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Research article

Potential impact of biochar types and microbial inoculants on growth of onion plant in differently textured and phosphorus limited soils

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ABSTRACT

Non-renewable phosphorus (P) resources are intensively declining and recyclable P is high in demand for agricultural sector. Biochar as a renewable source of P and its physicochemical properties may improve the nutrients condition in the soil for plant availability. This study was designed to evaluate the interaction of biochar with soil microbes in differently textured and P-limited soils for P availability, root colonization and nutrient uptake by plants. Onion plants were grown in two differently textured soils with two types of biochar, with or without P application, three microbially inoculated treatments and uninoculated control. Plants were grown for 65 days and root-shoot biomass, nutrient concentration and mycorrhizal root colonization were analyzed. The WinRhizo was used to evaluate root attributes such as length, surface area and volume of roots. Biochar addition enhanced the nutrient uptake and plant biomass in the presence of P and microbial inoculants. Root colonization was notably increased in biochar + mycorrhizal inoculated plants. Biochar and soil type interactions may develop a unique behavior of nutrient uptake, root colonization, plant growth and root attributes. Biochar in combination with microbial inoculants could be considered a potentially renewable source of P fertilizer.

1. Introduction

Extensive application of chemical fertilizers to the arable soils for better plant growth and yield has become a serious problem at the world level nowadays. Phosphorus (P) is one of the essential macro-nutrient for plants and its concentration in soils is continuously decreasing due to extensive agriculture. Aggressive cultivation of crops may deplete nutrients in the soil, which are required for plant growth (Faucon et al. 2015). Soil nutrient availability could be maintained by tailoring alternative resources (Qayyum et al., 2017: Rehman et al., 2018). The major supply of P is through agrochemicals and these chemicals may remain in the soil for a longer duration, while 75–90% P fertilizer sources are not taken up by the crops. Due to over fertilization, the P may accumulate in soils (Cordell and White, 2014) especially in plough layer. This excessive use of chemical fertilizers in the fields may cause deleterious effects on soil quality and its micro-flora (Tripti et al., 2015). The efficient application of P fertilizers in a sustainable way is of utmost importance in the future for better crop yield along with reduction of environmental hazards (Withers et al., 2014). Plants can only accumulate P ions ($H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}) from the soil solution (Brady and Weil, 2013) which needs continuous replenishment of P ions. The low total P in soil due to the higher fixation capacity of soils may result in deficiency of bioavailable P forms in soil solution. This may hinder crop production in a sustainable way (Bortoluzzi et al., 2015; Fink et al., 2016).

Recently, great attention has been given to biochar (a pyrolyzed product of organic waste) as a soil amendment under various environmental conditions (Rizwan et al., 2016; Azhar et al., 2019). Biochar can enhance soil fertility, improve crop productivity and sequester carbon in soils for longer duration (Lehmann, 2007; Atkinson et al., 2010; Rehman et al., 2016; Rizwan et al., 2019. The concentration of P in biochar as compared to nitrogen (N), and potassium (K) has received

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Abbreviation

PhB	phragmites biochar
SDB	sawdust biochar
C	control
B	bacterium (L. fusiformis 31MZR)
M	mycorrhizal fungus (R. clarus)
B + M	bacterium and mycorrhizal fungus
NO3-N	nitrate-nitrogen
CECest	estimated cation exchange capacity
SOM	soil organic matter
PSB	phosphorus solubilizing bacteria

greater attention and reasons can be: assimilation of P in the biochar during pyrolysis of feedstocks (Rehman et al., 2018), and thermo-chemical transformations of P species in feedstocks during biochar preparation (Bruun et al., 2014). Published literature demonstrated that P sorption capacity decreased in soil through biochar application which may stimulate P available forms in the soil solution (Jiang et al., 2015; Qayyum et al., 2015). However, this trend is not consistent and there is a significant amount of P in biochar which depends upon feedstocks, pyrolysis temperature and resident time duration (Enders and Lehmann, 2012; Rehman et al., 2018). The future P depletion crisis may be handled by using biochar as a potential source of P in soil. Promising agronomic impacts of biochar soil applications have been reported in a wide range of soils with low fertility status (Biederman and Harpole, 2013; Liu et al., 2013; Abbas et al., 2018; Ali et al., 2019), which might be associated with improvements in soil nutritional status and positive impacts on biological, physical or chemical characteristics of the soil (Cornelissen et al., 2013; Lehmann and Rondon, 2006). Amendment of soil with biochar may improve the arbuscular mycorrhizal fungi activities, and colonization rates (Yamato et al. 2006). DeLuca et al. (2015) enhanced nutrient uptake capacity of roots (Atkinson et al., 2010) and upsurged P level in soil. It also mediated the P availability to plants from soil either by direct supply of P or altering cation exchange capacity (CEC) and soil pH (Atkinson et al., 2010; Chan et al. 2008; Enders and Lehmann, 2012; Peng et al., 2012). The level of total and available P in biochar mainly depends on the feedstock, pyrolysis temperature and residence time (Kloss et al., 2012; Mukome et al., 2013). Biochar application in soil may alter the availability of P in soil by affecting CEC (DeLuca et al., 2015; Xu et al., 2014; Zhang et al., 2016a). Biochar releases available-P into the soil slowly, presence of soil bacteria and mycorrhizal fungi in rhizosphere possibly increase P availability to plant roots (He et al., 2014; Zwetsloot et al., 2016). Biochar from woody feedstocks such as sawdust, reed, eucalyptus or bamboo have a high concentration of nutrients. It can release nutrients into the soil slowly, where plant-available nutrients are already deficient (Mukherjee and Zimmerman, 2013; Yao et al., 2013).

Plant growth promoting rhizobacteria are among beneficial soil microorganisms, which may improve nutrients bioavailability and bioassimilation (Rajkumar et al., 2010; Vílchez et al., 2016). Inorganic forms of P in soil may be solubilized by bacteria which might be associated with the production of organic acids, growth regulating hormones, and chelating agents such as indole-3-acetic acid (IAA) and siderophores (Ma et al., 2011; Otieno et al., 2015). Biochar may provide a good environment in soil for microbial growth (Khan et al., 2016). According to Anderson et al. (2011) findings, P-solubilizing bacteria enhance P-mining activity in biochar under controlled conditions. Similarly, He et al. (2014) found in a study that some bacterial strains of *Lysinibacillus fusiformis* solubilize P from biochar by 47–54%. Biochar usually contains 0.2–0.8% of P and studies showed that environment has a strong influence on release of P from biochar where according to an estimate, < 50% P in biochar is released under natural environmental conditions (Qian et al., 2013; Schneider and Haderlein, 2016).

