Influence of biochar addition to fluvisol on maize yield and soil microbiota

Влияние на биовъглен върху добив от царевица и микробиална активност при Алувиално- ливадна почва

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Received: September 12, 2021; accepted: January 26, 2022

ABSTRACT

A three-year field experiment with maize was carried out on fluvisol. The aim of the present study was to evaluate the impact of biochar on maize yield at different levels of water stress and its effect on the amount of soil microorganisms. The following variants were studied: control with no biochar addition, and variants with biochar. Biochar was applied in 2 t/ha in 2016, produced by pyrolysis of rice straw, then in 3 t/ha in 2017 and 10 t/ha in 2018, both obtained by pyrolysis of oak bark. These variants were divided into three more depending on the irrigation regime: i.e. non-irrigated, irrigation at dT>0 °C, and irrigation at dT<-1 °C. Under non-irrigated conditions, the effect of biochar on maize yield was not pronounced over three years. Under irrigation conditions with water added according to crop water stress, higher yields of maize were obtained in the third year when biochar application to the soil was highest (10 t/ha). Also, yield was higher when faster (at lower water stress dT<-1 °C) irrigation rates were obtained for irrigation at dT<-1 °C in 2018. These results showed that the influence of biochar on soil microbiota was significantly greater, and the most stimulating effect of biochar was obtained for bacterial populations.

Keywords: biochar, grain, water regime, nutrient uptake, microbial activity

АБСТРАКТ

Проведен е тригодишен полски опит с царевица върху Алувиално – ливадна почва. Целта на настоящата разработка е да се направи оценка на влиянието на биовъглена върху добива на царевица при различни нива на воден стрес и върху популациите на основните групи почвени микроорганизми. Предвидени са следните варианти: Контрола без биовъглен и варианти с биовъглен. Биовъглена се внася, през 2016 в количество от 2 t/ ha, произведен от оризова слама, а през 2017 и 2018 в дози 3 and 10 t/ha, произведен при пиролиза на дъбови кори. Тези варианти се разделят на още три в зависимост от поливния режим: неполивен; поливен при dT>0 и поливен при dT<-1. Установено е, че при неполивни условия, ефектът на биовъглена по отношение на добива от царевица не се проявява и през трите години от добавянето му в почвата. Доказано е, че при условията на поливен воден режим, когато поливките са съобразени с водния стрес dT<-1) са подадени поливките, толкова по-висок е добивът. Почвената влажност се изменя по-бавно при вариантите с биовъглен в най-голямо количество (10 t/ha). При това, колкото по-скоро (при по-нисък воден стрес dT<-1) са подадени поливките, толкова по-висок е добивът. Почвената влажност се изменя по-бавно при вариантите с биовъглен като най-добри стойности на температурната разлика са получени при поливка при dT<-1 през 2018 година. Най-силно изразен стимулиращ ефект на биовъглена е получена е получен спрямо бактериалните популации.

Ключови думи: биовъглен, зърно, поливен режим, износ на елементи, микробиална активност

INTRODUCTION

Maize is the second most important agricultural crop in Bulgaria after wheat. The introduction of new developments and innovative environmentally friendly technologies provides opportunities to improve the productivity of agricultural crops. In this regard, the application of biochar (BC), which is the end product of the oxygen-free combustion of biomass in the pyrolysis process (Lehmann and Joseph, 2009), is important.

Most investigations focused on studying biochar as a soil amendment and therefore its effect on crop yields (maize, wheat, rice, barley) in different parts of the world and under different soil-climatic conditions. Several field experiments have been carried out to investigate the cultivation of maize on biochar amended soil. In most of them, the application of biochar increased yields of maize compared to control (Major et al., 2010; Sukartono et al., 2011; Islami et al., 2011; Zhang et al., 2012). Others reported no significant difference in yields due to biochar application (Gaskin et al., 2010; Jones et al., 2012; Liang et al., 2014). Several independent sources confirmed the positive effect of biochar on mycorrhizal root colonization and beneficial soil microbial activity soil (Blackwell et al., 2010; Solaiman et al., 2010; Jones et al., 2012; Sun et al., 2012). Studies show that biochar has a high porosity and surface area which is leading to an increase in the general soil porosity and water content, reducing water stress in plants (Downie et al., 2009; Abel et al., 2013; Batista et al., 2018).

In Bulgaria, the studies of biochar are scarce and insufficient (Mikova, 2014; Stoimenov et al., 2015; Kercheva et al., 2018; Petkova et al., 2018; Simeonova et al., 2019; Atanassova et al., 2020; Benkova et al., 2020). Globally, research has been done for about 15 years. Often the results are contradictory, but some trends are clearly outlined regarding the effect of biochar on crop yields and soil microbial activity.

