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Title of the paper

Effect of biochar application on mineral and microbial properties of soils growing different plant species

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ABSTRACT

Biochar is widely used as a soil amendment to increase crop yields. However, the details of its impact on soil properties have not been fully understood. A pot experiment was conducted using soybean and sorghum under four soil treatment combinations (cattle farmyard manure with or without biochar and rapeseed cake with or without biochar) to elucidate the mechanisms of its beneficial effects on plant growth in terms of the microbial community structure and mineral availability in soils with different types of organic manure application. The application of biochar significantly increased the growth of both species, particularly sorghum with rapeseed cake application by 1.48 times higher than that without biochar. Microbial activity in soil was also enhanced by biochar application in both species with rapeseed cake application, particularly in sorghum. Principal component analysis using Biolog EcoPlate^{TM} data indicated that biochar application changed the microbial community structure in soil, particularly sorghum grown soil. The changes in microbial community structure in sorghum were considered to be at least partly affected by changes in soil pH due to interaction between plant and biochar under organic manure application. Biochar application had little effect on the profile of ammonium-acetate-extractable mineral elements in soil including calcium, potassium, magnesium, sodium, and sulphur with both types of manure application under soybean. Under sorghum, however, biochar with rapeseed cake manure application altered the profile. This alteration is attributable to an increase in the extractable concentration of certain metals in the soil including aluminum, cadmium, and zinc, possibly caused by enhanced organic matter decomposition producing metal-chelating organic compounds. These different changes in the soil properties by biochar application may be directly or indirectly related to the different growth responses of different plant species to biochar application under organic manure application.

Keywords: microbial activity, mineral element, organic manure, sorghum, wood biochar

1. Introduction

Driven by population growth, increased human pressure on land has forced the conversion of natural landscapes into agricultural fields while simultaneously depleting the land under agricultural use (Lal 2009). Therefore, there is an urgent need to establish effective agricultural management practices that not only increase food production but also prevent the negative environmental impacts of intensive agriculture. There are various fertilizers and soil amendments that are able to improve soil fertility and crop productivity. Fertilizers are necessary to increase crop production, and are supplied mainly in the form of chemical amendments. However, the continuous and excessive use of chemical fertilizers may result in environmental pollution. In addition, agriculture largely depending on chemical fertilizers is not sustainable in terms of a shortage of their resources and high energy costs for their production (Ryan *et al.* 2012; Woods *et al.* 2010). Organic fertilizers may be alternatively used as chemical fertilizers; such organic fertilizers can also act as soil amendments which improve the physical, chemical, and biological properties of soil. However, organic fertilizers are less effective than chemical fertilizers because nitrogen (N) and phosphorus (P) mainly occur in organic forms in organic fertilizer.

Biochar is a product of the thermal degradation of organic material under oxygen-limited conditions. With respect to appearance, it is similar to charcoal produced by natural burning; however, it is distinguished by its use as a soil amendment (Sohi *et al.* 2009; Lehmann and Joseph 2009). Many studies have shown the beneficial effects of biochar on soil chemical properties such as pH (Topoliantz *et al.* 2007; Masulili *et al.* 2010; Yuan *et al.* 2011), nutrient availability (Chan *et al.* 2008; Haefele *et al.* 2008), nutrient retention (Glaser *et al.* 2002; Lehmann *et al.* 2003), and cation-exchange capacity (Glaser *et al.* 2002; Masulili *et al*. 2010; Yuan *et al*. 2011). Improvements in the growth and yield of plants following biochar application have also been reported in various crop species, including cowpea and rice (Lehmann *et al*. 2003), radish (Chan *et al*. 2008), soybean (Tagoe *et al*. 2007), and maize (Yamato *et al*. 2006).

Recently, the use of organic fertilizers in soil to increase crop productivity has received considerable attention. The incorporation of organic fertilizers is a useful approach for maintaining organic matter content in soil and thereby enhance soil biological activity and increase nutrient content, which, in turn, contributes to increasing crop productivity (Dikinya and Mufwanzala 2010; Diacono and Montemurro 2010). However, in order to supply available nutrients to plants, organic fertilizers need to be mineralized by soil microorganisms. Biochars have been shown to have a positive effect on soil fertility and plant growth (as described above); however, little information is available on their effects when combined with manure, particularly in terms of the mineral and microbial properties of soil. Therefore, this study assessed the effects of biochar on the microbial community structure and mineral availability in soils growing different crop species under different organic manure treatments.

2. Materials and methods

2.1. Experimental setup

A pot experiment was conducted using soybean and sorghum under four soil treatment combinations (cattle farmyard manure with/without biochar and rapeseed cake with/without biochar) to examine the effects of wood biochar on the microbial community structure and mineral availability in soils (Table 1). Soils (Gleyic Fluvisol) were collected from the 0–25 cm layer at the experimental farm of Hokkaido University. The soil was air-dried and passed through a 2.0 mm mesh screen. Then, 0.8 L of soil and 0.8 L of perlite were mixed and placed in a plastic pot (1.6 L). Since soil used in a preliminary experiment showed a compacted layer and little penetration of plant root, we mixed soil with perlite to improve soil physical conditions. The biochar used in this experiment was purchased from Shimokawa City Forest Organization Carbon Industry and was produced from broad-leaved trees at 400°C; the biochar had a Carbon (C) content of 71.8%. The amount of fine biochar (<0.25 mm) applied to each pot was 28 g (equivalent to a field application rate of 35 t ha^{-1}). Two different types of organic fertilizer were used: cattle farmyard manure (0.8% N, 1.7% P_2O_5, and 1.8% K_2O) and rapeseed cake (5.3% N, 2.0% P_2O_5, and 1.0% K_2O); 31.75 g of cattle farmyard manure (providing 0.254 g N, 0.540 g P_2O_5, and 0.572 g K_2O) or 4.793 g of rapeseed cake (providing 0.254 g N, 0.096 g P_2O_5, and 0.048 g K_2O) was applied to each pot. In treatments with rapeseed cake, 0.878 g of calcium superphosphate and 0.381 g of

K_2SO_4 were added to make the application rates of each of N, P_2O_5, and K_2O equal to 100 kg ha^{-1}. These organic fertilizers were used in the present study because they are very common in crop cultivation but their chemical properties differ remarkably. Chemical properties of biochar and each organic manure used in this study were shown in the Supplementary material, Table S1. After mixing of the soil, perlite, fertilizer, and biochar, the pots were incubated for 4 weeks in a greenhouse under moderately moist conditions (40–60% of field capacity depending on the soil condition).

Seeds of soybean (*Glycine max* (L.) Merr. cv. Toyoharuka) and sorghum (*Sorghum bicolor* (L.) Moench cv. Hybrid Sorgo) were sterilized with 10% (v/v) NaClO solution for 1 min and then rinsed in deionized water. The seeds were sown and germinated in vermiculite. After the first two leaves appeared (post 10–12 days), two of each species were transplanted to each pot. Depending on the soil condition during the experiment, all pots were then watered with deionized water to 40–60% of their field capacity. The pot experiment was performed for 30 days (November 15–December 12, 2015) for soybean and 40 days (November 15–December 23, 2015) for sorghum, according to their growth rates in the vegetative stage. The experiment was conducted in a greenhouse at an almost constant average temperature of 25°C.

