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Additive effects due to biochar and endophyte application enable soybean to enhance nutrient uptake and modulate nutritional parameters*#

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Abstract: We studied the effects of hardwood-derived biochar (BC) and the phytohormone-producing endophyte *Galactomyces geotrichum* WLL1 in soybean (*Glycine max* (L.) Merr.) with respect to basic, macro- and micronutrient uptakes and assimilations, and their subsequent effects on the regulation of functional amino acids, isoflavones, fatty acid composition, total sugar contents, total phenolic contents, and 1,1-diphenyl-2-picrylhydrazyl (DPPH)-scavenging activity. The assimilation of basic nutrients such as nitrogen was up-regulated, leaving carbon, oxygen, and hydrogen unaffected in BC+*G. geotrichum*-treated soybean plants. In comparison, the uptakes of macro- and micronutrients fluctuated in the individual or co-application of BC and *G. geotrichum* in soybean plant organs and rhizospheric substrate. Moreover, the same attribute was recorded for the regulation of functional amino acids, isoflavones, fatty acid composition, total sugar contents, total phenolic contents, and DPPH-scavenging activity. Collectively, these results showed that BC+*G. geotrichum*-treated soybean yielded better results than did the plants treated with individual applications. It was concluded that BC is an additional nutriment source and that the *G. geotrichum* acts as a plant biostimulating source and the effects of both are additive towards plant growth promotion. Strategies involving the incorporation of BC and endophytic symbiosis may help achieve eco-friendly agricultural production, thus reducing the excessive use of chemical agents.

Key words: Phytohormone-producing endophytic fungi; Nutrients uptake; Assimilation; Nutritional quality; Soybean
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1 Introduction

The conversion of biomass to a stable soil carbon pool negatively regulates atmospheric carbon (C) concentrations, and this phenomenon underlies the concept of biochar (BC) production (Lehmann, 2007). BC has been used to improve agricultural productivity in traditional systems, with examples of its use being traced in the terra preta of the Amazon Basin (Lehmann, 2007). BC is the product of the slow

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pyrolysis of biomass, and its properties vary depending on certain factors such as feedstock type, time, and temperature conditions during preparation (de Corato *et al*., 2015; Gwenzi *et al*., 2015; Li *et al*., 2015). However, incorporating BC into soil proved to operate as a conditioner, by improving soil fertility through increasing soil organic C levels and nutrient availability (releasing its own nutrients and cycling existing nutrients in the soil and preventing their leaching), transforming phosphorus (P), enhancing soil field capacity, and decreasing bulk density (Ahmed and Schoenau, 2015; Bayabil *et al*., 2015; Dong *et al*., 2015; Gwenzi *et al*., 2015; Zhang *et al*., 2015). The large surface area and negative charges of BC immobilize nitrogen (N) and reduce N pollution (Dong *et al*., 2015). Other important aspects that make BC a promising candidate for environmentally friendly agriculture are its ability to inhibit methane emission (Gwenzi *et al*., 2015), remediate heavy metal-contaminated soil, neutralize soil toxic compounds, and increase beneficial microbial activities (Waqas *et al*., 2014; Li *et al*., 2015). BC has also been found to reduce the incidence of crop disease and mitigate biotic stress (de Corato *et al*., 2015; Gwenzi *et al*., 2015; Haider *et al*., 2015).

Endophytic fungi are ubiquitous with plant species, and live asymptomatically inside healthy plant tissues in mutualistic association. This symbiotic association demonstrates the ecological and evolutionary importance for the establishment of plants in terrestrial ecosystems (Hartley *et al*., 2015; Zhou *et al*., 2015). Endophytes colonize all plant organs, and they have been isolated from the roots, stems, and leaves (Zhou *et al*., 2015). The occurrence of endophytes in specific tissues supports the view that they have a particular role in host plant tissues. For example, endophytes found in plant roots help with water uptake, nutrient acquisition, and plant growth promotion, while those found in the leaves and stems strengthen plant defense mechanisms (Khan *et al*., 2013; 2014; Waqas *et al*., 2014; 2015; Hartley *et al*., 2015; Zhou *et al*., 2015). Endophytes enhance the performance of host tissue functions by producing a vast array of biologically active secondary metabolites (Khan *et al*., 2012a; 2013; Waqas *et al*., 2014; 2015). Phytohormones and similar substances are examples of biologically active secondary metabolites, which are mostly responsible for enhancing plant growth and development under normal, abiotic, and biotic stress conditions. Endophytes (fungi and bacteria) have the ability to produce gibberellins and indole-3-acetic acid (IAA); these hormones have been extensively reported to enhance plant growth attributes (Khan *et al*., 2012b; 2013; 2014; Waqas *et al*., 2012).

The combined application of phytohormone-producing fungal endophytes and hardwood-derived BC ameliorates high Zn concentrations and enhances soybean plant growth in the presence and absence of heavy metal stress (Waqas *et al*., 2014). To move on from the results of the previous experiment, the current study was conducted and we hypothesized that: (1) the endophytic fungus and BC individually or in combination enhance the provisions and uptakes of macro- and micronutrients; (2) as a result, the nutritional and medicinal qualities of soybean are improved.

2 Materials and methods

2.1 Soil preparation and biochar addition

In this study, the substrate TBT (Soil and Fertilizer Technology, Korea) was used instead of common soil. Hence, the focus was to evaluate the roles of endophytes and BC in plant growth promotion. Therefore, other properties, such as bulk density and porosity, were not determined. The nutrient composition of the substrate consisted of coco peat (45%–50%), perlite (35%–40%), peat moss (10%–15%), and zeolite (6%–8%), and it contained P_2O_5 0.35 mg/g, K_2O 0.1 mg/g, NO_3^- 0.205 mg/g, and NH_4^+ 0.09 mg/g. The BC was obtained commercially from Kangwon Grasses Industries Ltd. (Kangwondo, Korea) and the company information provided on bags shows that the pine trees-derived BC via slow pyrolysis is alkaline in nature, has an average particle size of 5 mm or less and a moisture level of 10%, and contains 2%–3% ash. Chemical characterization of BC was performed with an elemental analyzer and inductively coupled plasma mass spectrometry (ICP). The analysis revealed the basic elemental composition of BC as 80% C, 1.62% N, and 1.49% hydrogen (H), and macro-/micronutrient (mg/kg) as 2.58 P, 3.64 potassium (K), 43.58 calcium (Ca), 2.95 sulfur (S), 1.81 manganese (Mn), 20.30 magnesium (Mg), 13.21 molybdenum (Mo), 2.09 aluminum (Al), 6.63 boron (B), 0.35 copper (Cu), 1.13 iron (Fe), and 6.88 sodium

(Na). BC was added at 10:90 (w/w) to the substrate, and was mixed well by stirring and rotating end-over-end in sealed plastic bags to incorporate it homogeneously. The substrates with and without BC were moistened in the amount of water of half of its weight, packed in plastic bags, and left for 7 d in dark conditions at room temperature to equilibrate. The substrates with and without BC were then sterilized (at 121 °C for 15 min) three times to create microbe-free conditions. The autoclavable plastic plant growth pots (30 cm×15 cm; sterilized at 121 °C for 15 min) were filled with 1 kg substrate. Plastic bases were placed in the pots to collect any leaching water, which was then added back to the pots to prevent the loss of BC. Culture broth containing mycelia (40 ml, (200±10) mg) of *Galactomyces geotrichum* WLL1, along with potato dextrose agar (PDA)-inoculated disks, was applied in the center of each substrate-filled pot in the endophyte treatment with and without BC. The substrate in pots with endophytes was then mixed by stirring with a sterile rod to uniformly distribute the mycelia and PDA-inoculated disks in the rhizosphere. Autoclaved 40 ml Czapek broth medium and 20 ml non-inoculated disks were added to the center of control and only BC pots as well for the purpose of balancing the nutrients status of the substrate compared to inoculated pots. The pots were then aseptically incubated for 5 d to be ready for transplantation.

