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Full Length Article

Impacts of Biochar Amended Soils on Ryegrass (*Lolium perenne*) Growth under Different Water Stress Conditions

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Abstract

Improvement of soil water capacity is a critical technology to decrease the plant growth restriction by water stress in the arid and semi-arid region. The influences of biochar amended soils (which were incubated for 11 months) on the ryegrass growth at different clipping stages and water stress conditions were determined. Biochar amendment did not influence the plant germination. Better improvement effects on plant height and biomass were found in the Shahuang soil (M soil) with lower fertility than that in the Dark Loessial soil (B soil) at the first clipping stage and under different water stress condition at the second clipping stage. The biochar application rate of 8 g kg^{-1} appeared to get better improvement effects than the control and 16 g kg^{-1}. This study showed that biochar application could be used as the soil amendment in the Loess Plateau to weaken the restriction of water shortage and promote plant growth. © 2016 Friends Science Publishers

Keywords: Biochar; Ryegrass; Soil amendment; Water stress; Loess plateau

Introduction

The application of biochar into soils showed both the ecological and agricultural benefits from its carbon stability and other physical and chemical properties. Biochar in agriculture is expected to lead to the formation of a carbon sink in soil and be an effective amelioration of the soil chemical properties in highly weathered infertile tropical soils (Masahide *et al.,* 2006). Biochar application had the improvement effects on soil water condition that increase the water retention capacity (Chen *et al.,* 2010; Laird *et al.,* 2010; Fellet *et al.,* 2011) and the effective soil water content (Chen *et al.*, 2010).

Positive effects of biochar application on plant growth have been reported in much of the former research (Masahide *et al.*, 2006; Oguntunde *et al.*, 2004; Vaccari *et al.*, 2011). The application of bark charcoal under a fertilized condition in tropical soils significantly increased the yields of maize and peanut, and the root amount in maize (Masahide *et al*., 2006). Huang *et al.* (2011) demonstrated that ryegrass biomass significantly increased along with the application rate when grew in the biochar treatment red soil with low organic content. The improvement of soil water holding capacity may be one of the main mechanisms for plant yield increase (Jeffery *et al.*, 2011; Chen *et al.*, 2010).

The soil desiccation research found that the dried soil layers widely spread in the Loess Plateau in the arid and semiarid region. Soil desiccation had negative effects on water cycle in soils, which would reduce the anti-drought capacity of plants, influence the plant growth and natural succession of vegetation, and limit the development and sustainability of the local ecological environment (Chen *et al.*, 2008; Wang *et al.*, 2010).

The amendment effect of biochar on soil water holding capacity and plant growth demonstrates that biochar could be an effective organic material in the field of agricultural production and ecological recovery in the Loess Plateau. Especially, the increased soil moisture capacity by biochar has the potential to increase crop yields for crops exposed to water stress during critical periods of the growing season (Laird *et al.*, 2010).

However, the effects of biochar applications on the grain and biomass yields were variable because of the varieties of plant, the properties of the biochars and the fertilized conditions based on previous studies (Van Zwieten *et al.*, 2010). Some studies had shown that no significant effects on the plant growth in the biochar application treatments without fertilizer (Van Zwieten *et al.*, 2010); other studies demonstrated that biochar application would cause the decrease in the grain yield in the fertilized conditions (Gaskin *et al.*, 2010). The effects of biochar

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application on soil water condition showed that the willing capacity can increase along with the biochar application rate (Fellet *et al.*, 2011). So, it is possible that biochar application could cause the effective water content decreased under the same soil water content condition, and then the growth of plant would be restricted.

Plant growth was greatly influenced by the water stress condition (Fedorenko *et al*., 1995; Bai *et al*., 2006). The plant growth in the water stress condition can directly reflect the influence of soil on the plant. Few studies focused on the effect of biochar application on plant growth under water stress condition, especially the soil amended by biochar at a lower application rate and applied for a relatively long period. Furthermore, so far little is known about the whole growth process of clipping plant, such as germination, growing and after-cutting, under different water stress and soil treated with biochar.

It is more effective to reflect the effect of soil amendment in the arid area under the stimulated water stress condition than sufficient water condition. Ryegrass, which is one of the most important kinds of grass for grassland animal husbandry and green-recovery, was chosen as the research object to investigate the influences of biochar amendment and water stress on the plant growth process in two kinds of soils from the Loess Plateau.

