Soil physical properties affected by biochar addition at different plant phaenological phases. Part II**

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A b s t r a c t. A great emphasis has been placed on biochar addition to soils to improve its physical, chemical, and biological properties in recent times in order to achieve improved crop growth and yields. The present study explored to soil physical changes through different plant growth stages caused by biochar addition to silt loam soil in a pot-experiment. Our research focused on changes in soil bulk density, aggregate size distribution, and saturated hydraulic conductivity. The soils were amended with different amounts of biochars (control with 0, BC0.5 with 0.5%, BC2.5 with 2.5%, and BC5.0 with 5.0% biochar, by weight). *Capsicum annuum* L. were planted at a two-four leaf stage. Soil samples were taken at 6, 10 and 12 weeks after planting. The biochar amendment resulted in a significant decrease in soil bulk density values. Soil saturated hydraulic conductivity values ranged between 5.5 and 7.9 times higher for all treatments compared to the controls.

K e y w o r d s: biochar, aggregate size distribution, hydraulic conductivity, bulk density

INTRODUCTION

Biochar is a substance with a high carbon content, which has been widely studied in recent decades as a soil enhancer material to promote soil quality, reduce greenhouse gas emissions, and to achieve improved crop yields (An and Huang, 2015; Gascó *et al.*, 2016; Helliwell, 2015). The addition of charcoal, ash, or biochar to soils can change soil chemical (Horel *et al.*, 2019a; Jien and Wang, 2013; Liang *et al.*, 2006), microbial (Anderson *et al.*, 2011; Horel *et al.*, 2018), and physical-hydrophysical properties (Jien and Wang, 2013; Liang *et al.*, 2006; Novak *et al.*, 2009; Sun and Lu, 2014). While biochar addition to soils may influence soil and plant health, the different stages of plant and root growth may also further affect soil hydrological properties, including the moisture regime (Angers and Caron, 1998; Surda *et al.*, 2015), therefore changes in soil properties over time should be carefully investigated before its use.

The soil pore network (shape, pore size distribution, connectivity, *etc*.) may have a great influence over the storage and movement of water within the soil matrix. Internal and external forces such as wetting and drying, climate, root penetration, soil fauna, agricultural management *etc*. may cause significant changes in soil structure (Ghezzehei, 2012). While biochar can affect soil characteristics, the type of soil is also a very important factor as biochar addition may result in varying effects on soil aggregates (Hartley *et al.*, 2016; Ouyang *et al.*, 2013; Sun and Lu, 2014).

Soil bulk density (ρ_b) is a good indicator of soil compaction, as the higher the bulk density of a given soil the more compact it is. High ρ_b values tend to negatively influence soil characteristics, significantly affecting soil aeration, water infiltration through the soil profile and the available water capacity which in turn affects root growth and plant nutrient availability. Studies have shown that biochar addition to soil can decrease its ρ_b values (Laird *et al.*, 2010a; Zhang *et al.*, 2012); thereby positively influencing the porosity of highly compacted soils, especially its macroporosity. Numerous studies found that in the case of coarse textured soils, the water holding capacity could be improved by biochar addition (Dugan *et al.*, 2010; Karhu *et al.*, 2011); however, studies concerning the connection of these changes to plant development phases are lacking in the literature.

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Soil saturated hydraulic conductivity (K_{sat}) measurements may provide information on the rate at which soil water can move within or leave the soil system, indicating possible nutrient leaching rates, which may affect plant growth and health and also lead to the contamination of groundwater. The rate of change in K_{sat} values as a result of biochar addition to soils may depend, among other factors, on the type of biochar, the rate at which biochar is applied, the particle size distirbution (*PSD*) of the biochar or soil (Lim *et al.*, 2016), or the physical and chemical characteristics of the soil used. Biochar additions to soils have varying effects on changes in their K_{sat} values. Barnes *et al.* (2014) observed a 50% decrease in K_{sat} in coarse textured sandy soils, while the K_{sat} values of clayey soils increased by over 300%. Laird *et al.* (2010b); however, did not observe any changes in the K_{sat} values of fine-loamy soils, neither did Ouyang *et al.* (2013) concerning silty clay soil.