2.2 Biological materials and culture condition

G. geotrichum WLL1 (NCBI GenBank accession number KJ817904) was previously isolated from *Trapa japonica* inhabiting Nak-Dong river (35°44′47.20″ N, 128°23′07.79″ E) with a rainfall catchment area from an abandoned zinc mine (Waqas *et al*., 2014). The *G. geotrichum* WLL1 was capable of producing gibberellins (GA_1=(7.83±0.40) ng/ml, GA_4=(54.11±1.50) ng/ml, GA_7=(4.12±0.13) ng/ml), indole acetic acid ((76.89±2.35) μg/ml), and reactive oxygen species (peroxide, superoxide), and found to extensively colonize soybean root in the presence and absence of BC with or without Zn heavy metal stress. For the current experiment, Czapek broth media (40 ml; 1% (0.01 g/ml) glucose, 1% (0.01 g/ml) peptone, 0.05% (0.5 g/L) KCl, 0.05% (0.5 g/L) $MgSO_4·7H_2O$, and 0.001% (0.01 g/L) $FeSO_4·7H_2O$; pH 7.3±0.2) in 50 ml flasks were inoculated with *G. geotrichum* and grown at 30 °C for 10 d at 120 r/min under dark conditions. At the same time PDA media (20 ml; 0.4% (4 g/L) potato starch, 2.0% (20 g/L) dextrose, and 1.5% (15 g/L) agar; pH 5.6±0.2) in 70 mm Petri dishes were also inoculated with *G. geotrichum* and kept for 7 d at 25 °C under dark conditions. At the time of each application into every pot, from these *G. geotrichum*-inoculated broth and agar media, one flask of Czapek culture broth and PDA plate disks divided into small pieces with sterilized blades were used.

2.3 Soybean growth experiment under controlled condition

Soybean seeds (*Glycine max* L. var. Hwangkeumkong) were obtained from the Soybean Genetic Resource Center (Prof. Jeong Deong LEE, Kyungpook National University, Korea). The seeds were healthy, with 6% moisture content and 95% germination. At the same time as pot preparation, the seeds were germinated (28 °C and relative humidity of 60%) for 5 d to get seedlings of identical size in the sprouting trays. Before germination, the seeds were surface-sterilized in autoclaved pots with 2.5% (25 g/L) sodium hypochlorite for 30 min, and were then rinsed with autoclaved double-distilled water. After germination, seedlings of equal size were randomly selected, screened for the presence of any microbes and transplanted to pots. To ensure that microbial free plants were transplanted, microscopic analysis was performed using an Olympus (BX50; Olympus Optical Co. Ltd., Shinjuku, Tokyo, Japan) light microscope according to Likar and Regvar (2013) and Waqas *et al*. (2014; 2015) before further experimentation. The roots of randomly collected soybean plants from the sprouting tray fragmented into 1-cm-long pieces were evaluated under a light microscope. The experimental treatments included: (1) soybeans without endophyte and BC, (2) endophyte-inoculated soybeans, (3) soybeans with BC (10%, w/w), and (4) both endophyte and BC (10%, w/w) applied to soybeans. The soybeans were left to grow for 22 d under controlled growth chamber conditions (day/night cycle: 14 h at 28 °C/10 h at 25 °C; relative humidity 60%–70%; light intensity 1000 μE/(m^2·s) from sodium lamps). The soybeans were irrigated daily with 30 ml of distilled water to minimize BC leaching from pots. During the growth period, 40 ml of Czapek broth with fungal mycelia ((200±10) mg) and inoculated PDA disks were applied to the root zone at the

beginning of V3 and V4 life stages of soybean for optimal endophytic infection after transplantation. Sterile Czapek and PDA media of the same amount were added to the control/only BC-treated plants to balance the nutrient status of the substrate with that of the endophytic treatment that might have occurred because of its application along with the endophyte. The plants were immediately stored after harvesting in liquid nitrogen and then freeze-dried for one week. Before harvesting, three plants per treatment (endophyte) were selected for the re-isolation and molecular identification of endophytes from the soybean secondary root pieces to check their inside colonization according to the procedure described by Khan *et al*. (2012b). The re-isolated purified endophytes were compared with those of the original plates and showed 100% morphological similarity. In case of molecular identification, the obtained sequences of the internal transcribed spacer (ITS) and large subunit (LSU) regions from ribosomal DNA (rDNA) were BLAST-searched and confirmed 100% sequence homology with *G. geotrichum* WLL1.

2.4 Macro- and micronutrient analyses in soil and plant tissues

To determine macro- and micronutrient uptakes, representative fresh plants were randomly selected from each replicated treatment. The plants were carefully harvested and divided into roots and shoots. The roots were carefully washed with double-distilled water to remove all debris and apoplastic contents (Waqas *et al*., 2014). After washing, the roots were dried with autoclaved Kimtech Science Wipers (Yuhan-Kimberly Inc., Seoul, Korea). The roots and shoots were immediately placed in liquid nitrogen, lyophilized at −50 °C for 3–4 d, and ground to a fine powder using a grinder. To analyze the macro- and micronutrient concentrations of the substrate, three samples were randomly collected from each replicated treatment. The three samples were mixed together, air-dried, sieved to 2 mm, and ground to a fine powder in an agate mortar for further analysis. Macro- and micronutrient levels, including P, K, S, Ca, Mg, Mn, Fe, and Cu, were analyzed in the shoots, roots, and substrates using ICP having 0.1 mg/kg limit of detection (Optima 7300DV, Perkin-Elmer, USA). The samples (0.1 g) were prepared with microwave (ETHOS 1, advanced microwave digestion system) assisted concentrated HNO_3, H_2O_2 and H_2SO_4 acid digestions. Here, H_2O_2 and H_2SO_4 have been used for safe, fast, and reliable sample digestion prior to ICP analysis due to their abilities for complete sample digestion and high boiling point, respectively. Although H_2O_2 completely decomposes the sample, its combination with HNO_3 is the best substitute for perchloric acid because of safety issues as well as the fact that it increases solubility of the sulphate salts, which are not obtained accurately with H_2SO_4. The digested samples were diluted with double distilled water before analysis. For the external proficiency testing scheme, ICP multi-element standard solution IV (Merck KGaA, 64271 Darmstadt, Germany) was used. The coefficient value was found to be 0.99 for all calibration curves. C, H, O, and N contents were determined by an elemental analyzer (Flash2000, ThermoFisher Scientific Inc., Waltham, MA, USA) in whole plant tissues. The elemental analyzer with 0.3 mg/kg limit of detection was calibrated with the standard (BBOT standard, Fisons Instruments SpA Strada Rivoltana, 20090 Rodano, Milan, Italy) for elemental analysis.