Until now, few studies have examined nutrient acquisition strategies of plant for P uptake under soil amendments in the presence of PSB and mycorrhizal fungi under controlled conditions (Fox et al., 2014; Rafique et al., 2017). Especially in the presence (also absence) of P fertilizers and different biochars, managing the foraging capacity of crops, bacterial and mycorrhizal associations may depend on the acquisition of P by plants. Limited research is available on the combined impacts of plant root-bacteria-mycorrhizae interaction, and biochar on uptake of P by plants in different biochar amended soils. We hypothesized that biochar along with microbial inoculation might be a potential P source for plant growth improvement and nutritional quality of crops. Thus, onion was grown in two differently textured soils, under greenhouse conditions and measured macro- and micronutrient uptake by plants, chlorophyll fluorescence, root morphology and root colonization in response to microbial inoculation in different biochars amended soils with and without P application. The key aims were to identify that (1) how the biochar types interact with microbial inoculants in P-deficient soils for root colonization and nutrients uptake and (2) whether the biochar and microbial inoculants mediate alteration in plant-soil systems coincide with emerging global issue of P-deficiency in soils.

2. Materials and methods

2.1. Soil selection and biochar preparation

Two types of soils were used in the 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. Soils were collected from research farms of Cukurova University (Table 1 shows initial soil properties). The detailed properties of soil B can also be found in a recent study (Rafique et al., 2019).

The feedstock of common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) and sawdust were collected from vicinity of the research area of Cukurova University, Turkey. Before biochar preparation, feedstock was ground and sieved by passing through a 50-mesh and dried at 110 °C for about 24 h. The feedstock was charred at 350 °C for 2 h at a rate of 10 °C increase per minute in a closed container under O_2 limited environment in a muffle furnace (RD50, REF-SAN, Turkey) (Sánchez et al., 2009). After attaining 350 °C temperature of the furnace, 1 h residence time was provided to the feedstock a temperature of 350 °C. Biochar was milled to pass a 2 mm and labeled properly for further analyses and utilization. The prepared biochar samples were characterized by proximate (moisture, ash, volatile matrix, and fixed carbon), ultimate and nutrient (C, N, P, K, Ca, Mg, Fe, Mn, and B) analyses (Table 2). Detailed procedure about measurement of these

Table 1
Physicochemical properties of the soils used in experiment.

	Soil A	Soil B
Texture analysis (%)		
Sand	6.0	25.0
Silt	67.0	17.0
Clay	27.0	58.0
Characteristics		
SOM (%)	1.10	1.6
pH_{water}	7.84	6.68
$CaCO_3$ (%)	22.16	1.33
Bulk Density (g cm^{-3})	1.0	1.3
CEC_{est} ($cmol_c$ kg^{-1})	21.23	20.65
Nutrient content (mg kg^{-1})		
NO_3-N	8.0	8.0
P_2O_5	1.45	1.87
K_2O	82.05	75.91

SOM soil organic matter, **CECest** estimated cation exchange capacity.

Table 2
Properties of biochar used as soil amendment.

	PhB	SDB
pH	7.49 ± 0.08	6.29 ± 0.09
EC (mS cm^{-1})	2.23 ± 0.16	0.33 ± 0.00
Proximate analysis (%)[c]		
Moisture[b]	3.91 ± 0.11	2.56 ± 0.99
Ash[a]	8.18 ± 0.02	3.11 ± 0.01
VM[b]	56.05 ± 1.50	66.99 ± 0.58
FC[c]	31.86 ± 1.59	27.34 ± 1.35
Ultimate/Nutrient analysis (%)		
Total C	67.89 ± 8.76	61.96 ± 3.96
Stable C	67.89 ± 8.74	61.96 ± 4.0
Unstable C	0.0004 ± 0.0001	0.0006 ± 0.0001
$CaCO_3$	0.30 ± 0.04	0.95 ± 0.10
N	0.57 ± 0.13	0.56 ± 0.05
P	0.09 ± 0.00	0.03 ± 0.00
K	2.07 ± 0.01	0.22 ± 0.01
Ca	0.12 ± 0. 00 [c]	0.79 ± 0.03
Mg	0.09 ± 0.00 [b]	0.08 ± 0.00
Fe	0.003 ± 0.00	0.02[a] ± 0.00
Mn (mg kg^{-1})	4.40 ± 0.00	28.60 ± 22.40
B (mg kg^{-1})	0.19 ± 0.01	2.00 ± 0.70

PhB Phragmites biochar, **SDB** Sawdust biochar **VM** volatile matrix, **FC** fixed carbon,
[a] ash on dry basis,
[b] volatile matrix on dry basis,
[c] fixed carbon on dry basis (means and standard deviation; $n = 3$).

parameters have been given in supplementary material. The biochars were characterized by FTIR (Table S1) and scanning electron microscopy was also done (Fig. S1).

2.2. Pot study setup

The experiment was conducted in the greenhouse of the Cukurova University. Andesitic tuff (local substrate with 0.5–1 mm granulometry) + peat moss (Potgrond P, Geeste, Germany) (1:1 v:v) mixture was prepared in plastic trays where onion seeds (Balkan Hybrid, Adana, Turkey) were broadcasted for seedling development. The surface of seeds was covered by spreading peat moss and irrigated with distilled water. To increase temperature and humidity, trays were covered with a polyethylene plastic sheet of 0.25 mm thickness and put in greenhouse, where environmental conditions were 25 ± 3 °C, 80 ± 3% relative humidity and 16/8 h day/night duration. After three weeks of seed sowing, vegetatively grown uniform seedlings were transferred to the pots according to respective treatments. All amendments applied were factorial arrangements of two soil types, two biochar, two phosphorus levels and three biological inoculants in addition to (uninoculated) control resulting in four treatments. For each soil, three replicates per treatment were prepared and there were 96 pots. All pots were arranged in CRD (completely randomized design). Nursery pots (21 cm diameter, 18 cm height) were filled with 3 kg soil treated with 1% biochar (Rafique et al., 2017). Two levels of P_2O_5 were established as 0 mg kg^{-1} (without-P) and 38 mg kg^{-1} (with-P). The dose of P was exactly half of locally recommended P_2O_5 dose for onion plant. The soil in pots was mixed with 1.0% *w/w* dry biochar and biological inoculants based on experimental treatments. The treatments were uninoculated control (C), *Lysinibacillus fusiformis* 31MZR (B), *Rhizophagus clarus* (M), *L. fusiformis* 31MZR + *R. clarus* (B + M). Soil, biochar, and fertilizer were mixed to ensure uniform distribution of applied amendments. In each pot, five healthy and uniform seedlings based on vegetative growth were transplanted. Treatments labeled with bacterial inoculation were inoculated with *L. fusiformis*, isolated from corn (Rafique et al., 2017). The *R. clarus* (BEG248) was obtained from The International Bank for the Glomeromycota and further multiplied and propagated using sorghum as a host plant in greenhouse of the Cukurova University. Infected roots, hyphae, spores, and substrates were obtained for further application in experiment. Each mycorrhizal inoculated pot was filled with 50 g (equivalent to ~ 700 spores) inoculum.