There are numerous examples of research studying the changes in soil physical properties after biochar addition to soils (Hartley *et al.*, 2016; Jeffery *et al.*, 2015; Ouyang *et al.*, 2013). The present study aims to investigate some of these characteristics over a period of plant growth, divided into different phaenological stages from planting to fruit harvest, under natural environmental conditions (*e.g.* sunlight, rainfall).

In this study we investigated the effects of biochar addition to silt loam on the physical and structural properties of the soil and its relationship to the state of the soil matrix (Horel *et al.*, 2019b). The second part of the study mainly focused on changes in i) aggregate size distribution; ii) saturated hydraulic conductivity; and iii) bulk density of the soils over time while varying the biochar doses. We hypothesized that i) the amount of biochar added to the silt loam soil would have significant effects on soil aggregate size distribution changes at different rates over time; and ii) other soil physical parameters such as soil K_{sat} or bulk density values are not only altered by environmental changes such as previous tillage, irrigation, plant growth, and root penetration, but are also affected by the rate of biochar addition.

MATERIALS AND METHODS

The soil used was an eroded Alfisol (according to USDA) freshly tilled, silt loam, from the upper 28 cm (A horizon). The basic soil parameters are presented in Table 1.

Biochar was obtained commercially; it was certified with a European Biochar Certificate (EBC). The biochar was made from paper fibre and grain husk materials using Pyreg-reactor technology at approximately 600ºC. We listed the main parameters of pure biochar in Table 1.

The experimental setup included 2 kg of homogenized soil per pot with dimensions of 14 cm height and 18 cm diameter with a drain outlet. Green pepper (*Capsicum annuum* L.) were planted at the two to four leaves stages.

Four different treatment types were used: a control and three biochar dosages, studied in three replicates per sampling time (Fig. 1). We calculated the biochar additions to

Table 1. Basic parameters of the pure biochar and the control soil sample

Material	Clay <6.6 μm (%)	Silt 6.6 – 52.5 μm (%)	Sand 52.5 – 2000 μm (%)	pH-H$_2$O	Organic C (%)	K$_2$O (mg kg^{-1})	P$_2$O$_5$ (mg kg^{-1})	Total N (%)	NH$_4^+$-N (mg kg^{-1})	NO$_3^-$-N (mg kg^{-1})	CaCO$_3$ (%)
Biochar	1.57	13.90	84.52	10.33	27.89	13570.32	5031.10	1.01	1.86	n.d.	–
Soil	24.13	50.03	25.84	7.94	0.93	443.14	977.87	0.14	5.84	8.76	10.4

n.d. – not determined.
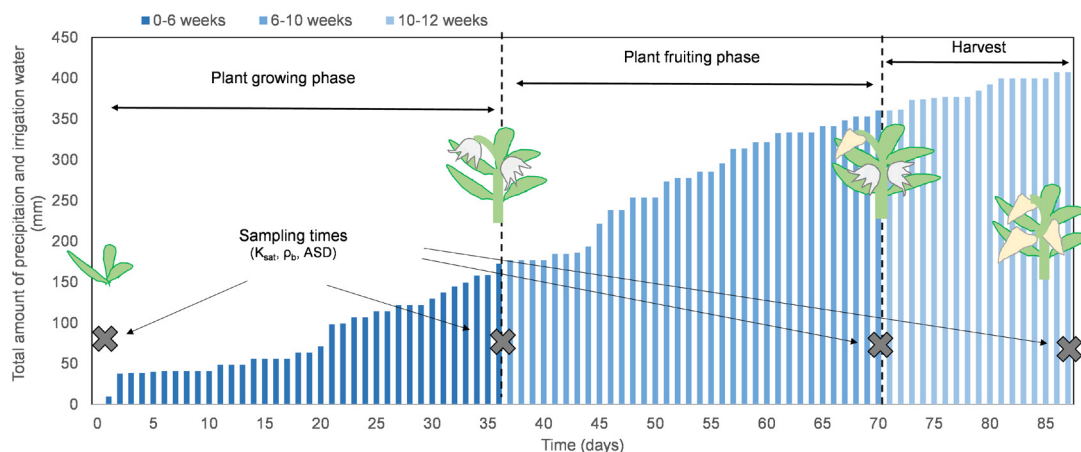
Fig. 1. Total amount of precipitation and sampling times.