2.5 Functional amino acid analysis

Functional amino acids were analyzed according to the method described by Khan *et al*. (2013) and Waqas *et al*. (2015) in freeze-dried plant samples of all treatment types (50 mg). An amino acid standard mixture solution (type H) for an automatic amino acid analyzer (L-8900 Hitachi, Japan) was used for the quantification of endogenous amino acids. Each treatment was replicated three times.

2.6 Flavonoid extraction and quantification

Flavonoids were extracted and analyzed from powdered freeze-dried plant samples (0.2 g) of each treatment as described previously by Khan *et al*. (2013). After extraction, the samples (10 μl) were injected in a PerkinElmer series 200 high-performance liquid chromatography (HPLC) system (USA) fitted with COL-CHOICE C18 column 4.6 mm×150 mm (5 μm) pack. The solvent flow rate of using gradient solutions, viz. acetonitrile and 0.1% (1 g/L) acetic acid in water, was 1.0 ml/min. The elution was monitored at 260 nm by using series 200 UV/vis detector. Isoflavones were identified on the basis of comparisons with retention time of genuine standards obtained from Sigma Chemical Co., USA and LC Laboratory, USA.

2.7 Fatty acid analysis

For fatty acid extraction, the method described in Khan *et al*. (2012b) was followed. The processed final extraction from each sample (fresh 0.2 g) of 1 μl was collected and injected for analysis, carried out on gas chromatography (GC) with an Agilent Model 7890A series (Agilent, Dover, DE, USA) equipped with an Agilent 5975C mass spectrometry (MS) detector with MS ChemStation Agilent v. A.03.00 (Table S1). The constituent fatty acids were recognized based on the evaluation of their relative retention time and mass spectra with those of the standards, Wiley7N, the NIST library data of the GC-MS system, and data from the published literature. The numerical data were expressed as percentage area.

2.8 Determination of total sugar content, total phenolic content, and DPPH-scavenging activity

To determine total sugar content, a modified method of DuBois *et al*. (1956) was followed, as described in Albalasmeh *et al*. (2013).

Total phenolic content was quantified using Folin-Ciocalteu reagent through an improved version of the assay described by Slinkard and Singleton (1977). The total phenolic values having absorbance at 765 nm for all samples were expressed in terms of gallic acid equivalents (μg/ml). The blank (dimethyl sulphoxide (DMSO)) was treated in the same way as the other samples. However, the dilution factor was taken into account for those samples where dilution was performed.

1,1-Diphenyl-2-picrylhydrazyl (DPPH)-scavenging activity was analyzed by an assay modified from Gulati *et al*. (2012) and the absorbance was recorded at 490 nm. The results were compared with the control, i.e. 50 μl ethanol instead of culture samples, and their respective antioxidant activity was expressed as percent of inhibition (I): $I\ (\%)=(A_{490,c}-A_{490,s})/A_{490,c}\times 100\%$, where $A_{490,c}$ and $A_{490,s}$ are the absorbances at 490 nm of control and samples, respectively.

2.9 Statistical analysis

The experiment was conducted in a completely randomized design and repeated three times with each treatment being replicated six times. Analysis of variance (ANOVA) was employed for statistical analysis and the mean values were compared with the Duncan's multiple range test (DMRT) ($P<0.05$) using the statistical software program SAS (Version 9.2, Cary, NC, USA).

3 Results

3.1 Basic/essential elemental composition and fresh biomass of soybean individually and in combination with biochar and *G. geotrichum*

Percentages of N, C, H, and O contents were analyzed in whole plant tissue samples to evaluate the effect of treatments on basic/essential elemental composition or their assimilation in soybean plants (Fig. 1). In comparison to the control ((4.75±0.09)%), N content significantly increased in soybean plants treated with the co-application of BC+*G. geotrichum* ((5.28±0.05)%), followed by *G. geotrichum* alone ((4.95±0.02)%), as shown in Fig. 1a. However, C, H and O compositions in treated and control soybean plant tissues were not significantly different (Figs. 1b–1d). Subsequently, the recorded fresh biomass revealed the same behavior and the highest values were documented in BC+*G. geotrichum* ((22.17±2.95) g) followed by *G. geotrichum* alone ((16.39±4.00) g) (Fig. 1e).

3.2 Macro- and micronutrient dynamics in the substrate, root, and shoot of soybean with the individual and combined applications of biochar and *G. geotrichum*

Macro- and micronutrient analyses of the control and treated substrates show that P, K, S, Ca, Mg, and Mn were significantly higher in individual BC application, followed by control and BC+*G. geotrichum* application (Table 1). The individual application of *G. geotrichum* produced the lowest substrate nutrient status for P, K, S, Ca, Mg, and Mn. Fe was significantly higher in the control substrate, followed by the substrate treated with BC alone. The lowest concentration of Fe was found in the substrate treated with *G. geotrichum* alone and BC+*G. geotrichum*. Cu was found to be similar in the substrate treated with BC alone and control, but was not detected in the substrate treated with *G. geotrichum* alone or BC+*G. geotrichum*.

The analysis of soybean root tissues (Table 2) revealed that P, K, S, Ca, Mg, and Mn were significantly higher in plants treated with *G. geotrichum* alone, followed by BC+*G. geotrichum*. The lowest

