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Residual effects of biochar and phosphorus on growth and nutrient accumulation by maize (*Zea mays* L.) amended with microbes in texturally different soils

Mazhar Rafique [a, b, c], Ibrahim Ortas [b], Muhammad Rizwan [d, *], Hassan Javed Chaudhary [a, **], Ali Raza Gurmani [c], Muhammad Farooq Hussain Munis [a]

[a] Department of Plant Sciences, Quaid-i-Azam University, Islamabad, 45320, Pakistan
[b] Department of Soil Science and Plant Nutrition, Cukurova University, Adana, 1150, Turkey
[c] Department of Soil Science, The University of Haripur, Haripur, 22630, Khyber Pakhtunkhwa, Pakistan
[d] Department of Environmental Sciences and Engineering, Government College University, Allama Iqbal Road, Faisalabad, 38000, Pakistan

HIGHLIGHTS

- Residual effect of biochar and phosphorus was studied to address soil P deficiency problem.
- Biochar and P significantly enhanced nutrients uptake and maize plant growth.
- Microbial inoculants further stimulated plant biomass and nutrients due to effective root colonization.
- Residual biochar + microbial inoculation might be an efficient source of P for plants.

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ABSTRACT

The purpose of study was to examine the residual effects of two types of biochar amendments, two phosphorus (P) fertilizer levels, phosphorus solubilizing bacteria (PSB) and arbuscular mycorrhizal fungs (AMF) on plant growth, nutrients absorption and root architecture of *Zea mays* L. in texturally different soils. Biochar signficantly increased nutrients absorption and plant biomass production with P-fertilization and microbial inoculantion. Texturally different soils enhanced the plant biomass and nutrients absorption in their independent capacity on addition of biochar, microbial inoculants and P-fertilization. It was shown that mycorrhizal inoculation had positive influence on plant root and shoot biomass in both soils irrespective to the biochar type used. Root colonization was notably increased in biochar + mycorrhizae (B + M) inocultaed plants. It was shown that mycorrhizal inoculation had positive influence on nutrients absorption by plant roots and it had high content of P, potassium, calcium and magnesium in plants at all biochar and P levels. Without P fertilization, biochar amendments significantly promoted shoot P content and root colonization. The P application significantly influenced soil microbial activity in terms of nutrient concentration and plant growth. Root attributes were significantly inclined by microbial inoculation. Residual effects of biochar and P significantly enhanced the nutreints absorption and maize plant growth. Thus, we concluded that residual biochar and P fertilizer showed positive effects on nutrients absorption and maize plant growth promotion in differently textured soils. Microbial inoculants further stimulated the plant biomass production and nutrients absorption due to effective root colonization.

1. Introduction

In productive agriculture, chemical fertilizer consumption is high in arable soils aiming to enhance plant growth and yield. Extensive use of chemical fertilizers is a worldwide emerging problem. Phosphorus (P) being an essential macronutrient for plant

* Corresponding author.
** Corresponding author.
E-mail addresses: mrazi1532@yahoo.com, mrizwan@gcuf.edu.pk (M. Rizwan), hassaan_javed@yahoo.com (H.J. Chaudhary).

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growth is applied in soils at a very high rate. Low P availability to plants is a global problem which is limiting crop productivity (Richardson and Simpson, 2011; Rafique et al., 2018). Soils face nutrients depletion due to aggressive cultivation of crops (Faucon et al., 2015; Rehman et al., 2018a). As a result, a number of agronomic practices have been proposed to enhance P availability and its use by crops under diverse climatic conditions (Simpson et al., 2011). Agrochemicals are key source of P which reside in soils for a longer period of time. It has been reported that over fertilization of P accumulates in soils and 75–90% of applied P fertilizer sources stay in the plough layer of soil and not taken up by crops (Cordell and White, 2014). This excessive use may have deleterious effects on soil microflora and soil quality (Tripti et al., 2015). Efficient application of P-fertilizers for better soil quality and crop yield is of utmost importance considering reduction in environmental perils (Withers et al., 2014). Plants may accummulate P in ionic forms such as $H_2PO_4^-$, HPO_4^{2-}, and PO_4^{3-}, which need continuous replenishment of the soil (Brady and Weil, 2013). High fixation capacity of soil may reduce P bioavailability to plants in soil solution and threatens sustainability of crop production (Fink et al., 2016).

Recycling of nutrients from organic sources into soil is a sustainable approach for improving soil physical, chemical and biological properties (Girmay et al., 2008; Rizwan et al., 2016; Abbas et al., 2018). Such practices are important to enhance soil fertility and crop productivity in soils with intrinsically low soil fertility (Anjum et al., 2011; Qayyum et al., 2017). Recently biochar has attained great attention as soil amendment due to its functionality in various environments (Rizwan et al., 2016; Azhar et al., 2019). Reportedly, biochar has multiple functions in enhancing soil fertility, crop productivity and mitgating climate change through carbon sequestration in soil for many years (Lehmann, 2007; Rizwan et al., 2019). Biochar can act as a source of soluble P in soil application (Parvage et al., 2013). During pyrolysis, biochar assimilates P and further transformation concentrates P in biochar (Bruun et al., 2014; Rehman et al., 2018b). The release of P from biochar has been noticed by a considerable amount of P desorption from biochar at zero P level in P sorption experiments (Morales et al., 2013). Addition of biochar into soil may reduce P sorption capacity which make it available for plant uptake through soil solution (Qayyum et al., 2015). Promsing effects of biochar application on soil physical, chemical and biological properties have been reported influencing soil fertility status (Cornelissen et al., 2013; Ali et al., 2019). Woody biochar such as bamboo, eucalyptus, reed and sawdust are rich in nutrients required to plants and they relase into soil slowly. Their residual effect of nutrients sustain for a longer time and multiple crops get benefits growing in same soil (Mukherjee and Zimmerman, 2013; Yao et al., 2013; Rizwan et al., 2018).

Biochar amended soil also improves colonization of mycorrhizal fungi inoculated roots and their P-uptake ability (Atkinson et al., 2010; DeLuca et al., 2015). Release of biochar P into soil and microbial inoculants presence in rhizosphere may increase P-availability for plant uptake (Zwetsloot et al., 2016). Residual P adsorbed on soil surface may solubilize by bacteria through release of organic acisds, chelating agents (indole-3-acetic acid and siderophores) and growth regulating hormones (Otieno et al., 2015). In presence of bcteria, P-minning activities increase from biochar and reportedly, 47–54% biochar P was solubilized through bacterial strain *Lysinibacillus fusiformis* (Anderson et al., 2011; He et al., 2014). Only <50% P in biochar is released under natural environmental conditions whereas residual P may release slowly into soil (Schneider and Haderlein, 2016). Addition of biochar with microbial inoculants have positive effects on maize plant height and nutrients concentration. Moreover, plants amended with sawdust biochar + *L. fusiformis* strain 31MZR inoculation increased N, P and K (Rafique et al., 2017).

