant species exhibited no direct response to any biochar rate (Fig. S4; Tables S8–S10). Compost amendments

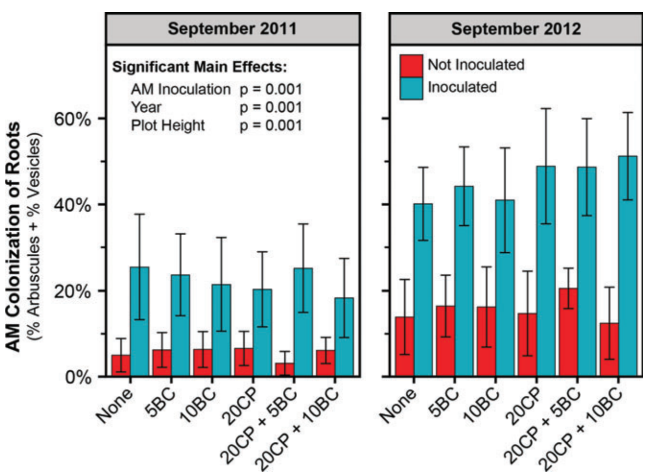

Figure 1. AM fungal colonization of the mixed community of field roots in the plant plug trial. Means with error bars ± 1 SD are given based on the most parsimonious linear mixed effects model. Experimental treatment replication = 9. The left panel represents growth after one growing season while the right panel represents growth after two seasons. Labels on the *x*-axis: None = no soil amendment, 5BC = 5 T/ha biochar, 10BC = 10 T/ha biochar, 20CP = 20 T/ha compost, 5BC + 20CP = 5 T/ha biochar + 20 T/ha compost, 10BC + 20CP = 10 T/ha biochar + 20 T/ha compost. Statistical output shows the significant main effect terms. Complete statistical information with significant interactions is given in Table S7.

Table 2. Statistical output for predicted total plant biomass in the plant plug trial (see Figure 2). Main effects included in the linear mixed effects model were: amendment, AM inoculation, plot height, and year. The % explained deviance is abbreviated as % Expl. Dev. in the output. Labels: 5BC = 5 T/ha biochar, 10BC = 10 T/ha biochar, 20CP = 20 T/ha compost. Post hoc comparisions of the interactions are given in the second half of the table.

Model Terms	p *Value*	*% Expl. Dev.*
Amendment	0.056	2.95%
Year	0.001	3.99%
Plot height (dry → wet)	0.068	0.90%
Significant Post Hoc Comparisons	p *Value*	
5BC → 20CP	0.039	n.a.
5BC → 20CP + 10BC	0.037	n.a.
10BC → 20CP	0.008	n.a.
10BC → 20CP + 5BC	0.052	n.a.
10BC → 20CP + 10BC	0.008	

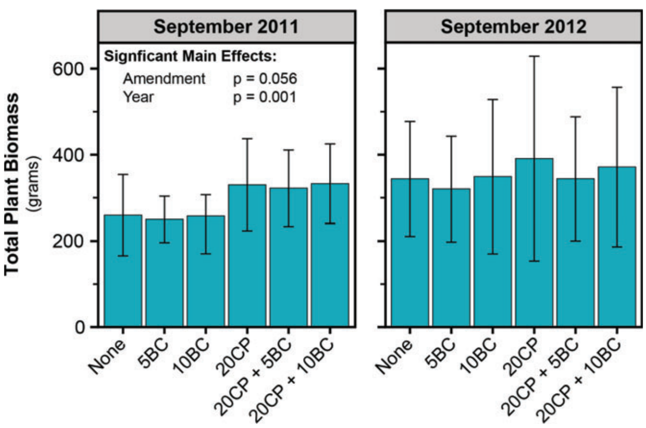

Figure 2. Estimated total plant biomass in the plant plug trial. Means with error bars ± 1 SD are given based on the most parsimonious linear mixed effects model (hence AM fungal inoculation was not a significant term and was dropped from the model). Experimental treatment replication = 9. The left panel represents growth after one growing season, and the right represents growth after two seasons. Labels on the *x*-axis: none = no soil amendment, 5BC = 5 T/ha biochar, 10BC = 10 T/ha biochar, 20CP = 20 T/ha compost, 5BC +20CP = 5 T/ha biochar + 20 T/ha compost, 10BC + 20CP = 10 T/ha biochar + 20 T/ha compost. Statistical output shows the significant main effect terms. Complete statistical information with significant interactions is given in Table 2.

led to a significant increase in plant biomass of *Desmodium canadense* ($p = 0.001$) in the plant plug trial compared to non-amended controls. *Andropogon gerardi* biomass was reduced in the presence of compost compared to non-amended control plots (Fig. S4A). No other direct compost-only effects were detected for the four other plant species in this trial.