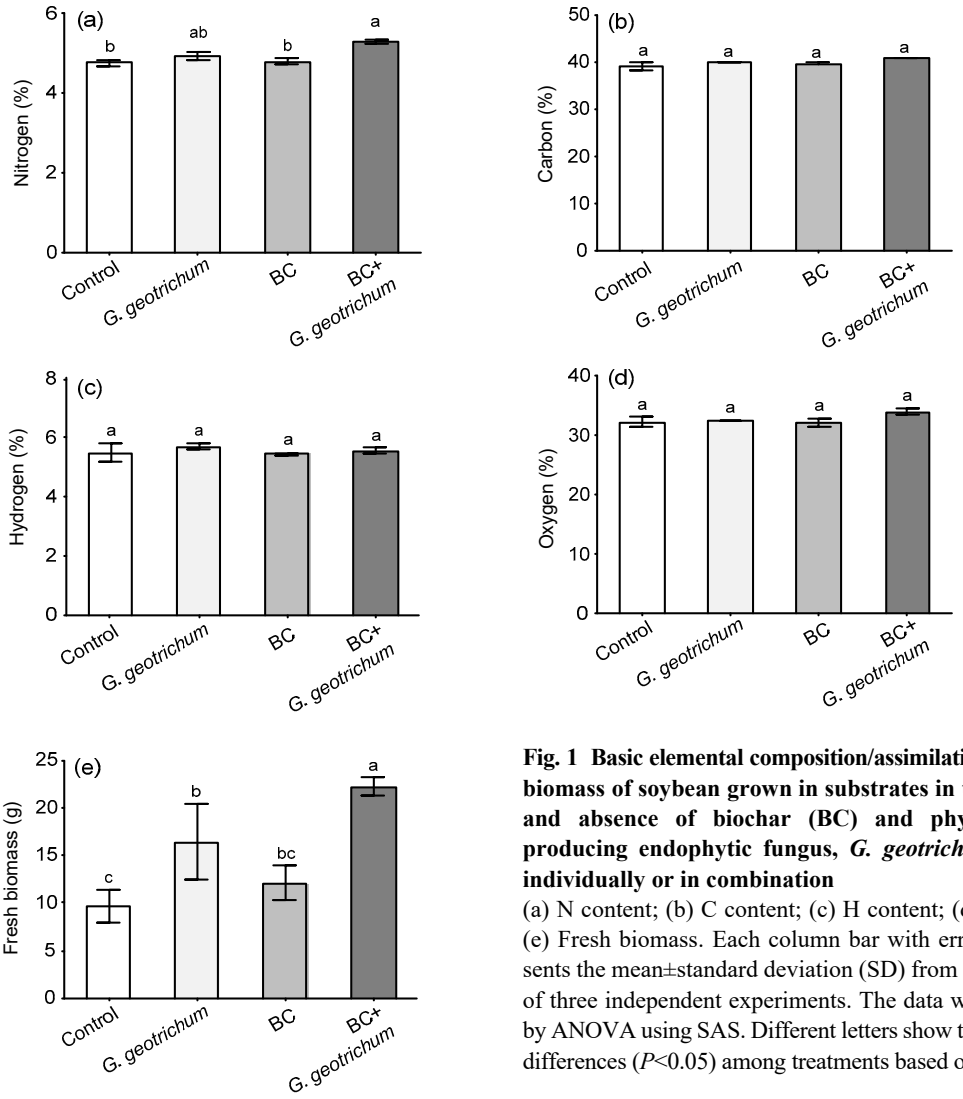

Fig. 1 Basic elemental composition/assimilation and fresh biomass of soybean grown in substrates in the presence and absence of biochar (BC) and phytohormone-producing endophytic fungus, *G. geotrichum*, applied individually or in combination

(a) N content; (b) C content; (c) H content; (d) O content; (e) Fresh biomass. Each column bar with error bar represents the mean±standard deviation (SD) from six replicates of three independent experiments. The data were analyzed by ANOVA using SAS. Different letters show the significant differences (P<0.05) among treatments based on DMRT

amounts of these elements were found in plants treated with BC alone and the control. Fe was significantly higher in plants treated with BC alone, followed by *G. geotrichum* and BC+*G. geotrichum* (Table 2). Moreover, Cu was not detected in any of the treatments, including the control.

Macro- and micronutrients were differentially regulated in the tissues of treated and control soybean shoots (Table 3). Compared to the control, P, K, and S were significantly higher in the BC+*G. geotrichum* and individual BC treatments. Furthermore, Ca, Mg, Mn, and Fe were significantly higher in plants treated with BC alone, followed by the BC+*G. geotrichum* treatment and the control. However, Cu was also not detected in any of treatments.

3.3 Functional amino acids of soybean in the presence and absence of biochar and *G. geotrichum*

The composition of functional amino acids from aliphatic, hydroxyl, aromatic, acidic, basic, and cyclic groups of soybean shoots in response to the individual and combined BC and *G. geotrichum* was analyzed, and the results are presented in Table 4. Compared to the control, from the analyzed amino acids, the isoleucine, glutamic acid, glycine, phenylalanine, methionine, cysteine, and arginine contents significantly increased in soybean tissues treated with BC+*G. geotrichum*. Aspartic acid, threonine, and lysine were significantly higher in soybean treated with *G. geotrichum* alone compared to the control. However, leucine and cysteine were significantly higher in

Table 1 Macro- and micronutrient dynamics in the substrate after plant growth in the presence and absence of biochar and phytohormone-producing endophytic fungus, *G. geotrichum*, when applied individually and in combination

Treatment	P (mg/kg)	K (mg/kg)	S (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Cu (mg/kg)
Control	9.45±0.39^{b}	91.73±0.50^{b}	44.51±0.76^{b}	384.30±0.07^{b}	106.90±0.11^{b}	2.72±0.81^{b}	185.90±0.56^{a}	0.15±0.08^{a}
GG	7.22±0.49^{c}	42.99±0.46^{d}	22.36±0.28^{d}	45.23±0.47^{d}	44.97±0.28^{d}	1.01±0.64^{d}	78.12±0.42^{c}	ND
BC	13.44±0.99^{a}	122.50±0.67^{a}	92.97±0.94^{a}	441.50±0.79^{a}	217.20±0.64^{a}	3.41±0.95^{a}	113.50±0.59^{b}	0.15±0.01^{a}
BC+GG	6.93±0.73^{d}	48.00±0.53^{c}	39.09±0.57^{c}	148.20±0.95^{c}	90.57±0.36^{c}	1.29±0.69^{c}	72.59±0.19^{d}	ND

BC: biochar; GG: *G. geotrichum*; ND: not detected or not determined in the samples. Each value is expressed as mean±SD of six replicates from three independent experiments. The data were analyzed by ANOVA using SAS. Values in a column followed by different letters show the significant differences (P<0.05) among the treatments based on DMRT

Table 2 Macro- and micronutrient dynamics of soybean roots grown in the presence and absence of biochar and phytohormone-producing endophytic fungus, *G. geotrichum*, when applied individually and in combination

Treatment	P (mg/kg)	K (mg/kg)	S (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Cu (mg/kg)
Control	16.15±0.50^{c}	68.31±0.43^{c}	12.78±0.38^{b}	12.10±0.28^{c}	4.28±0.91^{c}	0.30±0.57^{c}	1.32±0.53^{d}	ND
GG	20.71±0.11^{a}	83.26±0.67^{a}	17.73±0.67^{a}	19.69±0.11^{a}	8.71±0.52^{a}	0.38±0.09^{a}	6.89±0.11^{b}	ND
BC	14.99±0.56^{d}	66.13±0.83^{d}	12.54±0.24^{b}	16.28±0.12^{b}	7.14±0.53^{b}	0.29±0.02^{d}	11.68±0.72^{a}	ND
BC+GG	17.40±0.72^{b}	81.31±0.96^{b}	17.68±0.57^{a}	19.60±0.18^{a}	7.15±0.09^{b}	0.33±0.04^{b}	3.36±0.40^{c}	ND

BC: biochar; GG: *G. geotrichum*; ND: not detected or not determined in the samples. Each value is expressed as mean±SD of six replicates from three independent experiments. The data were analyzed by ANOVA using SAS. Values in a column followed by different letters show the significant differences (P<0.05) among treatments based on DMRT

Table 3 Macro- and micronutrient dynamics in soybean shoots grown in the presence and absence of biochar and phytohormone-producing endophytic fungus, *G. geotrichum*, when applied individually and in combination

Treatment	P (mg/kg)	K (mg/kg)	S (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Cu (mg/kg)
Control	14.63±0.01^{c}	63.60±0.96^{c}	13.77±0.71^{c}	44.29±0.74^{b}	16.44±0.11^{c}	0.37±0.34^{c}	0.59±0.08^{d}	ND
GG	19.47±0.78^{b}	90.32±0.37^{b}	17.90±0.11^{b}	29.32±0.21^{c}	13.12±0.55^{d}	0.25±0.83^{d}	0.68±0.36^{c}	ND
BC	23.44±0.88^{a}	105.00±0.44^{a}	20.32±0.96^{a}	51.06±0.09^{a}	22.20±0.86^{a}	0.44±0.36^{a}	0.81±0.03^{a}	ND
BC+GG	24.87±0.69^{a}	107.20±0.62^{a}	22.60±0.90^{a}	43.15±0.69^{b}	19.71±0.81^{b}	0.41±0.25^{b}	0.75±0.61^{b}	ND

