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A preliminary assessment of the potential of using an acacia—Biochar system for spent mine site rehabilitation

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Abstract Mining activities result in extensive soil degradation by removing the top soil, disturbing soil structure and altering microbial communities. Rehabilitation of spent mine sites through revegetation thus requires proper soil amendments. In this study, a pot trial was conducted to investigate the effects of a jarrah biochar on the growth and nutrient status of a native legume, *Acacia tetragonophylla*, grown in a mixture of topsoil and mine rejects. Two biochar application rates (37 and 74 t ha⁻¹) and two types of biochar, namely nutrient-enriched and non-enriched, were tested. We measured the soil pH and electrical conductivity, the carbon (C) and nitrogen (N) contents and C and N isotope composition ($\delta^{13}\text{C}$ and

$\delta^{15}\text{N}$) of soil and plants, the foliar phosphorus content and the growth and leaf biomass of the plants. Whilst no significant effect of biochar was observed on plant growth, biochar amendment affected soil properties and plant nutritional status. The highest rate of biochar application increased soil pH, C content and C/N ratio, and decreased soil $\delta^{13}\text{C}$. Biochar application also enhanced photosynthetic N use efficiency, as showed by the increase in foliar C/N ratio, and biological N fixation rates, as indicated by foliar $\delta^{15}\text{N}$. These positive effects were not observed when biochar was nutrient-enriched due to the associated increase in soil N. Revegetation of mine sites with acacia in combination with biochar amendment constitutes a plausible alternative to the wide use of N fertiliser through the supply of additional N to the system, even though other nutrients may be required in order to enhance plant early growth.

Keywords *Acacia tetragonophylla* · Mine rejects · Biological nitrogen fixation · Photosynthetic nitrogen use efficiency · Isotope composition

Introduction

Mineral resources represent an important component of Australia's economy (Hajkowicz et al. 2011) but mining operations also cause intensive land degradation, including the removal of vegetation cover, loss of top soil and production of a large amount of mine wastes (Wong et al. 2003). Government regulations require offsetting activities and rehabilitation of the degraded areas after the mining activity has ceased. Because the most fertile layers of soils are usually removed during the mining process (Mench et al. 2003), post-mining site revegetation is often challenging (Machado et al. 2013). The early stage of vegetation establishment is particularly crucial and requires good site preparation practices to

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maximise the chance of success of the revegetation process (Ruthrof et al. 2013; Hosseini Bai et al. 2014).

Site preparation practices have the potential to modify soil physicochemical properties (Hosseini Bai et al. 2012), thereby influencing nutrient availability for the establishing plants (Tan et al. 2005; Ibell et al. 2010). The application of soil amendments and fertilisers for example will facilitate the establishment of a broader range of vegetation (Bell 2001) by reducing competition for available water and nutrients (Ibell et al. 2013). Biochar, a carbon (C)-rich substrate and by-product of organic matter residue pyrolysis, is considered to be a promising soil amendment to enhance soil quality whilst reducing the global C footprint through soil C sequestration (Lehmann 2007; Barrow 2012). Biochar has been reported to improve soil structure and soil cation exchange capacity, as well as to retain plant available water due to its porous structure (Lehmann et al. 2003; Laird et al. 2010; Anderson et al. 2011). The application of biochar hence constitutes a site preparation method that could potentially increase the success of revegetation. However, some studies have shown that biochar only had a positive effect on plant growth when applied in conjunction with fertilisers (Rajkovich et al. 2012; Zhang et al. 2012), because high rates of biochar application can promote nitrogen (N) immobilisation (Van Zwieten et al. 2010a; Bruun et al. 2012). Since degraded soils are likely to have limited N pools (Wong 2003), it is essential to ensure that this decrease in N availability following biochar application would not impair revegetation success. Combining biochar amendment with leguminous plants could help offset the potential decrease in N availability by ensuring an additional source of N input into the system (Forrester et al. 2005; Quilliam et al. 2013).

