Canadian Journal of Soil Science
Revue canadienne de la science du sol

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Journal:	*Canadian Journal of Soil Science*
Manuscript ID	CJSS-2015-094.R2
Manuscript Type:	Article
Date Submitted by the Author:	16-Jun-2016
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Keywords:	Soil management, Chernozemic soil, Chemical properties, Fertility, Fertilzer

Woodchip biochar with or without synthetic fertilizers affects soil properties and available phosphorus in two alkaline, Chernozemic soils

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Short title: Biochar effects on soil fertility of alkaline chernozems

Abbreviations: **BC1**, Biochar at 10 g kg^{-1}; **BC2**, Biochar at 20 g kg^{-1}; **CEC**, Cation exchange capacity; **EC**, Electrical conductivity; **MWD**, Mean weight diameter; **OC**, Organic carbon; **P$_{MR}$**, molybdate reactive P; **SF**, Synthetic fertilizer

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Abstract

Fertility enhancement with biochar application is well documented for tropical acidic soils; however, benefits of biochar co-applied with synthetic fertilizers on soil fertility are not well documented, particularly for alkaline chernozems. We examined the short-term interactive effects of woodchip biochar amendment with fertilizers on selected soil properties, available phosphorus (P) and P fractions of two alkaline Chernozems from Manitoba. Treatments were (1) urea and monoammonium phosphate fertilizers, (2) biochar at 10 g kg^{-1}, (3) biochar at 20 g kg^{-1}, (4) biochar at 10 g kg^{-1} with fertilizers, (5) biochar at 20 g kg^{-1} with fertilizers, and (5) a control. Treated soils were analysed for pH, electrical conductivity (EC), and Olsen P concentration biweekly, and for P fractions, cation exchange capacity (CEC), organic carbon (OC) and wet aggregate stability after 70 d of incubation. Biochar amendment without fertilizers significantly increased soil pH and CEC, but had no effect on EC, while co-application with fertilizers significantly increased Olsen P and labile P concentrations. When co-applied with fertilizers, biochar did not significantly increase soil pH relative to the control. Results suggest that biochar improved soil properties and available P in alkaline Chernozems, and the beneficial effects were enhanced when co-applied with synthetic fertilizers.

Key words: biochar, Chernozemic soils, phosphorus availability, phosphorus fractions, soil properties, synthetic fertilizers

INTRODUCTION

Biochar has been used as a soil amendment for centuries, but during the last three decades, the use of biochar in agriculture had received renewed interest because of its potential role in enhancing soil fertility for crop production (Sohi et al. 2010; Jeffery et al. 2011; Gul et al. 2015). Soil fertility is defined as ‘‘the quality of a soil that enables it to provide nutrients in adequate amounts and in proper balance for the growth of specified plants or crops” (Soil Science Society of America 2001). Since the availability, mobility and uptake of nutrients are dependent on soil physical, chemical and biological properties, biochar effects on soil fertility is often evaluated by considering the improvement of these properties with biochar application (Kloss et al. 2014; Mandal et al. 2015).

Biochar is produced by incomplete combustion of organic biomass (Schmidt et al. 1999; Lehmann 2007) under anaerobic conditions (pyrolysis) and consists of condensed aromatic forms of OC. This stable form of carbon (C) does not decompose easily in soils (Atkinson et al. 2010), preventing C from returning to the atmosphere as carbon dioxide within a short period. The residence time of biochar in soils therefore can range from hundreds to thousands of years (Lehmann 2007; Gul et al. 2015). Generally, biochar has a higher pH, higher CEC, higher porosity and a greater specific surface area than the other un-charred organic amendments (Lehmann et al. 2006). However, these properties vary depending on the organic material, charring conditions and the formation process (Lehmann 2007; Kloss et al. 2012).

Application of biochar can increase the CEC of soils (Yamato et al. 2006; Novak et al. 2009) and thereby increase nutrient retention (Glaser et al. 2002; Major et al. 2010). Biochar application has increased soil OC concentration (Novak et al. 2009; Sukartono et al. 2011; Angst et al. 2014), and increased soil microbial and mycorrhizal activities (Warnock et al. 2007; Gul et al. 2015), thus promoting microbe-mediated processes in soils such as mineralization of organic matter and phosphorus (P) solubilisation, resulting in an enhanced bioavailability of nutrients. Biochar amendment to soil also improved water-holding capacity (Glaser et al. 2002), and reduced soil acidity (Van Zwieten et al. 2010; Peng et al. 2011), providing favourable conditions for crop growth. Wet aggregate stability, an indicator of soil resistance to erosion by water, increased with biochar application (Ouyang et al. 2013; Soinne et al. 2014), thus minimizing the loss of fertile topsoil due to water erosion. These effects of biochar application on soil physical, chemical and biological properties often improved soil fertility (Glaser et al. 2002; Novak et al. 2009; Major et al. 2010), and thereby increased crop yields (Yamato et al. 2006; Atkinson et al. 2010; Sukartono et al. 2011), while nutrient losses from the soil and environmental pollution were reduced (Laird et al. 2010; Wu et al. 2013).

Biochar effects on P availability have been inconsistent (DeLuca et al. 2009; Xu et al. 2014); in some soils, biochar application increased P availability, while in others, P availability was not affected or reduced, mainly due to increased P sorption (Novak et al. 2009; Kloss et al. 2014; Xu et al. 2014). Since biochar usually contains P in relatively larger concentrations compared to soil, its addition can directly release soluble P and increase available P concentration in amended soils (Atkinson et al. 2010). In addition, changes in soil properties with biochar amendment can alter P availability by influencing P reactions in soils such as adsorption, desorption, precipitation and dissolution (Xu et al. 2014). The mechanisms underlying the P availability changes remain poorly understood, particularly in alkaline and calcareous soils (Farell et al. 2014). Investigating changes in operationally defined P fractions with biochar amendment rather than changes in available P measured using a single extraction provides a better understanding on biochar effects on soil P availability (Farell et al. 2014).

Contrary to these positive effects, some researchers found negative effects of biochar application on soil fertility, especially in the short term, such as reduced bioavailability of nutrients such as nitrogen (N) and P (Novak et al. 2009; Zavalloni et al. 2011; Case et al. 2012) and increased EC in soil (Méndez et al. 2012; Kloss et al. 2014) resulting in reduced crop yields (Kloss et al. 2014). To mitigate the negative effects of biochar on soil fertility, recent work examined the effects of biochar applied to soil in combination with inorganic fertilizers. Synergistic effects were observed in some studies with co- application of biochar and synthetic fertilizers; however, this effect was inconsistent (Asai et al. 2009; van Zwieten et al. 2010; Saarino et al. 2013; Mete et al. 2015), and needs further investigation.

Most of the studies that showed enhanced crop yields and improved soil conditions with biochar application have focused on weathered, acidic, tropical soils with low OC contents and low CEC (Chan et al. 2008; Sukartono et al. 2011). Chernozemic soils (Mollisols) in temperate regions are generally considered fertile, because of their high OC content and high CEC. Laird et al. (2010) observed that biochar amendments have the potential to improve the quality and fertility status of Mollisols; however, results of Kloss et al. (2014) suggest short-term growth inhibition with biochar application in a chernozemic soil. Limited information is available on fertility improvement with biochar application to chernozemic soils, especially when it is co-applied with synthetic fertilizer.

In the above context, the objective of this study was to evaluate the effects of two rates of biochar produced from Manitoba Maple woodchips, with and without synthetic fertilizer, on selected soil properties, available P and P fractions, in two low-P Chernozemic soils from the Canadian prairies. Our hypothesis was that biochar has the potential to improve soil fertility of alkaline, low-P chernozemic soils, and the soil fertility improvement would be enhanced with the application of biochar in combination with synthetic fertilizers compared to biochar applied alone.

