Influences of Biochar and Biochar-Mineral Complex on Mycorrhizal Colonisation and Nutrition of Wheat and Sorghum

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ABSTRACT

The high price of synthetic fertilisers and the price barrier for biochar as a soil amendment have encouraged the exploration of using biochar in fertiliser replacement formulations. Biochars coupled with fertilisers can be applied at lower application rates to achieve benefits in plant growth and nutrition, as well as soil biological fertility. It is necessary to evaluate the use of biochar as a fertiliser substitute. Therefore, this study investigated the comparative influences of biochars, including Acacia saligna (AS), Simeco jarrah (SJ) and Wundowie jarrah (WJ), mineral fertiliser with microbes (MF + M), biochar-mineral complex (BMC) and their combination on mycorrhizal colonisation, growth and nutrition of wheat in a glasshouse experiment and sorghum in field conditions. BMC + MF + M treatment produced higher mycorrhizal colonisation than MF + M alone, indicating that BMC had a significant role in increasing mycorrhizal colonisation. SJ (treated with acetic acid) and MF + M treatments, as well as AS + MF + M application, showed similar effects on mycorrhizal colonisation, but lower colonisation than the BMC + MF + M treatment. Overall, the BMC + MF + M treatment supported the maximum shoot, root and total plant dry weight followed by AS + MF + M and WJ + MF + M. The MF + M treatment had the maximum shoot N and K concentrations, while BMC + MF + M application had the maximum shoot P concentration. AS + MF + M and WJ + MF + M treatments supported the maximum N uptake by wheat shoots, while BMC + MF + M supported the maximum P uptake. The results showed that biochars and BMCs could increase mycorrhizal colonisation, plant growth and nutrient uptake of wheat, particularly N, P, K, S and Zn. The field experiment confirmed that BMC application at a rate of 300 kg ha\(^{-1}\) could increase the yield of irrigated sorghum on a loam soil and provide better applied P use efficiency compared to a water-soluble fertiliser alone. These results indicated that biochar-based fertilisers might increase the resilience and sustainability of dryland cropping in environments such as in Western Australia and warrant further field evaluation.

Key Words: carbon sequestration, nutrient uptake, P use efficiency, soil biological fertility, wheat production


Biochar is a product of pyrolysis of either plant or animal biomass through heating at 300 to 600 °C under partial exclusion of oxygen (Antal and Grenli, 2003), resulting in highly aromatic organic material with C concentrations of 400 to 800 g kg\(^{-1}\) (Lehmann et al., 2002; Lehmann and Rondon, 2006). Biochar is a variable charge organic material, with a relatively high surface area and pore volume, that has the potential to increase soil water-holding capacity, pH, cation exchange capacity (CEC), surface sorption capacity, nutrient content, and base saturation (Glaser et al., 2002; Bélanger et al., 2004; Keech et al., 2005; Liang et al., 2006). These properties change in relation to the temperature of biochar formation and the feedstock used for biochar production (Gundale and DeLuca, 2006; Bornermann et al., 2007). The increased nutrient availability to plants is the results of both the direct nutrient additions in biochar and greater nutrient retention against leaching (Lehmann et al., 2003), but it can also be an effect of changes in soil microbial dynamics (Lehmann and Rondon, 2006). Interactions between biochar, soil, microbes and plant roots may occur within a short period of time after application to the soil (Joseph et al., 2010). Biochars undergo dissolution-pro-
cipitation, adsorption-desorption, acid-base and redox reactions after application to soil. Low-temperature biochars have a greater reactivity in soils and support higher crop yields than higher-temperature biochars, possibly due to their higher available nutrient contents that provide a greater contribution to soil fertility (Chan et al., 2008; Steinbeiss et al., 2009).

Joseph et al. (2010) stated that the following time-sequence of reactions takes place when biochars are added to moist soils: i) there is a change, within the first week, in the pH, electrical conductivity (EC), and CEC around the particle, as the minerals in biochar dissolve and/or ions are exchanged with the surfaces of surrounding clay particles; ii) a rain event can result in the inclusion and enhanced surface interaction of soil mineral and organic compounds with the biochar particles; iii) a considerable quantity of soluble organics can be released to the soil solution that can stimulate both seed germination and growth of fungi (Vigilante et al., 1998; Light et al., 2009); iv) enhanced CO$_2$ emissions during the first months after biochar addition to soil are partly attributed to biochar surface oxidation. There are also several different biotic and abiotic reaction mechanisms involved. High mineral ash biochars oxidise faster than low mineral ash biochars (Amonette et al., 2006); v) following surface oxidation of biochars, the potential for hydrophilic interactions of biochars with a range of soil organic and inorganic compounds increases. This is more significant in high mineral ash biochars (Lima and Marshall, 2005). Greater reactivity of biochars with mineral particles in soil could further promote physical protection of biochar and enhance long-term stability (Brodowski et al., 2006); and vi) once roots and root hairs interact with the biochars, a much wider range of reactions can occur, involving the uptake of nutrients and release of root exudates, which enhances both complexation reactions and microbial activity in the rhizosphere.