The aim of the present study was to evaluate the impact of biochar on maize yield at different levels of water stress and its effect on the amount of soil microorganisms.

MATERIALS AND METHODS

A three-year field experiment with maize was carried out on a fluvisol (FAO, 2006) in the experimental field of Tsalapitsa (Plovdiv) in the period 2016–2018. The soil is characterized with a sandy-loam texture (sand 68%, silt 23%, clay 9%), low content of total nitrogen (0.052%) and organic matter (0.78%), with slightly acidic reaction overall the profile (pH 5.3–5.6) and cation adsorption capacity of 15.9 cmol/kg. The mineral nitrogen content is low (19 mg/kg), available P_2O_5 is 17 mg/100g and K_2O is 24.1 mg/100 g, respectively.

Over three years, the maize from hybrid "Pioneer 9175" (330 according to FAO) was sown with crop density of 50000 plants/ha in the optimal term for crops (i.e. in April). The experiment was based on a block design, which consisted of 12 plots that all had dimensions of $15 \text{ m} \times 0.7$ m with three replications. The distance between the plots was 70 cm. Background fertilization was: 150 kg/ha N (as ammonium nitrate), 300 kg/ha P (as superphosphate), and 200 kg/ha K (as potassium sulphate). The maize was fertilized with 120 kg/ha N in the form of ammonium nitrate at the end of June.

Biochar was applied at rate of 2 t/ha in 2016, produced by pyrolysis of rice straw and at rates of 3 and 10 t/ha in 2017 and 2018, obtained by pyrolysis of oak bark (pH=7.9, C=37.7%, CEC=10.9 cmol/kg, exch. Ca=7.8 cmol/kg and exch. Mg=3.1 cmol/kg, min. N =47.2 mg/kg, available $P_2O_5 = 15.2$ mg/100g, and $K_2O = 427.2 \text{ mg}/100\text{g}$) before sowing of maize. The following variants were studied: control - with no biochar addition (C), and variants with biochar (BC2016, BC2017 and BC2018). These variants were further divided into three, depending on the irrigation regime: non-irrigated (BCDry); irrigation at dT>0 °C (BCIr0) and irrigation at $dT < 1 \circ C$ (BCIr1), where the dT = canopy temperature (Tc) - ambient air temperature (Ta). Each variant consists of three replications. During the vegetation period the plant water status was measured daily at 2 pm by infrared thermometer. The irrigation water was applied during the critical - period (July- August) for the maize vegetation.

Soil moisture was evaluated by gypsum blocks (Stoimenov and Kirkova, 2012). The precipitation and air temperature were evaluated by meteorological station situated on the experimental field.

After harvest, plant samples were taken. The maize grain was dried at 65 °C and grinded. First, the samples were analyzed by dry mineralization in muffle furnace at 500 °C and dissolution in 20% HCI. Then, potassium content was measured by flame photometer. Phosphorous was determined by phosphor molybdate - vanadate yellow method and measured by spectrophotometer at $\lambda = 460$ nm. The content of total nitrogen in plant samples was determined by wet combustion method of Ginzburg and Kjeldahl distillation (Peterburgskii, 1986). The grain yield (kg/ha), content of nutrients N, P, K (%) in dry weight (DW) and their uptake by production were determined by the methods of Peterburgskii (1986).

Microbiological analyses of samples from a field experiment with maize were done at the end of the plant vegetation. The following parameters were determined: number of microorganisms from some main groups (ammonifying bacteria, spore-forming bacteria, microscopic fungi, cellulose-decomposing microorganisms and actinomycetes) using the method of decimal dilutions on selective agar media (Grudeva et al., 2007). It was expressed as colony forming units (CFU) per gram of dry soil and total biological activity (CO₂ emission) – titrimetrically (Alef et al., 1998).

Statistical analysis of the data included determination of the least significant differences among the treatments (LSD) (P<0.05), One-way ANOVA and Multifactor ANOVA methods (STATGRAPHICS Centurion XV).

RESULTS AND DISCUSSIONS

Meteorological situation during the investigation period (2016 – 2018)

The climate in region of Plovdiv, Upper Tracian Plain, is transitional continental. Temperature follows a regular seasonal trend, with average low temperature in January (0 °C) and average high temperature in July (23.5 °C). For the crop season, May–September, the temperature sum is on average 2965 °C, sufficient for the development of crop as maize.