Little information is available regarding the combined effect of biochar and legumes for land rehabilitation. Biochar has previously been reported to increase biological N fixation (Rondon et al. 2006) through increases in nitrogenase activity (Quilliam et al. 2013), which would enhance the positive effects of legumes on N-poor soils. In addition, some legume species have been considered to be adequate species for the rehabilitation of degraded sites due to their high adaptability to harsh environmental conditions, including drought and low nutrient availability (Adams et al. 2010; Reverchon et al. 2012). The objective of this study was then to investigate the effect of biochar applied onto a mine site soil on the growth and nutrient status of early-stage *Acacia tetragonophylla* F. Muell. (hereafter referred to as acacia, unless otherwise specified). Since several studies have noted an increase in plant growth following biochar application only when fertilisers were applied (Major et al. 2010; Rillig et al. 2010), we also tested whether saturating biochar in a nutrient solution prior to its application would affect soil and acacia characteristics during the early stage of plant establishment.

Methods

Biochar preparation

The jarrah wood biochar (*Eucalyptus marginata* Donn ex. Sm.) used in this study is the same as the jarrah biochar used in our previous work (Reverchon et al. 2014). The biochar was produced in an ANSAC HK indirectly fired kiln, at a maximum pyrolysis temperature of 700 °C and a residence time of 20 min under depleted oxygen environment. We then boiled 7 kg of biochar in an equivalent volume of water for 60 min in an unpressurized 20 L pot in order to remove most of the toxic phenols. After boiling, the biochar was drained and oven-dried for 48 h at 70 °C. Biochar characteristics were reported in Reverchon et al. (2014). Briefly, biochar Brunauer–Emmett–Teller (BET) surface area was 252 m² g⁻¹, its micropore area was 228 m² g⁻¹ and its average pore diameter was 2.14 nm. Biochar pH measured in water (1:5 ratio) was 9.22, its total C content was 74.7 % C and its total N content was 0.11 % N.

In addition, we saturated the boiled biochar in a nutrient-rich solution (Hoagland's No. 2 Basal Salt Mixture, Sigma) prior to its application to test for the effect of fertiliser in combination with biochar application. Hoagland's solution is a widely used fertiliser that principally contains calcium and potassium nitrate, magnesium sulfate and ammonium phosphate. This additional treatment was also used in our previous study (Reverchon et al. 2014) which showed that the application of nutrient-enriched biochar enhanced nutrient availability and offset the competition between biochar and plants for nutrients.

Greenhouse pot trial

The greenhouse pot trial consisted of four treatments: (1) application of 2.5 % (w/w) biochar, which corresponds to a rate of 37 t h⁻¹; (2) application of 2.5 % (w/w) nutrient-enriched biochar; (3) application of 5.0 % (w/w) biochar, which corresponds to a rate of 74 t h⁻¹ and (4) application of 5.0 % (w/w) nutrient-enriched biochar. These biochar application rates were selected to be consistent with those used in our previous experiment (Reverchon et al. 2014). A control was included to the pot trial (no biochar application). Each treatment was replicated five times.

To investigate the feasibility of using biochar as a soil amendment for mine site rehabilitation, we used as a substrate a mixture of topsoil and mine rejects collected at BHP Billiton Iron Ore Pty Ltd's Mt Whaleback iron ore mine, located near Newman, in the Pilbara region of Western Australia. Soil and mine rejects were crushed and sieved to pass a 3-mm mechanical sieve and mixed homogeneously at a 1:1 (w/w) ratio. The different rates of biochar were added to 4.66 kg of the soil and