Biochar additions to soil alter soil microbial populations, increase the concentration of functional groups (Pietikäinen et al., 2000), and have the potential to reduce soil bulk density (Gundale and DeLuca, 2006). Biochars may act as a habitat for soil microorganisms involved in N, P and S transformations (Pietikäinen et al., 2000). Soil microbial diversity and activity may be affected by the amount and type of biochar added to the soil (Thies and Suzuki, 2003; Lehmann and Rondon, 2006). Microbes have a significant influence on nutrient cycles and nutrient availability to plants. Biochar can act as an inoculum carrier for arbuscular mycorrhizal (AM) fungi (Saito and Marumoto, 2002). A more rapid cycling of nutrients in soil organic matter and microbial biomass, as well as better AM fungi colonisation of roots, will improve nutrient availability and crop yields by retention of nutrients against leaching in highly weathered soils of the humid tropics that have low CEC, and by better access of plant roots to available P far from the rhizosphere due to mycorrhizal colonisation.

Biochar has the potential to increase net nitrification in acid forest soils (DeLuca et al., 2009). The mechanisms behind this stimulation remain the subject of ongoing debate; however, it is likely to be due to the sorption of compounds and/or changes in abundance of specific micro-organisms. In contrast, biochar does not increase ammonification, and although biochar applications have been found to increase plant N uptake, there is no increase in available N in soil. This may be a result of the capacity of biochar to adsorb NH$_4^+$, resulting in no measurable increases in net ammonification. Biochar additions to soils stimulate mycorrhizal colonisation, which may increase P uptake; but when biochar is applied with P fertiliser, this effect may disappear. Addition of biochar to soil increases the availability of major cations and P, as well as total N concentration in soil and finally to plants (Glaser et al., 2002; Lehmann et al., 2003). The response function is dependent on the properties of the biochar, soil properties (greater response occurs on nutrient-deficient sandy soils), concurrent nutrient and organic matter additions, and plant species.

Gross margins for dryland wheat production in south-western Australia tend to be small and profitability is at risk through price fluctuations for inputs and products (Kingwell, 2000). Changes to crop growing conditions under climate change also threaten to cause more frequent periods of decline in gross margin (Sasse, 2009). If biochar in Australia is, hypothetically, sold at US$ 300 t$^{-}$, biochar application to soil for dryland wheat production may not be economically viable, especially at application rates of 5–30 t ha$^{-1}$, which are commonly used in research trials (Chan and Xu, 2009). Common application rates for full topsoil incorporation of biochars are often 1–10 t ha$^{-1}$; with additional costs associated with application to the soil, this is seen as a significant price barrier to adoption. This price barrier for biochar as a soil ameliorant has encouraged the exploration of using biochar in fertiliser replacement formulations (Blackwell et al., 2010). The influence of biochar on crop growth and nutrient uptake changes when biochar is applied with fertilisers (Solaiman et al., 2010a, b). Application of deep-banded biochar with beneficial microbe-inoculated fertiliser significantly increased mycorrhizal colonisation in whe-
at roots and wheat growth in a low rainfall area in Western Australia (Blackwell et al., 2010). The results suggested that a low biochar application rate (about 1 t ha\(^{-1}\)) by banding might provide significant positive effects on crop yield and reduce fertiliser requirement. Banding was used to minimise wind erosion risk and to place biochar close to crop roots making the method most efficient for fertiliser use and thus increasing the perceived benefits (Blackwell et al., 2009a, b). Mycorrhizal hyphae associated with biochar may improve water supply to plants thereby reducing drought stress by extending exploration by plant roots for water from the wide inter-rows.

Sharp increases in fertiliser prices in 2007–2008 in Australia highlighted the need to develop more efficient fertiliser strategies, including less expensive nutrient sources. Use of waste materials, such as agricultural waste, biomass, animal manure, grape marc and treated human effluent, have been considered as a means to decrease the need for synthetic, water-soluble fertilisers, especially with regard to P supply to crops (e.g., Arvanitoyannis et al., 2006). Biochar prepared from these waste materials can be used as an alternative fertiliser alone, or in conjunction with chemical or mineral fertilisers (Lehmann and Rondon, 2006) to benefit plant nutrition and growth as well as enhance soil biology. Biochar application to soil may follow two main strategies; these are as either an infrequent soil ameliorant or regular additions as a fertiliser replacement. Evaluation of biochar as a fertiliser substitute is at a very early stage worldwide but it is able to provide several nutrient elements to plants. The main objective of this study was to compare the influences of biochar, mineral fertilisers with microbes, biochar-mineral complex (BMC) and their combination on mycorrhizal co-