Limited data is available for P absorption by plant tissues with residual biochar and residual P. We hypothesized that both residual biochar and P might have a key source of P for maize plant growth immediately after harvesting of onion plant in same soils. Thus maize plants were grown in present study after onion growth in differently textured soils (Rafique et al., 2019b) to monitor the residual effects of biochar and P. Moreover, microbial inocualnts were applied into soil and evaluation of macro- and micronutrients in plant, root colonization and root morphology were measured. The key objectives were to identify that (1) how residual biochar types and residual P interact with microbial inoculants in different soils for root colonization and nutrients absorption and (2) whether residual biochar and residual P with microbial inoculants mediate global changes in modern agriculture system where P-deficiency crisis is a major threat in future.

2. Materials and methods

2.1. Soil properties and biochar treatements

In this study, we used soil that was previously used for onion cultivation and detailed physicochemical properties of soil are reported in Rafique et al. (2019b). In brief, a greenhouse study was conducted in Cukurova Univerity, Adana, Turkey. Two types of soils were used in experiment. Soil A was localy named as Menekse (Typic xerorthent Ortehnt Entisol) and Soil B as Kiziltapir (Lithic Rpodoxeralf Xeralf Alfisol) soil series by USDA classification. Soil A was texturally classified as silty loam soil with sand 6%, silt 67% and clay 27%. Furthermore, soil pH was 7.84, and CEC 21.23 $cmol_c\ kg^{-1}$. Soil B was texturally classified as clay soil with sand 25%, silt 17% and clay 58%. Furthermore, soil pH was 6.68, and CEC 20.65 $cmol_c\ kg^{-1}$. Two types of biochar made from common reed (*Phragmites australis*) and sawdust feedstock were used in study. Biochar was prepared at 350 °C for 2 h at a rate of 10 °C increase per minute. After attaining 350 °C temperature in furnace, 1 h residence time was provided. Common reed biochar had pH 7.49, EC 2.23 mS cm^{-1}, ash content 3.9%, voltile matrix 56.05%, C 67.89%, N 0.57%, K 2.07%, and P 0.09% while sawdust biochar had pH 6.29%, EC 0.33 mS cm^{-1}, ash content 3.11%, volatile matrix 66.99%, C 61.96%, N 0.95%, K 0.22%, and P 0.03% (Rafique et al., 2019b). Biochar were applied as 1% *w/w* in soil and bioinoculants (*Lysinibacillus fusiformis* 3MZR and *Rhizophagus clarus*) were added as different treatments before transplantation of onion seedlings (Rafique et al., 2019b). After harvesting onion plants with roots, soil was left undisturbed in their pots for 15 days without any plant growth. Only bacterial and mycorrhizal bioinoculants were added before sowing maize seeds. No further addition of biochar and P was performed to evauate their residual effects.

2.2. Experimental setup

The study was conducted in greenhouse of Cukurova University, Adana, Turkey, under environmental conditions as 25 ± 3 °C, 80 ± 3% relative humidity and 16:8 h day:night cycle. Each pot had 3 kg soil and after harvesting of onion plants, soil was left undisturbed for 15 days till further sowing of maize seeds. Initially, five maize seeds (LG 37.10, Anadolu, Hybrid, Turkey) were added to every plastic pot (3 L, 21 cm diameter, 18 cm height) with three replicates following CRD (completely randomized design). Finally, three plants were maintained in each pot after 10 days of sowing. Pot had two levels of P for onion and no further addition of P was done for maize. Each pot was provided with a basal dose of ammonium nitrate (34% N), and Muriate of Potash (MOP, 60% K_2O) at recommended rates of 160 kg N ha^{-1}, and 60 kg K_2O ha^{-1}

equivalents based on soil test results to avoid nutrients deficiency. No further biochar and P were added in soil for maize experiment and previsous addition of biochar as 1.0% *w/w* was considered to evaluate residual effects. Bacterial and mycorrhizal inoculants were added based on experimental treatments. The treatments were; uninoculated control (C), *Lysinibacillus fusiformis* 31MZR (B), *Rhizophagus clarus* (M), and *L. fusiformis* 31MZR + *R. clarus* (B + M). The bacterial counts were 10^{8-9} cfu/mL. All pots were irrigated with distilled water to field capacity.

2.3. Plant harvesting and sample preparation

Plants were harvested after 65 days of sowing the seeds. Data related to aboveground biomass was collected through cutting stem at soil surface, dried to a constant weight at 60 °C and weighed to determine dry biomass. Root samples were extracted from each pot, rinsed with deionized water and dried to a constant weight at 60 °C and weighed to determine dry biomass. All dried plant tissues (above- and belowground) were ground with a Tema mill, RM100 (Retsch Solutions in Miling and Sieving, Haan, Germany) to pass through a 0.5 mm mesh sieve, samples were stored in sealed containers for analyses.

2.4. Roots characterization

Harvested roots were thoroughly washed using deionized water (Kachenko and Singh, 2006). Root system of plants was placed on a scanner (Epson Perfection V700, Photo Long Beach, CA, USA), in a transparent plastic tray filled with water. Root length, root surface area, and root volume were analyzed using WinRHIZO Pro 3.10 (Regent Instruments Inc.) (Himmelbauer, 2004).

2.5. Tissue nutrients analyses and mycorrhizal root colonization

Nutrient concentration (P, K, Cu, Mn, and Zn) in above- and belowground biomass was analyzed by ICP-OES (inductively coupled plasma optical emission spectrometry, PerkinElmer, USA) (Wheal et al., 2011). Root and shoot samples were heated up to 500 °C for 2 h and then retained at 500 °C for 8 h. HNO_3 (5 mL) was added to each vessel and digested at 120 °C until they dried. Then, tubes were removed from block and allowed to cool before adding 1.0 mL HNO_3 and 4.0 mL H_2O_2. Samples were placed back into a preheated block and processed at 120 °C until dryness, dissolved in 1.43 mL HNO_3, volume was then raised with 18.57 mL deionized water and filtered. Tomato leaves were used as Standard Reference Material 2711a (NIST) in whole process.