the soils based on the air-dried soil weight percentage. Soil and biochar amounts were measured separately for each setup and homogenized thoroughly before placing them in pots. Out of the four treatments one was used as a control and received no biochar (0%) only plants, while the other three were amended with biochar in the amount of 0.5, 2.5, and 5.0% by dry weight (bw); hereafter referred to as C, BC0.5, BC2.5, and BC5.0, respectively.

Afterwards, we placed the pots under natural environmental settings, *e.g.* natural sunlight and rainfall, to better simulate field conditions; however, during times of water deficiency irrigation (200 ml tap water per pot per irrigation event) was also applied to reduce plant stress. Natural rain water and irrigation were monitored using an ECRN-100 rain gauge (Decagon Devices Inc., Pullman, WA), where the cumulative amount of precipitation including irrigation water at week: six – 145.2 mm, ten – 187.3 mm, and at week 12 it was 248.8 mm (Fig. 1).

Selected pots were disassembled at week 0 (W0), 6 (W6), 10 (W10), and 12 (W12) in order to study the changes in the physical and structural characteristics of the soil corresponding to three distinguishable plant phaenological phases, with the main factors of interest being the aggregate size distribution (ASD), bulk density (ρ_b), and saturated hydraulic conductivity (K_{sat}). W0 data represents the starting parameters of all four treatments prior to biochar addition; W6 data signifies the end of exponential plant growth; W10 represents the fruit development of mature plants; and W12 when fruit harvesting was performed. We determined the different plant growth phases by observation, such as tiller and leaf numbers, flowering stages, fruit development, and root and shoot system biomass weight.

During the disassembling of selected pots, the roots were carefully removed, the soil samples were air dried, gently homogenized, hand-milled and sieved through a 2 mm sieve. All soil physical measurements were performed in three replicates.

We determined the aggregate size distribution (ASD) by shaker and dry sieve analyses using 100 g soil samples, where the mesh size of the sieves were 0.25 mm, 0.5 mm, and 1 mm, and the shaking time was 5 min (Kemper and Rosenau, 1986). In order to quantify the degree of gradation of the structured soils, we defined a modified coefficient of uniformity (U_{ASD}) as the ratio of aggregate diameters corresponding to 60 and 10% (d_{60} and d_{10}, respectively) finer on the cumulative ASD curve:

$$U_{ASD} = \frac{d_{60}}{d_{10}}. \quad \text{(mm)}$$

According to equation, smaller U_{ASD} values correspond to a higher level of soil compactibility (Kézdi, 1974; Natural Resources Conservation Services, 1994).

We measured the saturated hydraulic conductivity (cm d^{-1}) with an Eijkelkamp permeameter (in a closed system) using disturbed soil samples, in three replicates. Air dried soil samples (100 g) were filled into a 5 cm in diameter

and 5 cm height sample holder, layer by layer, and manually compacted with a rubber device from the top. It was assumed that the measured K_{sat} values were determined primarily by physical properties, such as compressibility, ρ_b, or the pore size distribution, of these artificial soil columns and all the treatment effects are reflected by these properties in hydraulic conductivity. Measurements were carried out using tap water, and either the constant or falling head method (Klute and Dirksen, 1986), depending on the flow rate. We determined the geometric mean values from the log-normally distributed K_{sat} values (Nielsen *et al.*, 1973). The ρ_b values were also calculated (presented as g cm^{-3}) of the artificial soil columns used for the K_{sat} measurements.