MATERIALS AND METHODS

Biochars, biochar-mineral complex and mineral fertiliser

Three biochars were used in these experiments, produced from Acacia saligna wood (AS), jarrah wood (Eucalyptus marginata), called here Simcoa jarrah (SJ), and a mixture of jarrah and wandoo (E. wandoo) wood (Wandowie jarrah, WJ). The AS biochar was produced at 380 °C in a rotating batch kiln and the SJ and WJ biochars in vertical kilns at 550–650 °C. The WJ biochar was 24 years old and was taken from a large open pile that had been leached by the annual rainfall of about 700 mm per year. Biochars used in this evaluation were sieved to ≤ 2 mm. More detailed properties are given in Blackwell et al. (2010) and Chia et al. (2014). The pH of the biochars was measured in water and 0.01 mol L\(^{-1}\) CaCl\(_2\), respectively, at a ratio of 1:5 (weight/volume). A subsample of biochar was finely ground before total C and N concentrations were determined by dry combustion using an elementar (vario MACRO CNS; Elementar, Germany). Available P in biochars was extracted using 2% formic acid and dissolved P was measured by the molybdenum-blue method (Murphy and Riley, 1962).

BMC is a Terra Preta (black earth)-like material usually produced by mixing biochar (made from high mineral ash biomass such as bagasse palm waste, manures, wood and bark, clean paper sludge, rice husks, bones, and waste from shrimp processing at temperatures between 300 and 600 °C), clay, minerals (e.g., calcium carbonate, rock phosphate, dolomite, and crushed granite), and ash from biomass burnt in a furnace. The BMC used in this experiment has been characterised in detail by Chia et al. (2014). It was produced by thermal treatment of a mixture of clay, organic matter and approximately 100 g kg\(^{-1}\) AS biochar (produced at 380 °C) with the above mineral materials at 220 °C in a steam environment. The total analysis was N 12 g kg\(^{-1}\), P 28.3 g kg\(^{-1}\), K 8 g kg\(^{-1}\), S 6 g kg\(^{-1}\), Al 16 g kg\(^{-1}\), Fe 15 g kg\(^{-1}\) and C 269 g kg\(^{-1}\). Mineral fertiliser (MF) was provided by Australian Mineral Fertilisers Pty Ltd., Tenterden, Western Australia and consisted of ground rock minerals which contained N 50 g kg\(^{-1}\), P 86 g kg\(^{-1}\), K 50 g kg\(^{-1}\), S 80 g kg\(^{-1}\), Ca 70 g kg\(^{-1}\), and Mg 17 g kg\(^{-1}\). The fertiliser also contained inoculated beneficial soil microorganisms (750 g t\(^{-1}\) fertiliser), including Glomus intraradices, and species of Azospirillum, Azotobacter, Bacilli, Cellulomonas, Pseudomonas, Streptomyces and Saccharomyces.

Glasshouse experiment

Wheat (Triticum aestivum L. var. Wyalkatchem) was grown for 8 weeks under glasshouse conditions. There were 9 treatments comprising: T1—SJ (5 t ha\(^{-1}\))
+ MF (50 kg ha\textsuperscript{−1}) + microbes (M); T2—SJ boil in water + MF (50 kg ha\textsuperscript{−1}) + M; T3—SJ treated with acetic acid + MF (50 kg ha\textsuperscript{−1}) + M; T4—WJ (5 t ha\textsuperscript{−1}) + MF (50 kg ha\textsuperscript{−1}) + M; T5—AS (5 t ha\textsuperscript{−1}) + MF (50 kg ha\textsuperscript{−1}) + M; T6—BMC (5 t ha\textsuperscript{−1}) + MF (50 kg ha\textsuperscript{−1}) + M; T7—MF (50 kg ha\textsuperscript{−1}) + M; T8—MF (50 kg ha\textsuperscript{−1}) + microbes (M); T9—control (with no amendments). There were 3 replicates of each treatment. Each pot contained 1.4 kg soil that had been mixed with biochar, MF and BMC or mixed without additives as a control. Six seeds of wheat were sown in a 1.3-L plastic pot and thinned to 2 plants per pot after germination. Each pot was watered to 70% of field water capacity through daily addition of water.