To determine extent of mycorrhizal colonization, maize roots were cut into small pieces (about 1 cm length) and stained with Trypan Blue following a modification of procedure described by Phillips and Hayman (Koske and Gemma, 1989). Mycorrhizal colonization in maize roots was determined using the method described by Giovannetti and Mosse (1980).

$$\textit{Mycorrhizal root colonization}(\%) = \frac{\textit{Number of root segments colonized}}{\textit{Number of root segments examined}} \times 100$$

2.6. Statistical analyses

Data were analyzed using PROC MIXED Version 9.0 of the SAS System for Windows (SAS Institute, Inc., Cary, NC, USA) (Robert et al., 1997). The residual P (0 and 38 kg ha^{-1}), and residual biochar type [phragmites biochar (PhB) and sawdust biochar (SDB)] effects and their interaction were treated as fixed effects in model; replicate nested within soil type was treated as a random factor. Dependent variables were soil N, P, K, Cu, Mn, and Zn concentrations, shoot and root dry biomass, additional dependent variable was root colonization. Data were pooled to include both soils for all dependent variables after performing a Fisher F-test to verify assumption of homogeneity of variances among sample population. Statistical significance was postulated at $p \leq 0.05$; biologically interesting differences with $0.05 < p \leq 0.10$ are also presented. Pearson's correlation of coefficient test was performed to estimate relationship among different factors and observed nutrients concentration.

3. Results

3.1. Root colonization

The root colonization in both soils; Soil A and Soil B showed similar behavior. In both soils, C- and B- treatments had <10% colonization instead of C-treatment in Soil-A amended with PhB (without-P) (Fig. 1a and b). Soil A had more colonization in without-P treatments in comparison to with-P treatments. The *R. clarus* inoculation had 78% colonization while it was 77% in B + M-treatment in PhB amended Soil A (without-P). The combination of B + M had more colonization in both soils (73–82%). In Soil B, PhB amended soil had 73–76% colonization whereas SDB amended soil had 80–82% colonization which shows that biochar type influenced root colonization. In all combinations, bacteria and *R. clarus* together ensured more colonization than *R. clarus* alone.

3.2. Root characteristics

The residual P in soil significantly enhanced root length which was endorsed in Soil B amended with SDB (with-P) having a 98% increase in root length than control (Table 1). Besides that, biochar type also influenced root attributes, and up to 43% increase in root length have been noticed for PhB amended Soil A in B + M treatment (without-P) in comparison to control. Similarly, Soil B amended with SDB (with-P) significantly enhanced root length by 22% in B + M treatment as compared to control. The root surface area was influenced by biochar types along with other factors. Plants had significant increase in root surface area in PhB amended Soil A (without-P) for B + M treatment by 46% while it increased only 20% for with-P (Table 1) in comparison to control. Besides that, root surface area enhancement in Soil B was less while maximum of 25% increase was noticed in B + M treatment in SDB amended Soil B (with-P). Root volume enhancement was also noticed in PhB amended soil up to 49% in B + M treatment (without-P) whereas only 22% root volume increased in SDB amended *R. clarus* inoculated plants (with-P) as compared to control (Table 1).

3.3. Shoot and root dry biomass

Dry shoot weight in without-P increased significantly by 50% in B + M treatment of Soil A (PhB) whereas significantly increase of 33% was observed in SDB amended Soil A with respect to control (Fig. 2). In contrary, 6% increase in weight was noticed in *L. fusimormis* 18MZR inoculated plant of PhB while 3% reduction in SDB amended Soil A (without-P) as compared to control. *R. clarus* inoculated plant (with-P) had 7% increase in PhB amended Soil A and 14% increase in SDB amended Soil A. In Soil B, PhB significantly enhanced dry shoot weight by 34% in *R. clarus* inoculated plant followed by 14% increase in B + M (without-P) with respect to control. Data showed that PhB was more responsive in dry shoot weight enhancement than SDB.

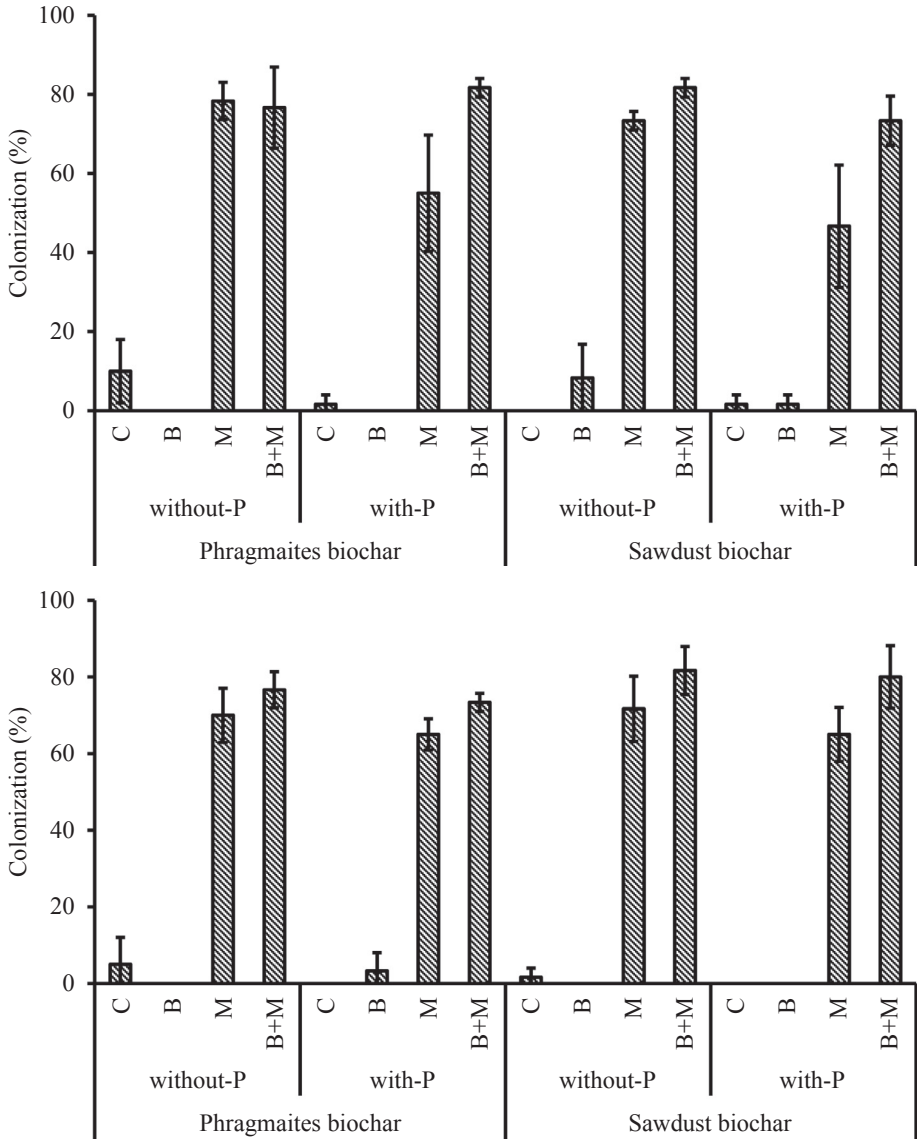

Fig. 1. a. Mycorrhizal root colonization (%) in Soil A with residual biochar and residual P, **b.** Mycorrhizal root colonization (%) in Soil B with residual biochar and residual P.