AMF Inoculation. AMF inoculation did not significantly influence total plant biomass in the plant plug trial (Fig. 2), although each species varied in plant biomass when inoculated with *Rhizophagus irregularis*: *Panicum virgatum* ($p = 0.001$) and *Lespedeza capitata* ($p = 0.021$) responded positively to *R. irregularis* inoculation, while *A. gerardi* biomass was significantly reduced in AMF inoculated plots ($p = 0.022$). No inoculation response was detected for *Liatris cylindracea*, *Symphyotrichum laeve*, and *D. canadense*. Altogether, interspecies variation in plant response to AMF inoculation resulted in a neutral effect on the total biomass response in the community.

Synergistic Effects of Biochar, Compost, and AMF Inoculation. No significant differences were detected when comparing plant biomass in compost versus compost + biochar. Rather, plants treated with 10 T/ha of biochar + 20 T/ha of compost significantly increased biomass compared to plots with 5 T/ha of biochar ($p = 0.037$) and 10 T/ha of biochar ($p = 0.008$). Plant biomass was not significantly affected by biochar and compost as compared to nonamended controls ($p = 0.117$). The interaction of AMF inoculum and soil amendments did not significantly influence total plant biomass (Fig. 2; Table 2).

When considering individual plants, *L. capitata* biomass increased by the interaction among soil amendments and AMF inoculation (Fig. S4D). *Desmodium canadense* experienced biomass gains only in the presence of compost + biochar treatments compared to biochar only and non-amended plots (Fig. S4C), particularly for the September 2011 harvest (Fig. S4C). Only *A. gerardi* responded negatively to the compost + biochar treatments compared to non-amended control plots (Fig. S4A).

Other Factors. Growing season explained the highest amount of variation in biomass. N-fixing forbs (Fig. S4C & S4D) and composite forbs (Fig. S4E & S4F) had significantly reduced biomass between September 2011 and September 2012. Comparatively, C_4 grasses (*A. gerardi* and *P. virgatum*) experienced biomass gains between September 2011 and September 2012 (Fig. S4A & S4B). C_4 grasses were among the largest contributors to total biomass, accounting for total biomass gains from September 2011 to September 2012 (Fig. S4A & S4B) (for all, see Tables S8–S10). Plot height was significant and positive for all measured plant species except for *D. canadense* and *P. virgatum*, but not for all plants together ($p = 0.068$) (data not shown).

Soil Nutrients. The sand substrate was acidic and contained low levels of C and nutrients (Table 3). Compost increased soil nutrients more than any other treatment ($p = 0.001$) All minerals were elevated under compost treatments, regardless of biochar or AMF treatment (Table 3). Biochar increased soil pH compared to control plants ($p = 0.001$).

Seed Application Trial

Non-seeded volunteer plant cover was negligible throughout the study (mean % cover: 1.1%, range: from 0.0 to 16.0%) (data not shown). Pooled C_3 and C_4 grasses largely dominated vegetative cover after three growing seasons (2013 mean % cover: 17.4%, range: 2.0–42.0%). N-fixing forbs were the second most abundant by the third growing season (2013 mean % cover: 3.0%, range: 0.0–19.0%). The establishment and survival of composite forbs was sparse (2013 mean % cover: 0.4%, range:

Table 3. Soil chemistry for the plug experiment. Values represent mean (SE). Soils were collected at the end of the second growing season (2012), and evaluated for pH, total C, total N, total P, Ca, Mg, and K. Treatments sharing the same letter are not significantly different at $p = 0.05$, following an analysis of variance and Tukey post hoc tests.

Treatment	*pH*	*Total C (g/kg)*	*Total N (μg/g)*	*Total P (μg/g)*	*Ca (μg/g)*	*Mg (μg/g)*	*K (μg/g)*
Control	5.4(.35)a	1.18(.18)a	163(20)a	348(36)a	116(12)a	51(9)ab	19(5)a
Control + AMF	6.0(.19)ab	1.11(.18)a	167(23)a	345(26)a	114(14)a	53(7)ab	17(3)a
Control + biochar	6.4(.32)b	1.34(.12)a	190(17)a	334(30)a	109(6)a	47(9)a	18(3)a
Control + AMF + biochar	5.9(.39)ab	1.21(.25)a	189(19)a	316(42)a	106(9)a	45(9)a	15(3)a
Control + compost	5.9(.38)ab	2.38(.52)b	326(96)b	502(77)b	152b	58(1,410)ab	37(9)b
Control + compost + AMF	5.8(.37)ab	2.54(.55)b	303(92)b	532(104)b	157(18)b	64(6)b	38(7)b
Control + compost + biochar	6.3(.32)b	2.17(.18)b	324(77)b	510(81)b	146(24)b	66(6)b	35(8)b
Control + compost + biochar + AMF	6.0(.40)ab	2.29(.77)b	258(86)ab	536(76)b	158(18)b	56(16)ab	38(15)b

0.0–7.0%). Two plots in the southeast corner of the seed application trial (5 T/ha biochar + AM fungi and 5 T/ha biochar − AM fungi) were removed from the analysis because of close proximity to the research site's water table.