All pots received an equal dose of N and K fertilizer according to farmer practice. Pots filled with Soil A and Soil B received 0.13 g of urea (equivalent to 160 kg N/ha) in all pots and 7.02 g potassium dihydrogen phosphate (equivalent to 38 kg P_2O_5 ha^{-1}) in with-P pots. To balance K in rest of the pots (without-P), 3.84 g of potassium chloride was added. The plants were watered to ensure 70% of soil water holding capacity. It was managed according to the water requirement of plants.

2.3. Harvesting

Every plant in the pot was harvested at vegetative maturity, 65 days after transplantation. Aboveground tissues were harvested by cutting the plant at the soil-plant interface, dried at 65 °C till constant dry biomass and weighed. Root samples were separated from soil, rinsed with tap water followed by deionized water. Roots were dried at 60 °C till constant weight and weighed. All plant samples were ground with Tema mill, RM100 (Retsch Solutions in Miling and Sieving, Haan, Germany) and passed through a 0.5 mm mesh.

2.4. Chlorophyll fluorescence measurement

Chlorophyll fluorescence was measured at the tips of leaf 5 to leaf 6. Measurement was performed at midday through a FluorPen FP 100 (Photons Systems Instruments, Drasov, Czech Republic) following the protocol (Ritchie and Bunthawin, 2010). Before measurement, plants were kept in dark conditions for a period of 30 min, minimal fluorescence in dark-adapted state (F_0) was noted. A saturating pulse of irradiation (2 mmol/m^2/s) for 3 s was then administered to record maximal fluorescence in dark-adapted state (F_m) (Gong et al., 2013). The leaf was then put in actinic light (300 mmol/m^2/s) to record maximal fluorescence (F_m 0), minimal fluorescence in light-adapted state (F_0) and steady-state fluorescence (Fs). Chlorophyll fluorescence attributes (F_v/F_m) were calculated using the method of Zai et al. (2012).

2.5. Tissue nutrients analyses and AMF root colonization

An elemental analyzer (Thermo Fisher Scientific FLASH 2000 Series CN Elemental Analyzer, Thermo Fisher Scientific, Waltham, U.S.A.) was used to measure total N in plant samples. 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). 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 the 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 the whole process. For the estimation of AMF colonization, roots of onion were stained with Trypan Blue method with modifications Phillips and Hayman (Koske and Gemma, 1989). The AMF colonization in onion plant roots was estimated using a specified protocol (Giovannetti and Mosse, 1980).

2.6. Calculations and statistical analyses

Data collected 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 application of P (0 and 38 kg/ha), biochar type (PhB and SDB) effects and their interaction were considered as fixed attributes in model; replicate nested within soil type was considered as a random factor. Soil N, P, K, Cu, Mn, and Zn concentration, shoot and root dry biomass, N and P uptake per plant were considered as

dependent variables, additional dependent variables were root colonization, chlorophyll fluorescence and root related parameters. Data obtained were pooled to include both soils for all dependent variables after performing a Fisher F-test to verify the assumption of homogeneity of variances among sample populations. Statistical significance was noted at $p \leq 0.05$; biologically interesting differences with $0.05 < p \leq 0.10$ are also presented. Pearson's correlation coefficient test was conducted to estimate the relationships among different factors and observed nutrients concentration. The major trends across multivariate data-set of 21 evaluated parameters related to plants were summarized using a Principal Components Analysis (PCA) on centered and standardized data.

3. Results

3.1. Root colonization and root traits

The root colonization in both soils; Soil A and Soil B had similar behavior. In all soils, C- and B- treatments had < 10% colonization that could be due to transfer of mycorrhizal spores through air or irrigation (Fig. 1). Soil A had more colonization in without-P treatments in comparison to with-P treatments. The treatment of B + M had highest colonization as 73 and 70% for without-P and with-P application in PhB, while with SDB it was 58 and 68% respectively. In Soil B, B + M-treatment had highest colonization of 82 and 85% for without-P and with-P application under PhB amendment. A similar trend was observed in SDB amended Soil B, as 80 and 82% for without-P and with-P respectively. In all combinations, *L. fusiformis* 31MZR and *R. clarus* together ensured more colonization than *R. clarus* alone.

In both soils, treatment B + M (*L. fusiformis* 31MZR + *R. clarus*) enhanced plant root length in comparison to respective controls (Fig. 1). In Soil A amended with PhB, the B + M treatment increased root length by 48% (without-P) in comparison to control followed by M-treatment as 19% (without-P). When Soil A was amended with SDB, 18% root length was enhanced in B + M treatment (without-P). Soil B amended with both types of biochar did not enhance root length in presence and absence of P in comparison to the control. In terms of root surface area, 43% enhancement was noticed for B + M treatment in Soil A amended with PhB (without-P) while it increased to 13% in with-P (Fig. 1). Moreover, 19% enhancement was noticed for B + M treatment in Soil A amended with SDB (without-P) while it increased to 30% in with-P. Soil B couldn't influence root surface area positively in comparison to the control. In terms of root volume, 74% enhancement was noticed for B + M treatment in Soil A amended with PhB (without-P) while it increased to 14% in with-P (Fig. 1). Moreover, 50% enhancement was noticed for B + M treatment in Soil A amended with SDB (without-P) while it increased to 43% in with-P. Soil B could not influence root volume positively in comparison to control.