Figure 1. Average temperature and precipitation for investigation period (2016 -2018) compared to the climatic norm (1960-1990)

Precipitation during the maize cropping season (May-September) varied from 120 to 520 mm over the period 1960–1990 (Popova and Pereira, 2011). The average temperature and precipitation for the period from November 2016 to September 2018 are shown in Figure 1. They are compared with the climate normal (1960–1990), defined by the World Meteorological Organization.

The investigation period was characterized by a higher-than-average temperature of the climate normal in the field area. Increased air temperature and the intensity and frequency of drought are becoming a real problem in the Plovdiv region (Popova et al., 2015). The highest mean monthly temperatures were recorded in 2018. During the summer months (June, July, August), the temperatures in all experimental years exceeded the long-term period average temperatures for 1960-1990. The amounts of summer precipitations were significantly below the average for the region, except for 2018. The highest rainfall was recorded in June of the same year (two times higher than normal). Winter and summer precipitations exceed the normal values significantly, which provided good soil moisture at the beginning of the maize growing season.

In short, rainfall was below normal in the experimental period (except for June 2018), especially in the critical period (July-August), for maize growth. This, in combination with higher temperatures and lower available water capacity of the soil, did not create good conditions for the crop. Meteorological conditions during the three years were also essential for the growth and development of the cultivated crop due to the fact that irrigation is a basic condition for good agricultural production in the region.

Effect of biochar on the soil moisture in maize cultivation in 2018

Growth processes during maize vegetation proceed normally when the soil moisture is 70–80% of the maximum soil water content. During the vegetation period, two or three irrigations were carried out, depending on the irrigation regime, with a norm of 80 m³ per 0.5 hectare to maintain the irrigation humidity of 75%.

Under non-irrigated variants, the soil moisture in both layers (0-20 and 20-40 cm) decreased from 15% to 6% in summer, which was below the wilting point and the plants experience water stress (Figure 2A). Under irrigated variants at dT>0 (Figure 2B), soil moisture in the upper layers (0–20 and 20–40 cm) remained at 13–14% in late June and early July, due to rainfall. Soil moisture dropped to 8.5% in the middle of July and rose a little after the first irrigation and was around 8% throughout August.

Under irrigated variants at dT<-1 (Figure 2C), soil moisture varied significantly in both layers (0–20 and 20–40 cm). After irrigation, it increased to 14-15%, and then decreased to 7-8% due to the less rainfall (up to 11 I/m^2) during this period. In early September, as a result of most precipitation (42 I/m^2), humidity increased again compared to the other variants. Soil moisture dynamics during the vegetation of maize changed depending on the amount of precipitation and different levels of watering regime. The biochar variants maintained higher humidity, especially at dT<-1.







Figure 2. Dynamic of soil moisture measured by gypsum blocks for 0–20, 20–40, 40–60 and cm 60–100 soil layer during maize growth at different irrigation mode (A. non–irrigated, B. irrigated at dT>0 and C. irrigated at dT<-1)

The values of differences between the canopy temperature and air temperature (dT) are shown in Figure 3. It was found that these values were the highest in the variants without biochar i.e. plants experienced the highest degree of water stress. The average values of the temperature differences decreased when biochar was applied. The data showed that the effect of biochar applied in 2016 was negligible. Its influence was more favourable in the variants of 2018, where the values of dT were lower. Soil moisture changed more slowly in the biochar variants. The best values of the temperature differences were obtained for the irrigated variants at dT<-1 °C in 2018. The large adsorbing surface area of biochar was the likely reason for the improved water holding capacity of the soil and hence less water stress to the plants. Kercheva et al. (2022) (in press) investigated the physical characteristics of the same type of biocharamended soil (fluvisol) and found that the high adsorption properties of biochar (e.g., S $_{N2ads}$ = 205 m²/g and W $_{pF5.6}$ = 12.8% of oak bark) increased the soil specific surface area by $4.5 \div 8 \text{ m}^2/\text{g}$ and $W_{\text{pF5.6}}$ by $0.3 \div 0.5\%$.

Another reason could be the coarse soil texture of the investigated soil, which also determines a lower available

water capacity, so that the application of biochar could affect water retention in the soil. Studies by Hansen et al. (2016) and Razzaghi et al. (2020) found that biochar could be of greater benefit to coarse-textured soils as it significantly increased available water content (by 45%) compared to the medium- and fine-textured soils. Similar results were also reported by Wang et al. (2019) where high rate (\geq 10 t/ha) biochar with large pore volume (\geq 1 mm) improved water retention in soil of coarse-texture and limited water storage capacity.