An Independent-Sample T Test, One-Way ANOVA (Duncan test or Tamhane's test depending on the homogeneity of variances examined by Levene's test) and Boxplot analysis (SPSS 13.0) were conducted to compare the effects of biochar amendment (Treatment factor) and time (Time factor) on the measured soil physical parameters. The combined effects of treatment (Treatment * Time factor) and time on the investigated soil physical properties were also tested with an analysis of variance, ANOVA (Univariate General Linear Model; SPSS 13.0). The relationships between the selected soil properties were further investigated with a linear regression method (Linear regression, SPSS 13.0).

RESULTS

Four aggregate size ranges of below 0.25, 0.25-0.5, 0.5-1, and 1-2 mm were investigated and analysed in the present study over time. The ASD values of the C W12 control had the highest percentage of aggregates with 1-2 mm aggregate sizes compared to each collected soil samples from all of the other treatments, including controls (Table 2). We observed both the smallest and the largest aggregate values in the control samples. The frequency of the 1-2 mm fraction increased in all biochar treated samples. In contrast, the frequency of the fraction under 0.25 mm decreased in all cases at the end of the experiment. The most notable difference in aggregate sizes between treatments were observed in the 1-2 mm aggregate range. Aggregate sizes of between 0.5 and 1 mm showed the least changes within treatments over time (Table 2). The deviations for all of the other investigated aggregate sizes were more pronounced, indicating that changes in the lower (<0.25 mm) and higher (1-2 mm) aggregate size range were most likely to occur due to biochar addition and/or external environmental factors, such as irrigation.

During the construction of artificial columns to measure K_{sat} and ρ_b values, the soil samples showed varying ρ_b values despite the exact same column filling method being applied (Fig. 2a). The average ρ_b of the artificial soil columns was 1.39±0.03 g cm^{-3} at the beginning of the study. After adding biochar to the soils, the soil ρ_b values showed a significant decrease with increasing biochar amount. At W6 these values

Table 2. Aggregate size distributions at different plant growth phases, mean values, standard deviations in brackets

Sample	Aggregate size distribution (*ASD*, %)							
	<0.25 mm		0.25 – 0.5 mm		0.5 – 1 mm		1 – 2 mm	
C W0	34.66	(3.01)	5.80	(3.28)	18.05	(5.06)	41.49	(7.22)
C W6	39.53	(5.09)	14.01	(1.84)	24.84	(3.32)	21.62	(7.45)
C W10	23.08	(3.46)	15.70	(3.55)	27.44	(4.61)	33.78	(7.54)
C W12	28.83	(4.83)	6.90	(1.79)	17.50	(6.35)	46.78	(11.39)
BC0.5 W6	41.79	(1.96)	15.07	(1.05)	25.51	(4.47)	17.63	(5.80)
BC0.5 W10	32.51	(2.76)	12.99	(5.22)	24.20	(4.21)	30.29	(10.76)
BC0.5 W12	28.87	(6.21)	16.16	(0.81)	25.09	(4.71)	29.89	(7.94)
BC2.5 W6	37.54	(1.16)	16.47	(0.85)	25.80	(5.25)	20.18	(5.18)
BC2.5 W10	26.29	(1.97)	20.85	(2.25)	25.99	(4.73)	26.88	(8.36)
BC2.5 W12	33.91	(3.56)	16.08	(0.62)	24.73	(6.29)	25.29	(2.98)
BC5 W6	37.83	(2.93)	14.89	(0.22)	26.25	(4.82)	21.03	(7.20)
BC5 W10	20.41	(2.19)	20.11	(1.82)	28.36	(5.06)	31.13	(4.46)
BC5 W12	19.57	(2.88)	18.21	(3.80)	27.16	(4.93)	35.06	(11.59)