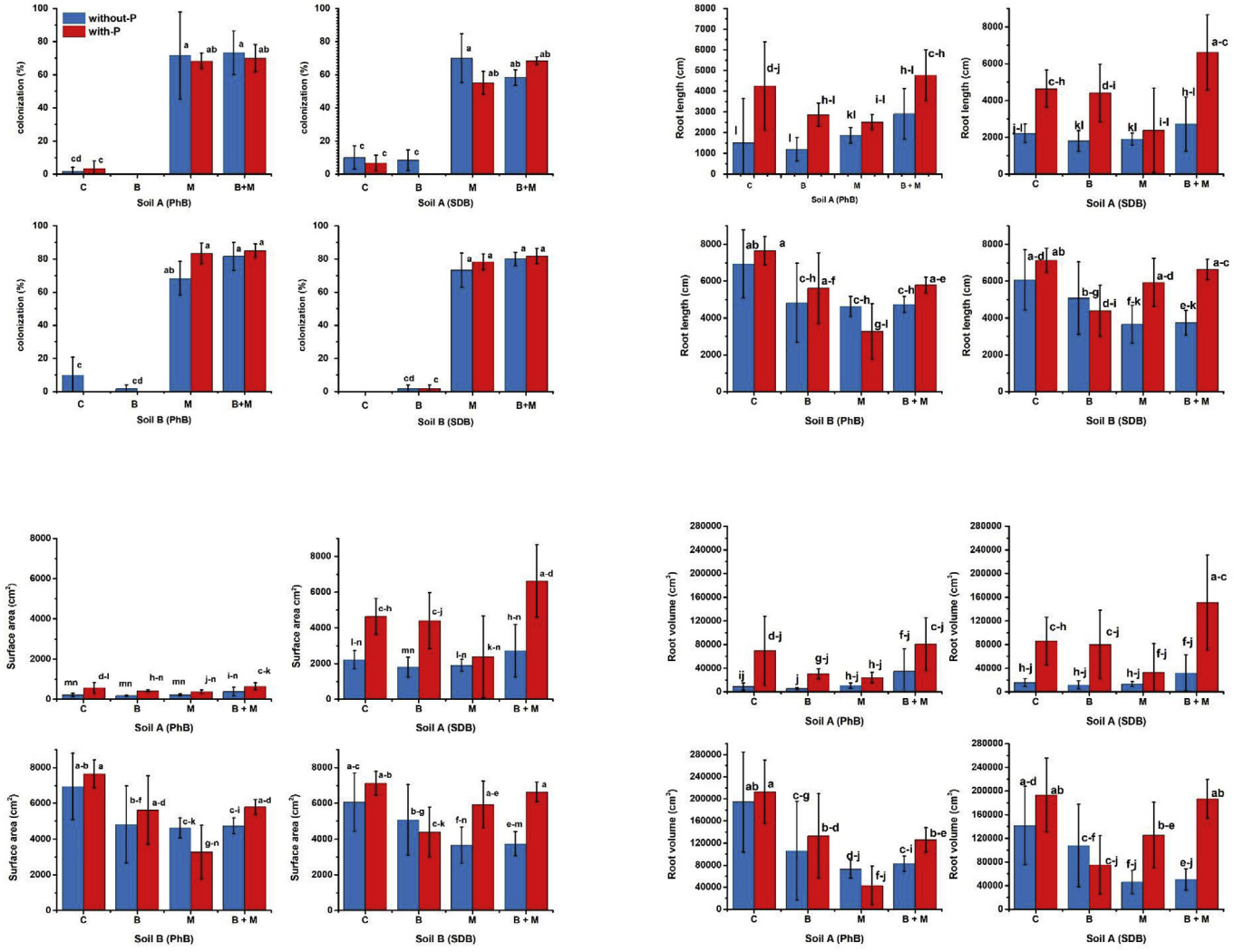


Fig. 1. Root colonization, root length, specific root length and root volume of onion plant in soils under different types of biochar (phragmites biochar (PhB), sawdust biochar (SDB)) and treatments as control (C), bacteria (B), mycorrhiza (M), bacteria + mycorrhiza (B + M). Lower letters show the difference between means (LSD).

3.2. Chlorophyll fluorescence

Soil A and Soil B amended with SDB showed high values of F_v/F_m in comparison to PhB (Table 3). There was a significant difference between same treatments of both soils. Moreover, treatments without-P had more value than with-P applied treatments. In Soil A, B + M combination had highest F_v/F_m value than rest of the treatments. Besides that, a notable difference was observed between B and M treatments of both soils amended with PhB.

3.3. Tissue macronutrient concentration

The Soil A, amended with PhB increased 2% shoot N without-P in B-treatment whereas with-P, it decreased by 15% (Table 4). Maximum reduction of 60% was observed in M-treatment, without-P whereas with-P it was only 2%. In shoot, B + M-treatment with-P, 8% increase was observed. Similarly, when SDB was applied, B + M-treatment with-P had a similar increase of 8% while a maximum increase of 31% was observed in B-treatment without-P. In Soil B, amended with PhB, 10 and 3% increase was noted in B-treatment of without-P and with-P respectively. Whereas, only B + M-treatment had a 8% increase with-P in SDB amended Soil B. The root N was observed on higher side in Soil A amended with PhB (with-P) than without-P, whereas on addition of SDB, this change was nonsignificant. In Soil B, SDB application without-P enhanced root N in comparison to other combinations.

In Soil A, M- and B + M-treatments significantly enhanced shoot P by 76 and 17% respectively without-P for PhB, while only B-treatment enhanced it by 25% in with-P (Table 4). When SDB was applied in the same soil (without-P), M-treatment enhanced P concentration in a shoot by 10% and with-P it was 15% in the B-treatment. The response of Soil B was quite different and 61% increase was observed in M-treatment of PhB amended soil (without-P), while it was only 8% in with-P. With the addition of SDB in Soil B, 61% shoot P was depicted in B-treatment whereas 11% increase in B + M-treatment (without-P). Only 12% was observed for B-treatment with-P. Soil microbes significantly increased root P for Soil A amended with PhB without-P, whereas it decreased in with-P except for B-treatment as 33%. A similar trend was observed for Soil B amended with PhB. Besides that, on application of SDB, root P was enhanced in M- and B + M-treatments which showed contribution of mycorrhizal fungs in both combinations of P application.