2.2. Soil sampling and analysis

Soil samples were collected at the time of plant sampling (30 and 40 days after sowing for soybean and sorghum, respectively). After removing the plants (as described later), the soil in each pot was mixed and remaining roots were removed. Fresh soil was taken for determining the microbial community structure and activity analyses using EcoPlate^{TM} (Biolog Inc., CA, USA). The remaining soil was air-dried and sieved for chemical analysis. EcoPlate contains three replicate wells of 31 of different carbon sources and water (no substrate; tetrazolium dye only as a blank) for soil community analysis. For assessing microbial carbon utilization patterns, a 1 g soil sample was thoroughly shaken by hand with 10 ml of sterile saline solution (0.85% NaCl) and diluted 1000 times with the same saline solution. A subsample of 150 μL was inoculated directly into each well of the EcoPlate. Three replicate suspensions were prepared for each soil sample. The EcoPlates were then placed in an incubator at 25 °C; purple color was formed when the microbes utilized the carbon source and began to respire. The color development was measured every 24 h for 5–6 days using a microplate reader (Sunrise Remote, TECAN A-5082, Austria) at 595 nm. Changes in the pattern were compared and analyzed using principle components analysis (PCA). The average well color development (AWCD) in each plate, which indicates microbial activity, was calculated as follows:

$$AWCD = [\Sigma(R_i - C)]/31,$$

where R_i and C are the optical density (OD) values at 595 nm of the response wells (containing sole carbon sources) and the control well (water), respectively. A high value of AWCD reflects high microbial activity.

Soil pH (H_2O) was determined at a soil/water ratio of 1:2.5 using a pH meter (Mettler Toledo, MP220, 2005). For determination of inorganic N (NH_4-N and NO_3-N) concentration in the soils, 4 g samples were extracted with 40 mL of 2 M KCl by shaking for 1 h. The soil extracts were passed through filter paper (No. 6, Advantec, Tokyo, Japan).

The NH_4-N and NO_3-N concentrations were determined by colorimetric methods. Available P was extracted with Truog’s solution and measured by spectrophotometry (U-5100, HITACHI, Japan) at 710 nm. Excluding inorganic N and available P, the concentrations of mineral elements in the soil were determined by extracting 2 g of soil with 40 mL of 1 M ammonium acetate, shaking for 30 min, and passing through fine filter paper (No. 5C, Advantec, Tokyo, Japan) to exclude fine soil particles. Thereafter, 5 mL of the filtrate was digested with 2 mL of 61% HNO_3 as described later. The concentrations of aluminum (Al), arsenic (As), boron (B), calcium (Ca), cadmium (Cd), cobalt (Co), cesium (Cs), copper (Cu), iron (Fe), potassium (K), lithium (Li), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), nickel (Ni), rubidium (Rb), sulphur (S), selenium (Se), strontium (Sr), vanadium (V), and zinc (Zn) in the digested solution were measured using inductively coupled plasma mass spectrometry (ICP-MS) (ELAN, DRC-e, Perkin Elmer, MA, USA).

2.3. Plant sampling and analysis

Plants were harvested at the end of the vegetative growth. Roots of the plants were washed clean with tap water. The plants were then separated, washed with de-ionized water, and dried in an oven at $70^{o}C$ for 7 days, before being weighed and ground for mineral analysis. The concentrations of mineral elements in the plant samples were determined as described in Watanabe *et al.* (2015). Briefly, plant samples were digested in 2 mL of 61% HNO_3 (EL grade, Kanto Chemical, Tokyo, Japan) at $110^{o}C$ in a DigiPREP apparatus (SCP Science, QC, Canada) for approximately 2 h until the solution had almost disappeared. After the samples had cooled, 0.5 mL H_2O_2 30% (semiconductor grade, Santoku Chemical, Tokyo, Japan) was added, and the samples were heated at 110^{o}C for a further 20 min. Once digestion was complete, the tubes were cooled and made up to a volume of 10 mL by adding 2% HNO_3. The concentrations of elements were measured using ICP-MS.

2.4. Data analysis

All experimental data were statistically analyzed using Minitab 16 (Minitab, Inc, United States). Analysis of variance (ANOVA) followed by Tukey's test were used to detect significant differences among treatments. To compare the results of the treatments with and without biochar, paired Student's t-tests were applied. PCA was used to profile the microbial communities and minerals in the soils.

3. Results

3.1. Growth and mineral accumulation of plants

The total dry weight of both the plant species grown with rapeseed cake significantly increased by the biochar application, particularly for sorghum (1.21 and 1.48 times higher than that without biochar for soybean and sorghum, respectively) (Fig. 1). A similar trend was also found for cattle farmyard manure; however, this was not statistically significant (Fig. 1). The concentrations of some metal elements in leaf material are shown in Table 2. Overall, irrespective of the plant species and manure type, the biochar application decreased or did not affect the concentration of these elements.

3.2. General chemical properties of soil

Soil pH was higher in soil receiving cattle farmyard manure (Table 3). The biochar application significantly increased soil pH under sorghum with each type of organic manure, but not in soybean. The concentration of inorganic N (NH_4-N and NO_3-N) did not differ significantly between the treatments (Table 3). Available P concentration was higher in soils receiving cattle farmyard manure; however, no biochar effect was observed for either plant grown soil (Table 3). The concentration of ammonium-acetate-extractable S in soil increased under sorghum because of biochar application with rapeseed cake (Table 3).

3.3. Microbial activity and community structure of soil

The microbial activity in soil was estimated by AWCD. A high value of AWCD reflects higher microbial activity. Figure 2 presents the AWCD values obtained from the EcoPlate. For both soybean and sorghum grown soils with rapeseed cake, biochar application significantly increased AWCD compared to the case without biochar. When PCA was conducted to assess the utilization patterns of the different carbon sources, the total variance explained by the first two components was 39% and 44% for soybean and sorghum, respectively. In the soybean grown soil, the score plot of PCA showed a separation between the B0M2 treatment and the other treatments in PC1 (Fig. 3). Meanwhile, in the score plot of the sorghum grown soil, the soil with biochar application shifted negatively along PC1 in both the cattle farmyard manure and rapeseed cake treatments (Fig. 3).

3.4. Mineral profile of soil

PCA was also used to examine the treatment effects on the mineral profile of the soils. Figure 4 shows the score plot of the first two components from the ammonium-acetate-extractable concentrations of each mineral element in soils growing soybean or sorghum. The first two components accounted for 67% and 92% of the total variance for the soybean and sorghum soils, respectively. In both species, a clear separation in the score plot was observed between the different types of organic fertilizer applied to the soil (Fig 4). Moreover, the biochar application altered the profile of the extractable mineral elements in the sorghum grown soil with rapeseed cake (Fig. 4), whereas it did not affect that in the soybean grown soil (Fig. 4).

4. Discussion

It has been reported that the positive effect of biochar on plant growth may be related to the nutrient-retention capacity of biochar (Glaser *et al*. 2002) and its sorption capacity for toxic metals and some phytotoxic compounds (Hille and den Ouden 2005; Lair *et al*. 2006). In the present study, the biochar application significantly enhanced the growth of both the plant species grown in soils with rapeseed cake, and the same trend was also observed in their growth with cattle farmyard manure (Fig. 1), indicating that the combined application of organic manure with biochar is effective at increasing crop yield. This growth promotion effect was more remarkable in the soil with rapeseed cake for sorghum. Therefore, different plant species as well as different types of organic manure may affect soil-biochar interactions differently. This raises the question regarding the factors causing these differences.