The soil used was collected from a field under the rotation of subterranean clover and wheat at Mingenew, Western Australia (29°19′ S, 115°44′ E). Mingenew has a Mediterranean climate and a mean annual rainfall of 400 mm with 80% falling in the growing season from May to October. The soil was a Tenosol (sand over gravel; Isbell, 1996) and the 0–10 cm layer was collected and analysed for basic properties. This soil was chosen because its loamy sand texture gave a low capacity to retain P and the low available P (7.5 mg kg\textsuperscript{−1}) was suitable for obtaining a mycorrhizal response for both subterranean clover and wheat.

At harvest, after washing with tap water, sub-samples of roots (1 g) were cut into approximately 1 cm pieces and cleared in 100 g L\textsuperscript{−1} KOH, acidified and stained with Trypan blue (0.05%) in lactoglycerol (1:1:1.2 lactic acid:glycerol:water) and destained in lactoglycerol (Abbott and Robson, 1981; Solaiman and Abbott, 2008). Root length colonisation (%) by AM fungi was assessed using the gridline intercept method under an optical microscope at 100 × magnification (Newman, 1966; Giovannetti and Mosse, 1980).

At harvest, shoots were cut from each plant and roots were washed free of soil and organic matter. Shoots and roots (after a defined weight of roots was removed for assessment of AM fungal colonisation) were dried at 60 °C for at least 72 h to determine shoot and root dry weights (DW). Oven-dried shoots were ground and digested in 3:1 HNO\textsubscript{3}:HClO\textsubscript{4} (Johnson and Ulrich, 1959) and the P concentration in the digest was measured by the molybdemnum-blue method (Murphy and Riley, 1962). Shoot P uptake was calculated by multiplying shoot P concentration by shoot weight.

Field experiment

The field experiment was conducted in a farm at Moonyoomooka, Western Australia. BMC was applied at a rate of 300 kg ha\textsuperscript{−1} and deeply banded in the sowing row. Mineral fertiliser (MF) was obtained from Australian Mineral fertilisers Pty Ltd. and used at the rate of 300 kg ha\textsuperscript{−1}, as well as a mixture of BMC and MF (75 and 225 kg ha\textsuperscript{−1}, MBC75 + MF225). The BMC was inoculated with the same inoculum of beneficial microbes as was the MF fertiliser. All these treatments were compared to 60 kg ha\textsuperscript{−1} of a water-soluble fertiliser named ZincStar (Summit fertiliser) applied at sowing and to a nil treatment. The sorghum crop was grown in an irrigation facility from February to April, 2009. The soil was a red deep loamy duplex with Colwell P 30 mg kg\textsuperscript{−1} (total P 80 mg kg\textsuperscript{−1}). Standard rates of N, K, S and trace elements (Ca, Mg, Zn, Mn, Cu, Fe, B and Mo) were applied in a water-soluble form between emergence and flowering to ensure adequate supply of nutrients for all of the treatments. Irrigation was applied every 2 d to minimise drought stress during crop growth and maturity.

Biomass weight, grain yield, nutrient concentration and content of shoots were determined at the anthesis stage. Mycorrhizal colonisation was also measured at the anthesis stage. Sub-samples of the washed roots containing biochar and MF particles were dried and the remaining sub-samples of these roots were then mounted in resin and then polished until a section of the root surrounded by biochar was visible. Assessment of the surface of BMC particles in the root system was undertaken using a Hitachi S3400 scanning electron microscope (SEM). Elemental analysis was carried out using an energy dispersive spectroscopy (EDS) detector interfaced to the SEM. The chromium coating was used to make the sample electrically conductive. This analysis was carried out to determine if any significant changes in nutrients could have occurred at the biochar-root interface.

Statistical analysis

Statistical analyses were carried out using Genstat version 12. One-way analysis of variance was used to identify significant effects of the treatments on all measured parameters. Least significant difference was calculated to test the significance of difference between means. The significant Pearson’s correlations between the mycorrhizal colonisation and measured parameters of wheat plants after 8-week growth were calculated.

RESULTS

Characterisation of biochars and BMC

Basic properties of the biochars and BMC are shown in Table I. It can be seen that there was a wide range of properties especially for pH, EC and C/N ra-
The WJ biochar had a low C content and pH, indicating that it had undergone significant changes over 20 years since it was aged and exposed in the open environment. BMC had the lowest C content, the highest N content and high EC. It had a slightly acidic pH, whereas the SJ biochar had a high pH and a low EC and AS biochar had high pH and EC values.

**Glasshouse experiment**

Table II summarises the N, P, K, S and Zn concentrations in wheat shoots for the different fertiliser treatments in the pot experiment. The MF + M treatment (T7) led to the maximum N concentration followed by the AS + MF + M (T5) and WJ + MF + M (T4). But the BMC + MF + M (T6) had the maximum P concentration followed by SJ (treated with acetic acid) + MF + M (T3) and AS + MF + M (T5). The MF (T8) had the maximum K concentration followed by AS + MF + M (T5). The AS + MF + M (T5) and WJ + MF + M (T4) resulted in the highest N uptake by wheat shoots, while the BMC + MF + M (T6) resulted in the maximum P uptake by wheat shoots followed by AS + MF + M (T5).