In Soil A, amended with PhB, a significant increase in shoot K was observed than for M- and B + M-treatments (without-P) (Table 4). A similar trend was noted for soil amended with SDB (with-P) while, change in shoot K was small in Soil B against both amendments and P application. Root K in Soil A amended with PhB (without-P) increased in comparison to without-P. In rest of the combinations of Soil A and Soil B, root K was negligible to mention.

3.4. N- and P- uptake

Nutrients uptake in onion plant was measured, and results illustrated that in Soil A amended with SDB showed highest N-uptake in B + M-treatment followed by PhB in without-P (Table S2). A similar trend with-P application was observed by the sequence of B + M > B > C > M while for PhB, it was B + M > C > B > M. When these treatments were applied to Soil B, response was quite different in terms of N-uptake amount, and that was M > C > B + M > B for the PhB (without-P) and a similar trend was observed in SDB amended soil (without-P). When P was applied to PhB amended soil, N-uptake increased significantly. Bacterium and mycorrhizal fungus worked synergistically in PhB, and SDB amended (without-P) Soil A. Whereas, with-P soil, B-treatment had highest P-uptake in both biochars for Soil A. Moreover, in Soil A, this trend was different while, P-uptake was more than in Soil A.

3.5. Tissue micronutrients analysis

The presence of Cu in onion shoot for M- and B + M treatments was > 5 mg kg^{-1} in PhB amended Soil A without-P, whereas, SDB amended soil it was 6.5 and 6.2 mg kg^{-1} respectively (Table 5). Inconsistent results were observed in treatments with-P for both biochar types amended Soil A. Generally, in both soils (without-P), Cu concentration decreased in order of B + M > M > C > B and in treatments of with-P, order was C > B > (M and B + M alternate) independent of biochar type used. The highest concentration of Cu in plant root was observed in SDB amended Soil A with-P. In case of Mn, soil type had a significant effect on shoot Mn concentration (Table 5). Soil B provided manifold more Mn then Soil A, moreover, its concentration was prominent in with-P treatments then without-P. Soil B strongly influenced Mn concentration in plant roots in comparison to Soil A. A similar trend was shown by Zn concentration in shoot and plants grown in Soil B had more Zn concentration in their shoots (Table 5).

Pearson's correlation analysis was done for macro- and micronutrients in relation to different sources of variance (Table S2) and (Table S3). Results showed that soil and biochar types strongly correlate with observed parameters. The PCA biplot in Fig. 2 showed correlation among different parameters of the study observed. They include roots and shoot nutrients concentration and root colonization. If two variables are close enough to form a small angle, it shows positive correlation. Besides that, variables with the difference of 180° angle are considered to be negatively correlated.

4. Discussion

The greenhouse study was conducted in different soil conditions, biochar types, and P-application levels. The results indicated that

Table 3
Chlorophyll fluoresence (F_v/F_m) of the onion plant under different soil conditions.

	Phragmites biochar		Sawdust biochar	
Treatments	without-P	with-P	without-P	with-P
Soil A				
C	0.64 ± 0.01 c-g	0.65 ± 0.02 b-g	0.72 ± 0.02 ab	0.69 ± 0.04 a-e
B	0.62 ± 0.07 f-h	0.65 ± 0.07 b-f	0.70 ± 0.01 a-d	0.69 ± 0.02 a-f
M	0.67 ± 0.03 a-f	0.68 ± 0.03 a-f	0.69 ± 0.02 a-e	0.70 ± 0.04 a-e
B + M	0.67 ± 0.02 a-f	0.49 ± 0.07 i	0.73 ± 0.02 a	0.68 ± 0.01 a-f
Soil B				
C	0.64 ± 0.02 e-g	0.68 ± 0.02 a-f	0.71 ± 0.01 a-c	0.68 ± 0.02 a-f
B	0.65 ± 0.03 b-g	0.68 ± 0.01 a-f	0.69 ± 0.02 a-e	0.67 ± 0.02 a-f
M	0.58 ± 0.09 gh	0.55 ± 0.02 hi	0.65 ± 0.00 b-f	0.65 ± 0.02 c-g
B + M	0.67 ± 0.01 a-f	0.66 ± 0.03 a-f	0.65 ± 0.03 c-g	0.64 ± 0.01 d-g

C control, **B** *L. fusiformis*, **M** *R. clarus*, **B + M** *L. fusiformis* + *R. clarus* (means and standard deviation; $n = 3$), lower letters show the difference between means (LSD).

Table 4
Concenteration of N, P and K (%) in plant shoot and root under different soil conditions and P application.