In both soybean and sorghum grown soils, AWCD-estimated microbial activity was increased by biochar application with rapeseed cake (Fig. 2). It has been reported that biochar provides a suitable habitat for microorganisms (Pietikainen *et al*. 2000) and produces substances that stimulate the growth of microbes (Kasozi *et al*. 2010). Rapeseed cake may have suitable characteristics for the exertion of these positive effects of biochar on microorganisms. The enhanced microbial activity can be expected to enhance the mineralization of rapeseed cake applied to soils. However, significant differences were not found for both plant species in inorganic N and available P concentrations between soils with and without biochar in the rapeseed cake treatment. By contrast, a significant increase was found in concentrations of extractable S, including SO_4^{2-} and soluble organic S such as S-containing amino acids, because of biochar application under sorghum in the rapeseed cake treatment (Table 3). Moreover, significant positive correlation was found between extractable S concentration in soil and utilization (absorbance) for phenylethyl-amine in the EcoPlate in sorghum with rapeseed cake application (r = 0.94, Supplementary material, Figure S1), but not in soybean. These results imply that the biochar enhanced the microbial decomposition of organic matter, containing organic S in this soil, resulting in superior growth of the sorghum. In fact, for the rapeseed cake treatments, S concentration in the leaves of sorghum significantly increased by biochar application (data not shown, Student's *t*-test, $P < 0.05$). Considerable difference in soil extractable S between soybean and sorghum (Table 3) may imply considerable difference in the impact of plant root on microbial community structure between different plant species as described later. Although we cannot know exactly why significant effect of biochar application was observed only in S but not in N and P, leaching and active absorption by root in N and fixation by soil in P may be involved in these different results.

It has also been suggested that biochar may change the soil microbial community structure (Lehmann *et al.* 2011). In the present study, PCA of the EcoPlate data demonstrated that biochar application clearly changed the microbial community structure, particularly in sorghum grown soils (Fig. 3). Correlation analysis was conducted to determine the factor(s) responsible for biochar-induced changes in the microbial community structure. In the PCA, the PC1 scores using the EcoPlate showed a strong negative correlation ($r = -0.89$, $n = 16$) with soil pH for sorghum but not for soybean (which showed a weak positive correlation, $r = 0.72$, $n = 16$) (Fig. 5). In fact, biochar application did not significantly affect soil pH under soybean but increased it under sorghum (Table 2). Although biochar is commonly alkaline and has a potential to increase the soil pH, the effect depends on the types of biochar (Yang *et al.* 2016). Moreover, changes in soil pH by biochar application may also be affected by microbial activities such as nitrification which produces H^+ (Yuan *et al.* 2011). The positive correlation between soil pH and NH_4/NO_3 ratio observed only in sorghum grown soil (Supplementary material, Figure S2) supports the involvement of nitrification in different responses of soil pH to biochar application between soybean and sorghum. Together, some interactions between the sorghum rhizosphere and biochar may affect soil pH; this may be the primary factor altering the microbial community structure in soils. Under soybean, biochar application had little effect on the profile of ammonium-acetate-extractable mineral elements of the soil for both types of manure application (Fig. 4). For sorghum, however, biochar application altered the profile of the extractable elements in the soil applied with rapeseed cake (Fig. 4). This alteration was mainly due to the increase in the extractable concentrations of certain metals in soils due to biochar application (Table 4). The biochar application increased soil pH in sorghum grown soil applied with rapeseed cake (Table 3), which cannot explain the results of the extractable metals in this study because increasing the pH normally decreases the availability of certain metal cations such as Al and Cd (von Uexküll and Mutert 1995; Xian and In Shokohifard 1989). In order to increase the solubility of these metal cations even at increased pH condition, some chelating compounds could be needed.

In contrast to the effects of biochar on soil, concentrations of these metals in the leaves of sorghum grown in the soil with rapeseed cake did not change, or they tended to show a decrease due to the biochar application, particularly in Al (Table 2). In case of Al, it is well known that organic ligands, such as organic acids can solubilize Al (Huang and Keller 1971) but also inactivate it for plant uptake (Ma and Hiradate 2000) in soil. Because biochar application increased microbial activity in the soils applied with rapeseed cake (Fig. 2), it possibly enhanced organic matter decomposition in this soil, producing chelating organic compounds such as organic acid that solubilized some metals but also made those metals less available to sorghum roots. In fact, when analyzing the correlation between extractable concentration of each of these metal elements and utilization (absorbance) for each carbon source in the EcoPlate, highly significant correlation ($P < 0.01$) was found in several carbon sources (4-hydroxy benzoic acid and Al/Ba; phenylethyl-amine and Zn; α-D-lactose and Zn) only in sorghum grown soil with rapeseed cake application (Supplementary material, Figure S1). These carbon utilization characteristics of microbial community in sorghum grown soil with rapeseed cake might be related to the production of chelating compounds from soil organic matter to solubilize certain metals in soil.

In conclusion, biochar application can be an important agricultural practice for increasing the efficiency of organic manure for crop cultivation. However, its effects differ depending on the plant species and organic manure type. These differences may be attributed to the complicated interactions between the plant rhizosphere, biochar, organic manure, and soil microorganisms. In order to elucidate these interactions, detailed analysis of the dynamics of microorganisms and organic/inorganic substances in the rhizosphere of soils applied with different types of organic manure is needed.

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Figure legends

Figure 1. Dry weight of soybean and sorghum. ■ and □ indicate root and shoot, respectively. The error bars represent the standard error of the mean (n = 4). * indicates a significant difference between treatments with and without biochar in each organic manure treatment (M1 or M2) ($P < 0.05$, Student’s *t*-test). Relative value of the B1 treatment to the B0 treatment is indicated on the bar of B1 in each manure treatment. B0: without biochar; B1: with biochar; M1: cattle farmyard manure; M2: rapeseed cake.

Figure 2. AWCD values of soybean and sorghum grown soils. Error bars represent the standard error of the mean (n = 4). * indicates a significant difference between treatments with and without biochar in each organic manure treatment (M1 or

M2) ($P < 0.05$, Student's *t*-test). B0: without biochar; B1: with biochar; M1: cattle farmyard manure; M2: rapeseed cake. Data recorded 144 and 120 h after the incubation started were used for soybean and sorghum, respectively.

Figure 3. Principal component analysis of carbon source utilization activity (EcoPlate) in soybean and sorghum grown soils. B0: without biochar; B1: with biochar; M1: cattle farmyard manure; M2: rapeseed cake. Data recorded at 144 and 120 h after the incubation started were used for soybean and sorghum, respectively.

Figure 4. Principal component analysis of ammonium acetate-extractable mineral elements in soybean and sorghum grown soils. B0: without biochar; B1: with biochar; M1: cattle farmyard manure; M2: rapeseed cake.

Figure 5. Correlation of soil pH with PC1 or PC2 of PCA ($P < 0.01$) in the EcoPlate for soybean and sorghum grown soils.

Table 1. Treatments in this study.

Treatment	Description
B0M1	no biochar + cattle farmyard manure
B1M1	biochar + cattle farmyard manure
B0M2	no biochar + rapeseed cake
B1M2	biochar + rapeseed cake

Table 2. Concentration (mg kg^{-1} dry weight) of some metal elements in plant leaf.

		Al			Ba			Cd			Co			Sr			Zn		
Soybean	B0M1	55.33	±6.48	c	79.4	±5.9	a	0.261	±0.018	ab	0.175	±0.006	b	91.2	±5.5	a	154.0	±8.4	a
	B1M1	70.71	±3.55	bc	48.7	±5.0	b	0.164	±0.013	c	0.181	±0.006	b	71.2	±2.6	b	124.7	±8.3	b
	B0M2	94.16	±4.29	a	13.3	±0.3	c	0.303	±0.007	a	0.223	±0.011	a	79.4	±1.7	ab	173.9	±3.1	ab
	B1M2	85.61	±4.97	ab	20.2	±0.6	c	0.244	±0.004	b	0.204	±0.007	ab	70.5	±0.7	b	148.9	±9.5	c
Sorghum	B0M1	65.38	±7.90	b	14.8	±0.9	a	2.11	±0.22	bc	0.080	±0.006	b	24.7	±1.1	ab	108.6	±3.9	a
	B1M1	40.71	±2.42	b	14.5	±0.8	a	1.71	±0.09	c	0.055	±0.003	b	22.0	±1.3	b	79.4	±3.9	b
	B0M2	360.54	±41.32	a	8.4	±0.8	b	2.81	±0.13	a	0.308	±0.028	a	28.3	±0.5	a	114.3	±3.2	a
	B1M2	61.92	±4.77	b	7.7	±0.4	b	2.61	±0.07	ab	0.068	±0.004	b	24.9	±0.7	ab	93.9	±1.9	c

Different letters indicate significant difference at $P<0.05$ in each species (Tukey's multiple range test).

