

Soil physical properties affected by biochar addition at different plant phenological phases. Part I**

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Abstract. Soil amendment usage can substantially modify soil structural and hydraulic properties, with the aim of improving its water, air and nutrition management along with crop growth. The purpose of the present study was to investigate the physical changes in soil through different plant growing stages caused by biochar addition to silt loam soil. This research focused on changes in structural stability, and macro- and microaggregate stability. The soils were amended with different amounts of biochar (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 results showed increasing macroaggregate stability values with increasing biochar addition; however, higher values were also detectable in control treatments over time. Increased microaggregate stability values were observed during the plant maturing phase and the decrease, which occurred during fruit development was more pronounced. The largest microaggregate stability value was observed in the case of BC2.5 among all treatments, which corresponded better to plant growth rather than to the amount of added biochar. It was also found that the laser diffraction method is a suitable alternative technique to the sieve-pipette method for analysing biochar and biochar-amended soil particle size distribution and structure.

Keywords: structural stability, macro- and microaggregate stability, laser diffraction method

INTRODUCTION

The development of new soil amendments has been of scientific interest in recent decades to promote crop growth and yield by providing more favourable soil conditions for plants. Charcoal, ash, or biochar addition to soils may cause changes in soil chemical (Jien and Wang, 2013;

Liang *et al.*, 2006), microbial (Anderson *et al.*, 2011; Horel *et al.*, 2018a; Schiewer and Horel, 2017), and physical and hydrophysical properties (Jien and Wang, 2013; Liang *et al.*, 2006; Novak *et al.*, 2009; Sun and Lu, 2014), and these modifications may ultimately influence soil productivity, yield, and plant health (An and Huang, 2015; Gascó *et al.*, 2016; Helliwell, 2015). Plant growth at different stages further influences soil hydrological properties, especially the moisture regime (Angers and Caron, 1998; Surda *et al.*, 2015), whereas the addition of biochar, further affects soil and plant health and consequently needs to be carefully investigated prior to its agricultural use.

Soil structure may be considered as the shape, size and spatial arrangement of individual and variously stable aggregated soil particles. It plays an important role in the creation of the soil pore network (shape, size distribution, connectivity, *etc.*), which also affects the storage and movement of water. Aggregate size and stability are important factors in understanding soil erosion and surface sealing (Nimmo, 2004). Soil structure may change dynamically due to various internal and external causes (*e.g.* wetting and drying, climate, root penetration, soil fauna, agricultural management) (Ghezzehei, 2012); however limited studies have been conducted investigating changes in soil aggregate stabilities based on different plant growth stages, especially in the case of microaggregate stability studies (Totsche *et al.*, 2018). Among other soil structure characterization methods, the aggregate stability measurements facilitate the determination of the persistence of soil structure under destructive impacting factors. The destructive

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activity of water is most frequently measured by wet sieving or a simulated rainfall system (Amézketa, 1999). The particle size distribution (PSD) analysis for determining the stability of microaggregates belongs to this group of testing methods (Amézketa *et al.*, 2003; Bieganski *et al.*, 2010). Depending on the type of soil, biochar may have varying effects on aggregate stability, which may increase after biochar addition to silty clay and clayey soils (Hartley *et al.*, 2016; Ouyang *et al.*, 2013; Sun and Lu, 2014).

When a large amount of organic material is present in a given soil matrix, commonly used PSD methods such as the sieve-pipette method (based on sedimentation) cannot be used with a high degree of reliability. In the case of pure biochar, its specific gravity is lower than 1 so it wouldn't settle, the sieve-pipette method is very difficult to utilize; therefore, in the present study the laser diffraction method (LDM) for both PSD measurements and soil microaggregate stability studies was applied. Currently, the LDM in aggregate stability measurements is not a commonly used technique yet (Amézketa *et al.*, 2003; Mukherjee and Lal, 2013), therefore the LDM is a relatively new approach when investigating ash, charcoal, or biochar amended soils. Although many studies have investigated changes in soil physical properties as a result of biochar addition to soils (Hartley *et al.*, 2016; Jeffery *et al.*, 2015; Ouyang *et al.*, 2013), the present study examines many of these characteristics over a period of a vegetable growing season, in different phenological stages, under regular irrigation and natural rainfall conditions.

The aim of the present study was to investigate changes in the physical and structural properties of soil after biochar addition to agricultural silt loam soil over time and its interactions within the soil system. Our interests were focused on the changes in i) macroaggregate stability; ii) microaggregate stability; and iii) aggregate stability indices. The study was based on the hypotheses that i) macro- and microaggregate stability changes will intensify over time at different plant development stages, as a response to plant root development; ii) these soil physical parameters 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 samples used in the present study were collected from a silt loam, freshly tilled arable soil (46.92936°N, 17.67033°E; USDA: eroded Alfisol with long-term vineyard history) from the upper 28 cm (A horizon). The basic soil parameters are presented in Table 1.

Biochar was bought commercially for the experiment, from a manufacturer holding a European Biochar Certificate (EBC). Based on information provided by the manufacturer and a certificate of analysis, the biochar was made from paper fibre and grain husk materials using Pyreg-reactor technology at approximately 600°C. The PSD of the pure biochar amendment used in the experiment is shown in Table 1 along with its chemical properties. At the time of purchase the following additional properties were noted based on the manufacturer's certificate: bulk density 0.224 g cm⁻³, hydrogen to carbon molar ratio (H/C) = 0.39, total ash = 38.9%, and surface area correlation = 199 m² g⁻¹ (dry weight).