W – represents the number of weeks in the experiment, while C, BC0.5, BC2.5, and BC5.0 represents the control and 0.5, 2.5, and 5.0% biochar additions to the soils, respectively.
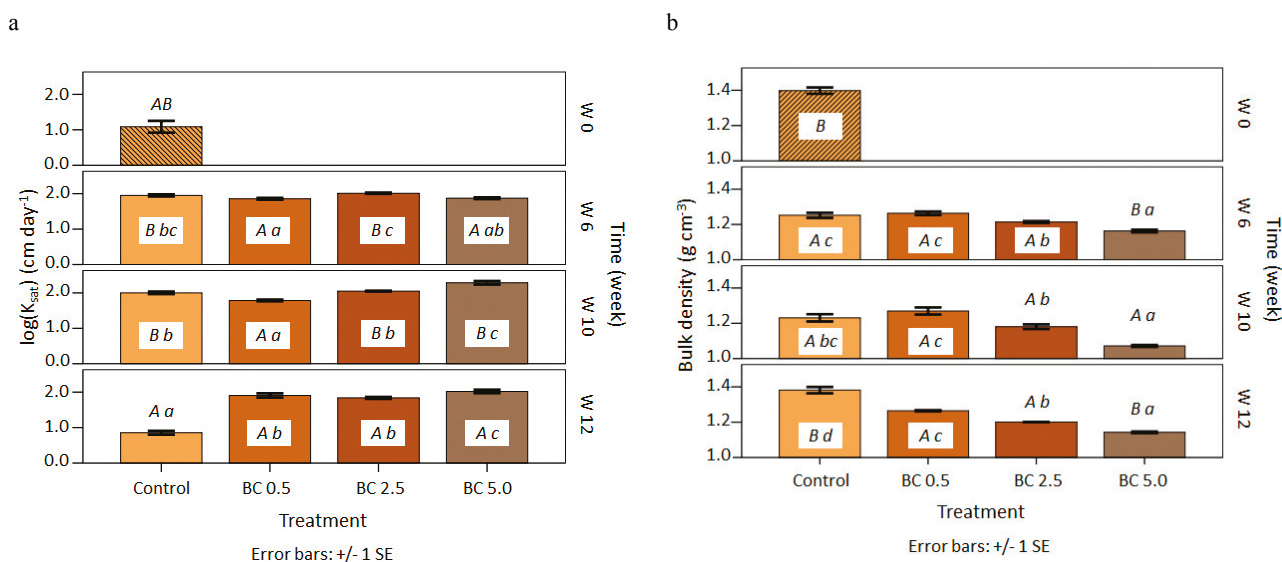
Fig. 2. Differences in: a – bulk density, b – saturated hydraulic conductivity data (means denoted by the same letter did not significantly differ at p < 0.05 according to the multiple range test; lowercase letters denote the comparison within biochar treatment, while uppercase letters compare the means of the time periods). W represents the number of weeks in the experiment, while C, BC0.5, BC2.5, and BC5.0 represent the control, 0.5, 2.5, and 5.0% biochar additions to soils, respectively. Error bars indicate the ±1 standard error of the mean for each prediction group.

ranged from between 1.25±0.03 and 1.16±0.01 g cm^{-3} for C and BC5.0, respectively. When comparing the W12 results, a further decrease in soil ρ_b was observed at high biochar additions (1.38±0.03 and 1.14±0.01 g cm^{-3} for C and BC5.0, respectively). However, these changes did not reflect a clear trend related to plant phaenological phases.

K_{sat} values showed a general increase with increasing biochar amount, with the extent of the increase decreasing over time (Fig. 2b). An increase in K_{sat} in the control treatments were also found during W6 and W10, while W12 data showed even lower average K_{sat} values than those measured in W0. The most stable K_{sat} values were measured in the case of BC0.5, where W6, W10, and W12 showed 5.5, 4.7, and 6.3 times higher values, respectively, indicating a beneficial combination of plant growth and a small amount of biochar in silt loam soil.

We analysed the effect of biochar treatment and time on bulk density, saturated hydraulic conductivity and the coefficient of uniformity with ANOVA. Treatment, Time and Treatment * Time factors were statistically significant for all soil physical parameters at $p < 0.01$. An exception to this trend occurred for the effect of the treatment on U values, where $p < 0.02$. In examining the possible causes of various degrees of compaction, we used the modified coefficient of uniformity (U_{ASD}) which showed a close relationship ($R^2 = 0.82$-0.91) with both the ρ_b and K_{sat} values of the samples (Fig. 3a, 3c). Similarly, a good correlation between ρ_b and the measured K_{sat} values were found ($R^2 = 0.77$) (Fig. 3b).