		Phragmites Biochar				Sawdust biochar			
		Without-P		With-P		Without-P		With-P	
	Treatments	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Nitrogen	***Soil A***								
	C	3.2 ± 0.2 ab	1.9 ± 0.0 lm	1.8 ± 0.2 h-j	2.0 ± 0.1 kl	2.0 ± 0.2 gh	1.7 ± 0.2 m	1.9 ± 0.1 hi	1.9 ± 0.0 lm
	B	3.3 ± 0.0 ab	2.0 ± 0.1 j-l	1.6 ± 0.1 k	2.5 ± 0.1 e-g	2.9 ± 0.0 c	2.0 ± 0.2 kl	1.5 ± 0.0 k	2.2 ± 0.0 h-j
	M	2.0 ± 0.1 gh	2.2 ± 0.2 h-j	1.6 ± 0.0 k	1.9 ± 0.1 k-m	2.0 ± 0.1 gh	2.0 ± 0.0 j-l	1.5 ± 0.1 k	2.1 ± 0.1 j-l
	B + M	1.9 ± 0.2 hi	2.4 ± 0.1 f-h	1.7 ± 0.2 i-k	2.4 ± 0.1 f-h	2.0 ± 0.0 gh	2.1 ± 0.1 i-k	1.6 ± 0.0 jk	2.1 ± 0.1 j-l
	Soil B								
	C	3.4 ± 0.3 a	2.6 ± 0.2 c-e	2.7 ± 0.1 d	3.2 ± 0.1 a	3.4 ± 0.1 a	2.3 ± 0.1 g-i	2.6 ± 0.1 d	2.8 ± 0.0 bc
	B	2.4 ± 0.1 ef	2.8 ± 0.1 b-d	2.5 ± 0.1 de	3.1 ± 0.1 a	3.1 ± 0.1 bc	2.6 ± 0.3 d-f	2.5 ± 0.1 de	2.8 ± 0.2 bc
	M	2.6 ± 0.2 d	2.7 ± 0.1 b-d	2.6 ± 0.1 de	2.8 ± 0.1 b-d	3.1 ± 0.2 bc	2.9 ± 0.1 b	2.2 ± 0.1 fg	2.5 ± 0.1 e-g
	B + M	2.6 ± 0.1 de	2.5 ± 0.1 e-g	2.5 ± 0.1 de	2.6 ± 0.1 d-f	2.6 ± 0.2 de	2.9 ± 0.1 b	2.3 ± 0.1 ef	2.7 ± 0.1 c-e
Phosphorus	***Soil A***								
	C	0.1 ± 0.0 op	0.1 ± 0.0 m	0.5 ± 0.0 b	0.4 ± 0.1 ij	0.1 ± 0.0 l-o	0.1 ± 0.0 k-m	0.4 ± 0.1 d-f	0.6 ± 0.1 g-i
	B	0.0 ± 0.0 p	0.1 ± 0.0 lm	0.7 ± 0.1 a	0.7 ± 0.1 e-g	0.1 ± 0.0 m-p	0.1 ± 0.1 k-m	0.5 ± 0.1 b-d	0.5 ± 0.1 h-j
	M	0.1 ± 0.0 m-p	0.2 ± 0.0 k-m	0.5 ± 0.0 b-d	0.5 ± 0.1 h-j	0.1 ± 0.0 k-o	0.1 ± 0.1 k-m	0.4 ± 0.2 d-f	0.6 ± 0.2 f-h
	B + M	0.2 ± 0.0 k-n	0.2 ± 0.0 k	0.4 ± 0.0 e-g	0.5 ± 0.0 h-j	0.1 ± 0.0 k-o	0.2 ± 0.0 k-m	0.3 ± 0.1 f-i	0.4 ± 0.1 j
	Soil B								
	C	0.2 ± 0.1 j-m	0.1 ± 0.0 k-m	0.4 ± 0.0 e-g	0.9 ± 0.1 c	0.1 ± 0.0 m-p	0.1 ± 0.0 k-m	0.4 ± 0.1 c-e	1.5 ± 0.1 a
	B	0.9 ± 0.0 n-p	0.1 ± 0.0 k-m	0.4 ± 0.0 e-g	1.4 ± 0.1 a	0.1 ± 0.0 m-p	0.1 ± 0.0 k-m	0.5 ± 0.1 bc	1.3 ± 0.1 a
	M	0.2 ± 0.0 i-k	0.2 ± 0.0 k-m	0.4 ± 0.0 d-f	1.0 ± 0.0 b	0.3 ± 0.0 h-j	0.2 ± 0.0 kl	0.4 ± 0.0 d-f	0.7 ± 0.1 ef
	B + M	0.2 ± 0.0 i-l	0.2 ± 0.0 k-m	0.3 ± 0.0 f-h	0.8 ± 0.0 cd	0.3 ± 0.0 g-j	0.2 ± 0.0 k	0.3 ± 0.0 f-h	0.7 ± 0.2 de
Potassium	***Soil A***								
	C	3.9 ± 0.1 n	1.7 ± 0.2 p	4.3 ± 0.2 l-n	7.5 ± 0.8 a	4.9 ± 0.1 e-i	2.7 ± 0.5 op	4.4 ± 0.4 j-m	7.4 ± 0.4 ab
	B	2.9 ± 0.2°	2.6 ± 0.2 p	4.4 ± 0.2 k-n	5.6 ± 0.5 f-l	5.1 ± 0.2 d-g	4.1 ± 0.9 mn	4.4 ± 0.3 i-m	6.0 ± 0.3 d-i
	M	4.6 ± 0.1 g-m	4.8 ± 0.3 k-n	4.1 ± 0.1 mn	6.8 ± 1.1 a-e	5.7 ± 0.1 ab	3.9 ± 0.3 mn	4.9 ± 0.4 e-j	5.4 ± 0.3 g-l
	B + M	5.0 ± 0.2 e-h	5.0 ± 0.4 i-m	4.1 ± 0.0 mn	6.9 ± 0.6 a-d	4.3 ± 0.1 l-n	3.7 ± 0.0 no	4.7 ± 0.3 f-l	5.4 ± 0.8 g-l
	Soil B								
	C	4.8 ± 0.3 e-k	6.3 ± 0.6 b-g	5.5 ± 0.2 bc	6.3 ± 0.2 b-g	5.1 ± 0.4 c-f	6.8 ± 0.4 a-e	6.2 ± 0.2 a	7.1 ± 0.4 a-c
	B	4.7 ± 0.3 f-l	6.2 ± 0.5 c-h	5.2 ± 0.1 c-e	6.5 ± 0.8 a-f	4.8 ± 0.3 e-k	5.6 ± 1.0 f-l	5.5 ± 0.3 b-d	5.8 ± 0.3 e-k
	M	4.8 ± 0.2 e-l	4.8 ± 0.6 k-m	4.5 ± 0.16 h-m	4.8 ± 0.4 j-m	5.1 ± 0.4 c-f	5.5 ± 0.1 f-l	5.3 ± 0.2 b-e	5.6 ± 0.7 f-l
	B + M	5.0 ± 0.1 e-h	4.6 ± 0.3 l-n	4.9 ± 0.5 e-k	5.5 ± 0.5 f-l	5.2 ± 0.1 c-e	5.2 ± 0.3 h-l	4.9 ± 0.16 e-k	5.9 ± .0.6 d-j

C control, **B** *L. fusiformis*, **M** *R. clarus*, **B+M** *L. fusiformis* + *R. clarus* (means and standard deviation; $n = 3$), lower case letters show the difference between means (LSD).

biochar has diverse effects on plants under same environmental conditions. It may influence soil microbes in a way to effectively participate for plant growth promotion by enhancing nutrients uptake in plants. This strategy might be an alternative and environmental friendly source of renewable P fertilizers (Rehman et al., 2018).