159	mine reject mixture (hereafter referred to as soil), and mixed	transformed. A Tukey post hoc test was used where the	208
160	thoroughly before being placed in 4 L pots.	ANOVA was significant to compare means between treat-	209
161	Seeds of acacia, a legume occurring naturally in the mining	ments. All analyses were considered significant at $p < 0.05$.	210
162	area where the soil and mine rejects were collected, were	Statistics were computed in SPSS version 21 (SPSS, Inc.).	211
163	immersed in boiled water for 24 h to break the seed shell		212
164	and favour germination. At the first sign of hypocotyl emer-		
165	gence, the seeds were transferred to 36-mm peat pellets, in		
166	order to minimise the transplant shock. One seed was sown	Results	213
167	per pot at 20 mm depth. The seedlings were watered and soils	Effect of biochar on soil properties	214
168	were kept at 60 % water holding capacity under the green-		
169	house conditions.		
170	Soil and plant analyses		
171	Soil and foliar samples were collected after 6 months for	The addition of biochar to the soil increased soil pH signifi-	215
172	physical and chemical analyses. Soil samplers of 20 ×	cantly compared to the control, regardless of the rate of	216
173	250 mm were used to obtain the soil samples. For each pot,	application. On the other hand, biochar addition did not influ-	217
174	two samplers were inserted diametrically opposite to each	ence soil EC (Table 1). As expected, the total C content of the	218
175	other, at the midpoint between the centre and the edge of the	soil increased significantly with the rate of biochar addition,	219
176	pot, 30 min after watering. The cores were then mixed thor-	with a sevenfold increase in the soil total C when 74 t ha ⁻¹ of	220
177	oughly to obtain one soil sample per pot. Approximately, 50 g	biochar was applied. The total N content of the soil was not	221
178	of each soil sample was air-dried and ground to a fine powder	significantly influenced by the biochar application rate but	222
179	using a Rocklabs ring grinder. We determined the total C, total	was affected by the nutrient-enrichment of biochar. At the	223
180	N and C and N isotope composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respec-	highest rate of biochar application, the total N content of the	224
181	tively) using mass spectrometry (GV Isoprime; Manchester,	soil was higher when biochar was nutrient-enriched than when	225
182	UK), as described in He et al. (2008). Soil pH and electrical	non-enriched biochar was applied. Significant differences in	226
183	conductivity (EC) were measured in water (1:5 ratio).	the C/N ratio of the soil were also found amongst treatments,	227
184	After 6 months, plants were measured for height and total	the highest C/N ratio being found when 74 t ha ⁻¹ of non-	228
185	number of leaves. Seedlings were still too small at 6 months	enriched biochar was applied, while the lowest C/N ratio was	229
186	for stem diameter to be accounted for. Only aboveground	found under the control conditions.	230
187	parameters were measured as plants were not harvested. One	Biochar application also influenced soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.	231
188	seedling in the 74 t ha ⁻¹ application of non-enriched biochar	Soil $\delta^{13}\text{C}$ decreased significantly with the rate of non-enriched	232
189	treatment died during the experiment, decreasing our total	biochar addition (Table 1). In addition, soil $\delta^{13}\text{C}$ after the	233
190	plant number to 24. In addition to plant measurements, ten	application of enriched biochar was lower than that of the	234
191	fully expanded leaves were collected from each plant, oven-	control. Biochar application also affected soil $\delta^{15}\text{N}$, the	235
192	dried at 60 °C to a constant weight and ground finely to	highest value for soil $\delta^{15}\text{N}$ being found when 74 t ha ⁻¹ of	236
193	measure the total C, total N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ using mass	enriched biochar was added (Table 1).	237
194	spectrometry. Total plant phosphorus (P) was determined		
195	following Kjeldahl digestion method (Taylor 2000). The total	Effect of biochar on acacia growth and foliar nutrient status	238
196	leaf dry mass was inferred by multiplying total number of		
197	leaves from the average weight of one leaf.	Biochar, regardless of the application rates and nutrient en-	239
198	Statistical analyses	richment, did not have a significant effect on acacia height or	240
199	An analysis of variance (ANOVA) was carried out to deter-	leaf biomass (Table 2). However, significant differences were	241
200	mine the significant differences between the various treat-	observed in acacia nutrient status amongst the treatments	242
201	ments. The variables that were included in the ANOVA were	(Table 3). The acacia total C content was significantly higher	243
202	soil pH and EC, soil and plant total C, total N, C/N ratio, $\delta^{13}\text{C}$	when biochar was applied to the soil than under the control	244
203	and $\delta^{15}\text{N}$, plant total P and plant height and leaf biomass. Prior	conditions, especially when biochar was not nutrient-	245
204	to the ANOVA, we used the Shapiro–Wilk test to verify	enriched. Whilst no significant effect of biochar was detected	246
205	variable normality and the Levene's test to verify homogene-	on acacia total N content, the acacia C/N ratio was significant-	247
206	ity of variance. Variables that did not meet these assumptions	ly higher when 37 t ha ⁻¹ of non-enriched biochar were added	248
207	(soil pH, soil total C and total N and soil $\delta^{15}\text{N}$) were log-	to the soil than under the control conditions. Differences were	249
		also observed in the acacia P status, with the plant total P	250
		being higher when 74 t ha ⁻¹ of enriched biochar was added to	251
		the soil than that when 37 t ha ⁻¹ of nutrient-enriched biochar	252
		was applied.	253