Effect of biochar on maize production

Multifactor ANOVA analyses of data for maize yield depending on the time of irrigation (moment) and the year of biochar application (year) are presented in Table 1. The results showed that both factors - the time of irrigation (A: moment) and year of biochar application (B: year) have a significant effect (P<0.05) on the yields in the three years. The factor time of irrigation had a significant effect on maize yields (66%). The factor year of biochar application a combination of the two factors contributed the least (2%).



Figure 3. Average values of the temperature difference (dT) during the critical period for maize growth by variants in the tree years

There was a strong correlation between maize yield and irrigation regime during the three years of the study. The highest yields were observed in the irrigated variants at lower water stress values (dT<-1). Yields of the biochar variants were lower when irrigated at dT>0, i.e. at the beginning of water stress when plant and air temperature were equalized. Maize grain yields for the three-year experimental period are presented in Figure 4.

The results obtained for maize yield in the nonirrigated variants were very low. In all three years, there were no statistically significant differences between the non-irrigated variants with biochar applied and nonirrigated controls (without biochar). It can be concluded that the use of biochar as a soil additive when growing maize without irrigation did not affect yields during the three-year period. There were statistical differences (P>95%) between the non-irrigated and all other biochar variants, regardless of irrigation timing (Figure 4). Under irrigated variants at dT>0, yields were the highest when biochar was applied in 2018, followed by the biochar variants in 2016, which differed significantly (95% confidence level) from each other as well as from other variants (Figure 4). For the irrigated treatments at dT<-1, the highest yields were observed with biochar applied in 2018, i.e. the highest amount of biochar application. Besides that, these variants were significantly different from the controls and the other biochar variants.

In 2016, the effect of the irrigation regime at dT<-1 was greater than the application of biochar, as there was no difference between the irrigation without and with biochar treatments. This was probably due to the type of

biochar and the lowest rate applied. In 2017 and 2018, differences were found between the control treatments (without biochar) and the corresponding treatments with biochar added at irrigation dT<-1. Differences between treatments (with BC added) irrigated at different times (at dT>0 and at dT<-1) were statistically significant across the three years. The lowest maize yield values were recorded in all treatments in 2017. This may be due to both the low biochar rate and the climatic conditions during the critical moisture period of maize development.

Under non-irrigated conditions, the effect of biochar on maize yield was not pronounced in all three years. Under irrigated conditions, when irrigation was matched to the plant water stress, higher maize yields were observed in the third year after biochar application. Furthermore, the earlier (at lower water stress dT<-1 °C) the irrigations were carried out, the higher was the yield. Similar results, but in pot experiments (Ahmed et al., 2018), showed that drought reduced maize yields in all treatments, regardless of the amount of biochar, compared to the control and fully irrigated treatments. Danso et al. (2019) found that in both seasons, maize grain yield in the 15 t/ha biochar treatment was statistically similar to that without treatment, whether irrigated or not.

Content of nutrients (N, P and K) and uptake with maize grain

The contents of major nutrients varied within a narrow range typical for the crop, and the differences between all treatments were negligible. The biochar application apparently affects growing conditions, resulting in



Figure 4. Maize (grain) yields (kg/ha) at different treatments: C-Dry (control without biochar and non-irrigated), C-IrO- (control without biochar and irrigated at dT>0), C-Ir1 (control without biochar and irrigated at dT<-1), BC-Dry (with biochar and non- irrigated, BCIrO (with biochar and irrigated at dT>0) and BCIr1 (with biochar and irrigated at dT<-1), 2016-2018

*a, b, c, d - different letters indicate significant differences at P<0.05 level; every box represents five statistical values: median, minimum and maximum values and 25/75 quartiles

significantly higher yields. However, it did not affect the chemical composition of the grain as can be seen in Table 2.

Based on maize yield and the percentage of elements in it, the uptake of nitrogen, potassium and phosphorus was determined (Table 2). Over the three-year period, yield of maize was highest under irrigation at dT<-1 (10391, 10923 and 12887 kg/ha). The uptake of macroelements was the highest in these variants, respectively (Table 2). The variants without irrigation had lower yields (3252-3341 kg/ha) and nitrogen uptake was 41-43 kg/ ha. Potassium uptake was lower than nitrogen and was within the maize norm for the variants from 16 to 56 kg/ ha. The amount of phosphorus exported with the yield ranged from 6.5 to 36 kg/ha. The higher nutrient uptake in the BC-2018 variants was due to the higher yields and correspondingly higher nutrient content in crop tissues.

Similar results that plant phosphorus and potassium levels were not affected and did not decrease in biochar treatments during a long-term maize experiment in western Kenya were obtained by Kimetu et al. (2008). Solaiman et al. (2010) found that biochar amended soils with higher rate (6 t/ha) showed early nutrient export, which could be explained by the increase of maize yield.