Table 3. pH and concentration (mg kg^{-1} dry soil) of NH_4-N, NO_3-N, Truog-P, and ammonium acetate-extractable S in soil.

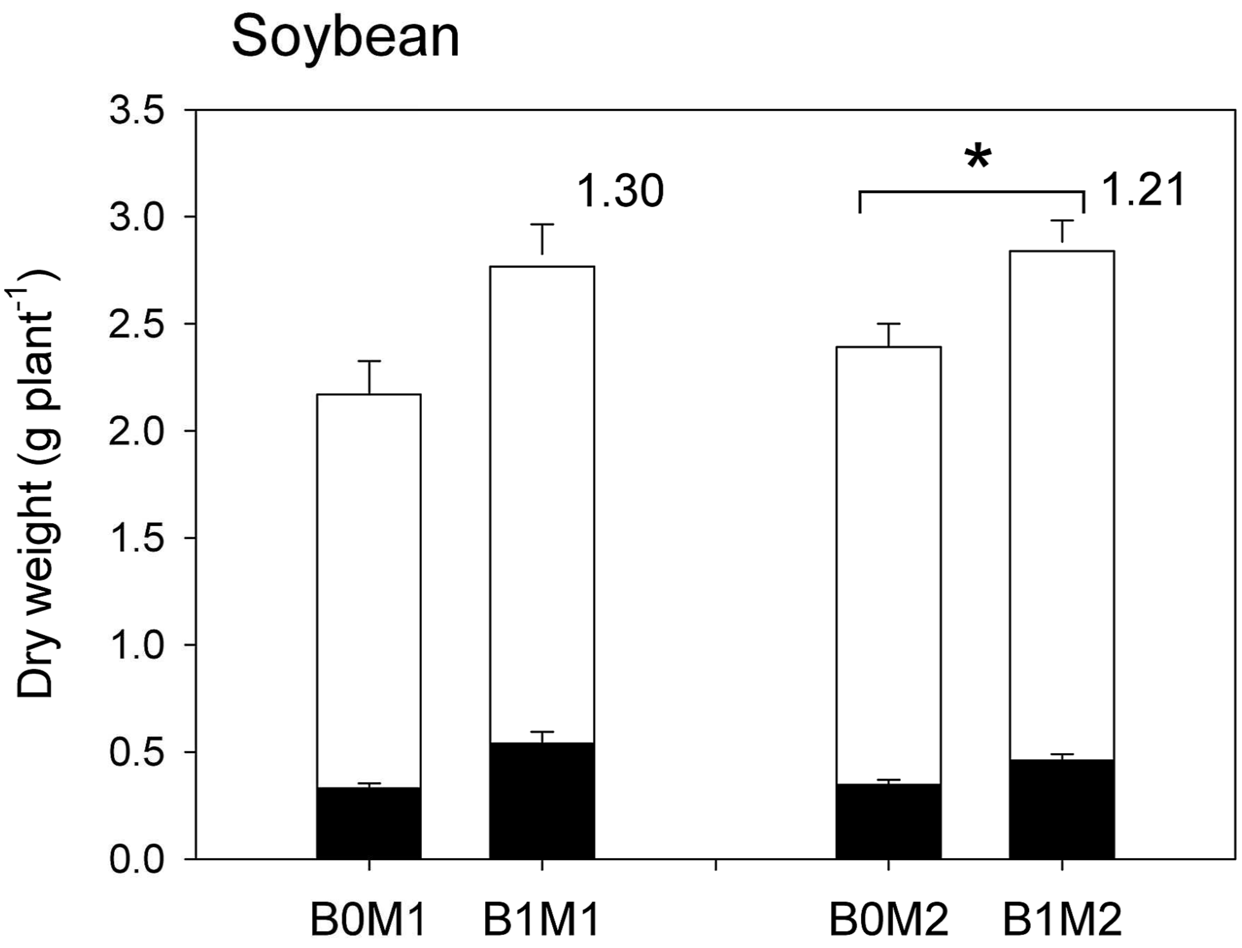
		pH			NH_4-N			NO_3-N			P			S		
Soybean	B0M1	5.26	±0.04	a	105.3	±20.1	a	14.9	±0.9	a	546	±17	a	91	±5	c
	B1M1	5.41	±0.06	a	85.6	±20.8	a	9.0	±2.0	b	568	±22	a	98	±2	c
	B0M2	4.88	±0.02	b	38.7	±10.3	a	13.3	±1.1	ab	287	±12	b	362	±13	a
	B1M2	4.99	±0.02	b	47.6	±8.2	a	10.4	±1.3	ab	267	±5	b	303	±6	b
Sorghum	B0M1	5.23	±0.03	b	38.1	±5.2	a	21.0	±1.3	ab	595	±24	a	92	±2	b
	B1M1	5.47	±0.03	a	33.1	±1.7	a	12.2	±0.7	b	601	±20	a	109	±7	b
	B0M2	4.81	±0.02	d	22.8	±3.1	a	28.8	±3.9	a	293	±23	b	1506	±418	b
	B1M2	4.98	±0.01	c	34.9	±5.0	a	21.8	±2.4	ab	278	±9	b	9245	±2390	a

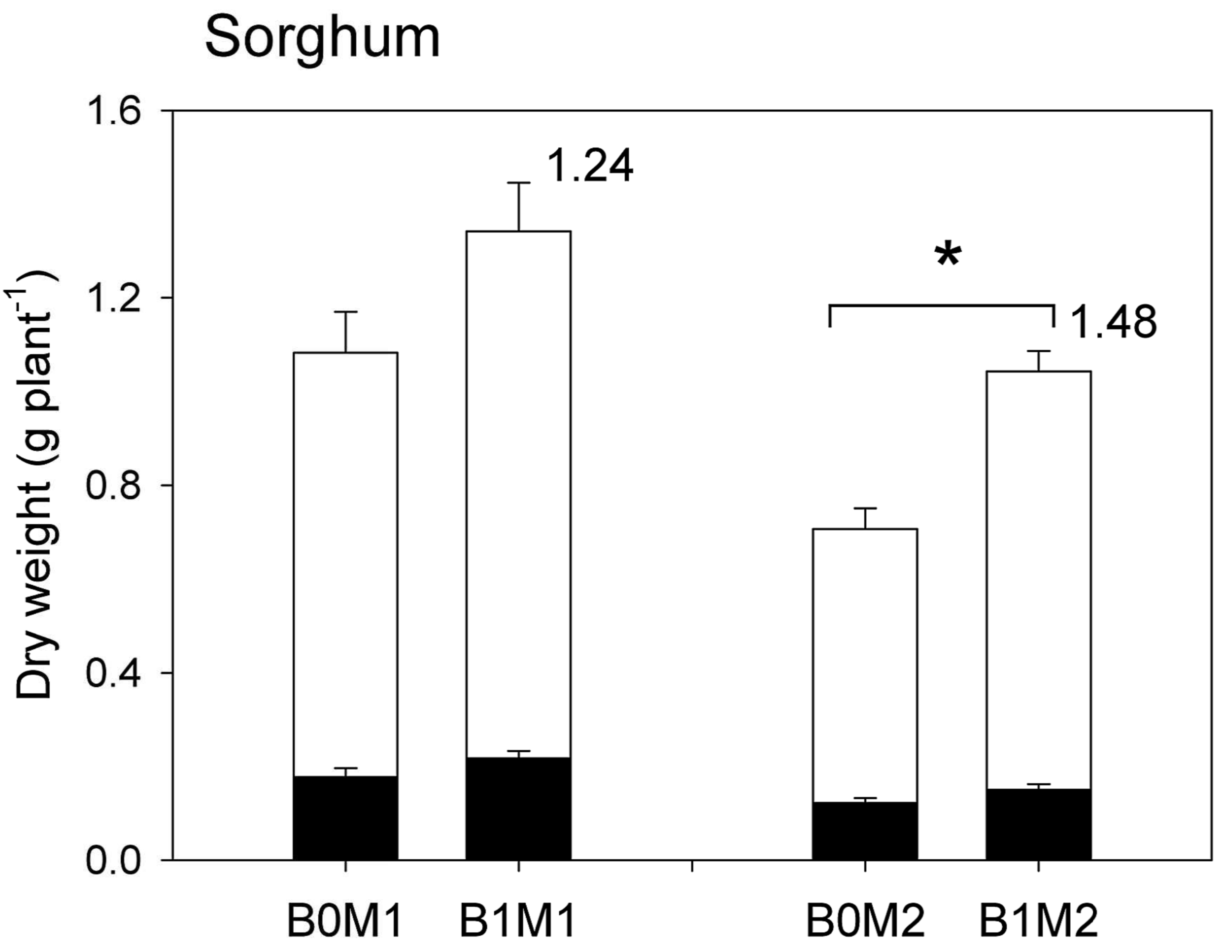
Different letters indicate significant difference at $P<0.05$ in each species (Tukey's multiple range test).