Materials and Methods

Biochar Amended Soils

Biochar used in the experiment was bought from Yonghong Charcoal Factory, located in Hu County, Shaanxi Province, China. The C and N contents of the biochar were 66.27% and 2.21%, respectively, a total ash content 12.50%, a pH (H_2O) 8.38, CEC (cation exchange capacity) 31.28 cmol kg^{-1}. Two types of soils, Shahuang soil (recorded as M soil) and Dark Loessial soil (recorded as B soil) which widely spread in the Loess Plateau, were collected from the 0–20 cm horizon. Main properties of the soils used in the experiment are listed in Table 1 (Liu and Zhang, 2012). The main processes were as follows: the biochar and air-dried soils were all ground to pass 2 mm sieve and mixed together to get the ratios of biochar to soil with 0 g kg^{-1}, 8 g kg^{-1} and 16 g kg^{-1} for the incubation experiment, respectively. The mixture was put into the pots (16 cm in diameter, 20 cm in depth) to get the designed bulk density of 1.3 g cm^{-3} for the treatments. Soil moisture was adjusted to 60–70% of water holding capacity every 4 to 5 days in all columns and no water was leached out from the pots. The subsamples were collected to determine soil properties after 11 months incubation and the rest soils were passed 5 mm sieve and used in the ryegrass growth experiment.

Ryegrass Growth Experiment

The ryegrass growth experiment was from April to June in 2012. Six replications were used for each of the soil treatments. 18 seeds were sowed to every experimental pot (10 cm in diameter, 10 cm in depth), which contained 1 kg natural air-dried incubated soil passed 5 mm sieve on April 15^{th}, 2012. The bottom of the pots was sealed to prevent water loss and facilitate water control. The distance from the position of the seeds to the surface of the soil is about 5 mm.

The first growth stage lasting for 26 days after the germination was divided into two intervals. The first interval lasted for 1 week. In this interval of all the pots, water was added every day to 100% field capacity to meet the germination requirement of seeds. After eliminating other undesirable shoots by hand, 6 plants were left for per pot. The second interval of the first growth stage lasted for 19 days. In the interval, all the pots were added water every day to 85–90% field capacity until the end of the second interval.

The second stage lasted for about 1 month. Three water stress treatments were designed for each biochar treatment soil: CT (control treatment, keep the water content to 90–85% field capacity), WS (water stress, 50–45% field capacity), and RW (rewatering after water stress, watered to 90–85% field capacity when the water content decrease to 50–45% field capacity). CT and WS treatments were watered every day, while the irrigation cycle for RW was about 5 days.

Heights of the plants in both of the two stages were recorded every 5 days. Above ground biomass of the first stage was harvest and dried (60^{o}C) at the end of the stage with 6 replications. Above ground and underground biomass of the second stage were collected and dried (60^{o}C) at the end of the research. All the values of parameters were calculated to that of per plant.

Analysis

Analyses of variance were carried out using SPSS 16.0 statistical software. One-way ANOVA was used to analyze the effect of different biochar amendments or water stress conditions on plant growth. Two-way ANOVA was used to analyze for the interaction effects of biochar and water stress on the plant dry weight in the second stage. Duncan's multiple-range comparison test was used to compare means.

Results

Germination Time and Rate

For all the treatments, the germination of plants appeared on the third day after sowing, the second leaf period on the ninth day. There is no significant difference in the time of germination and second leaf period between the different treatments of the two kind soils.

The germination rates of all the treatments on the forth and twelfth day are presented in Table 2. For all the treatments, the germination rates were about 60–73% on the forth day, about 70–75% on the twelfth day. No difference was found for all the treatments.