MATERIALS AND METHODS

Soil characteristics

We collected surface soil samples (0- to 15-cm layer) from two locations, Roseisle (N 49^o 33.577′; W 098^o 24.824′) and Justice (N 49^o 58.590′; W 099^o 52.908′) in Manitoba, Canada. The soils were (i) an Almasippi loamy sand (Gleyed Rego Black Chernozem) and (ii) a Newdale clay loam (Orthic Black Chernozem) according to Canadian system of classification (CASCC 1998). A composite soil sample was collected from a farmer's field in each location. Soils were stored at room temperature and field moisture content. A homogenized subsample of soils were air-dried and sieved (<2mm) and used for analysis of soil properties. Particle size analysis was done using the pipette method (Gee and Bauder 1986). Soil pH (1:2, soil: water) and EC (1:2, soil: water) were measured using an Accumet AB15 pH meter and an Accumet AB30 conductivity meter (Fisher Scientific Ltd, Ottawa, Canada), respectively. Soil organic matter determined by the loss-on-ignition at 550 ^{o}C was converted to OC by multiplying by the conversion coefficient of 0.58 (Davies 1974). To determine exchangeable cation concentrations, soils were extracted using 1.0 M ammonium acetate (Rhoades, 1982), and exchangeable calcium (Ca), potassium (K), magnesium (Mg) and sodium (Na) concentrations in extracts were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Thermo iCAP 6500 Duo, Cambridge, UK). The sum of exchangeable Ca^{2+}, K^+, Mg^{2+} and Na^+ was reported as the CEC of the soils. Olsen extractable P in soils was determined by shaking 1-g soil with 20 mL of a 0.5 M $NaHCO_3$ solution at pH 8.5 with 0.25 g P-free charcoal for 30 min and filtering through a Whatman No. 40 filter paper (Olsen et al. 1954). Inorganic P concentration in extracts was determined by the molybdate blue method (Murphy and Riley 1962) at a wavelength of 882 nm using an Ultraspec 2100 Pro UV/visible spectrophotometer (Biochrom, Cambridge, UK). Available N (Nitrate-N) concentration was determined in 2 M KCl extracts using the Cd reduction method

(Mulvaney 1996). To determine total P, soil samples were digested with H_2O_2/H_2SO_4 in a block digester (Akinremi et al. 2003) and P concentration was measured using the molybdate blue method.

Biochar characteristics

We used biochar produced by a local Manitoba farmer using woodchips of Manitoba Maple (*Acer negundo* L.) from a logging area of Eastern Manitoba for this study. The biochar was produced using top-lit-up-draft technique in 200-L drums as described by Munkhbat et al. (2013) with a pyrolysis temperature in the range of 500 – 650 °C. Total C concentration in the homogenized biochar samples was measured using a combustion analyser at 1100 °C. (Carlo Erba NA1500, Milan, Italy). To determine total element concentrations in biochar, representative biochar samples were digested with HNO_3/H_2O_2 using the hot block digestion procedure (United States Environmental Protection Agency, 1986) and concentrations of total P, K, Ca, and Mg in digests were analysed using the same procedure described above. Olsen P, and exchangeable cation concentration (Ca^{2+}, K^{+}, Mg^{2+} and Na^{+}) and CEC of biochar samples were determined using the same procedures described above for soil analysis.

Incubation studies

The first incubation experiment was laid out in a completely randomized design with a 2 × 6 factorial treatment structure and three replicates per treatment. The factors were the two soils described above and six amendments: (1) synthetic fertilizer applied at a rate of 50 mg N and 15 mg P kg^{-1} soil (equivalent to 100 kg N and 30 kg ha^{-1} to a 15-cm depth) using urea and monoammonium phosphate (SF), (2) biochar applied at 10 g kg^{-1} (or 1% w/w, BC1), (3) biochar applied at 20 g kg^{-1} (or 2% w/w, BC2), (4) biochar at 10 g $kg^{-1}$with synthetic fertilizer (BC1+SF), (5) biochar applied at 20 g $kg^{-1}$with synthetic fertilizer (BC2+SF), and (6) an unamended control soil (C). Even though our research mainly focused on P availability, we supplied N as urea to investigate the interactive effects of fertilizers and biochar amendment on soil properties and P availability. Biochar rate of 10 g kg^{-1} and 20 g kg^{-1} represent 15 and 30 t ha^{-1}, considering a soil depth of 15 cm and a bulk density of 1.0 g cm^{-3}. The lower rate of biochar application was selected based on the findings of previous researchers (Sukartono et al. 2011; Uzoma et al. 2011) that the rate, 15 t ha^{-1} is optimum for crop production, and we used 2 x this rate for our higher rate of application. The biochar was sieved and particles of 0.2-4.47 mm size were added to soils, with or without fertilizers and thoroughly mixed. Amended and unamended soils were watered to field capacity using reverse osmosis water, and 500 g of the moistened soil was packed into 1.5-L glass incubation vessels to a bulk density of 1.1 g cm^{-3}. Soils were incubated for 70 d at 20 °C and maintained at field capacity moisture content throughout the incubation period by weighing and adding reverse osmosis water twice a week. Soil samples were taken at 14-d intervals from all incubation vessels and analysed for pH, EC, and Olsen P concentration. At the end of the incubation period, CEC, OC and wet aggregate stability were quantified for control, 10 and 20 g kg^{-1} biochar treatments assuming these soil properties did not change substantially with the addition of synthetic fertilizer. Phosphorus fractionation of soil samples from all treatments was conducted at the end of the incubation period using a modified Hedley procedure (Ajiboye et al. 2004; Kashem et al. 2004). Soil samples (0.5 g) were sequentially extracted with 30 mL of deionized water, 0.5 M $NaHCO_3$, 0.1 M NaOH and 1 M HCl. In each extraction step, soil with the extraction solution was shaken at 120 oscillations min^{-1} for 16 h, and then centrifuged at 12500 g for 10 min. The supernatant was collected through vacuum filtration using 0.45 µm membrane filters and the P concentration in each extract was determined by the molybdate blue method (Murphy and Riley 1962). The sum of water-extractable and $NaHCO_3$-extractable P was considered as “labile P” (Kumaragamage et al. 2012). Residual soil remaining after the extraction was digested with H_2O_2/H_2SO_4 in a block digester and total P concentrations of digests were determined.

At the end of the incubation period, soils were sieved through a 4-mm sieve. Wet aggregate stability was measured using a wet-sieving device (Yoder 1936). A 50-g sample was placed on a filter paper and wetted up slowly on a tension plate at 10 cm tension for one day. Sieves were assembled in the order of 2.0, 1.0, 0.5 and 0.25 mm. The sample was placed onto the top sieve and then wet-sieved for 10 min at a frequency of 30 strokes min^{-1}. Soil remaining on each sieve was removed and oven-dried at 105 ^{o}C overnight to obtain the mean weight diameter (MWD) (van Bavel 1949) using the equation:

$$X = \sum_{i=1}^{n} x_i w_i$$

Where X = Mean Weight Diameter (mm), n= number of size fraction+1, x_i= mean diameter of a given size range of aggregates separated by sieving, and w_i= weight of aggregates in that size range as a fraction of total dry weight of sample.

A second, separate incubation study that ran in parallel, determined treatment effects on soil microbial activity during incubation by measuring microbial respiration. Moist soil (10 g) from each treatment (same as in the previous incubation study) was placed in an incubation vessel with a vial containing 5 mL of freshly prepared 0.1 M NaOH to trap evolved CO_2. Another incubation vessel was maintained without soil as a blank treatment. Incubation vessels were kept covered using Parafilm (Fisher Scientific, Pittsburgh, PA). During the first 14 d, the amount of CO_2 trapped in NaOH was quantified by titrating the NaOH remaining in the vial with 0.1 M HCl on 2, 7 and 10 d. Thereafter, trapped CO_2 was quantified every 14 d for 70 days.

Statistical analysis

Analysis of variance (ANOVA) of pH, Olsen P concentration, EC and microbial respiration data was performed using the MIXED procedure in SAS v9.3 (SAS Institute 2011) with soil and amendment as fixed effects and time as the repeated measure factor. A two-way ANOVA was conducted for CEC, organic matter, wet aggregate stability and P fractions, with soil and biochar rate as fixed effects. Normality of data was tested using the Shapiro-Wilks test (W< 0.9) from PROC UNIVARIATE. For data that were not normally distributed (wet aggregate stability, and P fractions extracted with NaOH, HCl and residual P), natural log transformed data were used for analysis to meet the assumption of normality of residuals. The LSMEANS function in SAS was used to compare treatment means, with adjustments made using Tukey's pairwise-comparison method for soil properties and available P, and using Fisher's protected LSD method for P fractions.