BC: biochar; GG: *G. geotrichum*; ND: not detected or not determined in the samples. Each value is expressed as mean±SD of six replicates from three independent experiments. The data were analyzed by ANOVA using SAS. Values in a column followed by different letters show the significant differences (P<0.05) among treatments based on DMRT

Table 4 Functional amino acid contents of soybean in response to the presence and absence of biochar and *G. geotrichum* when applied individually and in combination

Treatment	Asp (μg/g)	Thr (μg/g)	Met (μg/g)	ILE (μg/g)	Ser (μg/g)	Glu (μg/g)	Leu (μg/g)	Tyr (μg/g)	Gly (μg/g)
Control	20.73±2.13^{d}	7.09±0.37^{c}	1.52±0.56^{c}	11.42±0.71^{b}	4.64±0.84^{d}	13.96±1.96^{d}	24.16±2.10^{b}	5.37±0.22^{a}	5.70±0.38^{d}
GG	45.70±5.95^{a}	11.51±1.15^{a}	1.79±0.49^{b}	11.79±1.32^{ab}	9.03±0.75^{a}	20.55±1.45^{b}	26.42±2.64^{b}	5.79±0.14^{a}	8.28±0.15^{b}
BC	31.99±3.78^{c}	9.51±0.76^{b}	1.41±0.07^{d}	8.28±0.96^{c}	5.99±0.25^{c}	18.04±1.15^{c}	30.49±2.90^{a}	0.00±0.00^{b}	6.39±0.95^{c}
BC+GG	36.12±4.71^{b}	9.62±1.45^{b}	2.12±0.86^{a}	12.45±1.24^{a}	6.26±0.31^{b}	30.66±1.76^{a}	22.95±0.00^{c}	0.00±0.00^{b}	11.52±1.08^{a}

Treatment	Phe (μg/g)	Lys (μg/g)	Cys (μg/g)	Val (μg/g)	His (μg/g)	Arg (μg/g)	Pro (μg/g)	Total amino acids (μg/g)
Control	11.39±1.94^{ab}	8.62±0.62^{ab}	2.78±0.63^{d}	10.34±1.51^{b}	3.88±0.41^{b}	10.59±0.92^{c}	7.86±2.81^{b}	150.05^{c}
GG	8.36±1.64^{b}	13.00±1.02^{a}	2.86±0.73^{c}	14.02±3.85^{a}	5.30±0.24^{a}	15.14±1.94^{b}	10.66±2.26^{a}	210.05^{a}
BC	11.03±1.30^{ab}	7.08±0.29^{b}	3.57±0.55^{a}	14.51±2.75^{a}	5.23±0.34^{a}	15.42±2.26^{b}	10.42±1.05^{a}	179.36^{b}
BC+GG	12.22±1.17^{a}	9.80±0.68^{ab}	3.66±0.35^{a}	14.98±2.94^{a}	6.18±0.47^{a}	17.07±1.16^{a}	11.32±2.65^{a}	206.57^{a}

BC: biochar; GG: *G. geotrichum*; Asp: aspartic acid; Thr: threonine; Met: methionine; ILE: isoleucine; Ser: serine; Glu: glutamic acid; Leu: leucine; Tyr: tyrosine; Gly: glycine; Phe: phenylalanine; Lys: lysine; Cys: cysteine; Val: valine; His: histidine; Arg: arginine; Pro: proline. The amino acids were analyzed from freeze-dried soybean plants in the presence and absence of biochar and *G. geotrichum* applied individually and in combination, to identify the beneficial effects of their interactions. Each value is expressed as mean±SD of six replicates from three independent experiments. The data were analyzed by ANOVA using SAS. Values in a column followed by different letters show the significant differences (P<0.05) among treatments based on DMRT

plants treated with BC alone. Furthermore, compared to the control, valine, histidine, and proline levels were found to be statistically the same in treated soybean tissues, and remained significantly higher. In addition, tyrosine was only detected in soybean plants treated with *G. geotrichum* alone and the control.

3.4 Isoflavone regulation in soybean plants in the individual and combined applications of biochar and *G. geotrichum*

Compared to the control plants and those treated individually with *G. geotrichum*, total isoflavone content significantly increased in individual BC and BC+*G. geotrichum* treatments (Table 5). Of the aglycone class of isoflavones, daidzein and glycitein significantly increased in plants treated with BC alone, followed by BC+*G. geotrichum*, compared to the control. However, genistein was only detected in the control, but not in any of the treated soybean plants.

In the malonyl class of isoflavones, malonyldaidzin and malonylgenistin significantly increased compared to the control in the plants treated with BC+*G. geotrichum*, while malonylglycitin increased in plants treated with BC alone. However, none of the isoflavones in the acetyl class was detected in any of the treatments, including the control.

In the glucoside class of isoflavones, only glycitin was detected in all of the treatments, and was significantly higher in plants treated with BC alone and BC+*G. geotrichum* than in the control. Daidzin was not detected in any of the treatments, while genistin was only detected in control plants.

3.5 Saturated and unsaturated fatty acid composition

The analysis of free fatty acid composition showed that palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1) significantly increased in soybean plants treated with BC alone compared to the control (Figs. 2a–2c). In contrast, these fatty acids (C16:0, C18:0, and C18:1) were significantly reduced in soybean plant tissues treated with BC+*G. geotrichum*. However, linoleic (C18:2) and linolenic (C18:3) acids were significantly higher in the BC+*G. geotrichum* treatment compared to the control (Figs. 2d and 2e).

3.6 Total sugar and total phenolic contents and DPPH-scavenging activity

Compared to the control ((0.62±0.02) mg/g), total sugar content was significantly higher in soybean plants treated with BC+*G. geotrichum* ((1.71±0.33) mg/g) and *G. geotrichum* alone ((1.62±0.32) mg/g) (Table 6). Similarly, compared to the control ((300.71±5.13) mg/g), total phenolic content (Table 6) was significantly higher in soybean plants treated with BC+*G. geotrichum* ((407.50±12.90) mg/g) and *G. geotrichum* alone ((389.20±8.30) mg/g).

Table 5 Isoflavone contents of soybean in response to the presence and absence of biochar and *G. geotrichum* applied individually and in combination

Treatment	Isoflavone aglycones (μg/g)			Malonyl isoflavones (μg/g)		
	Daidzein	Glycitein	Genistein	Daidzin	Glycitin	Genistin
Control	95.40±0.03^{c}	144.07±0.04^{b}	1.11±0.67^{a}	4.53±0.08^{c}	20.07±0.88^{b}	31.80±0.42^{c}
GG	95.85±0.17^{c}	148.81±0.22^{b}	ND	4.45±0.00^{c}	20.31±1.03^{b}	34.15±0.24^{c}
BC	111.15±0.08^{a}	183.30±0.26^{a}	ND	11.99±0.02^{b}	24.64±0.06^{a}	42.81±0.10^{b}
BC+GG	105.18±0.07^{b}	148.00±0.31^{b}	ND	29.37±0.20^{a}	21.51±0.21^{b}	77.00±0.35^{a}