The BMC + MF + M (T6) produced the maximum increase in mycorrhizal colonisation (Fig. 1). The BMC + MF + M (T6) had a higher mycorrhizal colonisation than the MF + M (T7), indicating that BMC had a significant effect to increase the mycorrhizal colonisation. Treatments T1–T6 showed a similar effect on mycorrhizal colonisation (Fig. 1). The MF (T8) and control (T9) treatments showed the lowest mycorrhizal colonisation in wheat roots. Out of all the treatments applied, the BMC + MF + M (T6) treatment had the maximum shoot, root and total plant dry weight, but there were no statistically significant differences among treatments T2–T6 (Fig. 2).

**TABLE I**

Basic characteristics of biochars, including *Acacia saligna* (AS), Simcoa jarrah (SJ) and Wundowie jarrah (WJ), biochar-mineral complex (BMC) and soil (for pot experiment) used in this study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>AS</th>
<th>SJ</th>
<th>WJ</th>
<th>BMC</th>
<th>Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source of biomass</td>
<td>Acacia plants</td>
<td>Jarrah tree</td>
<td>Wandoo plus other woods</td>
<td>AS, clay, minerals</td>
<td></td>
</tr>
<tr>
<td>Production temperature (^{\circ}C)</td>
<td>380</td>
<td>550–650</td>
<td>550–650</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td>pH (H(_2)O)</td>
<td>8.65</td>
<td>9.52</td>
<td>4.80</td>
<td>6.80</td>
<td>5.70</td>
</tr>
<tr>
<td>pH (CaCl(_2))</td>
<td>7.63</td>
<td>8.41</td>
<td>3.72</td>
<td>5.70</td>
<td>4.80</td>
</tr>
<tr>
<td>Electrical conductivity (dS m(^{-1}))</td>
<td>5.70</td>
<td>0.50</td>
<td>0.04</td>
<td>3.60</td>
<td>0.05</td>
</tr>
<tr>
<td>Carbon (g kg(^{-1}))</td>
<td>582</td>
<td>784</td>
<td>505</td>
<td>3.60</td>
<td>0.05</td>
</tr>
<tr>
<td>Nitrogen (g kg(^{-1}))</td>
<td>4.4</td>
<td>4.0</td>
<td>4.9</td>
<td>12.0</td>
<td>1.0</td>
</tr>
<tr>
<td>C:N ratio</td>
<td>132</td>
<td>196</td>
<td>266</td>
<td>22</td>
<td>12</td>
</tr>
<tr>
<td>Total P (g kg(^{-1}))</td>
<td>0.5</td>
<td>0.6</td>
<td>0.5</td>
<td>28.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

**TABLE II**

Effects of biochars, mineral fertilisers (MF) and biochar-mineral complex (BMC) on nutrient concentrations and uptake by wheat shoots under glasshouse conditions

<table>
<thead>
<tr>
<th>Treatment(^{a)})</th>
<th>Nutrient concentration</th>
<th>Nutrient uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>P</td>
</tr>
<tr>
<td>T1</td>
<td>14.4</td>
<td>3.6</td>
</tr>
<tr>
<td>T2</td>
<td>16.5</td>
<td>3.4</td>
</tr>
<tr>
<td>T3</td>
<td>15.1</td>
<td>4.1</td>
</tr>
<tr>
<td>T4</td>
<td>16.8</td>
<td>3.7</td>
</tr>
<tr>
<td>T5</td>
<td>16.8</td>
<td>4.0</td>
</tr>
<tr>
<td>T6</td>
<td>15.5</td>
<td>5.4</td>
</tr>
<tr>
<td>T7</td>
<td>17.7</td>
<td>4.0</td>
</tr>
<tr>
<td>T8</td>
<td>15.6</td>
<td>3.3</td>
</tr>
<tr>
<td>T9</td>
<td>8.6</td>
<td>1.7</td>
</tr>
</tbody>
</table>
| LSD\(^{b)}\) (\(P < 0.05\)) | 2.6 | 1.2 | 2.8 | 0.5 | 0.01 | 28.6 | 11.6 | 23.2 | 4.9 | 0.05

\(^{a)}T1 =* Simcoa jarrah* (SJ, 5 t ha\(^{-1}\)) + MF (50 kg ha\(^{-1}\)) + microbes (M); T2 = SJ boil in water + MF (50 kg ha\(^{-1}\)) + M; T3 = SJ treated with acetic acid + MF (50 kg ha\(^{-1}\)) + M; T4 = Wundowie jarrah (5 t ha\(^{-1}\)) + MF (50 kg ha\(^{-1}\)) + M; T5 = *Acacia saligna* (5 t ha\(^{-1}\)) + MF (50 kg ha\(^{-1}\)) + M; T6 = BMC (5 t ha\(^{-1}\)) + MF (50 kg ha\(^{-1}\)) + M; T7 = MF (50 kg ha\(^{-1}\)) + M; T8 = MF (50 kg ha\(^{-1}\)) + M; T9 = control (with no amendments).