In this study, biochar-amended soil increased root colonization and improved plant growth (Fig. 1). The plant growth promoting bacteria can solubilize nutrients in soil and enhance nutrients uptake using different biochemical mechanisms (Afridi et al., 2019). Previous studies have shown that mycorrhizal abundance depends on biochar type and soil properties (Dobo et al., 2018; Rafique et al., 2019; Solaiman et al., 2019). Porous structure of biochar can be a potential refugee for bacteria, mycorrhizal hyphae and its spores increasing the surface area for P uptake (Solaiman et al., 2010). Mycorrhizal abundance may increase in biochar-amended soil as this study had two divers soils and biochar types which increased mycorrhizal colonization as reported previously (Lehmann et al., 2011; Rafique et al., 2019). The mechanism behind reduced utility of symbiosis in presence of nutrients explained previously (Lehmann et al., 2011). This study also exhibited a similar response and mycorrhizal colonization varied with P levels in soils. As biochar is considered soil conditioner, it improves soil physiochemical properties. Mycorrhizal inoculation has a linear trend with increasing dose of biochar (Elmer and Pignatello, 2011).

In healthy plants, usually F_v/F_m values are over 0.8, but in this study, it ranged from 0.5 to 0.73 which are slightly lower. This range of value indicates that plants during mycorrhization system were under suboptimal conditions. The F_v/F_m value less than 0.7 in C- and B-treatments showed that photosynthesis in plants was suboptimal (Herrmann et al., 2004). It can also lead to a point that plants may have lower Chla $a + b$ content that is consistent with decreased ΦPSII values. Our data highlight an important aspect that, treatments with mycorrhizal colonization had high (ΦPSII) data close to 0.8. It correlates with the high photosynthetic activity of plant resulting to photosynthetic carbon assimilation (Seaton and Walker, 1990). These results are in accordance with high demand of carbon by fungi (Hutchison and Piché, 1995). The data further highlight importance of pre-mycorrhizal phase with plant roots which need high carbon assimilation rate and should not be interrupted by forced reduction which may include growth regulators. They unbalance the system and reduces plant growth.

Biochar and mycorrhizal fungi effects on plant growth and nutrients uptake are limited in chemically fertilized crops. Such plants already have sufficient amount of nutrients and suppress mycorrhization and biochar effects; therefore in such conditions, mycorrhizal fungi cannot harvest complete benefits as enjoy in P-deficient environment (Gazey et al., 2004; Solaiman et al., 2010). Mycorrhizal fungi have capability to significantly create a pathway for P-uptake into roots (Smith et al., 2004). Nutrients availability to plants is strongly dependant on soil properties and water use efficiency. Mycorrhizal fungi use in Soil A improved P-uptake (Neumann and George, 2004). When biochar interacts with bacteria, particularly PSB such as *L. fusiformis* 31MZR, it enhances N-concentration up to 23% more than control, and P-concentration as up to 63% (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 present in soil ecosystem, interact with plant roots in terms of nutrients uptake (Zhang et al., 2016b). *L. fusiformis* 31MZR was already reported as P-solubilizer and promote plant growth confirmed by various biochemical tests (Chauhan et al., 2016). It may solubilize P in biochar-amended soil and make it available for plant roots and mycorrhizal fungi for further transportation into the roots.

Table 5
Concenteration of Cu, Mn and Zn (mg/kg) in plant shoot and root under different soil conditions and P application.