Treatment	Acetyl isoflavones (μg/g)		Isoflavone glucosides (μg/g)			Total (μg/g)
	Daidzin	Genistin	Daidzin	Glycitin	Genistin	
Control	ND	ND	ND	0.00±0.00^{c}	0.60±0.09^{a}	297.58±55.74^{b}
GG	ND	ND	ND	2.61±0.06^{b}	ND	306.18±58.84^{b}
BC	ND	ND	ND	3.86±0.24^{a}	ND	377.75±70.38^{a}
BC+GG	ND	ND	ND	4.16±0.19^{a}	ND	385.22±55.69^{a}

BC: biochar; GG: *G. geotrichum*; ND: not detected or not determined in the samples. The isoflavones were analyzed from freeze-dried soybean plants in the presence and absence of biochar and *G. geotrichum* applied individually and in combination, to identify the beneficial effect of their interaction. Each value is expressed as mean±SD of six replicates from three independent experiments. The data were analyzed by ANOVA using SAS. Values in a column followed by different letters show the significant differences (P<0.05) among treatments based on DMRT

Table 6 Biosynthesis of total sugar content, total phenolic content, and DPPH inhibition activity of soybean grown in the presence and absence of biochar and phytohormone-producing endophytic fungus, *G. geotrichum*, when applied individually and in combination

Treatment	Total sugar content (mg/g)	Total phenolic content (mg/g)	DPPH inhibition activity (%)
Control	0.62±0.02[b]	300.70±5.13[b]	51.28
GG	1.62±0.32[a]	389.20±8.30[a]	68.92
BC	0.62±0.15[b]	306.90±4.10[b]	55.28
BC+GG	1.71±0.33[a]	407.50±12.90[a]	73.34

BC: biochar; GG: *G. geotrichum*. Each value is expressed as mean±SD of six replicates from three independent experiments. The data were analyzed by ANOVA using SAS. Values in a column followed by different letters show the significant differences (P<0.05) among treatments based on DMRT

DPPH-scavenging activity is used to measure the antioxidant properties of plants or their food product and hence the nutraceutical importance of plants is indexed and recommended for human food consumption. In the light of this importance, this parameter was measured. Therefore, compared to the control (51.28%), the highest percentage of DPPH-scavenging activity was obtained for soybean plants treated with BC+*G. geotrichum* (73.34%) and *G. geotrichum* (68.92%) alone (Table 6).

4 Discussion

The significant increase in a basic element, namely N (Fig. 1a), in soybean treated with BC+

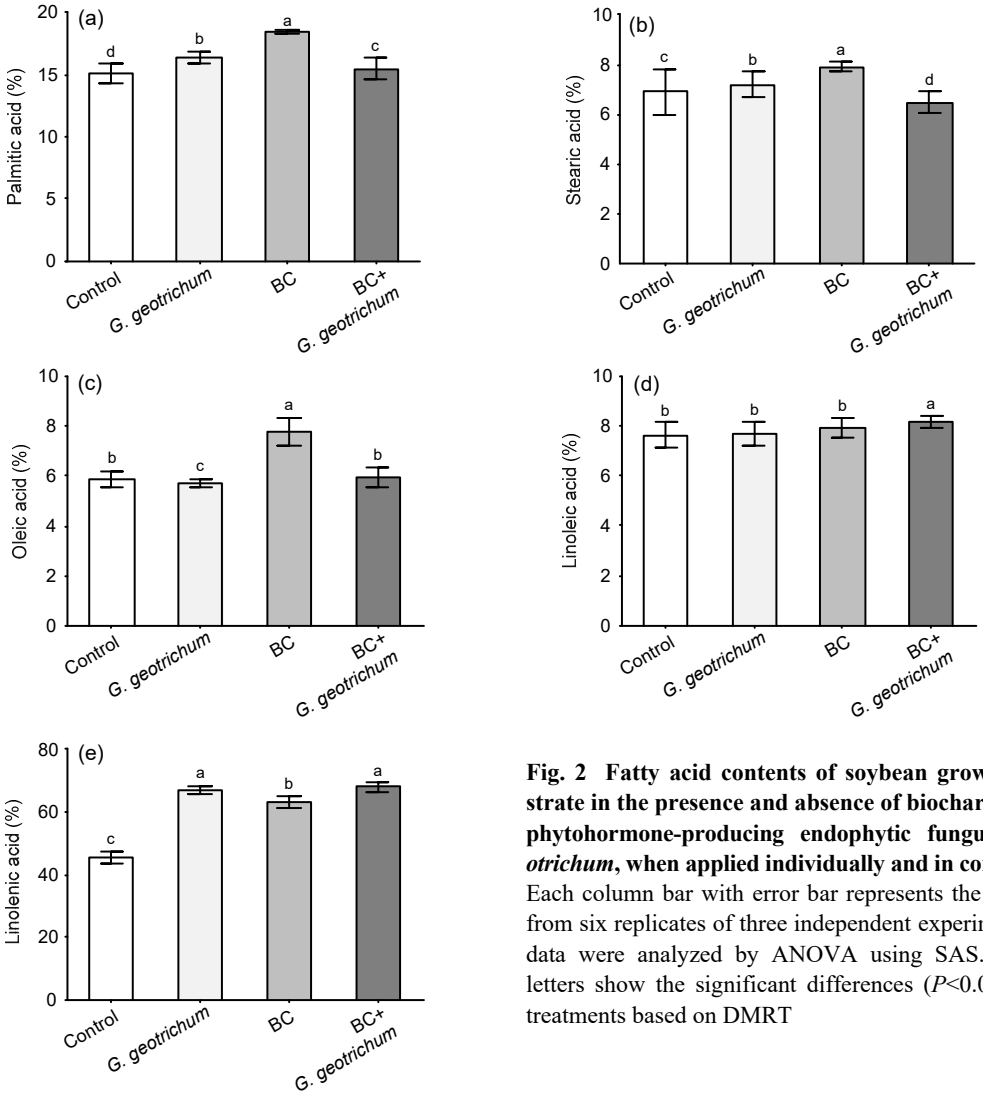

Fig. 2 Fatty acid contents of soybean grown in substrate in the presence and absence of biochar (BC) and phytohormone-producing endophytic fungus, *G. geotrichum*, when applied individually and in combination
Each column bar with error bar represents the mean±SD from six replicates of three independent experiments. The data were analyzed by ANOVA using SAS. Different letters show the significant differences (P<0.05) among treatments based on DMRT

G. geotrichum may be attributed to the additive effect of the plant growth promoting effect of BC and the phytohormone-producing endophytic fungus *G. geotrichum* (Waqas *et al*., 2014). Both BC and, particularly, endophytes have been reported to improve basic elemental composition under normal and stressful conditions (Waqas *et al*., 2012; Khan and Lee, 2013; Zhang *et al*., 2014). For instance, it was found that the optimization of composted green waste for optimum plant growth of *Calathea insignis* was best obtained with 20% BC and 0.7% humic acid, which, compared to the control, significantly increased the total N content of *C. insignis* leaves by 66.4% (Zhang *et al*., 2014). In addition to this property of BC, the combined application of endophytes in the current study may further enhance the assimilation of N, as also reported in Khan and Lee (2013). This paper reported that endophytic association offered by *Penicillium funiculosum* increased the N assimilation of the host soybean plants, maintaining high growth under normal and stressed conditions (copper heavy metal). Furthermore, high N levels would enhance plant metabolism and fix more atmospheric C via photosynthesis (Evans, 1989; Newman *et al*., 2003; Heinonsalo *et al*., 2015). The high assimilation of CO_2 depends on N and its allocation in plant organs. This phenomenon was observed in Scots pine, in which more C was allocated to the roots than to the shoots, which was because of the presence of more N in the roots (Heinonsalo *et al*., 2015).