RESULTS

Initial soil properties and biochar properties

Initial soil and biochar properties are presented in Table 1. Based on the OC and total N concentrations of biochar, the added rates of biochar at 10 and 20 g kg^{-1} provided OC at rates of 3.1 and 6.2 mg kg^{-1} soil (4.7 and 9.4 t ha^{-1}) respectively, while the amount of total N added was 10.8 and 21.6 mg kg^{-1} soil, respectively. Based on the Olsen P concentration in biochar, the added rates of 10 and 20 g kg^{-1} provided available P at rates of 3.0 and 6.0 mg kg^{-1} soil, respectively.

Both soils had neutral to slightly alkaline pH (Table 1) indicating a favourable pH for crop production. Both soils were non-saline, and had low available P concentrations. The loamy sand had lower soil OC content, CEC, and available nutrients, and was therefore less fertile in general than the clay loam. According to the rating system used in Manitoba (Manitoba Soil Fertility Advisory Committee 2007), loamy sand had "moderate" available N and "very low" available P, while the clay loam had "high" available N and "low" available P. Thus both soils showed a severe P-limitation for crop production and P is the main focus for the evaluation of fertility effects in this experiment. Biochar used in the current study had an alkaline pH, which was substantially greater than the pH of both soils. The total C and OC contents of biochar were lower than values reported previously for wood based biochars (Lehmenn et al. 2002; Glaser et al. 2002) but greater than the values reported by Major et al. (2010). Cation exchange capacity was similar to CEC reported previously for biochar made from various sources (Major et al. 2010; Sukartono et al. 2011; Kloss et al. 2014). Available P concentration (measured as Olsen P) for the woodchip biochar in the current study was greater than available P measured as Mehlich 3 and Bray 1 extractable P (Major et al. 2010; Yamato et al. 2006) for other wood based biochars, but less than or comparable to available P measured using a sequential water extraction (Angst et al. 2014) for pine woodchip biochar.

Soil pH and EC in unamended and amended soils

Initial pH values in the two soils were neutral to slightly alkaline (Table 1). Soil pH changed with amendment application and during incubation. A significant ($P=0.005$) interaction was observed for soil × time x amendment (Table 2), indicating that the effect of biochar and fertilizer application on pH varied depending on the soil type and incubation time. In general, application of biochar without fertilizers resulted in higher soil pH compared with the unamended control soil. At 70 d of incubation, the increase in pH was significant ($P< 0.05$) in the loamy sand for the BC2 treatment (Fig. 1). For the clay loam, pH increase was significant for BC2 at 42 d after incubation, and for BC1, BC2 and BC2+SF after 70 d of incubation. The increase in pH with biochar application became more apparent with time of incubation and was greatest at 70 d of incubation. At 70 d of incubation, the pH difference between BC2 and the control was greater in the clay loam (0.46 pH units) than in the loamy sand (0.23 pH units). Application of synthetic fertilizer, on the other hand, reduced soil pH (Fig. 1) compared to the control, but the differences were significant ($P<0.05$) only for the loamy sand at 14 and 70 d of incubation.

Soil, amendment and incubation time main effects and the soil × time interaction were significant ($P<0.0001$) for EC (Table 2). As expected, synthetic fertilizer application increased EC in both soils, on average by about 120 µS cm^{-1} compared to the control treatment. Application of biochar alone (BC1 and BC2) did not show significant changes ($P<0.05$) in EC relative to the control treatment in both soils. Application of biochar with fertilizer increased soil EC relative to the control treatment by about 95 µS cm^{-1} for BC1+SF and by 105 µS cm^{-1} for BC2+SF, on average (Table 2). Further, EC values of the BC1+SF and BC2+SF treatments did not differ significantly from EC values of the SF treatment. In the loamy sand, EC values did not significantly ($P<0.05$) change over time of incubation, but in the clay loam EC values increased up to about 56 d of incubation and then significantly ($P<0.05$) decreased between 56 and 70 d of incubation (Fig. 2).

Olsen P and P fractions in unamended and amended soils

The soil x time x amendment interaction was significant ($P< 0.0001$) for Olsen P concentration (Table 2). Application of P fertilizer and/or biochar increased the Olsen P concentration significantly ($P<0.05$) in both soils with a few exceptions (Fig. 3). At all sampling times, the BC2+SF treatment had greater Olsen P concentration than all the other treatments, whereas the lowest Olsen P concentration, in general, was observed in the control treatment. Olsen P concentrations were significantly ($P<0.05$) greater in the BC1+SF and BC2+SF treatments than in the other treatments for both soils at most sampling times (Fig. 3). The BC2 treatment significantly ($P< 0.05$) increased Olsen P concentration compared with the SF treatment at 14 and 28 d of incubation in the loamy sand but not in the clay loam.

For all treatments, concentrations of water-extractable molybdate reactive P (P_{MR}) were smaller (<10 mg kg^{-1}) compared to other fractions, while HCl-extractable and residual P concentrations were largest (>100 mg kg^{-1}in the loamy sand and >250 mg kg^{-1}in the clay loam) (Fig. 4). Biochar and fertilizer amendment increased P_{MR} concentrations in most fractions with a few exceptions. The soil × amendment interaction was significant ($P<0.05$) for P_{MR} extracted by water, $NaHCO_3$, NaOH and HCl. Biochar amendment (at both rates) with and without fertilizer amendment significantly ($P<0.05$) increased water extractable P_{MR} in the loamy sand, but not in the clay loam (Fig. 4a). The BC2+SF treatment in the loamy sand significantly ($P<0.05$) increased water-extractable P_{MR} concentration compared with synthetic fertilizer alone (SF), indicating that the application of fertilizer in combination with biochar enhanced the water-extractable P concentration in the soil. Both fertilizer and biochar amendments did not significantly increase $NaHCO_3$-extractable and NaOH-extractable P_{MR} in the loamy sand soil. However, in the clay loam, fertilizer amendment significantly ($P<0.05$) increased $NaHCO_3$-extractable P_{MR} compared to the control, while both fertilizer and biochar amendments significantly ($P<0.05$) increased NaOH-extractable P_{MR} (Fig. 4b and 4c). In both soils, "labile" P concentration (water extractable plus $NaHCO_3$-extractable P_{MR}) was significantly ($P<0.05$) greater with biochar amendment at 20 g kg^{-1} with fertilizer. In the loamy sand, HCl-extractable P_{MR} concentration in the BC1 treatment was significantly less compared to the control, SF, BC1+SF and BC2+SF treatments. But in the clay loam, both BC1+SF and BC2+SF treatments had significantly greater HCl-extractable P_{MR}, than control and BC2 treatments. Residual P concentrations were not significantly affected by any of the treatments in either soil (Fig. 4d and 4e).

Soil OC, wet aggregate stability and CEC

Soil OC, wet aggregate stability and cation exchange capacity were determined in only three treatments (control, BC1 and BC2) at the end of incubation. Organic C concentration in the clay loam was significantly ($P<0.001$), and nearly 3-fold greater than that in the loamy sand at the end of the 70-d incubation period. However, the main effect of biochar rate and interaction effect of biochar rate × soil on soil OC were not significant (Table 3). Significant increases in OC concentrations were not observed for either rate of biochar treatments, compared to the unamended control.

Wet aggregate stability of soils determined as MWD, differed significantly between the two soils while the soil × biochar rate interaction was also significant ($P< 0.05$) for MWD (Table 3). The clay loam had a higher MWD than the loamy sand. Even though both rates of biochar (10 and 20 g kg^{-1}) increased MWD numerically in both soils compared to un-amended control, the differences were statistically significant ($P<0.05$) only in the loamy sand at the highest biochar rate of 20 g kg^{-1} (Table 3).