Table 1: Main characteristics of the M and B soils (Liu and Zhang , 2012)

Parameter	M	B
Sand (%)	32.48	17.35
Silt (%)	61.76	68.85
Clay (%)	5.75	13.80
OC (g kg^{-1})	2.33	6.31
N (g kg^{-1})	0.30	0.69
pH (1:2.5 H_2O)	8.76	8.71
CEC (cmol kg^{-1})	8.05	13.95

M: Shahuang soil; B: Dark loessial soil; OC: Organic carbon content; CEC: Cation exchange capacity

Table 2: Germination rates of ryegrass

Treatments	Germination rates (%)	
	Date: 4.18	4.23
M0	59.26 a	75.93 a
M8	66.67 a	70.37 a
M16	73.15 a	75.93 a
B0	62.96 a	71.30 a
B8	67.59 a	73.15 a
B16	69.44 a	70.37 a

Within the same soil, treatment means followed by the different letter are significant different at p=0.05

Height Growth at the First Stage

The plant heights of the two soils at the same determination time and biochar treatment were similar (Fig. 1). At the end of the first stage (23 days after germination), the plant heights of the three biochar treatments were 11.8, 12.8, 12.3 cm for M soil, while 11.7, 12.9, 12.2 for B soil.

Biochar treatment improved seedling growth for both of the two soils under the first stage with sufficient water condition. And the improvement effect could be found in the initial growing period. The significant effect ($P<0.05$) could be found on the eighth day after germination for M soil, on the third day for the B soil. The increase effects were kept and the effects were more significant at the later of the first stage. For the two kinds of soil types, we also found that the 8 g kg^{-1} treatment always got the highest mean value of plant height.

Height Growth at the Second Stage

The details of ryegrass height growth under different water stress conditions at second stage are shown in Table 3. For the M soil, the significant decreasing effect on plant height under the same biochar treatment only could be found in M0. The significant low plant height was shown in that treatment under WS condition. No significant difference of plant height was found in the M8 and M16 treatments under all the 3 different water stress conditions, although that the lowest value always shown in the WS condition.

Under the sufficient water supply condition, the M8 treatment got the significant increasing effect on plant height at the whole growing stage, while the M16 treatment increasing in the later stage. Under the serious water stress condition, both the M8 and M16 treatment got the significantly increasing effect on plant height at the 30 days growing stage. Meanwhile, the M8 treatment always got the highest average plant height, except that in the initial 5 days. Under the RW condition, the M16 treatment always got the highest plant height in all the stage, and significant higher than that in the M0 treatment in most time of the stage. The heights of M8 treatment were higher than that in the M0 treatment, while the significant difference only in the initial stage. From Table 3, we also found the plant height of M8 and M16 soils under WS condition were always significant higher than that in the M0 treatment under the CT and RW condition.

For the B soils, the restrict effect on plant growth by water stress was found after the 10^{th} day, and the decreasing effect by water stress firstly showed in the B8 treatment. No significant difference of plant heights was found between the WS and RW conditions of B8 treatment, while the plant heights of the two conditions were all lower than that in the well water condition in all the stage. In the B16 treatments, the plant height under WS condition always got the lowest value.

For the same water stress condition, significant increasing effect of biochar treatment on plant height was only found under well soil water condition. And the significant increasing effect only showed in the initial period, which disappeared later. Biochar application into B soil had no significant influence on plant height under different water stress conditions.

Above Ground Dry Biomass per Plant at First Clipping

The plant grown in the biochar amended soil got the higher average above ground dry weight (Table 4), and the increasing effect could be found in the two soils. But only the M soil got the significant increasing effect at 8 g kg^{-1} biochar application rate.

Above Ground Dry Biomass per Plant at Second Clipping

The growth of ryegrass under different water stress conditions was shown in Table 5. The well water treatment always got the highest value in all the biochar treatments of both M and B soils, and it is significantly higher than that in the WS condition which was always the lowest. The significant difference was also found between the control treatments and the RW condition of all the B treatments.

The M8 and M16 treatments got the significant higher aboveground biomass under different water stress conditions than that in M0. And under the WS condition, the value of the M8 treatment, which was always the highest, was significant higher than that of the M16.