Root dry weight followed a similar trend and a significant increase of 55% was noticed for B + M treatment of Soil A amended with PhB (without-P) whereas, in SDB amended soil it significantly contributed by 47% more than control (Fig. 3). When P_2O_5 was added to soil A, 15% enhancement in *R. clarus* inoculated plants was observed. Mycorrhizal inoculation in Soil B significantly increased root dry weight by 35% in PhB amended Soil B (without-P) and 32% in SDB amended Soil B (without-P). Addition of P_2O_5 had a positive influence on root weight and SDB amended Soil B had 32% and 34% more dry root weight in M − and B + M − treatments respectively. The PhB amended Soil A (with-P) had 27% and 28% enhancement for M − and B + M − treatments respectively than control.

3.4. Root and shoot tissue nutrients analyses

In Soil A, M − and B + M-treatments significantly enhanced shoot P by 33% and 20% respectively in PhB amended soil (without-P), while in with-P, only B-treatment significantly enhanced by 52% and M−treatment by 24% than control. When SDB was applied in Soil A (without-P), B + M-treatment enhanced P concentration in shoot by 12% in comparison to control (Table 2). Soil B response was different as 27% increase was observed in M-treatment of PhB amended soil (without-P) than control. On addition of SDB in Soil B, 13% shoot P was observed in B-treatment whereas 32% increase in M-treatment (without-P) than control. Only 2% increase was observed for B-treatment with-P than control. Soil inoculants significantly increased root P for Soil A amended with PhB (without-P). The P uptake in roots was also increased by addition of P_2O_5 in soil. Significant increase of 31% P was observed in *R. clarus* inoculated plants in comparison to control for Soil B amended with SDB (without-P).

In PhB amended Soil A, a significant increase in shoot K was observed for B- (20%) and B + M − (11%) treatments (without-P) in contrary to control (Table 2). Change in shoot K was significant in Soil B against both amendments and P application as M−treatment (16%) in without-P, while with-P it was 9% (B-treatment), and 8% (M-treatment) in PhB amended the soil. On application of SDB, this change was negligible while 15% increase was observed in B-treatment than control. Root K in Soil A amended with PhB (with-P) increased 2.9% in comparison to without-P. In plant roots, *R. clarus*

Table 1
Root length, root surface area and root volume of maize plant influenced by the biochar-microbial interaction in soils with residual biochar and residual P.

	Treatment	Phragmites biochar		Sawdust biochar	
Root length (cm)	***Soil A***	without-P	with-P	without-P	with-P
	C	8178.6 ± 1444.2 l	26,625.4 ± 1778.7 c-h	8244.5 ± 1390.5 l	24,774.3 ± 2718.8 e-h
	B	6743.8 ± 1156.5 l	27,437.8 ± 2636.8 c-h	8817.4 ± 1803.9 l	24,964.7 ± 4044.6 e-h
	M	12,914.9 ± 1047.8 j-l	28,131.7 ± 4600.0 c-h	12,199.3 ± 3098.7 kl	31,037.2 ± 3788.1 b-g
	B + M	20,237.8 ± 5993.3 h-k	28,174.2 ± 2963.9 c-h	14,079.6 ± 4628.4 i-l	23,209.9 ± 5138.9 f-i
	Soil B				
	C	22,773.7 ± 5395.2 g-j	20,051.3 ± 1176.7 h-k	35,105.3 ± 6525.0 a-d	32,826.71 ± 12,238.83 b-g
	B	23,286.7 ± 890.2 f-i	31,827.6 ± 5701.5 b-g	25,846.2 ± 8971.3 d-h	34,824.59 ± 7354.78 a-e
	M	39,214.5 ± 6101.2 ab	33,134.4 ± 2022.2 a-f	32,577.9 ± 729.2 b-g	34,553.00 ± 1720.10 a-e
	B + M	28,520.0 ± 4217.6 c-h	33,637.8 ± 10,333.2 a-e	36,264.8 ± 711.4 a-c	43,105.04 ± 8757.85 a
Root surface area (cm^2)	***Soil A***				
	C	758.8 ± 79.9 h	2661.8 ± 431.0 b-e	786.7 ± 82.0 h	2037.0 ± 116.2 ef
	B	694.3 ± 118.0 h	2431.8 ± 230.4 b-f	852.6 ± 102.5 h	2190.1 ± 444.6 c-f
	M	1246.5 ± 76.1 gh	2414.1 ± 375.2 b-f	1067.3 ± 297.9 gh	2885.8 ± 338.1 b-d
	B + M	1788.1 ± 361.0 fg	2612.3 ± 343.2 b-e	1222.4 ± 254.1 gh	2175.4 ± 312.3 c-f
	Soil B				
	C	2029.0 ± 270.9 ef	1749.2 ± 186.2 fg	2375.0 ± 268.87 b-f	2644.81 ± 691.33 b-e
	B	2120.5 ± 72.2 d-f	3013.3 ± 527.7 ab	2161.0 ± 552.21 c-f	2932.76 ± 639.56 bc
	M	3104.5 ± 342.4 ab	3043.3 ± 321.9 ab	2615.3 ± 140.49 b-e	2697.50 ± 99.64 b-e
	B + M	2509.8 ± 409.5 b-f	2771.0 ± 789.3 b-e	2892.63 ± 187.46 b-d	3720.86 ± 935.72 a
Root volume (cm^3)	***Soil A***				
	C	0.5 ± 0.0 l	2.0 ± 0.8 ab	0.6 ± 0.0 kl	1.2 ± 0.1 d-i
	B	0.5 ± 0.1 l	1.6 ± 0.3 b-f	0.6 ± 0.1 j-l	1.4 ± 0.4 c-g
	M	0.9 ± 0.0 g-l	1.5 ± 0.3 b-f	0.7 ± 0.2 i-l	1.9 ± 0.2 a-c
	B + M	1.2 ± 0.2 e-j	1.8 ± 0.3 a-e	0.8 ± 0.1 h-l	1.5 ± 0.2 b-f
	Soil B				
	C	1.3 ± 0.1 d-h	1.1 ± 0.2 f-k	1.17 ± 0.05 e-j	1.59 ± 0.29 b-f
	B	1.4 ± 0.1 c-g	2.1 ± 0.4 ab	1.32 ± 0.22 d-h	1.79 ± 0.42 a-d
	M	1.8 ± 0.2 a-d	2.0 ± 0.3 ab	1.52 ± 0.14 b-f	1.53 ± 0.18 b-f
	B + M	1.6 ± 0.5 b-f	1.7 ± 0.4 b-f	1.67 ± 0.18 b-f	2.33 ± 0.71 a

C control, **B** *L. fusiformis*, **M** *R. clarus*, **B + M** *L. fusiformis* + *R. clarus* (means and standard deviation; $n = 3$).