Most published studies have shown that the BC contains significant amounts of macro- and micronutrients, with the amount depending on the feedstock source, which leads to variation in the extent to which soil nutrient status is improved (Martinsen *et al*., 2014; Waqas *et al*., 2014; Zhao *et al*., 2014; Butnan *et al*., 2015; Haider *et al*., 2015). The high amount of these nutrients in BC-amended substrate (Table 1) may be because they were present in the BC (see materials and methods) applied in this experiment and, therefore, had an advantage over the control and only endophyte-treated soils (Zhao *et al*., 2014; Butnan *et al*., 2015). However, the TBT substrate used does not simulate the real situation as the common soil constituents could interact with plant and endophytic fungi. BC also indirectly enhances soil nutrient availability by modifying soil structure, reducing Al^{3+} solubility, increasing soil pH, and increasing cation exchange capacity (Martinsen *et al*., 2014; Waqas *et al*., 2014; Zhao *et al*., 2014; Butnan *et al*., 2015). However, the high P availability in BC-amended soil detected in our study supports the findings of Zhai *et al*. (2015) but contradicts those of Ahmed and Schoenau (2015). The reason for this discrepancy with Ahmed and Schoenau (2015) may be differences in the characteristics of the feedstock used, the pyrolysis process used for BC preparation, or soil temperature conditions. BC derived from various feed stock sources exhibits high variation in nutrient content; thus BC sourced from a different feed stock may show the same or opposite behavior. Therefore, the hardwood-derived BC in the current study showed the same behavior of increasing soil P availability as that of the maize residue BC used by Zhai *et al*. (2015), but is different from the BC derived from wheat straw, flax straw, and willow stems used by Ahmed and Schoenau (2015). Compared to the control, the lowest amount of nutrients was obtained in the substrate of soybean plants treated with *G. geotrichum* alone, followed by BC+*G. geotrichum*, and finally the control. These results demonstrate the nutrient uptake ability of *G. geotrichum*. As a result, the macro- and micronutrient statuses of endophyte-amended substrate in the presence and absence of BC were diminished, while their concentrations increased in soybean, as shown by the analysis of the root and shoot tissues (Tables 2 and 3). Here, the *G. geotrichum* may have acted as a biostimulating organism and therefore more macro- and micronutrients were assimilated in the shoot from the additional provided source in the form of BC. These findings support those of Waqas *et al*. (2012), Khan and Lee (2013), and Hammer *et al*. (2014), who reported that endophytes increase the ability of host plants to uptake macro- and micronutrients, particularly P, K, S, and Ca. For example, Hammer *et al*. (2014) provided the first evidence of the ability of a plant-associated fungus to enhance nutrient transfer from BC to the host plant.

In the current study, most nutrients were retained in the roots of *G. geotrichum* and BC+*G. geotrichum* treatment, as shown in the analysis comparing the amount of nutrients present in the shoots of the same plants. The translocation of nutrients inside the plant organs of endophyte-infected plants supports our previous results regarding the uptake of Zn during heavy metal stress (Waqas *et al*., 2014). Zn was

significantly higher in endophyte-treated roots compared to the roots in the other treatments (Waqas *et al*., 2014).

Amino acids are the building blocks of proteins and contribute to several important metabolic functions during normal and stress conditions in plants (Khan *et al*., 2013; Tegeder, 2014; Waqas *et al*., 2015). Amino acid synthesis depends on photosynthetic activity, which, in turn, depends on other factors, including nutrient availability (Weckopp and Kopriva, 2015). N and S are the two major nutrients that plants use for the synthesis of amino acids (Khan *et al*., 2013; Tegeder, 2014). Here, compared with the control, higher amounts of amino acids were synthesized (Table 4) in soybean plants treated with BC+*G. geotrichum* and individually with *G. geotrichum* and BC, which may be because of the greater uptakes of S and N and other supporting nutrients, like Mg (component of chlorophyll compound structure), due to greater availability. The significant amounts of amino acids in soybean shoots treated with BC+endophyte (*G. geotrichum*) and *G. geotrichum* alone corroborate the results of Khan *et al*. (2013), Khan and Lee (2013), and Waqas *et al*. (2015). The studies by these authors showed that the application of endophytes extends their beneficial effects by regulating amino acid production under normal and stress conditions. The regulation of amino acid synthesis subsequently mitigates and confers (abiotic and biotic) stress resistance in pepper, soybean, and sunflower, in addition to enhancing their anti-oxidative and hormone signaling activities (Khan *et al*., 2013; Khan and Lee, 2013; Waqas *et al*., 2015). In the case of amino acid up-regulation, soybean shoots treated with BC via individual application contributed more nutrients to plants (Elad *et al*., 2011). An increase in amino acid levels allows soybean plants to carry out secondary metabolite formation efficiently, and synthesize more vitamins (like vitamins B_1, B_2, B_3, B_5, B_7, B_9, and E) and proteins (Tzin and Galili, 2010; Miret and Munné-Bosch, 2014). The up- or down-regulation of both N and S limits the function of other nutrients (McGrath and Zhao, 1996). The application of BC may also serve as an additional balanced source of S and N provisions (Elad *et al*., 2011; Cheah *et al*., 2014; Waqas *et al*., 2014). The role of both nutrients is central and interrelated to amino acid biosynthesis and, hence, proteins (McGrath and Zhao, 1996).

Isoflavones are the main secondary metabolites of legumes, and are synthesized through the phenylpropanoid pathway (Khan *et al*., 2013; Algar *et al*., 2014). Isoflavones have several important functions during host plant environmental interactions. For example, isoflavones induce nodulation gene expression to facilitate the association of biological nitrogen fixing bacteria with legume crops, and they produce phytoalexins for defense against insects and pathogens (Algar *et al*., 2014; Ramos-Solano *et al*., 2015). Furthermore to reduce stress damaging effects, flavonoids act as antioxidant agents to neutralize reactive oxygen species and maintain the normal functioning of the plant cellular membrane (Khan *et al*., 2013). The enhanced production of isoflavones (Table 5) in soybean treated with BC and BC+*G. geotrichum* demonstrates treatment efficacy, and that they may contribute to plant nutritional value and defense requirements. Because they are secondary metabolite products, isoflavonoids are strongly induced by external stimuli to keep plants in prime condition (Hao *et al*., 2010; Ramos-Solano *et al*., 2015). Here, the enhanced production of isoflavonoids in the presence of BC alone or in combination with the fungal endophyte (*G. geotrichum*) demonstrates the priming effects of both treatments, supporting the findings of Hao *et al*. (2010), Khan *et al*. (2013), and Waqas *et al*. (2014). Waqas *et al*. (2014) reported that the priming effect of BC alone or in combination with fungal endophytes keeps soybean plants in a steady state, and improves growth and development under high Zn heavy metal stress. Moreover, Elad *et al*. (2011) and Harel *et al*. (2012) explored the possible involvement of the BC priming effect in eliciting the systemic acquired resistance (SAR) and induced systemic resistance (ISR) pathways. The results of these two studies showed that BC removes biotic stress caused to strawberry plants by necrotrophic, hemi-biotrophic, and biotrophic pathogens by inducing general defense pathways. Thus, in addition to fungal endophytes, BC may contribute to the elicitation of the flavonoid/phenylpropanoid pathway, which may explain the enhanced defense mechanism in strawberry observed by Harel *et al*. (2012).