The clay loam had significantly greater CEC ($P<0.0001$) than the loamy sand (Table 3). Cation exchange capacity significantly ($P<0.0001$) increased with the addition of biochar in both soils when compared with the respective unamended soil. However, there was no significant difference in CEC between the two rates of biochar (10 and 20 g kg^{-1}) in both soils (Table 3).

Changes in carbon dioxide emission during incubation

The main effects of soil and time and soil × time interaction were significant ($P< 0.05$) for cumulative microbial respiration but the amendment effect was not significant (Fig. 5). Therefore, incorporation of biochar with or without fertilizer did not significantly ($P>0.05$) influence the cumulative microbial respiration. Cumulative microbial respiration was significantly ($P<0.01$) greater in Almasippi loamy sand than in Newdale clay loam at most sampling times. In both soils, the rate of increase in cumulative microbial respiration was greater during the first 14 d of incubation, and continued to increase but at a slower rate thereafter (Fig. 5).

DISCUSSION

Soil pH changes during incubation in un-amended and amended soils

Application of biochar resulted in greater soil pH compared to the control treatment at all times of incubation, with significant differences at 70 d of incubation. Increased soil pH with biochar application has been previously observed with biochar derived from various sources

(Van Zwieten et al. 2010; Peng et al. 2011; Rabileh et al. 2015), but in most of those studies, acidic soils were used. Thus, biochar is considered an effective liming agent for acidic soils. In alkaline soils, Van Zwieten et al. (2010) and Busch and Glaser (2015) did not observe a significant increase in soil pH with biochar application, because the pH of soil and biochar were similar. In the current study with alkaline soils, the greatest pH increase was observed in the BC2 treatment for both soils at 70 d of incubation. The magnitude of pH increase for the BC2 treatment after 70 d of incubation was greater in the less alkaline clay loam (initial pH=7.6), than in the more alkaline loamy sand (initial pH=8.0). The pH increase with biochar amendment in the current study was often less than previously reported values for acidic ferrosol but greater than those reported for alkaline ferrosol soils (Van Zwieten et al.2010; Méndez et al. 2012). Contrary to our findings, Ahmed and Schoenau (2015) did not observe a significant effect on soil pH when biochar was amended at 1-2 t ha^{-1} in black Chernozems, probably due to the very low rate of biochar application.

The fertilizer amendment in the current study consisted of a combination of monoammonium phosphate and urea. Monoammonium phosphate is an acidic P fertilizer, resulting in an acidic saturated solution with a pH of 3.5, thus decreasing soil pH when applied to soil (Sample et al. 1980). The release of H$^+$ from the nitrification of ammonium with increasing incubation time may further decrease soil pH (Hanson and Westfall 1985; Al-Showk et al. 1987), as observed in the loamy sand. This effect was not observed in the clay loam likely due to the greater pH buffering ability in soils with greater clay concentration (Xie and Mackenzie, 1990). As expected, the increase in pH with application of biochar (BC1 and BC2) was smaller when biochar was added with fertilizer (BC1+SF and BC2+SF) because of counteracting effects of biochar and fertilizer on soil pH. Similar observations were made in acidic soils (Van Zwieten et al. 2010) where the pH increase was greater with biochar application in the absence of fertilizer than with fertilizer.

In alkaline soils, pH>7.5 may negatively influence plant growth due to reduced nutrient availability and microbial activity (Davidson, 2014). Further increase in pH with biochar addition may aggravate these negative effects and may offset the beneficial effects of biochar application. Biochar application with synthetic fertilizer, however, could be a better approach for alkaline chernozems, since the increase in soil pH was less than when biochar was applied alone.

Soil electrical conductivity changes during incubation in unamended and amended soils

The effect of biochar on soil EC was negligible and not significant in these alkaline chernozesm. Using a 30 t/ha rate of biochar, Kloss et al. (2014) observed similar results in chernozems, but at a higher rate of 90 t/ha of biochar, EC increased significantly immediately after application. Soinne et al. (2014), on the other hand, observed a decrease in soil EC with biochar addition. As expected, synthetic fertilizer consisting of monoammonium phosphate and urea increased EC in both soils because of the added cations and anions. Even though the increase in EC with synthetic fertilizer was statistically significant, it was not agronomically significant since the soil EC remained in the non-saline range.

Changes in soil Olsen P and P fractions during incubation

In both soils, the BC2+SF treatment increased the Olsen P close to or above 12 mg kg^{-1}, thus raising the available P rating from very low/low to medium (Manitoba Soil Fertility Advisory Committee 2007). Olsen P concentration increased about 2-fold with the application of biochar with synthetic fertilizer in the loamy sand compared to the control soil at 14 d of incubation, but the increase in Olsen P concentration with biochar and fertilizer application was less pronounced in the clay loam. A similar influence of soil texture on enhancing P availability with manure amendment has been previously reported for alkaline chernozems in Manitoba (Kumaragamage et al. 2011).

Biochar application affects the P cycle directly and indirectly through various mechanisms, and as a result, the effect of biochar on soil P availability has been inconsistent (DeLuca et al. 2009; Xu et al. 2014). Greater P availability could result from the direct supply of P by the biochar, as well as decrease in P retention and/or increased P mineralization due to enhanced microbial activity (DeLuca et al. 2009; Laird et al. 2010). In our study, increased P availability could result mainly from the direct supply of available P by the biochar, as its Olsen P concentration was 297 mg kg^{-1}, which is very high compared to the soil Olsen P concentration. At the rates of biochar application, the added Olsen extractable P with biochar was 3.0 and 6.0 mg kg^{-1} for BC1 and BC2, respectively. Similar to our findings, Kloss et al. (2014) observed an increase in P availability in a chernozemic soil immediately after biochar application; however after 7 months of application, available P in biochar amended and unamended soils did not show significant differences in the chernozem, unlike in a planosol and a cambisol they used in the same study. Xu et al. (2014) observed contradictory effects of biochar (total P of 2773 mg kg^{-1}) application on P retention in different soils depending on soil pH; P retention increased in two acidic soils and slightly decreased in an alkaline soil. They concluded that the initial level of soil acidity determines the response of P retention to biochar addition. However, it is interesting to note that the acidic soils in their study were sandy loams whereas the alkaline soil was a loam to silty loam. The results of the current study using two alkaline chernozems with similar pH levels indicate that soil properties other than soil pH, such as soil texture and organic matter, may influence P availability changes with biochar addition, which warrant further investigations using alkaline chernozemic soils.

Apart from Olsen P, concentrations of some P fractions in soils were influenced by biochar and fertilizer amendment. Co-application of fertilizer with biochar enhanced the water-extractable P concentration in the loamy sand. This observation has implications for improving P availability in this loamy sand, which had a very low initial Olsen P concentration. Concentrations of $NaHCO_3$-extractable and NaOH-extractable P were not significantly affected by amendments in the loamy sand. However, application of biochar and fertilizer, alone, or in combination, often increased the $NaHCO_3$-extractable and NaOH-extractable P concentration in the clay loam. Overall, the results indicate that application of biochar at 20 g kg^{-1}rate with or without synthetic fertilizer is an effective means of enhancing labile P concentration. In a Calcisol, biochar with P fertilizer showed a significant and interactive effect on soil P fractionation with the fertilizer having the main influence on changes in P fractions (Farrell et al. 2014). In two acidic soils, biochar application increased concentrations of Ca-bound P (HCl-extractable) but this was not observed in an alkaline soil (Xu et al. 2014). In the same study, biochar application slightly decreased the Fe-bound P (NaOH-extractable) in both alkaline and acidic soils. Results of HCl-extractable P in biochar-amended soils in the current study are consistent with the findings of Xu et al. (2014) and further suggest that effects of biochar application on soil P availability may be influenced by soil texture and soil pH, by influencing P sorption and desorption. This warrants further investigation, as it would have important implications for improving soil productivity on a large scale.