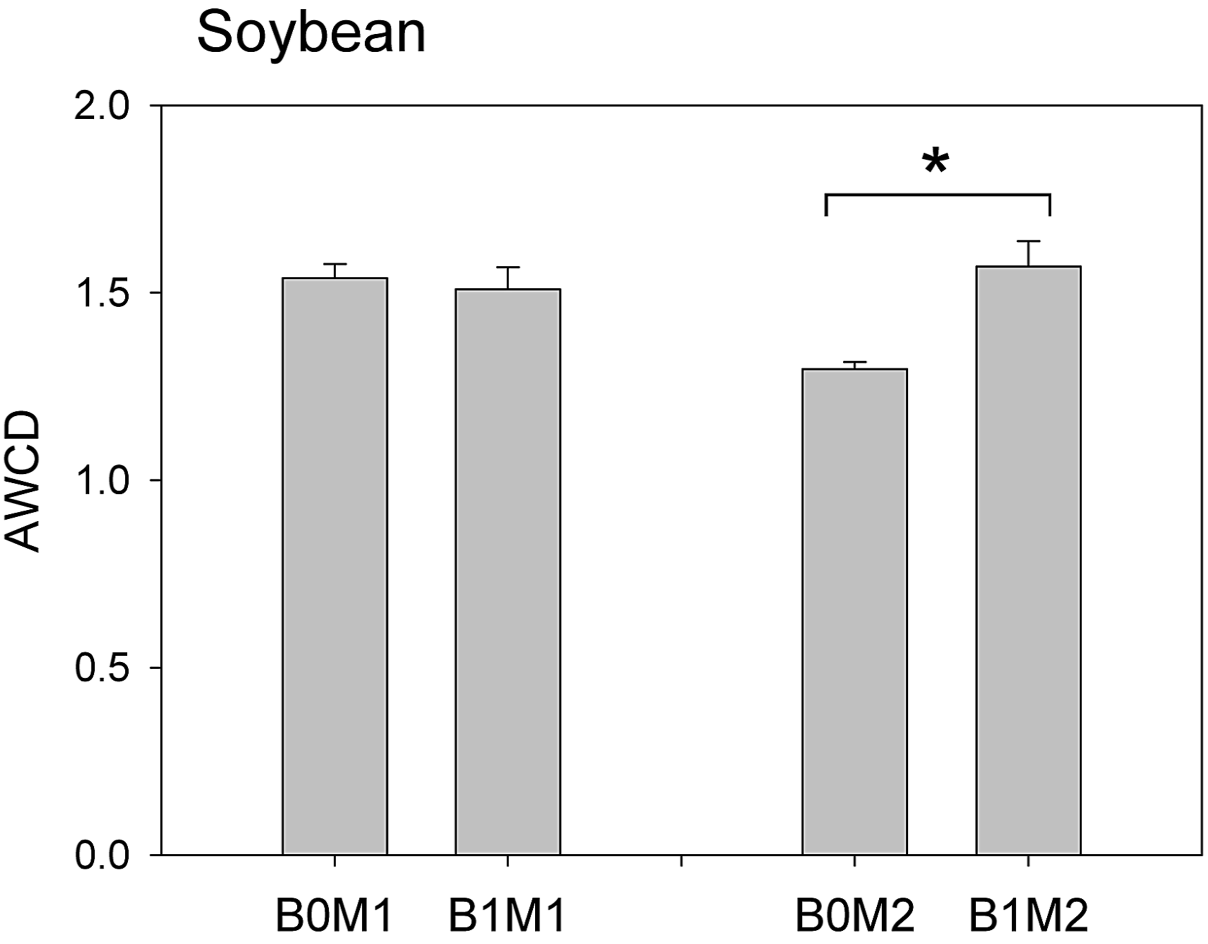
Table 4. Concentration (mg kg^{-1} dry soil) of some metal elements in the soil extract.