Plant Cover. Compost rate ($p = 0.025$) and growing season ($p = 0.001$) were the most influential drivers of total plant cover. Significant increases in total plant cover were largely driven by plots with three-way and four-way interactions among biochar, compost, AMF inoculation, and growing season (Table S11). The plot height covariate significantly influenced total cover where plots higher on the landscape had more plant cover regardless of treatment when accounting for growing season ($p = 0.002$) (Table S11).

Compost Amendments. Compost addition significantly increased plant cover in the seed application trial ($p = 0.025$) (Table S11). The compost × year interaction ($p = 0.001$) was driven by largely by variation in plots adding 40 T/ha compost.

Biochar Amendments. Biochar did not significantly influence total plant cover in the seed application trial ($p = 0.494$) (Table S11). In addition, the plot height covariate and growing season did not alter the influence of biochar on plant cover in the field ($p = 0.325$).

AMF Inoculation. AMF inoculation alone did not influence plant cover ($p = 0.268$) (Table S11), but over time, AMF inoculation tended to decrease plant cover ($p = 0.067$).

Synergistic Effects of Biochar, Compost, and AMF Inoculation. Overall, plot inoculation with AMF was most effective for increasing total plant cover when combined with high rates of biochar and compost, while accounting for plot height and growing season influences ($p = 0.012$). Plots with biochar and compost had more plant cover when accounting for growing season ($p = 0.040$). When adding AMF, plant cover increased only when combined with compost + growing season ($p = 0.018$) or compost + biochar ($p = 0.093$).

Discussion

This study shows that restoration success depends on the interaction among soil additives, soil type and choice of plant propagule. Adding biochar alone to post-mine sandpits that are nutrient-poor did not increase plant biomass compared to compost treatments and biochar + compost treatments when planting greenhouse-raised plugs. Compost was the most important solitary amendment for improving plant establishment when sowing native seeds, and this was largely because of the low C and nutrient conditions of the sand substrate. Co-amending compost with biochar and a commercial AMF inoculant accentuated its effectiveness compared to control plots in the seed application trial. As predicted, biochar and AMF application as solitary amendments were not beneficial for plant growth. These results support the use of co-amendments of compost, biochar, and AMF inoculum, thus can be effective land management tools to restore plants in severely disturbed post-mine landscapes, such as sandpits.

Plant Response to Compost

Compost as a solitary amendment promoted plant growth the most in both trials. In the plug trial, this resulted in increased plant growth compared to non-amended and biochar amended plots. In the seed application trial, compost addition resulted in increased total plant cover. These results are not surprising, as compost has long been shown to promote plant production from seed in other severely disturbed mine restoration scenarios (Noyd et al. 1996; Kohler et al. 2014; Gil-Loaiza et al. 2016). Such benefits are likely a result of compost's recognized fertilizer effect, its ability to increase water retention, and create higher cation exchange capacity in soils (Shiralipour et al. 1992), which may persist into future growing seasons (Diacono & Montemurro 2010). The compost effects shown here are likely magnified because sandy substrate is so nutrient poor.

Plant Response to Biochar

As predicted, biochar as a solitary amendment was not effective in promoting plant growth in the field. Plant response improved for compost and compost + biochar amendments compared to biochar only treatments in the plant plug trial. The suppressive effect of biochar only treatments may be a result of the high cation exchange capacity of biochar; biochar may have bound what few nutrients existed and induced more nutrient stress into an already nutrient limited system (Liang et al. 2006; Adams et al. 2013).

Positive vegetative response to biochar application in disturbed mine substrates has been attributed to increased sorption (Fellet et al. 2011; Kelly et al. 2014; Beesley et al. 2014) including changes to pH and nutrient bioavailability (Xu et al. 2013). Such changes in soil chemistry in low nutrient soil may have been detrimental to rapidly growing plants. This phenomenon was observed by Adams et al. (2013) who showed that for some grassland species, biochar was detrimental to growth unless soils were supplemented with nitrogen.