Changes in soil organic carbon, wet aggregate stability, and cation exchange capacity

Biochar amendment at both rates did not significantly increase OC concentration in the two soils. Results from the current study are inconsistent with previous findings in acidic soils where biochar addition significantly increased the soil OC concentration in laboratory incubation (Novak et al. 2009; Sukartono et al. 2011). The rates of total C added with biochar amendment in those studies (Novak et al. 2009; Sukartono et al. 2011) were often greater (6.3 to 25 t of total C ha^{-1}) compared to the total C added with biochar amendment in the current study (4.7 and 9.4 t ha^{-1}). Thus, the significance of the increase in soil OC in biochar-amended soil seems to depend on the total C added with biochar application.

A high MWD value is an indication of the predominance of larger and more stable aggregates over smaller and less stable aggregates, thus indicating greater wet aggregate stability. Application of biochar at the 20 g kg^{-1} rate to the loamy sand increased water stable aggregates and thereby reduced the vulnerability of the fertile topsoil loss through erosion, thus maintaining long term foil fertility. Ouyang et al. (2013) observed similar results in an incubation study with two soils, a sandy loam and a silty clay, both with slightly acidic pH. They found that the MWD values were significantly enhanced by biochar addition in the sandy loam on most of the sampling days, whereas in a silty clay soil, significant differences in MWD were observed only at later stages of incubation between unamended and biochar-amended treatments. Soinne et al. (2014), however, observed that biochar addition increased aggregate stability in acidic clay soils, implying that the influence of biochar in improving wet aggregate stability is not limited to sandy soils. The type of biochar used may also influence the effect on MWD. For example, aggregate stability measured as MWD in clay soils with a neutral pH (7.2) was enhanced by wastewater sludge biochar and straw biochar amendment, but not with woodchip biochar amendment (Sun and Lu 2014).

The cation exchange capacity was significantly increased with the addition of biochar in both soils when compared to the respective unamended soil. There was no significant difference in CEC between the two rates of biochar addition (10 and 20 g kg^{-1}) in either soil (Table 3). Contrary to our findings, Kloss et al. (2014) did not observe an increase in CEC with the application of biochar in temperate chernozems, while CEC increased with biochar application in a Planosol in the same study. Increase in CEC with biochar application has also been reported for strongly acidic tropical soils (Yamato et al. 2006; Sukartono et al. 2011). The presence of carboxylic and phenolic functional groups in biochar results in high surface negative charges, with a greater ability than other organic matter to adsorb cations (Liang et al. 2006). This characteristic of biochar may explain the increase in CEC in biochar amended soils compared to the control, however it does not explain the lack of a significant difference in CEC for the two rates of biochar. Even though the biochar used in the current study had CEC values similar to that of the Newdale clay loam, application of biochar significantly increased the CEC even in the clay loam. Increase of CEC with the addition of biochar will have both agronomic and environmental benefits. Greater retention of cationic nutrients through increasing CEC in the sandy soil would increase nutrient availability in soils as well as reduce nutrient leaching and runoff losses.

Changes in carbon dioxide emission during incubation

Increased soil microbial activity promotes microbe-mediated processes in soils such as mineralization, and P solubilisation, resulting in an enhanced bioavailability of nutrients that improves soil fertility, whereas increased immobilization of nutrients may also take place reducing nutrient availability in the short term, thus negatively affecting soil fertility. In our study, the cumulative microbial respiration pattern did not show a significant change with the incorporation of biochar in either soil. Our results are consistent with previous observations with black Chernozems (Wu et al. 2013; Cheng et al. 2012) showing that biochar addition did not result in a significant increase in CO_2 emissions, very likely because of the slow decomposition of biochar during incubation (Stainer et al. 2007; Jones et al. 2011). Biochars derived from manure or crop residue feedstock tend to promote microbial abundance more than wood-derived biochars.

CONCLUSIONS

The two alkaline Chernozems responded slightly differently to biochar amendment at different rates with and without fertilizers, with greater fertility benefits in the less fertile soil. The results indicate that biochar can be effectively used in combination with fertilizer for slightly alkaline soils to improve soil fertility parameters in alkaline chernozems without significantly increasing pH, but the initial pH of soils and biochar need to be considered.

Application of biochar resulted in higher wet aggregate stability in the loamy sand, but not in the clay loam. In general, biochar application resulted in an increased soil CEC, and enhanced P availability measured as Olsen P and labile P fractions in both soils. Overall, this study confirms that amendment of soils with biochar improved soil fertility parameters in the two alkaline chernozems, but biochar amendment at a high rate (20 g kg^{-1}) with synthetic fertilizer was more effective than applying biochar or fertilizer alone, or at a low rate (10 g kg^{-1}) with fertilizer. However, long-term studies under field conditions are needed to evaluate biochar effects on soil properties and sustainable crop production for alkaline Chernozems on the Canadian prairies.

ACKNOWLEDGEMENT

The authors acknowledge the Canadian Bureau of International Education for awarding a Canadian Commonwealth Scholarship to the senior author to conduct this study in Canada. We acknowledge Ryan Banman for providing the biochar for the study, Anthony Buckley for the technical support and Geethani Amarawansha for assisting with the statistical analysis.

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Table 1. Properties of biochar and two soils used for the study

Property	Almasippi loamy sand[a]	Newdale clay loam[a]	Biochar[b]
Sand %	88.4 (0.1)	35.8 (1.4)	
Silt %	4.6 (1.4)	31.0(0.2)	
Clay %	7.0 (1.4)	33.2 (1.2)	
pH (1:2, soil: H_2O)	8.0 (0.1)	7.6 (0.1)	9.7
Cation exchange capacity (mmol $_c$ kg^{-1})	136 (2)	273 (23)	200
Electrical conductivity (dS m^{-1})	0.18 (0.01)[c]	0.41 (0.02)[c]	16.9[d]
Organic C (g kg^{-1})	9.3 (0.6)	33.4 (1.1)	312
Total C (g kg^{-1})			314
Olsen P (mg kg^{-1})	3.5 (0.7)	5.5 (0.7)	297
Nitrate N (mg kg^{-1})	11 (1)	53 (1)	
Exchangeable Ca (mg kg^{-1})	2348 (21)	3823 (384)	1500
Exchangeable Mg (mg kg^{-1})	192 (7)	907 (46)	410
Exchangeable K (mg kg^{-1})	49 (4)	203 (1)	3510
Exchangeable Na (mg kg^{-1})	23.5 (0.7)	16.5 (0.7)	30
Total N (mg kg^{-1})			1080
Total P (mg kg^{-1})	185 (8)	354(12)	569
Total K (mg kg^{-1})	ND	ND	8244
Total Ca (mg kg^{-1})	ND	ND	8476
Total Mg (mg kg^{-1})	ND	ND	2073

[a] Means of two replicates. Values in parentheses are standard deviations.

[b] Biochar properties were measured only in one replicate.

[c] Measured in 1:2 soil:water extract

[d] Measured in saturated extract.

Table 2. ANOVA for soil pH, electrical conductivity and Olsen extractable P during 70 d of incubation with different amendment treatments in Almasippi loamy sand and Newdale clay loam.