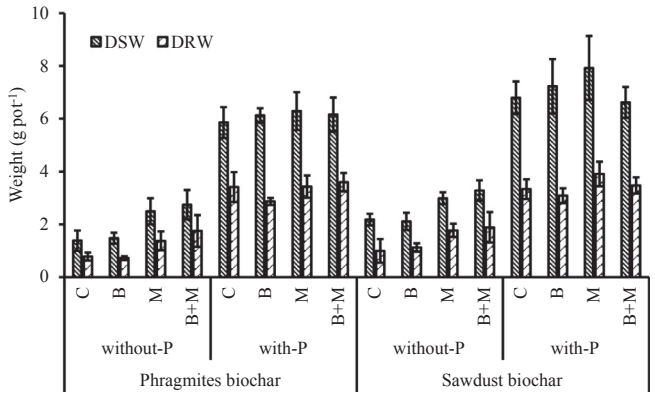

Fig. 2. Shoot and root dry weight of maize plant in Soil A with residual biochar and residual P.

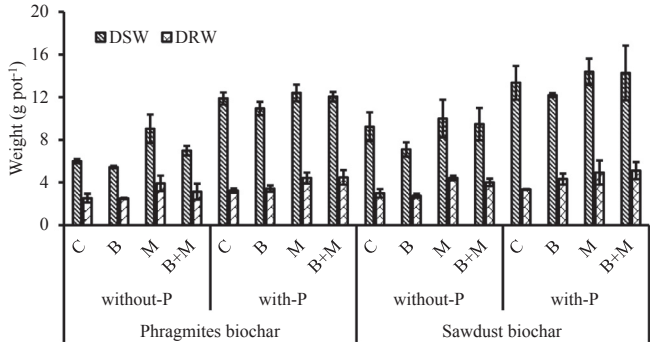

Fig. 3. Shoot and root dry weight of maize plant in Soil B with residual biochar and residual P.

enhanced K uptake in both soils and significant increase of 69% in K was observed for *R. clarus* inoculated plants in Soil A amended with SDB (without-P) than control. In rest of the combinations for Soil A and Soil B, root K enhancement was negligible.

A significant increase (41%) in Ca content was noticed in roots inoculated with B + M treatment in Soil A amended with SDB (with-P) in contrary to control (Table 2). While in Soil B, significant increase of 36% in Ca was noticed for PhB amended soil inoculated with B + M (with-P) in contrary to control. Besides that, addition of P_2O_5 in soil enhanced Mg uptake in roots irrespective to soil type (Table 2).

In Soil A, concentration of Mn in shoot was increased according to biochar type while use of SDB reduced Mn uptake in plant shoot (Table 3). Similar trend was observed in Soil B. The L. *fusiformis* significantly enhanced Mn uptake. Presence of Cu in different treatments of PhB amended Soil A without-P ranged as 5.27–8.07 ppm whereas in SDB amended soil it ranged as 4.37–22.33 ppm (Table 3). Biochar had a nominal impact on Cu uptake, SDB amended soil had more Cu in comparison to PhB amended soil. A significant increase (67%) was observed in B-treatment with-P soil amended with SDB. In the case of Mn, soil type had a significant effect on shoot Mn concentration. Soil B provided manifold Mn than Soil A, moreover, its concentration was prominent with-P treatments than without-P. A similar trend was shown by Zn concentration in shoot. Plants grown in Soil B had more Zn concentration in their shoots and soils with-P have less concentration of Zn than without-P (Table 3). A significant increase (36%) in Zn uptake was noticed in B + M treatment of Soil A amended with PhB (without-P) in comparison to control.

4. Discussion

In this study, two soils of different physiochemical properties

2
Concentration of P, K, Ca, Mg (%) in plant shoot and root under different soil conditions and P application in soils with residual biochar and residual P.