In the seed application trail, biochar did not affect plant growth. One reason that plug plants were more negatively affected by biochar may be because they grew faster than seeded plants and lacked sufficient access to soil nutrients. If so, biochar as a solitary amendment may only be beneficial when nutrients are not limiting. Therefore, restoration practitioners should approach the application of biochar to abiotically stressed mine areas with caution. Because plant response to biochar appears to depend, in part, on plant identity (Adams et al. 2013; Thomas & Gale 2015), biochar amendments in nutrient poor soil may have dramatic, long-term effects on plant community composition (van de Voorde et al. 2014).

Plant Response to AMF Inoculation

We found no evidence that inoculation by AMF increased plant biomass. This is surprising, because past studies have shown that AMF application in mine restoration generates positive plant responses from seed compared to non-inoculated controls (Noyd et al. 1996; Johnson 1998 , Richter & Stutz 2002; de Souza et al. 2010), particularly in severely degraded soils (Jin et al. 2013; Camprubi et al. 2015). However, AMF do not consistently increase plant response for all plant species or restoration scenarios: mycorrhizal associations range from mutualistic to parasitic depending upon the environmental context and host species (Johnson et al. 1997; Klironomos 2003). Thus, the universal application of a solitary AMF isolate may not benefit all target plants when restoring grassland habitat.

We failed to detect an AMF effect in the plug experiment but this is likely because we had no “non-AMF” controls (all plugs were colonized by AMF in the greenhouse, and thus all plugs were introduced to the field already colonized by fungi). For the seed trial, plants had a larger response to inoculation, likely due to the difference in inoculum potential between non-inoculated controls and amended treatments. In our system, inoculum potential in the mine substrate was likely to be very low as most infective propagules would have been removed during mining (Allen & Allen 1980; Stahl et al. 1988). Any established hyphal networks present before the disturbance would have been destroyed (Jasper et al. 1989).

We cannot determine if the commercial isolate established, or if naturally occurring fungi were responsible for the observed effect in the plug trial. The fact that there was a large mycorrhizal effect in the seed trial suggests that our inoculant did establish; however, we are not able to rule out the presence of other AMF, which may have tolerated mining disturbance or recovered due to dispersal from local species pools (Jasper 2007).

Synergism Among Biochar, Compost, and AMF

When added as solitary amendments, compost addition outperformed the addition of AMF or biochar in terms of plant response. The synergistic effect of all three amendments together accounted for the largest plant response in seed trial, but this was not true for the plug trial. This may be because the plants in the seed trial had more to gain than plant plugs.

Plants in the seed trial were forced to germinate and establish a mycorrhizal network in already nutrient impoverished state, whereas plant plugs were introduced into the sandpit with intact mycorrhizal networks. Under low nutrient conditions, AMF may suppress plant growth by competing with the plant for nutrients (Hart & Forsythe 2012), particularly when developing AMF create a significant carbon sink for young plants. Therefore, the interaction of increasing rates of compost with AMF inoculation should alleviate plant stress in the post-mine environment and facilitate greater response from plants germinated from seed on-site (Hammer et al. 2011).

Alone, the amendments may have imposed stress on germinating seedlings. The use of compost to ameliorate the harsh edaphic conditions of post-mine sites is highly recommended to accelerate plant community development. Land managers should approach the addition of biochar amendments with caution as this may induce further stress in post-mine substrates. Under less extreme conditions, however, plants may gain less from co-amendments, and practitioners may benefit more from a site specific, targeted approach.

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Supporting Information

The following information may be found in the online version of this article:

Table S1. Chemical analysis of urban yard waste compost added to the experimental plots in June 2010.
Table S2. The eight grassland plant species used in the plant plug trial and seed application trial.
Table S3. Morphological characters measured in the field for the six plant species in September 2011 and September 2012.
Table S4. Morphological characters selected for plant species measured in September 2011 and September 2012.
Table S5. Partial least squares regression diagnostics for the six plant species measured in September 2011 and September 2012.
Table S6. Seed addition in grams (g) per plot for the eight grassland plant species used in the seed application trial.
Table S7. AM fungal colonization of the mixed community of field roots in the plant plug trial.
Table S8. Statistical output for C_4 grass biomass (*Andropogon gerardi* and *Panicum virgatum*) in the plant plug trial.
Table S9. Statistical output for N-fixing biomass (*Desmodium canadense* and *Lespedeza capitata*) in the plant plug trial.

Table S10. Statistical output for forb biomass (*Symphyotrichum laeve* and *Liatris cylindracea*) in the plant plug trial.
Table S11. Statistical output for total native plant cover in the seed application trial based on the most parsimonious linear mixed effect model.

Figure S1. Diagram of the plant plug layout with plant positioning.
Figure S2. Collecting photographic data to analyze percent plant cover.
Figure S3. Percent AM fungal colonization of greenhouse-grown plant plug roots.
Figure S4. Predicted plant biomass for each species in the plant plug trial.

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