EFFECT	pH	Electrical conductivity[a] (µS cm^{-1})	Olsen extractable P (mg kg^{-1})
Soil			
Almasippi loamy sand	7.97	244	8.8
Newdale clay loam	7.62	541	9.2
Amendments			
Control	7.76	333^b	6.3
SF	7.61	456^a	8.4
BC1	7.89	341^b	7.6
BC2	7.96	348^b	8.7
BC1 + SF	7.75	448^a	10.7
BC2 + SF	7.83	429^a	12.2
		P values	
Soil	<0.0001	<0.0001	0.02
Time	<0.0001	<0.0001	<0.0001
Treatment	0.03	<0.0001	0.0004
Soil ×time	<0.0001	<0.0001	<0.0001
Soil × treatment	0.002	0.08	<0.0001
Time × treatment	0.01	0.62	<0.0001
Soil × time × treatment	0.005	0.09	<0.0001

[a] Mean comparison shown only when the interaction is not significant. Means within the same column followed by the same letter are not significantly different at $P<0.05$

SF – synthetic fertilizer; BC1- biochar at 10 g kg^{-1}; BC2 – biochar at 20 g kg^{-1}

Table 3. ANOVA and mean soil organic carbon concentration (OC), wet aggregate stability measured as mean weight diameter (MWD), and cation exchange capacity (CEC) in the control, biochar at 10 g kg^{-1} (BC1) and biochar at 20 g kg^{-1} (BC2) treatments after 70 d of incubation in Almasippi loamy sand and Newdale clay loam

EFFECT	**OC[z] (%)**	**MWD[ab] (mm)**	**CEC[a] (mmol$_c$ kg^{-1})**
Main effects			
Soil			
Almasippi loamy sand	1.22^{b}	0.36	116^{b}
Newdale clay loam	3.43^{a}	1.33	247^{a}
Biochar rate			
Control (0 g kg^{-1})	2.30	0.76	174^{b}
BC1	2.32	0.83	205^{a}
BC2	2.35	0.97	207^{a}
Interaction Effects			
Almasippi loamy sand			
Control	1.18	0.27^{b}	99
BC1	1.18	0.28^{b}	124
BC2	1.26	0.54^{a}	126
Newdale clay loam			
Control	3.41	1.24^{a}	249
BC1	3.41	1.38^{a}	285
BC2	3.47	1.34^{a}	287
ANOVA		P value	
Soil	<0.0001	<0.0001	<0.0001
Biochar rate	0.10	0.0004	<0.0001
Soil × biochar rate	0.58	0.02	0.44

[a] Means within the same column followed by the same letter are not significantly different at $P < 0.05$ according to the Tukey–Kramer test. Mean separation for main effects presented only in the absence of significant ($P < 0.05$) interaction effects.

[b] Back-transformed (geometric) means

Figure captions

Fig. 1. Mean soil pH showing the three-way interaction between soil, amendment and incubation time (a) Almasippi loamy sand and (b) Newdale clay loam. (SF – Synthetic fertilizer; BC1 - Biochar at 10 g kg^{-1}; BC2 – Biochar at 20 g kg^{-1}). Error bars represent the standard error of the mean (n=3).

Fig. 2. Mean EC values showing the two-way interaction between soil and incubation time. (a) Almasippi loamy sand and (b) Newdale clay loam. (SF – Synthetic fertilizer; BC1 - Biochar at 10 g kg^{-1}; BC2 – Biochar at 20 g kg^{-1}). Error bars represent the standard error of the mean (n=3).

Fig. 3. Mean Olsen P concentration showing the three-way interaction between soil, amendment and incubation time (a) Almasippi loamy sand and (b) Newdale clay loam. (SF – Synthetic fertilizer; BC1 - Biochar at 10 g kg^{-1}; BC2 – Biochar at 20 g kg^{-1}). Error bars represent the standard error of the mean (n=3).

Fig. 4. Interaction of soil and amendment for molybdate reactive P (PMR) concentrations in difference fractions at the end of 70-d incubation period (a) water extractable P, (b) $NaHCO_3$ extractable P, (c) NaOH extractable P (d) HCl extractable P and (e) Residual P (C - un-amended; SF - Synthetic fertilizer; BC1 - Biochar at 10 g kg^{-1}; BC2 – Biochar at 20 g kg^{-1}). Lowercase letters above the bars indicate significant differences among treatments within a soil ($P < 0.05$) according to Fisher's protected LSD method.

Fig. 5. Cumulative emission of CO_2 by microbial respiration during the 70-d incubation period for (a) Almasippi loamy sand (b) Newdale clay loam (C - un-amended; SF - Synthetic fertilizer; BC1 - Biohar at 10 g kg^{-1}; BC2 – Biochar at 20 g kg^{-1}). Bars indicate the standard error of the mean (n=3).

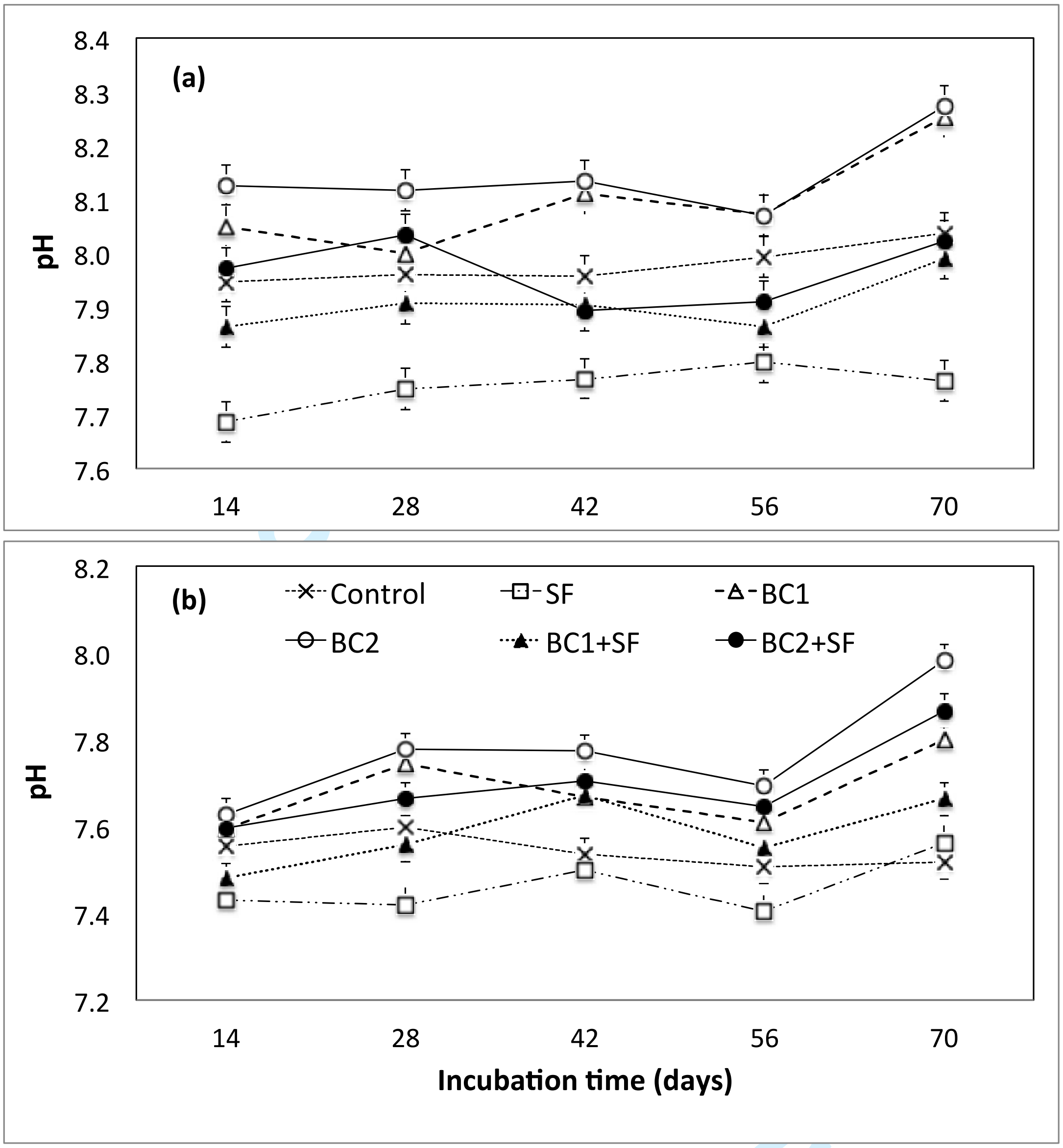
(a)
pH
8.4
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
14
28
42
56
70
(b)
Control
SF
BC1
BC2
BC1+SF
BC2+SF
pH
8.2
8.0
7.8
7.6
7.4
7.2
14
28
42
56
70
Incubation time (days)