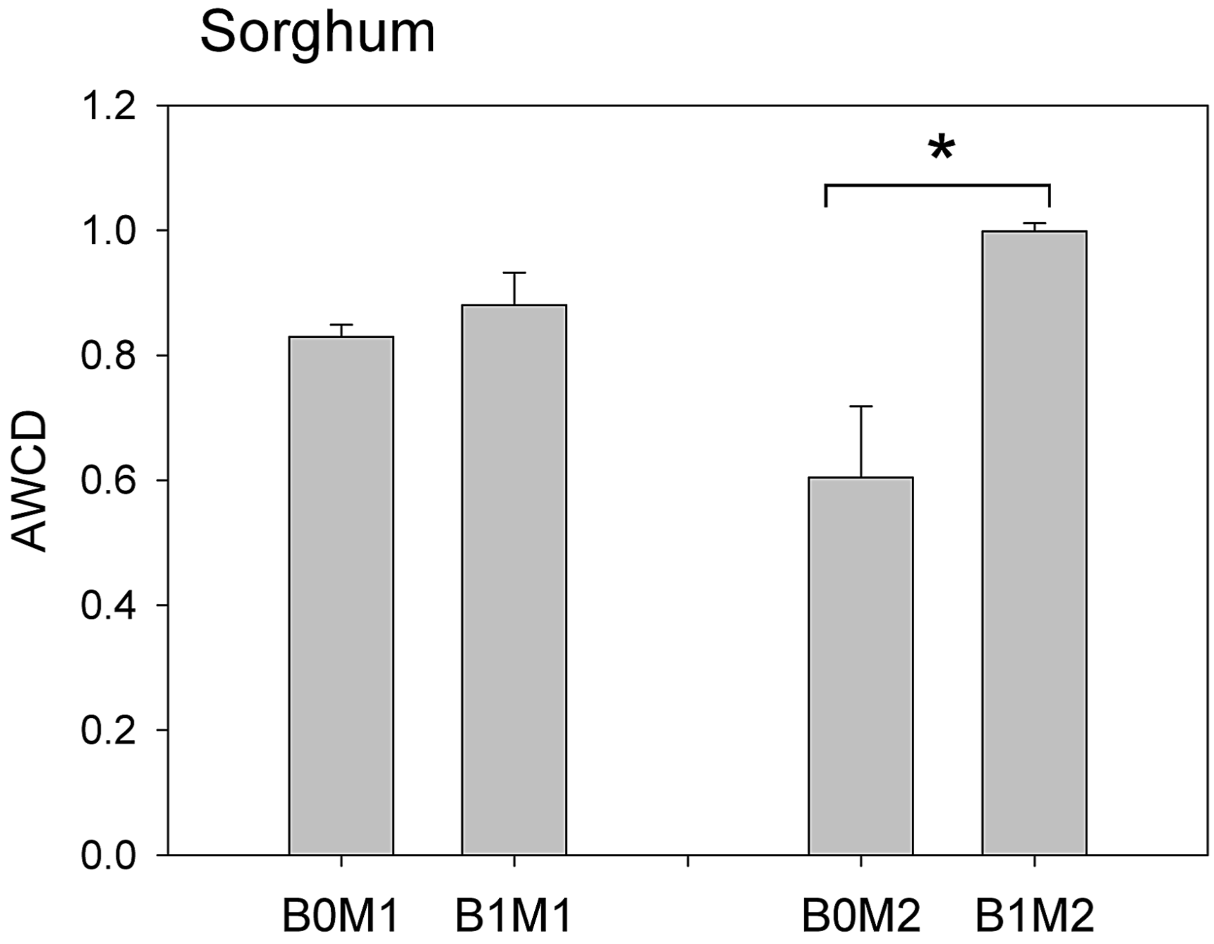
		Al			Ba			Cd			Co			Sr			Zn		
Soybean	B0M1	5.20	±0.59	a	30.0	±1.3	b	0.064	±0.003	b	0.026	±0.002	bc	14.0	±0.6	b	1.19	±0.29	c
	B1M1	4.23	±1.26	a	26.7	±1.8	b	0.060	±0.002	b	0.022	±0.000	c	14.0	±0.7	b	3.95	±0.67	a
	B0M2	3.85	±0.81	a	38.3	±1.9	a	0.100	±0.006	a	0.035	±0.002	ab	19.5	±0.9	a	1.49	±0.44	bc
	B1M2	4.11	±0.37	a	38.4	±1.5	a	0.098	±0.009	a	0.032	±0.003	ab	19.4	±1.4	a	0.63	±0.20	c
Sorghum	B0M1	3.84	±0.47	c	31.6	±0.5	b	0.084	±0.001	c	0.033	±0.001	b	17.2	±0.3	c	1.86	±0.47	c
	B1M1	3.96	±0.59	c	38.4	±3.7	b	0.093	±0.011	c	0.030	±0.003	b	20.2	±1.8	c	2.16	±0.61	c
	B0M2	11.05	±1.20	b	173.9	±41.1	b	0.327	±0.037	b	0.103	±0.012	a	51.0	±5.4	b	11.47	±0.66	b
	B1M2	18.61	±1.80	a	1528.5	±403.8	a	0.543	±0.066	a	0.127	±0.014	a	83.7	±9.9	a	17.51	±1.68	a

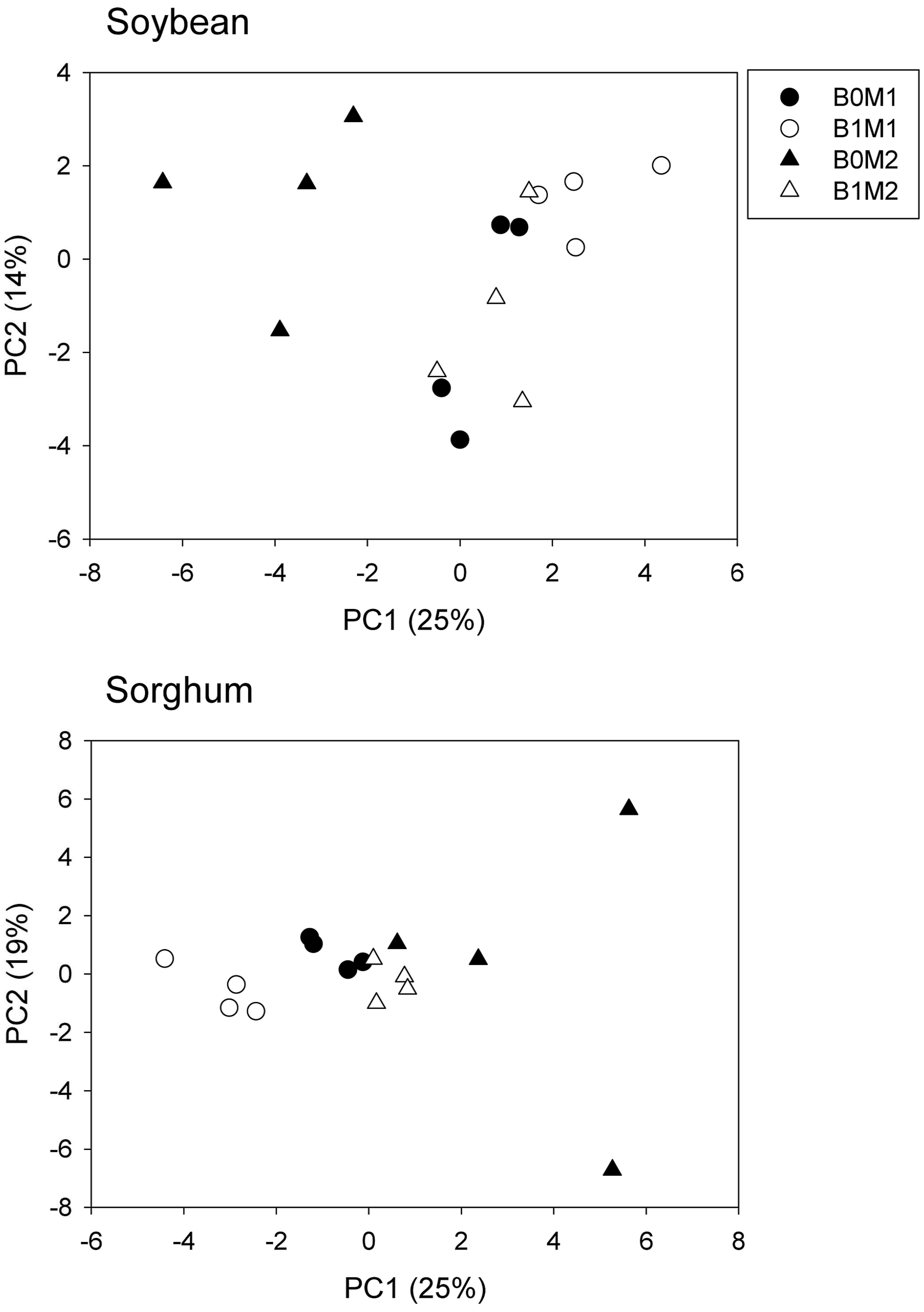
Different letters indicate significant difference at $P<0.05$ in each species (Tukey's multiple range test).