P (%)	Treatments	Phragmites Biochar				Sawdust biochar			
		Without-P		With-P		Without-P		With-P	
		Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
	Soil A								
	C	0.08 ± 0.0 n	0.08 ± 0.0 m-o	0.27 ± 0.0 i	0.15 ± 0.0 gh	0.15 ± 0.0 kl	0.10 ± 0.0 k-n	0.66 ± 0.0 f	0.30 ± 0.0 e
	B	0.09 ± 0.0 mn	0.08 ± 0.0 m-o	0.55 ± 0.0 g	0.21 ± 0.0 f	0.13 ± 0.0 l-n	0.08 ± 0.0 m-o	0.63 ± 0.0 f	0.30 ± 0.0 e
	M	0.13 ± 0.0 l-n	0.08 ± 0.0 l-o	0.35 ± 0.0 h	0.22 ± 0.0 f	0.13 ± 0.0 l-n	0.08 ± 0.0 m-o	0.61 ± 0.0 f	0.32 ± 0.0 e
	B + M	0.16 ± 0.0 kl	0.09 ± 0.0 k-o	0.21 ± 0.0 jk	0.13 ± 0.0 h-j	0.14 ± 0.0 lm	0.08 ± 0.0 l-o	0.33 ± 0.0 h	0.17 ± 0.0 g
	Soil B								
	C	0.16 ± 0.0 kl	0.08 ± 0.0 l-o	1.30 ± 0.0 b	0.67 ± 0.0 ab	0.14 ± 0.0 lm	0.07 ± 0.0°	1.07 ± 0.0 d	0.67 ± 0.0 ab
	B	0.16 ± 0.0 kl	0.08 ± 0.0 l-o	1.42 ± 0.0 a	0.59 ± 0.0 c	0.16 ± 0.0 kl	0.08 ± 0.0 no	1.10 ± 0.0 cd	0.65 ± 0.0 b
	M	0.22 ± 0.0 ij	0.10 ± 0.0 k-m	1.15 ± 0.0 c	0.46 ± 0.0 d	0.24 ± 0.0 ij	0.11 ± 0.0 i-k	1.02 ± 0.1 e	0.58 ± 0.0 c
	B + M	0.26 ± 0.0 ij	0.11 ± 0.0 j-l	1.01 ± 0.1 e	0.48 ± 0.0 d	0.26 ± 0.0 iS	0.13 ± 0.0 hi	0.97 ± 0.0 e	0.68 ± 0.0 a
K (%)	***Soil A***								
	C	5.33 ± 0.4 p	0.28 ± 0.1 p	8.22 ± 0.3 bc	1.48 ± 0.1 g-i	8.00 ± 0.1 cd	0.25 ± 0.0 p	7.04 ± 0.2 h-j	2.35 ± 0.2 ab
	B	6.67 ± 0.1 k-m	0.18 ± 0.0 p	9.23 ± 0.4 a	1.72 ± 0.2 e-g	6.30 ± 0.1 no	0.21 ± 0.0 p	6.84 ± 0.1 i-k	2.16 ± 0.1 bc
	M	6.44 ± 0.1 l-o	0.68 ± 0.1 n	8.14 ± 0.2 bc	1.90 ± 0.1 de	6.63 ± 0.1 k-n	0.69 ± 0.1 mn	6.47 ± 0.3 l-o	2.56 ± 0.2 a
	B + M	7.20 ± 0.0 gh	0.40 ± 0.0 op	8.03 ± 0.1 cd	1.56 ± 0.1 f-h	6.43 ± 0.3 l-o	0.94 ± 0.2 k-m	7.11 ± 0.3 hi	2.03 ± 0.1 cd
	Soil B								
	C	6.65 ± 0.2 k-m	0.96 ± 0.2 kl	7.70 ± 0.1 de	2.29 ± 0.1 b	6.35 ± 0.3 m-o	0.74 ± 0.0 l-n	6.55 ± 0.3 k-n	2.01 ± 0.0 cd
	B	6.15 ± 0.2°	0.94 ± 0.2 kl	8.48 ± 0.2 b	1.78 ± 0.1 d-f	7.49 ± 0.1 e-g	0.63 ± 0.0 no	6.35 ± 0.3 m-o	1.05 ± 0.2 jk
	M	7.29 ± 0.2 f-h	1.44 ± 0.2 hi	8.33 ± 0.1 bc	1.75 ± 0.3 ef	7.17 ± 0.1 g-i	1.27 ± 0.1 ij	6.43 ± 0.3 l-o	1.31 ± 0.1 hi
	B + M	7.31 ± 0.1 f-h	0.74 ± 0.0 l-n	7.61 ± 0.2 ef	1.46 ± 0.2 hi	6.71 ± 0.0 j-l	1.36 ± 0.1 hi	6.41 ± 0.1 l-o	1.31 ± 0.0 hi
Ca (%)	***Soil A***								
	C	1.58 ± 0.0 d-f	1.95 ± 0.0 f-i	1.27 ± 0.1 i-k	2.35 ± 0.1 bc	1.84 ± 0.0 c	1.99 ± 0.1 e-h	0.77 ± 0.0 q	2.35 ± 0.1 bc
	B	2.69 ± 0.1 a	2.59 ± 0.1 a	0.96 ± 0.1 no	1.37 ± 0.0 o-q	1.45 ± 0.1 f-h	2.06 ± 0.1 ef	0.79 ± 0.1 pq	1.73 ± 0.0 jk
	M	1.48 ± 0.1 e-g	2.03 ± 0.0 e-g	0.92 ± 0.0 op	2.17 ± 0.1 c-e	1.38 ± 0.0 g-i	2.43 ± 0.2 ab	0.73 ± 0.0 q	1.05 ± 0.1 s
	B + M	1.65 ± 0.0 d	2.02 ± 0.0 e-g	1.11 ± 0.0	2.59 ± 0.1 a lm	1.24 ± 0.0 i-l	1.67 ± 0.0 j-m	0.87 ± 0.0 o-q	1.76 ± 0.1 i-k
	Soil B								
	C	1.62 ± 0.2 de	1.33 ± 0.1 o-q	1.23 ± 0.1 j-l	1.71 ± 0.1 j-l	1.27 ± 0.0 ij	1.37 ± 0.1 o-q	1.08 ± 0.0 mn	1.16 ± 0.0 q-s
	B	2.07 ± 0.0 b	1.51 ± 0.2 l-o	1.32 ± 0.1 h-j	1.84 ± 0.1 g-j	1.60 ± 0.0 de	1.23 ± 0.1 p-s	0.97 ± 0.0 no	1.61 ± 0.3 k-n
	M	1.63 ± 0.1 d	1.47 ± 0.1 m-o	1.13 ± 0.1 k-m	1.30 ± 0.0 o-r	1.12 ± 0.1 lm	1.10 ± 0.0 rs	1.01 ± 0.0 m-o	1.79 ± 0.1 h-k
	B + M	1.82 ± 0.1 c	2.09 ± 0.1 d-f	1.31 ± 0.0 h-j	2.01 ± 0.1 e-g	1.29 ± 0.2 ij	1.43 ± 0.1 n-p	1.00 ± 0.0 m-o	2.27 ± 0.2 b-d
Mg (%)	***Soil A***								
	C	0.85 ± 0.0 c	0.87 ± 0.0 cd	0.65 ± 0.0 fg	1.18 ± 0.1 a	1.09 ± 0.1 a	0.78 ± 0.0 fg	0.51 ± 0.0 hi	1.02 ± 0.1 b
	B	1.07 ± 0.0 a	0.72 ± 0.1 gh	0.71 ± 0.1 ef	0.86 ± 0.0 de	0.94 ± 0.0 b	0.73 ± 0.1 gh	0.56 ± 0.0 h	0.94 ± 0.0 c
	M	0.75 ± 0.0 de	0.79 ± 0.1 e-g	0.63 ± 0.0 g	1.15 ± 0.0 a	0.73 ± 0.1 e	0.89 ± 0.0 cd	0.49 ± 0.0ij	0.69 ± 0.0 h
	B + M	0.80 ± 0.0 cd	0.85 ± 0.1 d-f	0.62 ± 0.0 g	1.14 ± 0.0 a	0.74 ± 0.1 de	0.75 ± 0.1 gh	0.53 ± 0.1 hi	0.72 ± 0.1 gh
	Soil B								
	C	0.36 ± 0.0 l-o	0.35 ± 0.0 i-l	0.31 ± 0.0 n-q	0.28 ± 0.0 lm	0.31 ± 0.0 n-q	0.31 ± 0.0 k-m	0.26 ± 0.0 q	0.27 ± 0.0 m
	B	0.42 ± 0.0 j-l	0.40 ± 0.0 ij	0.38 ± 0.0 k-m	0.33 ± 0.0 j-m	0.38 ± 0.0 k-m	0.28 ± 0.0 lm	0.28 ± 0.0 pq	0.31 ± 0.0 k-m
	M	0.37 ± 0.0 k-o	0.37 ± 0.0 i-k	0.34 ± 0.0 m-p	0.31 ± 0.0 k-m	0.30 ± 0.0 n-q	0.37 ± 0.0 i-k	0.27 ± 0.0 q	0.39 ± 0.0 ij
	B + M	0.44 ± 0.0 jk	0.42 ± 0.1 i	0.37 ± 0.0 k-o	0.39 ± 0.0 ij	0.34 ± 0.1 m-p	0.34 ± 0.0 j-m	0.31 ± 0.0 n-q	0.30 ± 0.0 k-m

C control, **B** *L. fusiformis*, **M** *R. clarus*, **B + M** *L. fusiformis* + *R. clarus* (means and standard deviation; $n = 3$).

were used to explore the residual effects of biochar and P on maize growth and nutirent accumulation in the presence of microbes. Both soils had residual biochar (PhB and SDB) and residual P applied previously in onion plant (Rafique et al., 2019b). Moreover, soils were inoculated with *L. fusiformis* 18MZR (B), *R. clarus* (M) and combination of both inoculants (B + M). In this study, biochar-amended soil increased the root colonization (Fig. 1a and b) that could be due to transfer of mycorrhizal spores by air (Ortas et al., 2017). Fluctuation of mycorrhizal fungi abundance depends on biochar type and soil properties. Besides that, increase in mycorrhizal abundance in biochar-amended soil is common as observed in previous studies with different types of soil and biochar (Lehmann et al., 2011; Rizwan et al., 2016).