Fig. 1

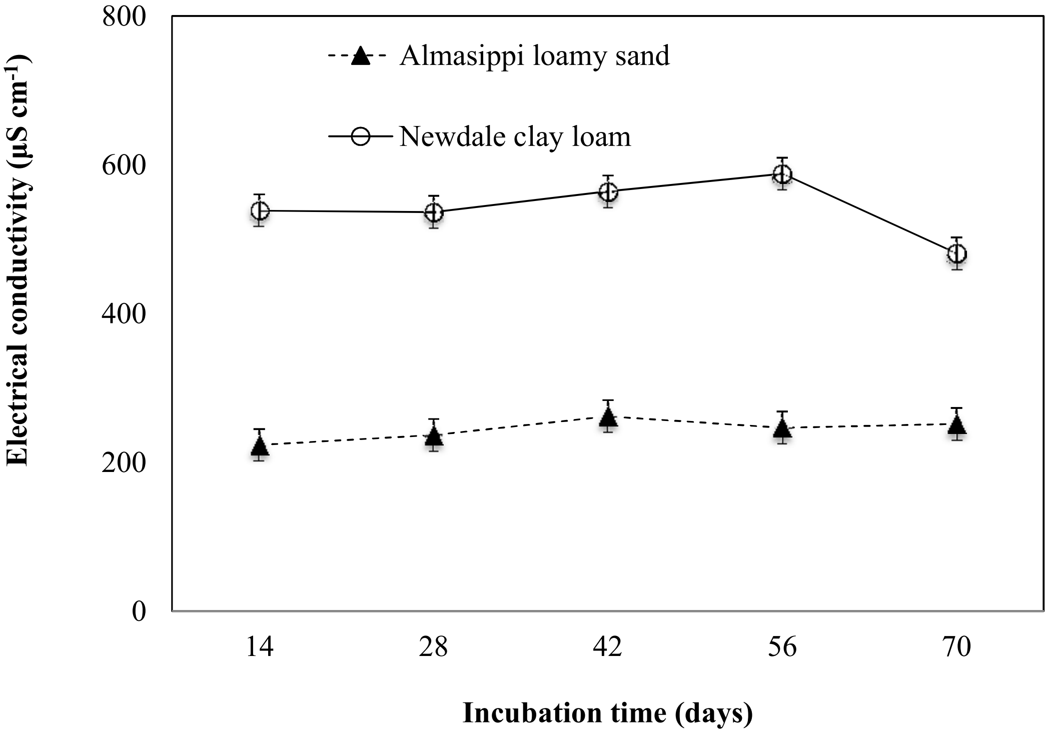
Almasippi loamy sand
Newdale clay loam
Electrical conductivity (µS cm-1)
800
600
400
200
0
14
28
42
56
70
Incubation time (days)

Fig 2

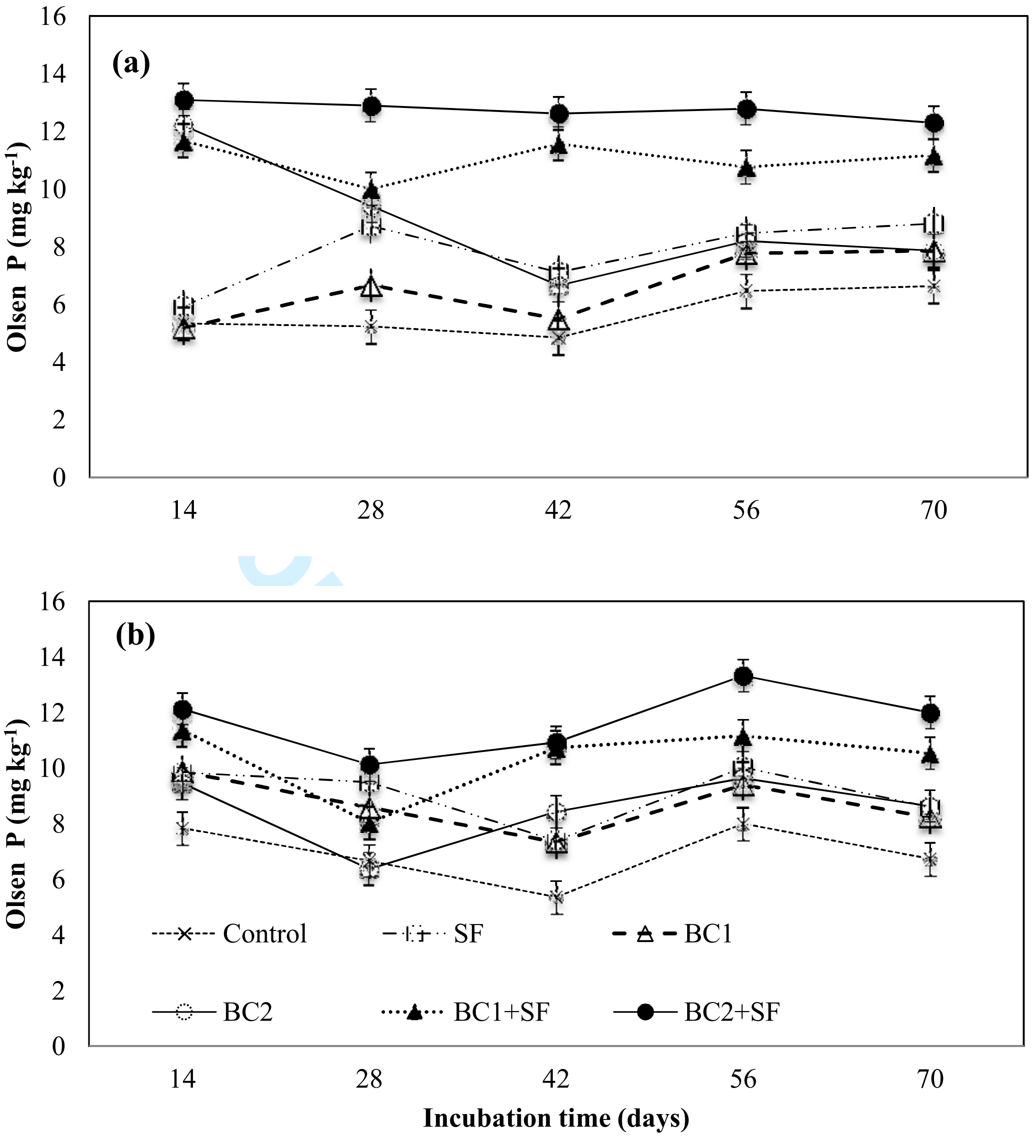
(a)
Olsen P (mg kg^{-1})
16
14
12
10
8
6
4
2
0
14
28
42
56
70
(b)
Olsen P (mg kg^{-1})
Control
SF
BC1
BC2
BC1+SF
BC2+SF
Incubation time (days)