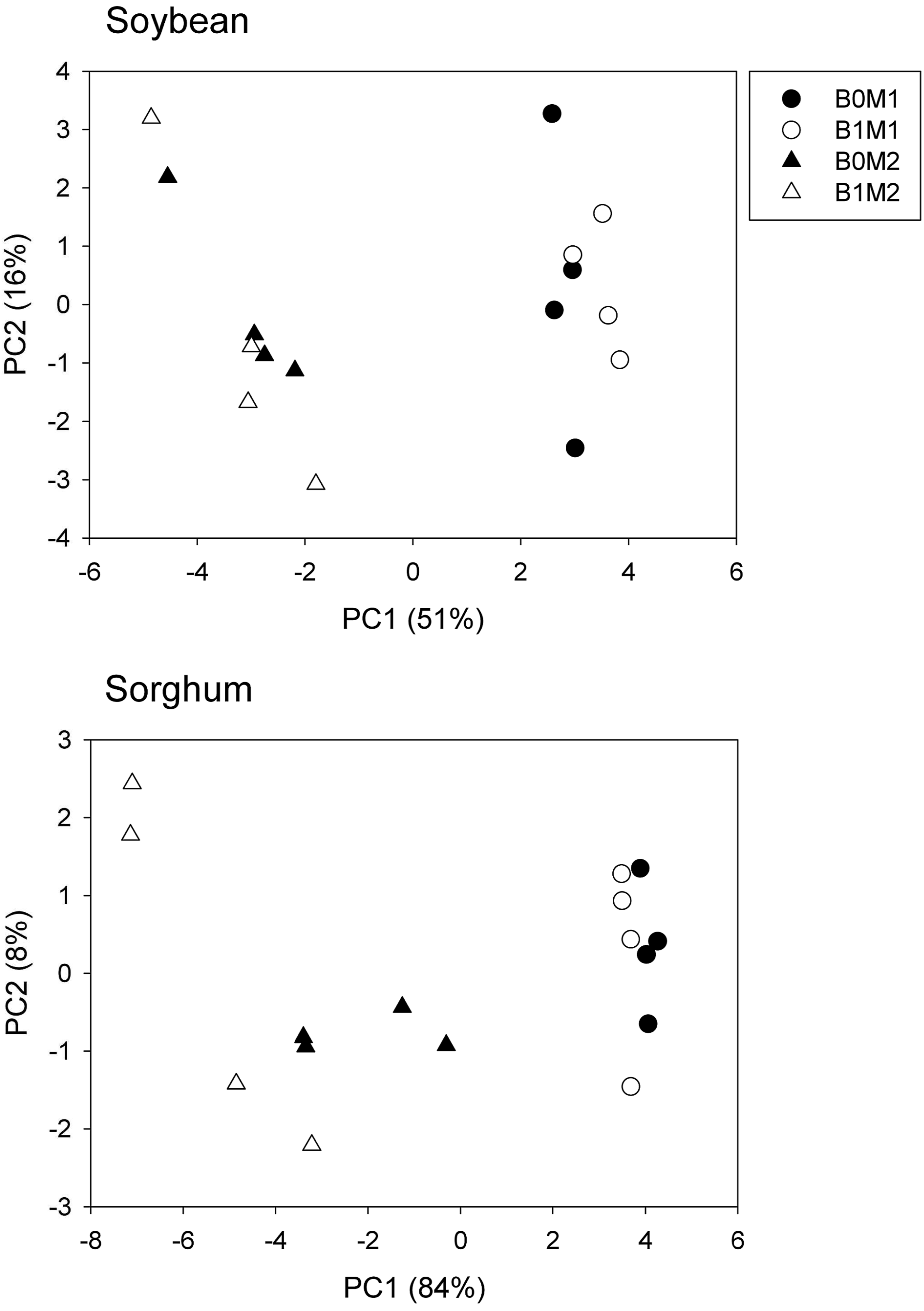

Soybean
Dry weight (g plant^{-1})
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1.30
*
1.21
B0M1
B1M1
B0M2
B1M2

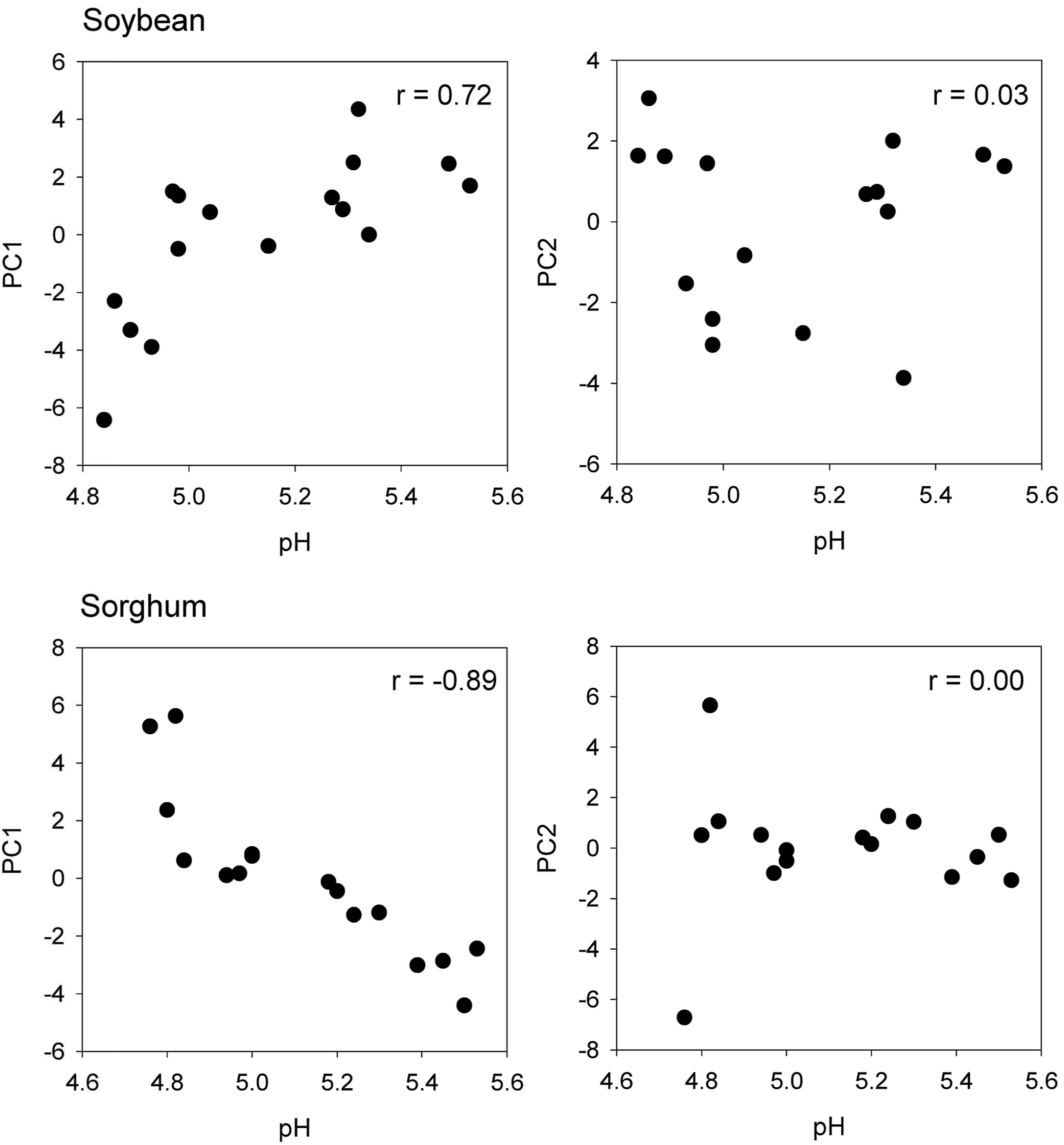

Sorghum
Dry weight (g plant^{-1})
1.6
1.2
0.8
0.4
0.0
1.24
*
1.48
B0M1
B1M1
B0M2
B1M2