\(^{b)}Least significant difference.
Effects of Biochars, Mineral Fertilisers (MF) and Biochar-Mineral Complex (BMC) on Crop

Mycorrhizal colonisation in sorghum roots was significantly increased by BMC alone (BMC300) and by BMC mixed with MF (BMC75 + MF225), whereas shoot biomass only increased with the BMC75 + MF-225 treatment at the anthesis stage of growth (Fig. 3). The sorghum grain yield without any applied fertiliser was 4.4 t ha$^{-1}$ (Fig. 3). Application of 60 kg ha$^{-1}$ of water-soluble SF (SF60) gave the highest grain yield of 6.6 t ha$^{-1}$, larger than any other treatments. The grain yield of sorghum with BMC75 + MF225 mixture application (5.3 t ha$^{-1}$) was less than the yield with BMC or MF alone (5.8 t ha$^{-1}$) at the same application rate but not statistically significant. The concentrations of N, P and K in plant shoots were not influenced by the treatments (Table III), but N and K uptake were increased for the application of BMC and MF mixture (Table IV). An image of mycorrhiza colonised plant roots presented in Fig. 4 showed that the roots were highly colonised by mycorrhizal fungi where roots were in contact with BMC.

Analysis of BMC using scanning electron microscopy

Fig. 5a is a scanning electron image of the surface of a root adjacent to a BMC particle that supported a high colonisation of filamentous fungi. Fig. 5b shows bacteria clustered on the surface of a BMC particle. Fig. 5c is a scanning electron image of fungi entering the pores of the BMC and Fig. 5d is a spot EDS analysis recorded from a region around the pores where fungi entered. The EDS spectrum showed that there were high concentrations of Fe, Mn, Si, Ca and Cr along with significant concentrations of P, Mg, Al, Mn and K. Fig. 6 is a backscattered electron image of a polished cross-section of a root hair that is surrounded by mineral particles and organic matter similar to that seen in Fig. 4. EDS analysis indicated the particles surrounding the root were mostly clay and quartz. The EDS spectrum was mostly Si plus clay, alumina and iron oxide. The high Cr was from a chromium coating used to make the sample electrically conductive.

DISCUSSION

Mycorrhizal colonisation was significantly increased by biochar materials, especially BMC compared to
Fig. 3 Effects of Summit fertiliser (SF), mineral fertilisers (MF) and biochar-mineral complex (BMC) on mycorrhizal colonisation, shoot biomass at anthesis stage, and grain yield of sorghum under field conditions. Water-soluble SF was applied at sowing stage at 60 kg ha\(^{-1}\) (SF60). The BMC was applied at a rate of 300 kg ha\(^{-1}\) (BMC300) and deeply banded in the sowing row. Australian MF was applied at 300 kg ha\(^{-1}\) (MF300) as well as a mixture of BMC and MF at 75 and 225 kg ha\(^{-1}\), respectively (BMC75 + MF225). Vertical bars indicate standard errors of the means (\(n=5\)). Bars with the same letter are not significantly different at \(P < 0.05\).