		Phragmites Biochar				Sawdust biochar			
		Without-P		With-P		Without-P		With-P	
	Treatments	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Copper	***Soil A***								
	C	3.5 ± 0.0 i-m	14.3 ± 1.6 d-h	4.2 ± 0.8 e-h	12.6 ± 0.7 f-j	6.1 ± 0.1 a	28.5 ± 2.9 a	3.5 ± 0.1 i-l	15.0 ± 2.2 d-g
	B	2.7 ± 0.0 n	12.5 ± 1.7 f-j	4.5 ± 0.0 d-f	14.2 ± 0.5 d-h	6.2 ± 0.0 a	28.3 ± 3.3 a	3.4 ± 0.1 j-m	11.6 ± 0.5 h-k
	M	5.4 ± 0.0 b	16.2 ± 1.3 de	3.2 ± 0.2 lm	15.5 ± 2.7 d-f	6.2 ± 0.1 a	23.4 ± 4.3 b	4.4 ± 0.8 d-g	13.6 ± 0.1 d-h
	B + M	5.1 ± 0.2 bc	24.9 ± 1.2 b	3.5 ± 0.3 i-l	12.8 ± 0.9 f-i	6.5 ± 0.0 a	23.9 ± 3.1 b	3.0 ± 0.5 mn	11.2 ± 0.0 h-k
	Soil B								
	C	3.8 ± 0.5 h-k	9.0 ± 0.1 k	4.0 ± 0.3 f-i	11.8 ± 0.9 g-k	4.5 ± 0.0 d-f	11.3 ± 0.1 h-k	4.6 ± 0.3 c-e	13.5 ± 0.9 d-h
	B	3.3 ± 0.0 k-m	9.4 ± 0.1 jk	4.0 ± 0.1 f-j	13.0 ± 0.7 e-i	3.6 ± 0.1 i-l	10.0 ± 0.0 i-k	3.9 ± 0.1 g-j	9.7 ± 0.8 i-k
	M	4.9 ± 0.2 b-d	12.5 ± 1.8 f-j	3.9 ± 0.3 g-j	11.0 ± 0.4 h-k	5.1 ± 0.8 bc	16.7 ± 1.2 cd	3.6 ± 0.2 i-l	12.0 ± 0.6 g-k
	B + M	4.3 ± 0.1 e-g	11.1 ± 0.8 h-k	3.2 ± 0.2 lm	11.2 ± 0.3 h-k	6.5 ± 0.2 a	19.5 ± 0.3 c	3.8 ± 0.2 h-k	13.9 ± 2.7 d-h
Manganese	***Soil A***								
	C	17.3 ± 0.2 i	25.7 ± 3.5 n	24.8 ± 2.0 i	37.2 ± 0.5 mn	25.4 ± 1.9 i	65.5 ± 1.9 l	24.7 ± 3.6 i	46.6 ± 8.0 l-n
	B	22.5 ± 2.6 i	54.3 ± 9.8 lm	24.7 ± 2.1 i	46.2 ± 6.0 l-n	23.6 ± 3.1 i	56.9 ± 0.0 lm	23.9 ± 4.3 i	48.0 ± 14.6 lm
	M	23.8 ± 3.3 i	57.0 ± 4.0 lm	25.7 ± 2.6 i	35.8 ± 5.9 mn	29.6 ± 1.6 i	56.7 ± 5.7 lm	20.6 ± 1.6 i	61.4 ± 7.7 l
	B + M	21.2 ± 1.0 i	53.4 ± 9.3 lm	20.9 ± 1.9 i	44.3 ± 1.4 l-n	25.7 ± 1.2 i	57.4 ± 10.3 lm	23.8 ± 4.1 i	53.5 ± 2.9 lm
	Soil B								
	C	78.8 ± 9.0 h	144.2 ± 0.7 ij	257.2 ± 36.0 b	279.5 ± 11.2 c	78.9 ± 2.9 h	190.8 ± 3.8 fg	117.6 ± 11.8 f	125.6 ± 4.7 j
	B	205.5 ± 1.5 c	370.7 ± 4.1 b	186.9 ± 9.4 c	170.8 ± 3.7 gh	106.7 ± 15.8 fg	167.0 ± 9.4 h	372.2 ± 46.3 a	442.5 ± 23.4 a
	M	159.1 ± 2.0 d	246.7 ± 43.4 de	148.1 ± 9.6 de	268.3 ± 9.6 cd	89.1 ± 4.0 gh	149.5 ± 2.2 hi	126.3 ± 10.9 ef	124.3 ± 11.9 j
	B + M	158.9 ± 4.7 d	204.7 ± 4.6 f	154.4 ± 8.8 d	228.2 ± 4.3 e	110.6 ± 4.1 fg	158.2 ± 11.2 hi	90.2 ± 3.3 gh	87.9 ± 6.6 k
Zinc	***Soil A***								
	C	4.3 ± 1.0 p	19.2 ± 5.2 d	5.3 ± 1.4 n-p	20.0 ± 3.3 d	25.2 ± 2.3 bc	87.0 ± 11.3 ab	6.5 ± 0.3 m-p	21.5 ± 3 d
	B	5.3 ± 0.9 n-p	24.9 ± 10.4 d	3.6 ± 0.5 p	96.8 ± 14.8 a	24.3 ± 3.6 cd	52.9 ± 21.4 b-d	7.3 ± 1.5 l-p	18.9 ± 1.7 d
	M	9.3 ± 0.1 k-m	34.1 ± 3.9 cd	4.1 ± 0.2 p	19.3 ± 3.0 d	36.6 ± 2.7 a	51.2 ± 2.6 b-d	21.0 ± 3.7 de	24.2 ± 4.1 d
	B + M	12.1 ± 2.0 h-k	56.5 ± 10.6 a-d	4.9 ± 1.0 op	18.7 ± 2.0 d	26.3 ± 6.7 bc	56.1 ± 20.5 a-d	9.0 ± 0.2 k-o	25.1 ± 3.1 d
	Soil B								
	C	10.2 ± 0.7 j-m	31.7 ± 3.3 d	11.2 ± 0.5 i-l	19.9 ± 0.7 d	19.4 ± 1.9 ef	42.4 ± 3.9 cd	15.1 ± 1.5 g-i	25.1 ± 2.7 d
	B	14.3 ± 1.1 h-j	42.4 ± 2.3 cd	10.9 ± 0.8 j-l	16.7 ± 1.9 d	15.7 ± 0.8 f-h	41.0 ± 1.9 cd	13.9 ± 2.3 h-j	23.5 ± 2.8 d
	M	18.9 ± 0.9 e-g	55.2 ± 4.1 a-d	9.6 ± 0.2 k-m	19.5 ± 1.5 d	27.0 ± 2.8 bc	75.7 ± 0.5 a-c	12.2 ± 0.2 h-k	21.9 ± 2.1 d
	B + M	19.3 ± 0.6 ef	55.4 ± 4.5 a-d	11.6 ± 2.0 h-k	21.1 ± 1.3 d	28.8 ± 3.4 b	89.9 ± 1.6 ab	11.0 ± 1.7 i-l	20.3 ± 1.7 d

C control, **B** *L. fusiformis*, **M** *R. clarus*, **B + M** *L. fusiformis* + *R. clarus* (means and standard deviation; $n = 3$), lower letters show the difference between means (LSD).

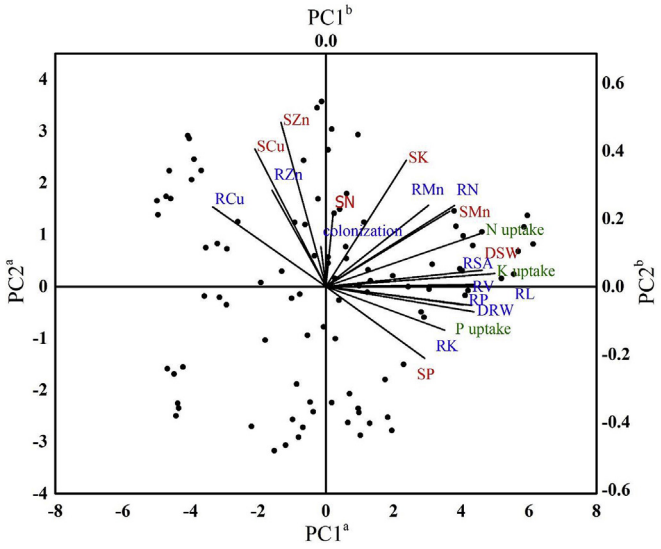


Fig. 2. BiPlot of principal component analysis of the variates, dry shoot weight (DSW), dry root weight (DRW), root colonization, N-uptake, P-uptake, K-uptake, N, K, P, Cu, Zn, and Mn in shoot (SN, SK, SP, SCu, SZn, SMn), N, K, P, Cu, Zn, and Mn in root (RN, RK, RP, RCu, RZn, RMn), root length (RL), root surface area (RSA) and root volume (RV).

5. Conclusion

The study showed that the addition of biochar in soil with microbial inoculants improved onion plant growth. In absence of P fertilizer, microbial inoculants boosted plant growth and nutrient uptake due to mycorrhizal networking in soil with plant roots. Observed physiological activities of plant were also enhanced in the presence of *R. clrus* and biochar. High rate of chlorophyll fluorescence in mycorrhizal associated plants showed rapid photosynthetic carbon assimilation. Observed macro- and micronutrient concentration in plant showed the influence of soil and biochar types as key contributors. Results showed that combination of *L. fusiformis* and *R. clarus* was more effective, irrespective to soil and biochar types. The improved plant growth, root colonization and nutrient uptake in study provides evidence that the interaction of biochar with microbial inoculants could be a futuristic approach for sustainable crop production. Reliance on chemical P can be reduced and study paves path towards the use of renewable P sources for increasing demand.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2019.06.123.

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