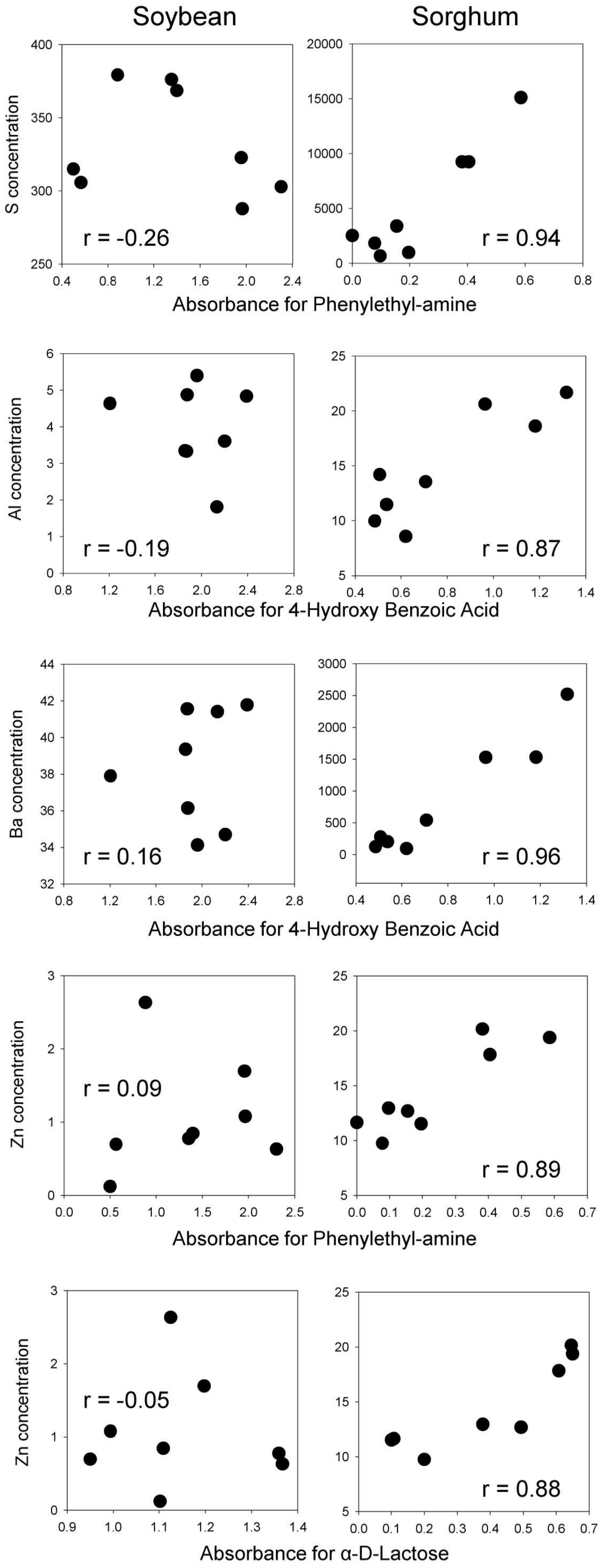

Soybean
AWCD
2.0
1.5
1.0
0.5
0.0
*
B0M1
B1M1
B0M2
B1M2


Sorghum
AWCD
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
B0M1
B1M1
B0M2
B1M2

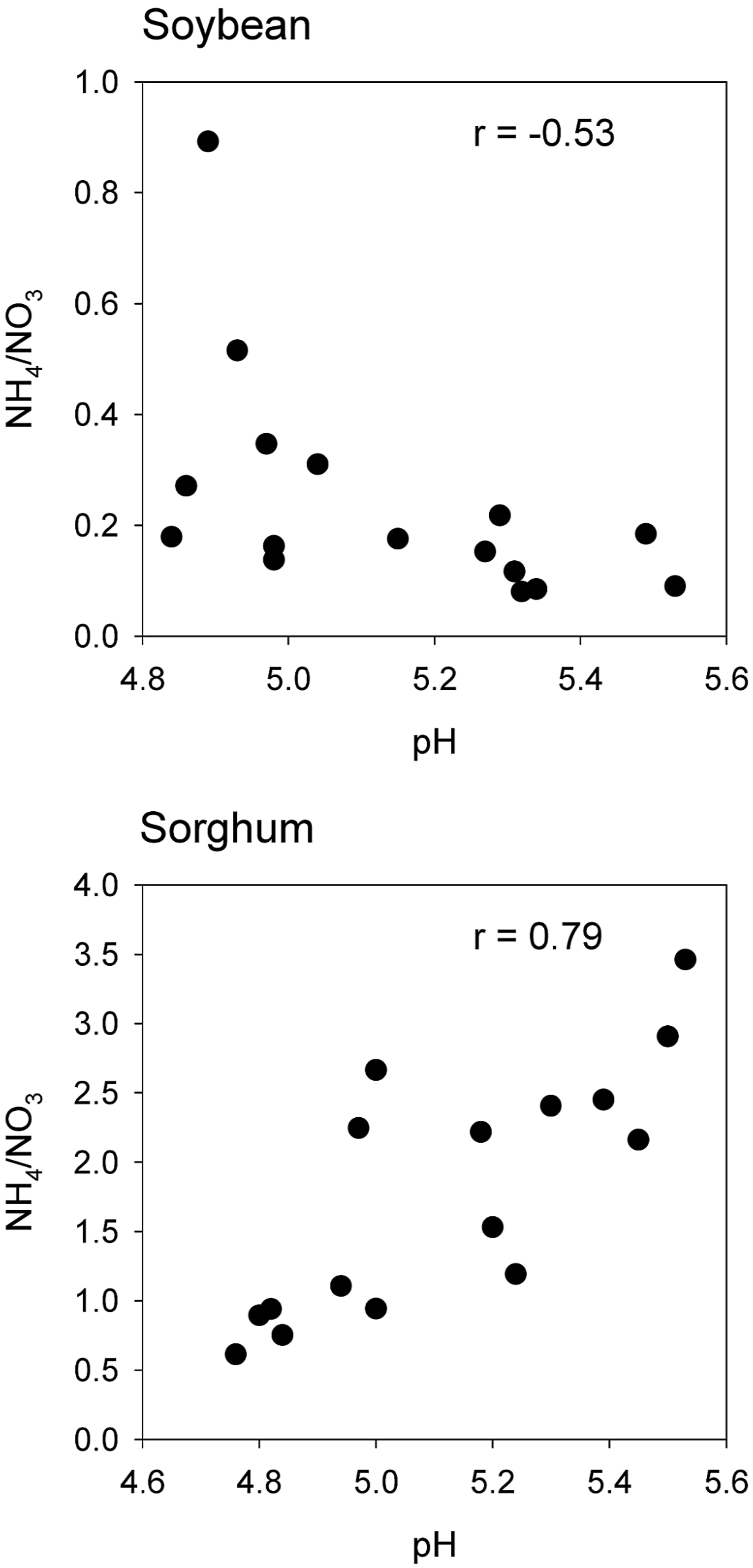

Soybean
B0M1
B1M1
B0M2
B1M2
PC2 (14%)
4
2
0
-2
-4
-6
-8
-6
-4
-2
0
2
4
6
PC1 (25%)
Sorghum
PC2 (19%)
8
6
4
2
0
-2
-4
-6
-8
-6
-4
-2
0
2
4
6
8
PC1 (25%)


Soybean
4
3
2
1
0
-1
-2
-3
-4
PC2 (16%)
-6
-4
-2
0
2
4
6
PC1 (51%)
● B0M1
○ B1M1
▲ B0M2
△ B1M2
Sorghum
3
2
1
0
-1
-2
-3
PC2 (8%)
-8
-6
-4
-2
0
2
4
6
PC1 (84%)


Soybean
r = 0.72
PC1
6
4
2
0
-2
-4
-6
-8
4.8
5.0
5.2
5.4
5.6
pH
r = 0.03
PC2
4
2
0
-2
-4
-6
4.8
5.0
5.2
5.4
5.6
pH
Sorghum
r = -0.89
PC1
8
6
4
2
0
-2
-4
-6
4.6
4.8
5.0
5.2
5.4
5.6
pH
r = 0.00
PC2
8
6
4
2
0
-2
-4
-6
-8
4.6
4.8
5.0
5.2
5.4
5.6
pH



Supplemental Figure S1. Correlation between concentration of extractable element (mg kg^{-1} dry soil) in soil and utilization (absorbance in Biolog Ecoplate) of carbon source in microbial community of soil with rapeseed cake (including both with and without biochar). Only pairs with significant positive correlation (*P* < 0.01) were shown.



Supplemental Figure S2. Correlation between pH and NH_4/NO_3 ratio in soils.