### TABLE III

Summit fertiliser (SF), mineral fertilisers (MF) and biochar-mineral complex (BMC) on nutrient concentrations of shoot at anthesis stage of sorghum growth in the field experiment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N (g kg(^{-1}))</th>
<th>P (mg kg(^{-1}))</th>
<th>K (mg kg(^{-1}))</th>
<th>S (mg kg(^{-1}))</th>
<th>Na (mg kg(^{-1}))</th>
<th>Ca (mg kg(^{-1}))</th>
<th>Mg (mg kg(^{-1}))</th>
<th>Cl (mg kg(^{-1}))</th>
<th>Cu (mg kg(^{-1}))</th>
<th>Zn (mg kg(^{-1}))</th>
<th>Mn (mg kg(^{-1}))</th>
<th>Fe (mg kg(^{-1}))</th>
<th>B (mg kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.35</td>
<td>2.35</td>
<td>31.38</td>
<td>1.87</td>
<td>0.23</td>
<td>3.58</td>
<td>2.77</td>
<td>16.39</td>
<td>48.1</td>
<td>105.6</td>
<td>229.4</td>
<td>254.0</td>
<td>9.2</td>
</tr>
<tr>
<td>SF60</td>
<td>23.49</td>
<td>2.27</td>
<td>29.75</td>
<td>1.86</td>
<td>0.22</td>
<td>3.88</td>
<td>2.76</td>
<td>14.92</td>
<td>43.2</td>
<td>96.5</td>
<td>218.1</td>
<td>260.3</td>
<td>8.6</td>
</tr>
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<td>BMC300</td>
<td>23.33</td>
<td>2.28</td>
<td>28.02</td>
<td>1.99</td>
<td>0.19</td>
<td>3.86</td>
<td>2.71</td>
<td>15.14</td>
<td>54.3</td>
<td>120.7</td>
<td>237.9</td>
<td>268.8</td>
<td>9.9</td>
</tr>
<tr>
<td>MF300</td>
<td>23.07</td>
<td>2.38</td>
<td>29.27</td>
<td>1.94</td>
<td>0.25</td>
<td>4.16</td>
<td>3.00</td>
<td>16.87</td>
<td>56.8</td>
<td>117.7</td>
<td>262.7</td>
<td>289.6</td>
<td>10.1</td>
</tr>
<tr>
<td>BMC75 + MF225</td>
<td>22.80</td>
<td>2.24</td>
<td>29.71</td>
<td>1.79</td>
<td>0.26</td>
<td>3.97</td>
<td>2.94</td>
<td>17.10</td>
<td>42.7</td>
<td>102.5</td>
<td>221.8</td>
<td>245.5</td>
<td>10.1</td>
</tr>
<tr>
<td>LSDb) ((P &lt; 0.05))</td>
<td>NSc)</td>
<td>NSc)</td>
<td>NSc)</td>
<td>0.15</td>
<td>0.06</td>
<td>0.25</td>
<td>0.21</td>
<td>NSc)</td>
<td>7.1</td>
<td>10.3</td>
<td>NSc)</td>
<td>30.1</td>
<td>NSc)</td>
</tr>
</tbody>
</table>

a) See Fig. 3 for the detailed descriptions of the treatments.
b) Least significant difference.
c) Not significant.

### TABLE IV

Effects of Summit fertiliser (SF), mineral fertilisers (MF) and biochar-mineral complex (BMC) on nutrient uptake by shoot at anthesis stage of sorghum growth in the field experiment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N (g m(^{-2}))</th>
<th>P (mg m(^{-2}))</th>
<th>K (mg m(^{-2}))</th>
<th>S (mg m(^{-2}))</th>
<th>Na (mg m(^{-2}))</th>
<th>Ca (mg m(^{-2}))</th>
<th>Mg (mg m(^{-2}))</th>
<th>Cl (mg m(^{-2}))</th>
<th>Cu (mg m(^{-2}))</th>
<th>Zn (mg m(^{-2}))</th>
<th>Mn (mg m(^{-2}))</th>
<th>Fe (mg m(^{-2}))</th>
<th>B (mg m(^{-2}))</th>
</tr>
</thead>
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<tr>
<td>Control</td>
<td>1.24</td>
<td>0.12</td>
<td>1.67</td>
<td>0.10</td>
<td>0.01</td>
<td>0.19</td>
<td>0.15</td>
<td>0.88</td>
<td>25.4</td>
<td>55.6</td>
<td>120.8</td>
<td>135.9</td>
<td>4.9</td>
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<td>1.42</td>
<td>0.14</td>
<td>1.81</td>
<td>0.11</td>
<td>0.01</td>
<td>0.24</td>
<td>0.17</td>
<td>0.91</td>
<td>26.2</td>
<td>58.6</td>
<td>132.4</td>
<td>158.3</td>
<td>5.2</td>
</tr>
<tr>
<td>BMC300</td>
<td>1.29</td>
<td>0.13</td>
<td>1.52</td>
<td>0.11</td>
<td>0.01</td>
<td>0.21</td>
<td>0.15</td>
<td>0.81</td>
<td>30.5</td>
<td>66.2</td>
<td>132.9</td>
<td>147.0</td>
<td>5.5</td>
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<td>MF300</td>
<td>1.21</td>
<td>0.13</td>
<td>1.56</td>
<td>0.10</td>
<td>0.01</td>
<td>0.22</td>
<td>0.16</td>
<td>0.91</td>
<td>30.2</td>
<td>62.0</td>
<td>136.3</td>
<td>153.4</td>
<td>5.2</td>
</tr>
<tr>
<td>BMC75 + MF225</td>
<td>1.44</td>
<td>0.14</td>
<td>1.90</td>
<td>0.11</td>
<td>0.02</td>
<td>0.26</td>
<td>0.19</td>
<td>1.12</td>
<td>27.4</td>
<td>65.6</td>
<td>140.5</td>
<td>157.3</td>
<td>6.3</td>
</tr>
<tr>
<td>LSDb) ((P &lt; 0.05))</td>
<td>0.15</td>
<td>NSc)</td>
<td>0.30</td>
<td>NSc)</td>
<td>0.06</td>
<td>NSc)</td>
<td>0.20</td>
<td>1.2</td>
<td>3.5</td>
<td>15.7</td>
<td>20.1</td>
<td>0.8</td>
<td></td>
</tr>
</tbody>
</table>