Table 1 Influence of biochar application on soil pH, EC, total C, total N, C/N and C and N isotope composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). Values represent means $\pm$ standard errors

Treatment	pH	EC (μS)	Total C (g kg^{-1})	Total N (g kg^{-1})	C/N	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Control	5.8 $\pm$ 0.05b	244 $\pm$ 46.6a	3.74 $\pm$ 0.11c	0.28 $\pm$ 0.006ab	13.6 $\pm$ 0.21c	-19.1 $\pm$ 0.12a	3.63 $\pm$ 0.07ab
37 t ha $^{-1}$ non-enriched biochar	7.6 $\pm$ 0.20a	332 $\pm$ 27.4a	18.3 $\pm$ 1.98b	0.25 $\pm$ 0.035ab	77.2 $\pm$ 8.03b	-25.6 $\pm$ 0.25b	2.38 $\pm$ 0.40b
37 t ha $^{-1}$ enriched biochar	7.2 $\pm$ 0.30a	339 $\pm$ 37.1a	18.5 $\pm$ 1.46b	0.27 $\pm$ 0.026ab	71.4 $\pm$ 11.85b	-25.7 $\pm$ 0.27bc	3.59 $\pm$ 0.23ab
74 t ha $^{-1}$ non-enriched biochar	7.8 $\pm$ 0.13a	398 $\pm$ 66.8a	31.8 $\pm$ 1.38a	0.23 $\pm$ 0.022b	149.4 $\pm$ 18.90a	-26.6 $\pm$ 0.06c	5.22 $\pm$ 1.10ab
74 t ha $^{-1}$ enriched biochar	7.0 $\pm$ 0.24a	228 $\pm$ 42.4a	30.4 $\pm$ 4.70a	0.34 $\pm$ 0.012a	90.1 $\pm$ 15.83b	-26.2 $\pm$ 0.12bc	6.81 $\pm$ 1.47a

Different letters within columns represent significant differences (Tukey post hoc test, $p < 0.05$)

The application of biochar did not affect foliar $\delta^{13}\text{C}$, but influenced foliar $\delta^{15}\text{N}$. Foliar $\delta^{15}\text{N}$ was significantly higher when nutrient-enriched biochar was applied, regardless of the rate of application, than when non-enriched biochar was added. Nevertheless, foliar $\delta^{15}\text{N}$ following nutrient-enriched biochar application did not differ from that of the control. Furthermore, foliar $\delta^{15}\text{N}$ was significantly lower under the highest rate of biochar application than when no biochar was added (Table 3).

Discussion

Effect of biochar on the soil properties

The effects of biochar application on the properties of the mixture of topsoil and mine rejects are consistent with findings reported for other types of soil. The increase in soil pH following biochar addition has been described previously (Van Zwieten et al. 2010b; Nelissen et al. 2012; Reverchon et al. 2014) and attributed to a higher pH of biochar (9.2 for the biochar used in this study) than that of the soil. The increment in pH after biochar addition was particularly significant in the acidic soil used in this study, as pH increased by up to 2 units under the highest rate of biochar application. Beesley et al.

(2010) and Luo et al. (2011) also described sharp increases in soil pH following biochar application to acidic soils (initial soil pH=5.5 and 3.7, respectively). Van Zwieten et al. (2010b) attributed the liming effect of biochar to the presence of carbonate complexes in the biochar, hence promoting its high acid neutralising ability. Soil pH has been reported to be a critical factor controlling revegetation success (Córdova et al. 2011), and biochar could be used as a liming amendment for enhancing nutrient availability and plant survival during the early stage of vegetation establishment. In addition to increasing the soil pH, biochar is usually reported to increase soil EC (Atkinson et al. 2010; Fellet et al. 2011; Smider and Singh 2014) through the input of wood ash containing readily soluble cations (Soinne et al. 2014). The lack of effect of biochar on soil EC in this study may be explained by the fact that biochar was boiled before being mixed with the soil, which could have led to cation loss.

The increase in the total C and C/N of the soil and the decrease in soil $\delta^{13}\text{C}$ with the application of increasing rates of biochar are most likely due to the intrinsic properties of biochar itself rather than to changes in the soil microbial activity (Ibell et al. 2010). Biochar is a C-rich product with a lower $\delta^{13}\text{C}$ (-26.2‰ for the biochar used in this study) than that of the soil under study. The initial C content of the soil mixture used in this study was particularly low, and biochar addition could constitute a means to restore some of the lost soil structure and stability (Atkinson et al. 2010), thereby favouring the revegetation process.