Fatty acids have many important roles in maintaining the normal functioning of plant physiological processes during biotic and abiotic stress (Upchurch, 2008; Steindal *et al*., 2015). During necrotrophic

pathogen and pests attack, the membrane lipid releases α-linolenic acid (a precursor molecule of phytooxylipin) to initiate the synthesis of jasmonic acids for defense purposes. Similarly, in *Arabidopsis*, oleic acid in the chloroplast mediates pathogen attack by the normal expression of the defense response (Upchurch, 2008). The results of the current investigation indicate that saturated fatty acids significantly increased in plants treated with BC alone, but showed the opposite trend for BC+*G. geotrichum* (Fig. 2). However, the unsaturated fatty acids were significantly higher in plants treated with BC+*G. geotrichum*. These observations show that the fatty acid profile is highly responsive to agronomic practices. Among agronomic practices, optimum nutrient management and the supply of adequate moisture are very important for plant growth and have a profound effect on plant yield and quality (Bellaloui *et al*., 2011; 2015). Bellaloui *et al*. (2011) conducted an experiment to evaluate the response of fatty acid composition to different agronomic (irrigated and non-irrigated) conditions along with the individual and combined dosages of S and N. It was concluded that the nutritional composition of soybean seeds was altered due to differences in the treatments, with it possible to tailor the desirable treatment to the specific requirement. The similar application and optimization of BC (i.e. in the presence and absence of endophytes in this study) to field crops could provide another way of improving the nutrient value of agricultural produce. In the current study, the combination of gibberellin and IAA-producing endophyte (e.g. *G. geotrichum*) with BC increased fatty acid content; however, various studies obtained the same results for the association of endophytic fungi with their host plants (Khan *et al*., 2012a; 2012b). In these and other studies, the production of secondary metabolites (mainly phytohormones) by endophytes was found to be the main reason for this effect (Khan *et al*., 2012a; 2012b; Jusoh *et al*., 2015). Furthermore, GA production by endophytes (Khan *et al*., 2012a; 2012b) and the exogenous application of IAA (Jusoh *et al*., 2015) have been reported to modulate fatty acids in plants under normal and stress conditions. Both types of phytohormones were detected in *G. geotrichum* (Waqas *et al*., 2014) and may be factors for the same results being reported.

Soybean is one of the most important sources of carbohydrate (sugar) and phenolic compounds with antioxidant activity (Malenčić *et al*., 2008; Bellaloui *et al*., 2015). It is important to mention that the modulation in phenolic compounds due to different treatments was measured in term of DPPH-scavenging activity and evaluation of the antioxidative properties. The DPPH free radicals readily accept reactive hydrogen or electrons and become a stable diamagnetic compound. In this manner, the unwanted production of reactive oxygen species is controlled to prevent damage to cellular organelles and the structural integrity of cells under stress conditions (Izuta *et al*., 2009). The enhancements of total sugar content, phenolic content, and DPPH-scavenging activity of soybean plants (Table 6) in response to BC+*G. geotrichum* and *G. geotrichum* alone support the results of Obledo *et al*. (2003), Huang *et al.* (2007a; 2007b), Pańka *et al*. (2013), and Patel *et al*. (2015). During a five-year experiment, Patel *et al*. (2015) reported that continuous organic amendments enhanced produce quality through building and sustaining soil health and productivity. The same building and conditioning characteristics have been reported for BC in agricultural soil, along with the long term provision of nutrients through the slow release mechanism (Lehmann, 2007; Elad *et al*., 2011; Martinsen *et al*., 2014; Zhang *et al*., 2014). This phenomenon might explain why BC applied in combination with an endophyte showed a synergistic effect in the current experiment. However, several studies have reported that endophytes alone improve plant primary and secondary metabolites (Obledo *et al*., 2003; Huang *et al*., 2007a; 2007b; Khan and Lee, 2013). The increased accumulations of total sugar content, phenolic content, and DPPH-scavenging activity in soybean biomass after treatment with BC+*G. geotrichum* or *G. geotrichum* alone demonstrate that both factors have a priming effect on plants, readying them for disturbance by abiotic and biotic stress (Porcel and Ruiz-Lozano, 2004; Huang *et al*., 2007b).

5 Conclusions

Sequestration of increasing atmospheric carbon (C) is highly desirable for a safe and sustainable environment in the current industrial era. BC and bioactive fungal endophytes have recently been confirmed as a stable source for mitigating C in terrestrial

ecosystems (Lehmann, 2007; Iqbal *et al*., 2012) and stimulating plant growth, but have been previously much less reported for their ability in combination to enhance plant nutrient uptake and the nutritional status. To investigate these issues, we determined from the results of this study that the combined and individual applications of BC (hardwood-derived) and a bioactive endophyte (*G. geotrichum* WLL1) significantly improved the assimilation and uptakes of basic, macro- and micronutrients in soybean. The ensuing beneficial effect was then observed by the improved biosynthesis of functional amino acids and nutritional characteristics, including isoflavone regulation, saturated and unsaturated fatty acid contents, total sugar and total phenolic contents, and antioxidative properties based on DPPH-scavenging activity. It may be concluded that the symbiotic association of bioactive fungal endophytes with BC generated additive effects, making them more effective in combination than individually. Thus, it is recommended that endophytes should be applied with BC to enhance soybean crop quality by creating a more desirable environment-friendly conditions in a highly enriched carbon concentrated atmosphere. However, to provide recommendations, field-scale studies should be conducted first to ensure that the BC used is compatible (type-wise by origin/substrate and preparation or pyrolysis condition) with the selected endophyte.

Compliance with ethics guidelines

Muhammad WAQAS, Yoon-Ha KIM, Abdul Latif KHAN, Raheem SHAHZAD, Sajjad ASAF, Muhammad HAMAYUN, Sang-Mo KANG, Muhammad Aaqil KHAN, and In-Jung LEE declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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List of electronic supplementary materials

Table S1 Details of GC-MS conditions for the analysis of fatty acids

中文概要

题　目：生物炭和内生菌促进大豆增加养分吸收和调节营养参数的叠加作用

目　的：探讨提高大豆作物品质的方法。

创新点：研究了生物炭和植物内生菌联合使用对大豆营养吸收和营养品质的叠加作用。

方　法：采用硬木生物炭和半乳糖霉菌（*Galactomyces geotrichum* WLL1）对大豆进行处理，按照处理方式的不同分成四组，包括对照组（无处理）、*G. geotrichum* 处理组、生物炭处理组和生物炭与 *G. geotrichum* 联合处理组。通过对比研究生物炭和内生菌对大豆宏量营养素和微量营养素的吸收和同化的作用，并观察其对功能性氨基酸、异黄酮、脂肪酸组成、总糖含量、总酚含量和 1,1-二苯基苦基苯肼（DPPH）自由基清除能力的影响。

结　论：研究结果发现生物炭和内生菌单独或联合处理均能增加大豆养分的吸收，促进功能性氨基酸的合成，并提升大豆营养品质。同时，生物炭是一种额外的营养源，而内生菌能产生生物刺激效应，两者联合使用具有叠加作用，比单独使用更加有效。

关键词：植物内生菌；养分吸收；同化；营养品质；大豆