a) See Fig. 3 for the detailed descriptions of the treatments.
b) Least significant difference.
c) Not significant.

the other treatments. Mycorrhizal colonisation and bacteria were observed on the BMC-treated roots. The addition of biochar materials, mainly BMC, has clearly increased the shoot, root and total dry weight of wheat plants over a control without biochar (Lehmann et al., 2003). The immediate beneficial effects of biochar ad-
conditions on plant nutrient uptake are largely due to higher N, K, P, S and Zn availability (Lehmann et al., 2003). In particular, the BMC has a much higher nutrient content than the woody biochar. It can be seen from the SEM analysis that the mycorrhizal fungi have entered into the pores of the BMC where the concentrations of nutrients are elevated. Longer-term benefits for nutrient availability include a greater stabilization of organic matter, concurrent slower nutrient release from added organic matter and better retention of all cations due to a greater cation exchange capacity. The results of this study reflected that improved crop yield and fertiliser use efficiency with biochar incorporation might relate to greater mycorrhizal colonisation of the crop roots. Blackwell et al. (2007) and Solaiman et al. (2010a) reported beneficial effects of biochar on root colonisation by mycorrhizal fungi and crop water and P supply for dryland wheat production in Western Australia. Solaiman et al. (2010b) also reported that biochar might assist mycorrhizal colonisation, in turn, influencing P and N uptake and the tolerance to drought stress in wheat.

The clearer understanding of the effects of biochar on soil fertility and crop production will facilitate estimation of potential benefits of biochar for C sequestration and improved fertiliser use efficiency in soil (Lehmann et al., 2003). In particular, the BMC has a much higher nutrient content than the woody biochar. It can be seen from the SEM analysis that the mycorrhizal fungi have entered into the pores of the BMC where the concentrations of nutrients are elevated. Longer-term benefits for nutrient availability include a greater stabilization of organic matter, concurrent slower nutrient release from added organic matter and better retention of all cations due to a greater cation exchange capacity. The results of this study reflected that improved crop yield and fertiliser use efficiency with biochar incorporation might relate to greater mycorrhizal colonisation of the crop roots. Blackwell et al. (2007) and Solaiman et al. (2010a) reported beneficial effects of biochar on root colonisation by mycorrhizal fungi and crop water and P supply for dryland wheat production in Western Australia. Solaiman et al. (2010b) also reported that biochar might assist mycorrhizal colonisation, in turn, influencing P and N uptake and the tolerance to drought stress in wheat.
Life cycle analyses show that biochar application to soil can reduce CO₂ emission (Roberts et al., 2010). If biochar application to soil enables decreased fertiliser use, this may further reduce emission due to fertiliser manufacture. Decreased fertiliser use would also mean financial savings for farmers if the cost of biochar application is less than the value of the fertiliser required to achieve the same grain yield. Such analysis assumes that decreased fertiliser use will not compromise long-term crop production by lowering the residual soil nutrient status.

Sorghum showed an evidence of increased yield and P uptake efficiency due to biochar application. These interpretations should be viewed with caution because the soil may have been equilibrating after initial disturbance of the field soil. A subsequent winter crop might provide a better evaluation of BMC effects because the soil will have attained equilibrium.

CONCLUSIONS

The benefits of biochars and biochar-mineral complex (BMC) were associated with increased mycorrhizal colonisation and nutrient uptake from a sandy soil with low nutrient availability. Evidence of better applied P use efficiency provided by BMC addition was shown by the results of the glasshouse experiment. Better P use efficiency was associated with higher mycorrhizal colonisation and might involve the contribution of helper bacteria to access pools of water-insoluble P forms. The field experiment also established evidence of biochar and BMC-based fertilisers providing more economical access to P stores in soil to help enable more profitable crops. The results indicated that BMC had a potential as a starter fertiliser for replacement of superphosphate. But more field researches are needed for a clear understanding of the potential of BMC as a fertiliser replacement. Use of BMC as a fertiliser will also enable increased C sequestration in wheat production with a lower cost barrier than the once-off use of biochar as a soil ameliorant.

ACKNOWLEDGEMENT

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