Biochar did not affect the N content and $\delta^{15}\text{N}$ of the soil, except when nutrient-enriched at the highest rate of application. The lack of effect of biochar on the soil N pools (Reverchon et al. 2014) or the decrease in N availability following biochar application in the short-term (Nelissen et al. 2012; Rajkovich et al. 2012) have prompted the recommendation of using biochar in combination with a fertiliser (Steiner et al. 2008; Van Zwieten et al. 2010a). This is particularly critical where nutrient retention could hamper plant early establishment on degraded soils that require stabilisation by revegetation (Beesley et al. 2011).

Table 2 Influence of biochar application on growth variables of *Acacia tetragonophylla*. Values represent means $\pm$ standard errors

Treatment	Plant height (cm)	Total leaf biomass (g)
Control	31.4 $\pm$ 3.0a	2.7 $\pm$ 0.5a
37 t ha $^{-1}$ non-enriched biochar	33.0 $\pm$ 4.2a	3.6 $\pm$ 0.7a
37 t ha $^{-1}$ enriched biochar	33.2 $\pm$ 4.0a	4.5 $\pm$ 0.7a
74 t ha $^{-1}$ non-enriched biochar	33.5 $\pm$ 4.9a	3.3 $\pm$ 0.5a
74 t ha $^{-1}$ enriched biochar	37.0 $\pm$ 4.4a	5.1 $\pm$ 1.0a

No significant differences were found amongst treatments, as shown by the same letters within columns (Tukey post hoc test, $p < 0.05$)

Table 3 Influence of biochar application on foliar nutrient status and C and N isotope composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of *Acacia tetragonophylla*. Values represent means $\pm$ standard errors

Treatment	Total C (g kg ⁻¹)	Total N (g kg ⁻¹)	C/N	Total P (g kg ⁻¹)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Control	437 $\pm$ 4.0c	21.6 $\pm$ 1.61a	20.7 $\pm$ 1.5b	0.74 $\pm$ 0.07ab	-32.31 $\pm$ 0.34a	4.56 $\pm$ 0.60ab
37 t ha ⁻¹ non-enriched biochar	464 $\pm$ 2.4a	17.4 $\pm$ 1.45a	27.5 $\pm$ 2.4a	0.64 $\pm$ 0.08ab	-32.93 $\pm$ 0.18a	1.69 $\pm$ 1.21bc
37 t ha ⁻¹ enriched biochar	446 $\pm$ 5.6abc	19.7 $\pm$ 1.05a	23.0 $\pm$ 1.5ab	0.62 $\pm$ 0.47b	-32.81 $\pm$ 0.14a	6.52 $\pm$ 1.39a
74 t ha ⁻¹ non-enriched biochar	459 $\pm$ 4.0ab	20.7 $\pm$ 0.69a	22.3 $\pm$ 0.8ab	0.76 $\pm$ 0.05ab	-33.25 $\pm$ 0.16a	0.31 $\pm$ 0.63c
74 t ha ⁻¹ enriched biochar	444 $\pm$ 5.3bc	22.5 $\pm$ 1.16a	19.9 $\pm$ 1.0b	1.07 $\pm$ 0.18a	-33.19 $\pm$ 0.33a	7.95 $\pm$ 0.57a

Different letters within columns represent significant differences (Tukey post hoc test, $p < 0.05$)

Effect of biochar on foliar nutrient status of acacia

Although biochar application did not have any significant effect on acacia height or leaf biomass, it positively influenced acacia leaf C and C/N ratio, especially when non-enriched biochar was applied. The C/N ratio indicates the C and N relationship in plants and is therefore an appraisal of photosynthetic N use efficiency (Mohale et al. 2014). This enhancement of N use efficiency was probably not associated with an increase in photosynthetic capacity, as no variations in leaf $\delta^{13}\text{C}$ were observed after biochar addition. Foliar $\delta^{13}\text{C}$ can be related to plant productivity through photosynthesis but also through water use efficiency (Farquhar and Richards 1984; Xu et al. 2000). The constant water holding capacity at which the pots were kept is hence likely to have hindered any effect of biochar on water use efficiency by acacias. The addition of nutrient-enriched biochar cancelled the increase in the photosynthetic N use efficiency observed through the foliar C/N ratio. The highest application rate of nutrient-enriched biochar constituted a significant additional supply of N as shown by the subsequent increase in the N content of the soil, which could have reduced the plant N use efficiency.