Fig 3

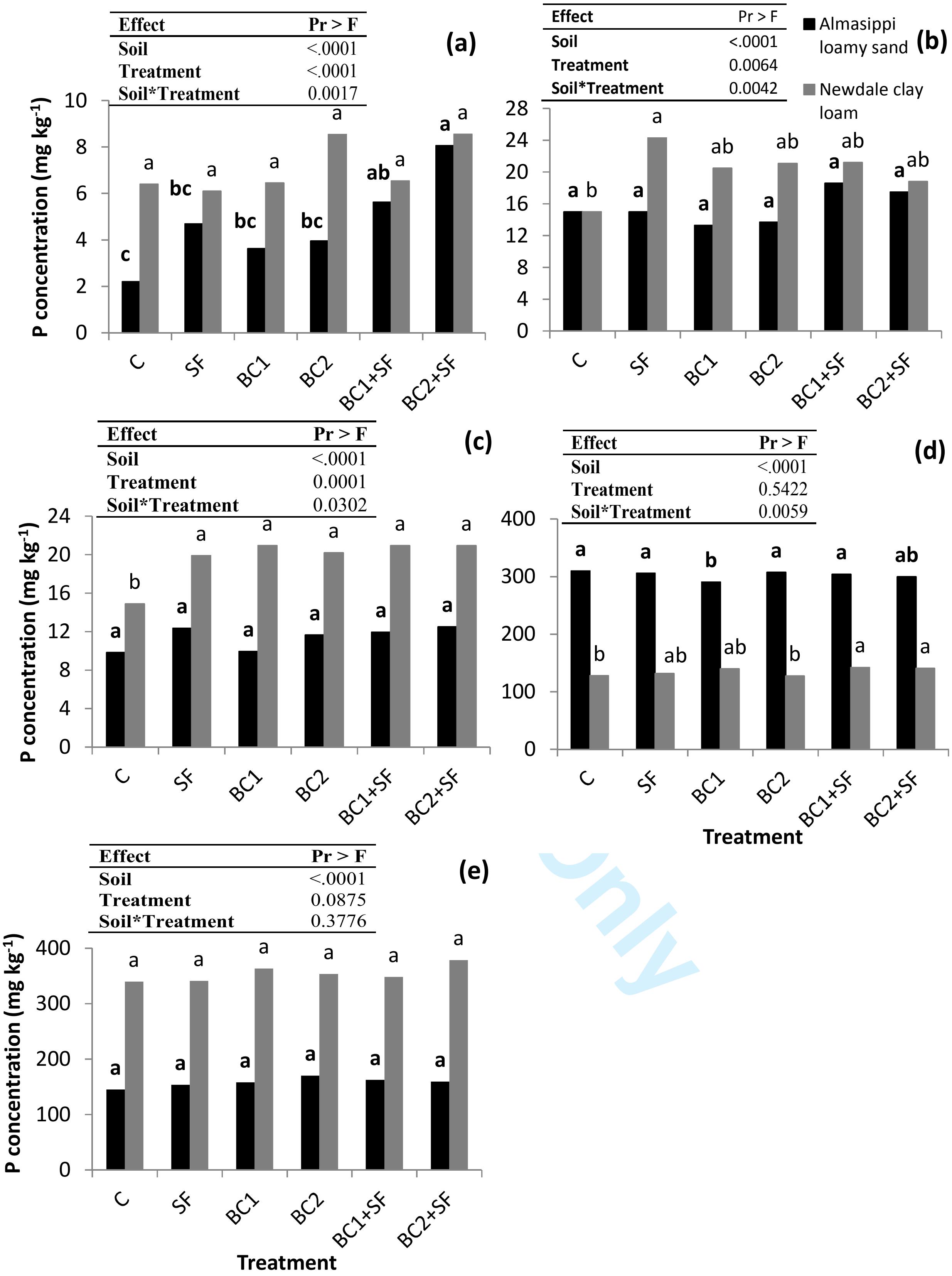
(a)
Effect Pr > F
Soil <.0001
Treatment <.0001
Soil*Treatment 0.0017
P concentration (mg kg-1)
0
2
4
6
8
10
c a
bc a
bc a
bc a
ab a
a a
C
SF
BC1
BC2
BC1+SF
BC2+SF
(b)
Effect Pr > F
Soil <.0001
Treatment 0.0064
Soil*Treatment 0.0042
■ Almasippi loamy sand
■ Newdale clay loam
0
4
8
12
16
20
24
28
a b
a a
a ab
a ab
a ab
a ab
C
SF
BC1
BC2
BC1+SF
BC2+SF
(c)
Effect Pr > F
Soil <.0001
Treatment 0.0001
Soil*Treatment 0.0302
P concentration (mg kg-1)
0
4
8
12
16
20
24
a b
a a
a a
a a
a a
a a
C
SF
BC1
BC2
BC1+SF
BC2+SF
(d)
Effect Pr > F
Soil <.0001
Treatment 0.5422
Soil*Treatment 0.0059
0
100
200
300
400
a b
a ab
b ab
a b
a a
ab a
C
SF
BC1
BC2
BC1+SF
BC2+SF
Treatment
(e)
Effect Pr > F
Soil <.0001
Treatment 0.0875
Soil*Treatment 0.3776
P concentration (mg kg-1)
0
100
200
300
400
a a
a a
a a
a a
a a
a a
C
SF
BC1
BC2
BC1+SF
BC2+SF
Treatment

Fig 4.

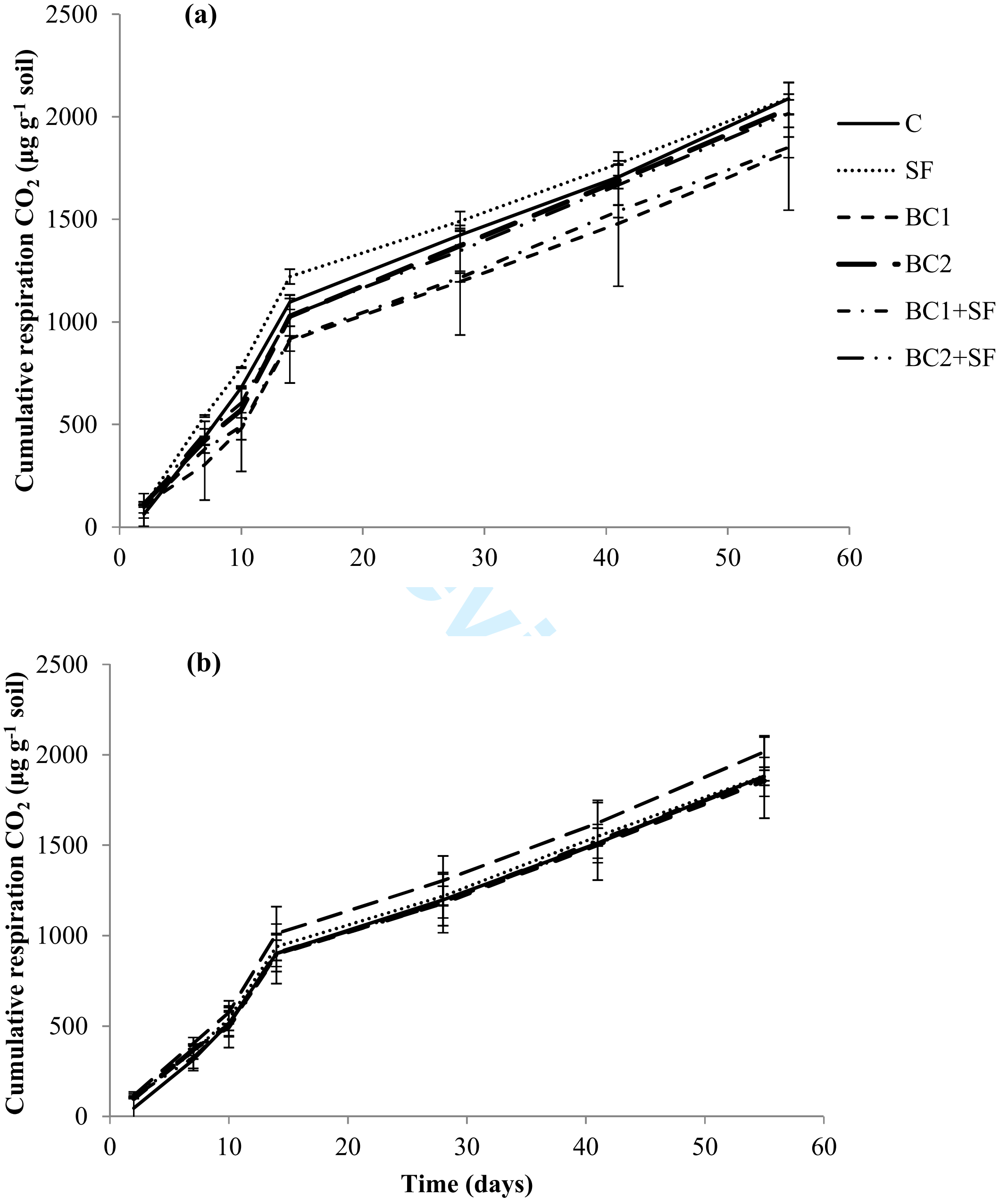
(a)
Cumulative respiration CO_2 (μg g^{-1} soil)
2500
2000
1500
1000
500
0
0
10
20
30
40
50
60
C
SF
BC1
BC2
BC1+SF
BC2+SF
(b)
Cumulative respiration CO_2 (μg g^{-1} soil)
2500
2000
1500
1000
500
0
0
10
20
30
40
50
60
Time (days)

Fig. 5.