DISCUSSION

The results of biochar addition to silt loam soil with a particular emphasis on soil structural and physical changes were studied over the course of plant growth and development. A higher grade of soil aggregation may be directly related to plant growth and health, as bacteria, fungi, and fungal hyphae can also help to hold together soil particles (Kelly *et al.*, 2017; Vergani and Graf, 2016; Warnock *et al.*, 2010). The formation of soil aggregate may be a response to soil organic carbon (Liu *et al.*, 2014), and with the support of binding agents present in the soils, microaggregates may develop into macroaggregates.

The possible compaction effect due to irrigation, or other environmental conditions, is likely to depend on the particle (*PSD*) and aggregate size distribution (*ASD*). In the present study, the same soil was used for all treatments; therefore it was expected that no changes or minimal changes would occur over time in *PSD*, and this was indeed found to be the case. On the other hand *ASD* is a good indicator of the aggregation processes within the soil matrix. Different degrees of the aggregation of soil samples may be explained through biochar treatment, irrigation, precipitation or the effects of the plant roots. The *ASD* in the present study did not show major differences between the control and biochar added treatments during the different plant growth stages. However, as a slight increase in the macro-
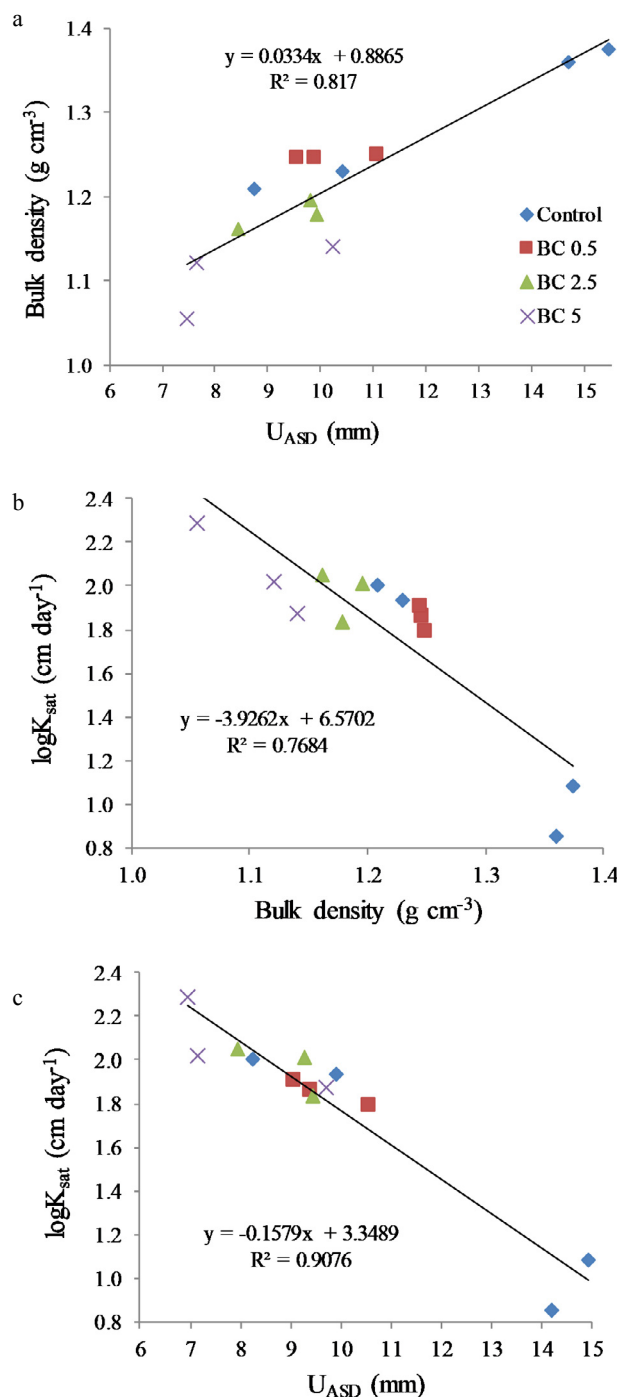
Fig. 3. Relationships are based on a linear regression between: a) the modified coefficients of uniformity (U_{ASD}) and bulk density (ρ_b), b) hydraulic conductivity (K_{sat}) and bulk density, and c) hydraulic conductivity (K_{sat}) and the modified coefficients of uniformity (U_{ASD}); (n = 13).