The additional N input into the system by enriching biochar in a fertiliser solution also affected leaf $\delta^{15}\text{N}$. Plant $\delta^{15}\text{N}$ indicates the conditions of N nutrition where and when the plants were grown (Reverchon et al. 2014) and, as such, is usually considered as an estimate of soil $\delta^{15}\text{N}$ at the time of growth (Xu et al. 2003; Ibell et al. 2013). In legumes, however, leaf $\delta^{15}\text{N}$ is an indication of biological N fixation (BNF) (Hosseini Bai et al. 2013) and has been frequently used in the so-called ^{15}N natural abundance method to estimate the percentage of N derived from the atmosphere (Shearer and Kohl 1986). A reduction in foliar $\delta^{15}\text{N}$ corresponds to a relative increment in BNF, although reference plants are necessary in order to assess the exact quantity of N fixed through BNF. In this study, the application rate of non-enriched biochar decreased leaf $\delta^{15}\text{N}$, thereby increasing BNF rates. The coupled increase in C/N values and decrease in $\delta^{15}\text{N}$ values (Fig. 1) suggest that the photosynthetic N use efficiency was enhanced by higher levels of BNF (Mohale et al. 2014). Nevertheless, the increment in BNF rates was not observed when nutrient-

enriched biochar was added (Fig. 1). Previous findings have shown that BNF increases under biochar application (Rondon et al. 2006) and decreases under fertiliser application (Ledgard and Steele 1992; Vessey and Waterer 1992). The BNF is a costly biological process that can promote plant survival under N-limited conditions (Hosseini Bai et al. 2013) but will not be needed when N supplies are sufficient for plant growth. In conclusion, whilst fertiliser addition in combination with biochar application may be required in order to guarantee plant growth in the early stage of plant establishment under a broad range of conditions, it may be unnecessary where acacias are to be used, which would hence decrease the associated risk of further environmental pollution (Graham and Vance 2000). Although the use of fertiliser could also benefit plant growth by increasing the foliar contents of other nutrients, especially P, in this study, we did not observe differences in foliar P when comparing non-enriched vs enriched biochar.

Recommendations for mining soil rehabilitation

In this study, we found that acacia combined with jarrah biochar could offer a plausible alternative for mining site rehabilitation. Although in this case biochar addition did not significantly improve plant growth, it enhanced BNF rates, thereby leading to the additional supply of N to the soil by litter fall. The additional input of N to the substrate may be crucial for the success of early establishment of plants on mine

Fig. 1 Effect of biochar application on foliar $\delta^{15}\text{N}$ (bars) and C/N ratio (triangles) of *Acacia tetragonophylla*. Non-enriched biochar application is represented in black and nutrient-enriched biochar in gray. Values represent means $\pm$ standard errors

rejects. Novak et al. (2009) suggested that biochar production could be adapted to fit the specific characteristics of a degraded soil in order to maximise its benefits. Wood biochars formed through slow pyrolysis were recommended by Jien and Wang (2013) as an efficient acid-neutralizing material for highly degraded soils and as a way to improve soil structure and erosion resistance through the increase aggregate stability. Some considerations should also be given to the specific mechanisms of contaminant-biochar retention and release over time to ensure that the success of revegetation coincides with an effective management of environmental pollution (Beesley et al. 2011). Finally, the impact of biochar on beneficial soil microorganisms should be further investigated, especially when the plants used for revegetation are known to form symbiotic associations. This is the case for acacias, which form associations with N-fixing bacteria and with mycorrhizal fungi. In the present trial, plants were not harvested in order to continue investigating longer-term effects of biochar on acacia growth. Therefore, we could not obtain data regarding the response of root biomass and rhizospheric microorganisms to biochar addition. Nevertheless, there are indications that biochar application has a positive short-term effect on N-fixing bacteria (Quilliam et al. 2013) and on arbuscular mycorrhizal fungi activity (Warnock et al. 2007). These positive effects of biochar on soil microorganisms still need to be assessed in the long-term and in field studies. Nevertheless, the potential of using biochar associated with acacias for revegetation of spent mine sites seems promising.

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