Table 3: Height of ryegrass in the second stage

Treatments	Average height of plant (cm)								
	5			10			15		
	CT	WS	RW	CT	WS	RW	CT	WS	RW
M0	2.43 Ba	2.43 Ba	2.40 Ba	4.54 Ba	3.93 Ba	4.41 Ba	6.09 Bab	5.29 Bb	7.28 Aa
M8	3.45 Aa	2.94 Aa	3.08 Aa	6.40 Aa	5.62 Aa	6.01 Aa	8.58 Aa	8.14 Aa	7.73 Aa
M16	2.69 Bb	3.23 Aab	3.43 Aa	5.00 Bb	5.38 Aab	6.22 Aa	7.38 ABa	7.28 Aa	8.08 Aa
B0	2.96 Ba	3.06 Aa	3.36 Aa	5.40 Ba	5.16 Aa	5.96 Aa	8.26 Ba	6.38 Ab	8.07 Aa
B8	3.78 Aa	3.27 Aa	3.22 Aa	6.63 Aa	5.47 Ab	5.51 Ab	9.58 ABa	8.01 Ab	7.73 Ab
B16	3.37 ABa	2.87 Aa	2.94 Aa	5.63 Ba	5.15 Aa	5.33 Aa	9.74 Aa	7.39 Ab	8.88 Aab
Treatments	Average height of plant (cm)								
	20			25			30		
	CT	WS	RW	CT	WS	RW	CT	WS	RW
M0	8.38 Bab	6.98 Bb	9.08 Ba	9.21 Bab	7.91 Bb	9.77 Ba	9.84 Ba	9.26 Ba	10.10 Ba
M8	11.64 Aa	10.54 Aa	9.93 ABa	12.87 Aa	11.30 Aa	10.99 ABa	12.99 Aa	12.05 Aa	11.55 ABa
M16	10.43 Aa	9.53 Aa	11.31 Aa	11.71 Aa	11.18 Aa	12.49 Aa	11.96 Aa	11.54 Aa	12.78 Aa
B0	9.93 Aa	7.81 Ab	10.52 Aa	11.55 Aa	8.43 Ab	11.73 Aa	12.51 Aa	8.75 Ab	11.48 Aa
B8	11.58 Aa	9.43 Ab	10.07 Aab	12.62 Aa	9.83 Ab	10.68 Ab	12.66 Aa	9.75 Ab	10.75 Ab
B16	11.43 Aa	8.16 Ab	11.14 Aa	12.68 Aa	8.18 Ab	11.77 Aa	12.98 Aa	8.81 Ab	11.84 Aa

Within rows of the same soil at the same determination day, treatment means followed by the different lowercase letter are significant different at p=0.05. Within columns of the same soil at the same determination day, treatment means followed by the different capital letter are significant different at p=0.05

CT: control treatment; WS: water stress; RW: rewatering after water stress

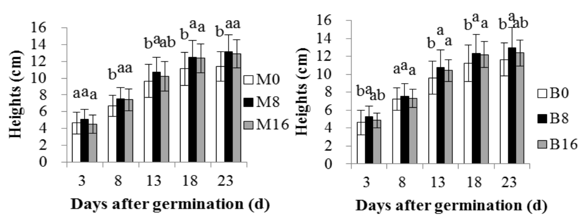

Fig. 1: Height growth of ryegrass at the first stage in the M soil (left) and B soil (right), respectively

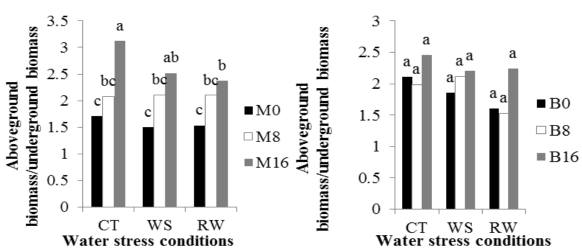

Fig. 2: Aboveground biomass/underground biomass under different water stress conditions in the M soil (left) and B soil (right), respectively. CT: control treatment; WS: water stress; RW: rewatering after water stress

The CT of water condition in the B soil got the significant higher value than that in the other water stress conditions, while the WS condition got the significant lower values. Biochar application only got the significant increase effect in the well water condition. No significant increase trend by biochar application was shown in the serious water stress condition, while no significant decrease effect found in the wet-dry water stress condition.

Underground Dry Biomass per Plant at Second Clipping

Masahide *et al.* (2006) found the significant increasing effect of biochar application on the root dry weight of maize. But the result was not found in this research of the two soils (Table 5).

For the underground biomass, the values influenced by different water stress conditions were similar to the trend of the above ground biomass. The sufficient water condition always got highest value for all the treatments of both the M and B soils, while the WS condition got the lowest value, except the M0, M16 and B0 treatments in which the RW treatment got the highest underground biomass.

The highest values of M soil under different water stress conditions were shown in the 8 g kg^{-1} treatment, while the lowest in the 16 g kg^{-1}. The results probably reflect that the high biochar application may cause restrict effect of the root growth. The value of M8 under the sufficient water condition was significant higher than that of M0 and M16. No significant difference was found in all the biochar treatments under RW condition.