Soil fertility status, particularly residual P plays an important role in mycorrhizal phenotype. Ususally, P-limmited soils develop mutualism with plant while parasitism and commensalism occur in P-rich soils (Johnson et al., 2015). The commonly known mechanism behind reduced utility of symbiosis in presence of nutrients is explained by Lehmann et al. (2011). Our study also showed a similar response and mycorrhizal colonization was comparatively less in with-P (residual) soils. Thus, mycorrhizal inoculation has a linear trend with increased dose of biochar (Elmer and Pignatello, 2011). Previous study indicated that B + M improved plant growth parameters and nutrients absorption (Rafique et al., 2019a). Microbial networks are the primary drivers in biochar mineralization, biotic degradation did not exceed 2% even after 96 days of mineralization, and the major loss mechanism of biochar was attributed to erosion fluxes (Mašek et al., 2013). Generally, poor, dry, compacted soils may impair total root length, increase root diameter and alter root hair length and density. Results showed that mycorrhizal symbiosis altered root architecture and P acquisition efficiency of maize, but the effects of *R. clarus* were dependant on residual P (Table 1). Moreover, availability of residual P reduced mycorrhizal colonization perhaps because the carbon cost associated with maintaining fungal tissue outweighed the benefit of obtaining additional P. Usually, mycorrhziation is beneficial for both host plants and colonizing mycorrhizal fungi which are influenced by number of factors (Hoeksema et al., 2010). Mycorrhizal symbiosis altered root architecture and increased P acquisition of plants in low P conditions.

Residual P was more effective than residual biochar in plant biomass production and elevation of P concentration (Figs. 2 and 3). A similar case was observed in a saline sodic soil where due to greater supply of P, the plant had more concentration of P (Xu et al., 2016). Application of biochar in soil induces phosphate binding with free cations (e.g., Ca^{2+}, Mg^{2+}, Fe^{3+} and Al^{3+}), and released P is

Table 3
Concentration of Cu, Mn, Zn (ppm) in plant shoot and root under different soil conditions and P application in soils with residual biochar and residual P.

	Treatments	Phragmites Biochar				Sawdust biochar			
		Without-P		With-P		Without-P		With-P	
		Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
	Soil A								
	C	5.60 ± 0.2 h-j	6.30 ± 2.50 k	7.67 ± 0.5 a-c	10.23 ± 1.09 e-j	7.80 ± 0.1 ab	11.07 ± 1.39 d-i	7.37 ± 1.5 a-d	9.00 ± 0.79 g-k
	B	6.60 ± 0.5 c-h	8.60 ± 0.49 h-k	6.90 ± 0.6 a-f	9.07 ± 1.32 g-k	6.80 ± 0.2 b-g	10.07 ± 1.23 e-j	5..33 ± 0.9 g-j	8.30 ± 0.45 i-k
	M	6.60 ± 0.7 c-h	9.87 ± 0.66 f-j	6.03 ± 0.0 e-i	9.70 ± 0.45 f-j	6.17 ± 0.0 e-i	10.70 ± 2.24 d-i	4.67 ± 0.9 jk	7.53 ± 0.97 jk
	B + M	8.07 ± 1.1 a	11.23 ± 1.16 d-h	5.27 ± 0.5 i-k	9.23 ± 0.45 g-j	6.47.33 ± 5.4 d-h	11.47 ± 0.76 d-g	4.37 ± 0.2 k	7.73 ± 1.29 jk
	Soil B								
	C	6.77 ± 0.5 b-h	16.07 ± 1.39 a	7.47 ± 0.3 a-d	10.77 ± 0.45 d-i	5.70 ± 0.4 g-j	13.23 ± 0.58 b-d	6.33 ± 0.5 d-i	11.57 ± 1.84 d-g
	B	6.47 ± 0.6 d-h	12.50 ± 1.47 c-f	8.07 ± 1.4 a	12.70 ± 2.01 c-e	6.73 ± 0.1 b-h	13.43 ± 1.62 a-d	6.10 ± 0.1 e-i	11.00 ± 1.79 d-i
	M	6.13 ± 0.2 e-i	11.27 ± c1.48 d-h	6.53 ± 0.2 c-h	12.23 ± 1.70 c-f	5.77 ± 0.5 f-j	15.67 ± 1.10 ab	6.00 ± 0.3 e-i	12.13 ± 2.16 c-f
	B + M	7.17 ± 0.7 a-e	13.27 ± 2.32 a-d	7.67 ± 0.5 a-c	14.90 ± 1.71 a-c	5.90 ± 0.8 f-i	14.77 ± 0.63 a-c	5.73 ± 0.7 f-j	12.37 ± 1.49 c-f
Mn	***Soil A***								
	C	56.80 ± 8.4 m-q	83.17 ± 3.4 i-k	59.63 ± 5.1 k-p	135.25 ± 12.3 gh	52.00 ± 6.7 n-q	66.90 ± 1.3 j-m	45.70 ± 4.1 pq	82.95 ± 5.6 i-k
	B	67.47 ± 6.8 j-o	88.80 ± 2.1 i-k	71.87 ± 6.4 i-n	56.90 ± 1.5 k-m	61.40 ± 4.8 k-p	65.10 ± 9.9 j-m	45.07 ± 5.2 pq	75.83 ± 7.2 i-l
	M	70.77 ± 13.1 i-n	61.80 ± 1.2 j-m	52.90 ± 3.9 m-q	83.00 ± 10.6 i-k	48.57 ± 2.6 o-q	79.47 ± 8.5 i-k	41.03 ± 2.1 pq	36.60 ± 1.8 m
	B + M	78.50 ± 10.3 h-l	72.50 ± 4.3 j-l	57.47 ± 4.3 l-q	92.30 ± 4.7 ij	53.73 ± 4.6 m-q	45.25 ± 1.5 lm	38.43 ± 3.4 q	44.10 ± 8.4 lm
	Soil B								
	C	130.27 ± 18.2 b-d	247.57 ± 36.3 a	175.67 ± 13.8 a	177.60 ± 20.4 c-e	102.73 ± 10.4 e-g	140.93 ± 7.8 fg	66.90 ± 3.8 j-o	107.30 ± 0.9 hi
	B	174.37 ± 22.0 a	231.10 ± 14.4 ab	144.07 ± 22.1 b	220.05 ± 12.1 ab	140.73 ± 3.8 bc	163.93 ± 4.0 d-g	148.27 ± 5.4 b	172.75 ± 32.2 d-f
	M	114.43 ± 8.3 d-f	186.05 ± 11.9 cd	95.73 ± 9.7 f-h	173.73 ± 14.3 de	87.30 ± 8.8 g-j	206.77 ± 6.4 bc	73.60 ± 9.1 i-m	137.95 ± 49.0 gh
	B + M	128.20 ± 20.8 b-d	237.00 ± 14.8 ab	120.47 ± 3.7 c-e	249.73 ± 34.5 a	89.57 ± 7.5 g-i	172.33 ± 11.2 d-f	79.17 ± 21.1 h-k	147.93 ± 9.4 e-g
Zn	***Soil A***								
	C	12.13 ± 2.2 kl	26.60 ± 0.01 g-j	5.37 ± 0.9 lm	29.60 ± 0.01 d-j	61.93 ± 10.2 a	33.47 ± 0.03 b-f	3.20 ± 1.0 m	25.63 ± 0.05 ij
	B	17.07 ± 2.3 i-k	24.53 ± 0.01 j	4.43 ± 1.2 lm	28.77 ± 0.02 e-j	45.50 ± 2.0 bc	36.10 ± 0.05 a-c	3.50 ± 0.4 m	27.87 ± 0.01 e-j
	M	27.13 ± 4.3 e-h	26.50 ± 0.03 g-j	4.97 ± 0.8 lm	35.53 ± 0.03 a-d	48.70 ± 3.8 b	32.93 ± 0.02 b-f	3.87 ± 1.0 lm	26.13 ± 0.02 h-j
	B + M	41.13 ± 8.0 b-d	41.65 ± 0.02 a	5.80 ± 1.1 lm	32.50 ± 0.02 b-g	46.87 ± 11.0 bc	37.47 ± 0.01 ab	6.00 ± 0.5 lm	24.70 ± 0.01 j
	Soil B								
	C	30.47 ± 4.9 e-g	33.13 ± 0.01 b-f	22.87 ± 1.6 g-j	36.90 ± 0.03 ab	25.27 ± 2.4 f-i	29.33 ± 0.00 e-j	21.63 ± 1.5 h-j	31.33 ± 0.04 b-i
	B	33.30 ± 6.0 d-f	27.83 ± 0.02 e-j	22.77 ± 3.1 g-j	31.60 ± 0.05 b-i	30.20 ± 0.4 e-g	30.10 ± 0.03 c-j	18.67 ± 2.1 h-k	29.95 ± 0.02 c-j
	M	33.90 ± 3.4 de	27.53 ± 0.02 f-j	17.93 ± 0.5 i-k	29.37 ± 0.03 d-j	40.33 ± 7.2 b-d	33.97 ± 0.02 b-e	19.80 ± 1.4 h-k	32.65 ± 0.00 b-g
	B + M	39.97 ± 0.7 cd	32.23 ± 0.04 b-h	19.53 ± 1.4 h-k	33.33 ± 0.09 b-f	39.50 ± 9.5 cd	36.00 ± 0.06 a-c	16.67 ± 0.4 jk	33.25 ± 0.02 b-f