aggregate sizes and a decrease in microaggregate sizes was observed in all treatments, the U_{ASD} values derived from *ASD* varied widely. Hartley *et al.* (2016) observed similar results, and the authors also found that the type of biochar also played a major role in aggregate formation, concluding

that some types of biochar addition may facilitate macro-aggregate formation but others may not affect the process significantly.

Through an investigation of the changes in soil bulk density decreased ρ_b values were observed compared to the control treatments in all biochar amended soils. In this experiment a decreasing ρ_b value was also noted in the case of control treatments during the first few weeks of the study, not only in biochar amended soils. However, after the initial reduction in ρ_b, soil compaction was also observed due to changes in environmental conditions. A significant decrease in soil bulk density was expected, as the specific gravity of the biochar was less than that of the soil, these results were similar to those found by other studies (Laird *et al.*, 2010b; Lim *et al.*, 2016; Zhang *et al.*, 2012). In studying the degree of these changes over plant phaeno-logical phases, a slight increase was observed in ρ_b at W12 (harvesting) compared to W10 data in the case of higher biochar amended treatments (BC2.5 and BC5.0). Rainfall and irrigation may influence soil properties, such as *ASD*, the water infiltration rate or ρ_b. A longer period of rainfall may cause a bulk density increase in the top portion of a soil layer (Fohrer *et al.*, 1999), which may help to explain the ρ_b values for the control treatment in the present experiment, because after the initial tilling, soil compactions take place. Concurrent with changes in ρ_b we observed a slight reduction in K_{sat} values, after initial increases. The higher K_{sat} values in the case of silt loam soil may be beneficial, as soil sealing is less likely to develop, hence soil water management may be improved. Similar results were observed by Barnes *et al.* (2014), where the authors noted a strong correlation between changes in ρ_b and K_{sat} values. The findings from the present study were also comparable with a study done by Jien and Wang (2013). Although increases in K_{sat} values were observed compared to W0 data, these changes were more likely to be related to initial soil preparation (additional soil tilling prior to planting), plant root growth at later times, and only marginally influenced by biochar addition as indicated by the noted extensive K_{sat} increase in the case of the control treatments as well. Therefore, biochar alone may not cause K_{sat} changes in soils directly, instead these changes may be caused by the indirect effects of biochar related enhancement in root development, or other factors such as rainfall and irrigation may cause changes in the *ASD* values and consequently in the U_{ASD} values. These environmental factors and changes in U_{ASD} may significantly influence soil ρ_b and the water movement through soil columns. All of these changes in the K_{sat} and ρ_b values cannot be explained by biochar addition only; there is also a quantifiable connection between U_{ASD}, ρ_b and K_{sat}. Therefore it was established that *ASD* values have substantial effects on ρ_b and K_{sat}, while changes in *ASD* are influenced by irrigation, precipitation, and plant growth. Consequently, a combination of biochar addition and changes in *ASD* have an impact on ρ_b and K_{sat} in soils.

CONCLUSIONS

1. The present study emphasizes the close connection between soil structure changes and plant development phases, it highlights the importance of soil and site specific analyses prior to amendment with biochar.

2. Biochar may positively influence aggregate formation and hydraulic conductivity, it may also reduce the bulk density of the studied silt loam soil over the course of *Capsicum annuum* L. growth and fruiting cycle.

3. There were distinct relationships between the physical and structural properties of the investigated soil, thus emphasizing soil biotic health.

4. Good correlations were found between the investigated soil structural parameters and changes in them due to biochar addition, including changes during different plant phaenological stages. While these results are confirmed for one type of biochar and soil type during a given plant growing phase, further investigation is required to draw larger-scale conclusions.

Conflict of interest: The Authors do not declare conflict of interest.

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