Though the B8 treatments under sufficient water condition got the highest value of underground biomass, biochar application didn't get any significant influence in all the three different water conditions.

Ratio of Aboveground Biomass to Underground Biomass

Robertson *et al.* (2012) found that the ratio of the shoot to root could not be changed by biochar application, which was approximately one. The ratios of aboveground biomass to underground biomass of the study were about 1.50 to

Table 4: Aboveground dry biomass per plant at first clipping

Treatments	Dry weight per plant (mg)
M0	3.72±0.45 b
M8	4.59±0.89 a
M16	4.04±0.29 ab
B0	3.91±0.37 a
B8	4.19±0.47 a
B16	4.50±0.53 a

Within the same soil, treatment means followed by the different letter are significant different at p=0.05

Table 5: Aboveground and underground biomass per plant at second clipping

	Above ground biomass			Underground biomass		
Treatments	CT	WS	RW	CT	WS	RW
M0	9.46 c	7.13 d	9.15 cd	5.53 bcd	4.75 cde	5.96 bc
M8	15.28 a	12.39 b	13.00 ab	7.33 a	5.89 bc	6.19 ab
M16	13.54 ab	10.18 c	12.94 ab	4.34 de	4.04 e	5.44 bcd
B0	15.56 b	7.31 d	13.13 c	7.39 abcd	3.94 d	8.19 ab
B8	19.40 a	8.83 d	12.38 c	9.78 a	4.17 cd	8.13 ab
B16	19.00 a	9.28 d	12.92 c	7.72 abc	4.21 cd	5.76 bcd

CT: control treatment; WS: water stress; RW: rewatering after water stress. Within the same soil and the same biomass type, treatment means followed by the different letter are significant different at p=0.05

Table 6: Two-way ANOVA for the effects of biochar and water stress on dry weight per plant at second clipping

Soil type	Factor	DF	Aboveground biomass			Underground biomass		
			SS	F	P	SS	F	P
M	Biochar	2	39.81	41.758	0.000	5.23	19.899	0.000
	Water stress	2	12.56	13.173	0.002	1.67	6.36	0.019
	B*W	4	0.87	0.909	0.498	0.67	2.541	0.113
	Error	9	0.95			0.26		
B	biochar	2	6.20	1.20	0.346	0.41	0.06	0.938
	water stress	2	141.48	27.28	0.000	41.29	6.46	0.018
	B*W	4	3.26	0.63	0.655	3.80	0.60	0.675
	Error	9	5.19			6.40		

3.12 for M soil, 1.52–2.46 for B (Fig. 2). And biochar amended soil would get the higher value than that in the control treatment for both of the two soils under different water stress conditions. The 16 g kg^{-1} treatment always got the higher ratio value. In both of the two soils, no significant difference was shown under the different water stress conditions in the same biochar amended soils. For the M soil, the ratio value of M16 was significant higher than that of the M0.

Interference Effect of the Biochar Amendment and Water Stress Conditions

Through the two-way ANOVA (Table 6), we found that both the biochar and water stress treatments had the significant influence on the plant aboveground and underground biomass in the M soil, while only water stress treatment had significant effect on the B soil. Meanwhile, the interference effect of the biochar and water stress was not significant for both the two soils.

Discussion

Biochar treatments have no significant effect on germination rates in both of the two kind soils (Table 2). The results were in accordance with Free *et al.* (2010) that maize seed germination and early growth were not significantly affected by biochars at less than 10 t ha^{-1} application rate. However, research was variable. Van Zwieten *et al*. (2010) found that most of the biochar treatment did not affect the germination of plants, while wheat seed germination was increased with a single dose (10 t/ha) of paper mill biochar in the ferrosol. However, Solaiman *et al.* (2012) found that biochars influence seed germination and early growth of seedlings. Bamberg *et al.* (1986) showed that activated charcoal enhanced seed germination of potato. Baronti *et al*. (2010) found germination was accelerated and it was interpreted with the increased soil temperature by biochar application. In summary, no evidence has been found so far for negative effects of biochars on seed germination. So we could conclude that biochar application could improve seed germination at some condition and never restrict germination.