C control, **B** *L. fusiformis*, **M** *R. clarus*, **B + M** *L. fusiformis* + *R. clarus* (means and standard deviation; $n = 3$).

used for plant growth again (Zhang et al., 2016a). Biochar can also play a role in adsorbing phosphate efficiently from solutions, implying that biochar could attribute to retaining P (Yao et al., 2013). A study reported that biochar addition increased nutrients (P and K) avaialability in soil (Prendergast-Miller et al., 2014). Our study indicated that, irrespective to microbial inoculants, biochar addition significantly increased P, K and Ca absorption to maize plant. The impacts of residual biochar and mycorrhizal fungi on plant growth and nutrients uptake are limited in crops fertilized with recommended dose of chemical fertilizer. Such plants already have sufficient amount of nutrients which can suppress mycorrhization and biochar effects; therefore mycorrhizal fungi cannot harvest benefits as exploited in P-deficient environment (Gazey et al., 2004; Solaiman et al., 2010). Recently, Liu et al. (2019) reported that addition of biochar and P into the soil increased available P level, consequently more plant growth. Mycorrhizal fungi have capability to significantly create a pathway for P-uptake into roots (Smith et al., 2004). As biochar can alter nutrients availability in soil, it can likely affect photosynthesis and plant production (Xu et al., 2015). Nutrient availability to plants is strongly dependant on soil properties and water use efficiency (Theodose and Bowman, 1997). *R. clarus* inoculation in Soil A improved P even in limited availability. Neumann and George (2004) also studied role of mycorrhizal fungi in P supply and water uptake which is inconsistent with this study. When biochar interacts with bacteria, particularly PSB such as *L. fusiformis* 31MZR, it increases P-concentration than control plants (Rafique et al., 2017). In this study, bacterial strain enhanced plant growth and nutrients uptake because of its ability to interact with plant roots positively. Such bacteria are present in soil ecosystem and they interact with plant roots in terms of nutrients uptake (Zhang et al., 2016b). *L. fusiformis* 31MZR is already reported as P-solubilizer which promotes plant growth confirmed by various biochemical tests (Chauhan et al., 2016). It may solubilize P in residual biochar-amended soil and make it available for the plant roots and mycorrhizal fungi for uptake. The P depletion crisis in future may be handled by evaluating residual biochar and P in soil and its availability to plants.

5. Conclusion

In present study, *L. fusiformis* 31MZR and *R. clarus* were used as microbial inoculants in two texturally different soils amended with two different residual biochars prepared form plant feedstock (herbaceous and woody material) used in previsous study. The residual P condition was also considered to evaluate performance of maize plants up to 65 days growth in terms of root colonization, plant biomass, root architecture, and macro-, micronutrients concentration. Soil amended with SDB significantly reduced the root colonization in presence of P. Moreover, the shoot and root dry biomass were enhanced in SDB amended soil which also elevated the nutrients uptake by plant. Both soil and biochar properties significantly influenced mycorrhizal root colonization. Combination of L. *fusiformis* 31MZR and *R. clarus* enhanced micronutrients concentration (Cu, Mn and Zn) in plant in residual biochar and residual P soils. Plant biomass enhanced in *L. fusiformis* 31MZR and *R. clarus* combinations significantly. Overall, biochar application in the soils might be a source of P for latter crops but the response varied with soil types and biochar feedstocks which needs in depth investigations at field levels in different climatic conditions before final recommendations.

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