Biochar effect on microorganisms

The number of ammonifying and spore-forming bacteria was higher in all treatments with biochar addition than in the control (Table 3). Significant differences among the treatments with respect to microscopic fungi were not established. For cellulose-decomposing microorganisms and actinomycetes the positive effect of biochar addition was observed only for the treatment with the longest period of biochar transformation in the soil. The different effect of biochar amendment on the growth of studied groups of microorganisms is probably related to its slow decomposition in the soil. During this decomposition heterocyclic compounds (including aromatic) present in varying amounts depending on the origin of the biochar, were converted into nutrients that are more readily available to microorganisms.

	Macroelements, %				Uptake, kg/ha		
_	Ν	Р	К	— Yield, kg/ha –	Ν	Р	К
C-Dry	1.25	0.33	0.48	4352	54.40	14.51	20.89
C-Ir0	1.13	0.29	0.44	7607	85.96	22.06	33.73
C-lr1	1.19	0.35	0.49	10242	121.88	36.19	50.18
BC2016Dry	1.27	0.20	0.51	3252	41.30	6.50	16.48
BC2016lr0	1.21	0.24	0.50	6554	79.30	15.51	32.55
BC2016lr1	0.91	0.17	0.45	10391	94.56	17.32	47.11
BC2017Dry	1.29	0.19	0.48	3341	43.10	6.24	16.15
BC2017Ir0	1.21	0.34	0.43	6864	83.05	23.34	29.29
BC2017Ir1	1.10	0.20	0.50	10923	120.15	22.21	54.98
BC2018Dry	1.25	0.34	0.52	4306	53.83	14.64	22.25
BC2018Ir0	1.22	0.34	0.44	9020	110.04	30.67	39.69
BC2018lr1	1.14	0.17	0.44	12887	146.91	21.91	56.27

Table 2. Content of main macro elements (% to DW), yield (kg/ha) and uptake (kg/ha) by maize grain

Table 3. Number of the microorganisms of alluvial meadow soil amended with biochar after maize harvesting

	Number of microorganisms (CFU/g)							
Variants	Ammonifying bacteria	Sporeforming bacteria	Microscopic fungi	Actinomycetes	Cellulosedecom- posing microorganisms			
	1.106	1.10 ⁵	1.10 ⁴	1.106	1.104			
1. Control	13.52ª	1.82ª	7.18ª	3.70ª	4.76 ^{ab}			
2. BC 2016	22.03 ^b	2.66 ^{bc}	6.81ª	5.02 ^b	5.80 ^{cd}			
3. BC 2017	28.09°	2.42 ^{bc}	7.25ª	3.23ª	5.12 ^{bc}			
4. BC 2018	22.10 ^b	2.70 ^c	7.17ª	4.34 ª	4.00 ^b			
LSD P≤0.05	5.89	0.45	0.68	0.73	0.87			

*Values in the same column, followed by different letters are significantly different at P<0.05





 * Different letters above bars show significantly different values at P≤0.05

The CO_2 emission was higher than that of the control in all biochar amended treatments, but the differences were not statistically significant (Figure 5). The results obtained confirmed the data reported by Kolb et al. (2008), Steiner et al. (2008) and Petkova et al. (2015), which also established favourable effect of biochar addition on the soil microorganisms. In this study, the highest stimulating effect was obtained for bacterial populations.

CONCLUSIONS

The present study investigated the influence of biochar added to fluvisol on maize yield and soil microbiota under different levels of water stress. The results showed that under non-irrigated conditions, the effect of biochar on the yield of maize was not pronounced during the three-year period. Under irrigated conditions with water addition according to the water stress of plants, higher yields of maize were obtained, especially in the third year, when the application of biochar into the soil was highest (10 t/ha). Also, the yield was higher when the irrigation rates were applied faster (at lower water stress dT<-1 °C). There was no effect of biochar on the macro-elements contents in maize grain and their uptake was associated with an increase in yield. Soil moisture changed slowly in the biochar variants and the best values of the temperature differences were obtained for irrigation at dT<-1 °C in 2018. The large adsorbing surface of biochar is the probable reason for the improvement of the water retention capacity of soil and, respectively, of the lower water stress of plants. These results showed that the influence of biochar on soil microbiota was significant, and the most stimulating effect of biochar was obtained for bacterial populations.

ACKNOWLEDGEMENTS

This work was supported by the National Science Fund, Ministry of Education and Science, Bulgaria, project: N° KP - 06 - H26 /7 (2018 - 2022).

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