Huang *et al.* (2011) found that biochar amendment soil, even with low biochar application rate, can cause the increasing of ryegrass biomass under sufficient water condition. The effect of biochar application on biomass production may be influenced by the different years. Jones *et al.* (2012) showed that the dry biomass productions of the first and second years were not influenced by the biochar application, while dactylis glomerata which was grown in the third year got the signifiant increasing of crop height and total dry biomass, especially at the higher application rate of 50 t ha^{-1}. Major *et al.* (2010) found that maize grain yield in the 20 t ha^{-1} biochar application treatment got significant increase in the following 3 years verse did not significantly increase in the first year. While, sustain positive effect on biomass production for two consecutive seasons by biochar application was shown by Vaccari *et al.* (2011).

In our research, we focused on the effect of biochar on ryegrass growth in different clipping stages under the sufficient water condition. The results were in line with findings Baronti *et al.* (2010) that the biochar treatment could increase the plant height and above ground biomass in the initial growing stage and the next clipping stages.

After one month study period, the plant height, the aboveground and underground biomass were always lower under WS condition than that under CT condition. Water stress would cause the restrict effect on plant growth. But the influence is not the same in the different soils. In the control treatment without biochar application, both the two soils showed that the plant height under the RW condition would get the highest mean value in some period, after 15 days in the M soil and about 15 days in the B soil.

Kammann *et al.* (2011) found that the high biochar application rates (100 and 200 t ha^{-1}) can influence the drought tolerance of plant. The result from Table 3 and Table 5 reflected that the biochar application in the M soil could weaken the declining effect on plant height growing of water stress and promote plant growth. But the increasing effect could not be found in the B soil.

Noguera *et al.* (2010) found that biochar application effect on rice growth depended on soil types, and the positive effect was only clear in the rich soil when compared with the poor soil or the poor soil with fertilization. However, Huang *et al.* (2011) showed that biochar application would increase ryegrass biomass significantly with the increasing application rate in the low fertility soils.

In our research, the B soil with high fertility only got the significant increase effect in the sufficient water condition of the second clipping stage. However, The M8 and M16 treatments got the significantly higher aboveground biomass and the ratio of aboveground biomass to underground biomass in the first and second clipping stage under all the three water conditions (Table 4 and 5; Fig. 2). The M soil with low fertility was influenced more significantly by biochar application. Thus, biochar amendment would get better improvement effect on plant growth in low fertility soils in the Loess Plateau.

Baronti *et al.* (2010) found that the higher application rate of biochar added to the soil, the higher dry matter of ryegrass got and up to a threshold of 1.7%. Negative effects of biochar were observed above of 1.7% (17 g kg^{-1}) biochar. Rajkovich *et al.* (2012) reported that effects of biochar on plant growth were influenced by biochar application rate and raw materials. Biochars produced from dairy manure, paper sludge or food waste would decrease corn growth when application rates above 2.0% (20 g/kg). And in Zhang *et al*. (2012) research which found that biochar application to calcareous and infertile dry croplands poor in soil organic carbon would get the increase of crop productivity, showed that the 20 t ha^{-1} application rate not the 40 t ha^{-1} got the better increased effect (Zhang *et al.,* 2012).

In our study, we found that the 8 g kg^{-1} biochar application rate would get the better increase effect on plant growth than that in the control and the 16 g kg^{-1} treatments at the two stages and in the two kinds of soil types. And the significant increase effect was found in the M soil. The 16 g kg^{-1} application rate would got the restrict effect on plant growth in some condition.

All of the results showed that biochar application effect on plant growth has the limited rate. The 8 g kg^{-1} would be the suitable biochar application rate in the soils of the Loess Plateau to get the increase effect on plant growth with well water condition and restrict the input of soil amendment materials.

In conclusion, biochar application treatment didn't show any influence on plant germination time and germination rate, but the plant height and dry weight would be significantly influenced in both of two soils and at the two application rates in the first clipping stage without water stress condition. Biochar amended soil would not restrict the plant growth under water stress conditions, and the improvement effect could be showed in some application rates and soil types. The 8 g kg^{-1} application rate could be used in the Loess Plateau to improve soil property. And the M soil with lower fertility would get better improvement effect on the plant height and biomass than that of B soil, even in the serious water stress condition. Biochar could be used in the Loess Plateau to release the soil water shortage restriction of agriculture production.

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