For the experiment, 2 kg of previously homogenized soil were placed in each pot, with dimensions of 14 cm in height and an 18 cm diameter with a drain outlet, *Capsicum annuum* L. (green pepper) were planted at the two to four leaves stages.

There were four different treatments: the control and three biochar dosages, these were studied in three replicates. Biochar additions to the soils were calculated based on an air-dried soil weight percentage. Soils 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%), while the other three were amended with biochar in the amount of 0.5, 2.5, and 5.0% by dry weight; hereafter referred to as C, BC0.5, BC2.5, and BC5.0, respectively.

The pots were then placed in natural environmental settings, e.g. natural sunlight and rainfall, in order to better simulate field conditions; however, during periods of water deficiency, irrigation (200 ml tap water per pot per irrigation event) was also performed to reduce plant stress. Natural rain water levels and irrigation activity were monitored using an ECRN-100 rain gauge (Decagon Devices Inc.,

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

Type	Particle size distribution (%)			pH-H ₂ O	Organic C (%)	K ₂ O	P ₂ O ₅	Total N (%)	NH ₄ ⁺ -N	NO ₃ ⁻ -N	CaCO ₃ (%)
	< 6.6	6.6-52.5	52.5-2000								
	µm					mg kg ⁻¹			mg kg ⁻¹		
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

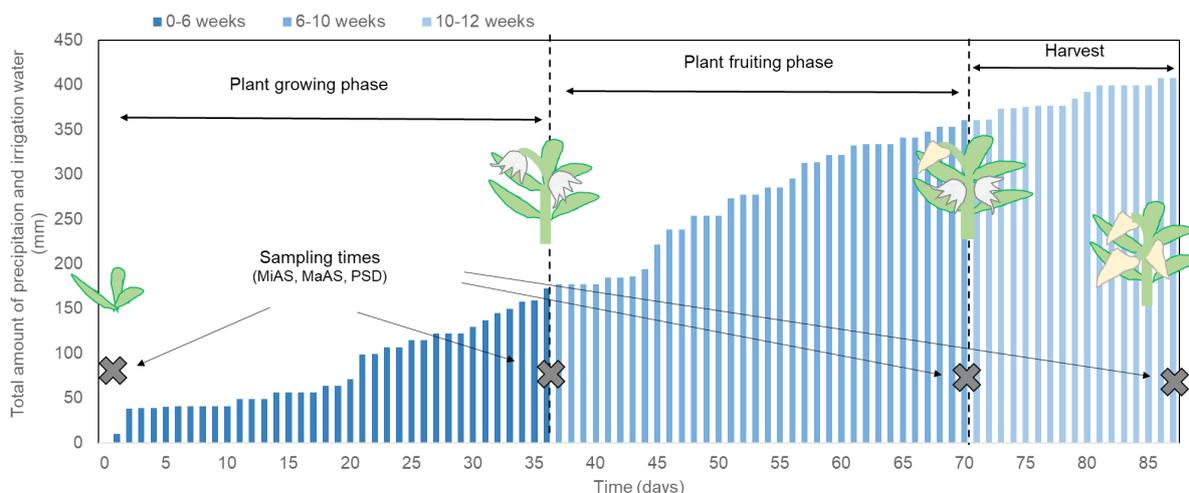


Fig. 1. The total amount of precipitation and irrigation (vertical bars) and sampling times (x signs) of the experiment. Plant phenological stages are presented by dashed lines and arrows.

Pullman, WA), where the cumulative amount of precipitation including irrigation water was 145.2 mm (week 6), 187.3 mm (week 10), and 248.8 mm (week 12) (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 soil corresponding to three distinguishable plant phenological phases, with the main focus being on micro- and macroaggregate stability. W0 data represents the starting parameters 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 represents the time period when fruit harvesting was performed. The different plant growth phases were determined by observation, such as tiller and leaf numbers, flowering stages, fruit development, and root and shoot system biomass weight. Fruit harvesting took place at W10 of the experiment from selected disassembled pots (two plants per treatment) and at W12 from all of the remaining pots (8 plants per treatment), and the data obtained were used to further evaluate micro- and macroaggregate stability changes in soil. The present study is a part of a more complex study investigating the effects of biochar addition to the biological, chemical, and physical parameters of soil (Horel *et al.*, 2018b).

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

Macroaggregate stability (*MaAS*, %) was measured by a wet sieving apparatus (Eijkelkamp), where 4 g of soil per sample from 1-2 mm size aggregates were used (Kemper and Rosenau, 1986). After careful pre-moistening, samples were placed into 0.25 mm mesh size sieves, immersed in containers filled with distilled water, and moved in an upward and downward direction for 3 min (stroke = 1.3 cm, at about 34 times min^{-1} , f_{nd}). After transferring the sieves

to alternative containers, they were immersed in sodium pyrophosphate dispersing solution (with a concentration of 2 g l^{-1}) and moved up and down for 8 min, at the end of the operation, the non-dispersed, very stable aggregates were gently separated by a rubber stick (f_d). Both sets of containers (soil suspensions with distilled water and pyrophosphate solution) were dried through evaporation before being oven dried (24 h, 105°C) prior to mass measurements. The percentage of the stable fraction was calculated as a ratio of the weight of the soil obtained in the distilled water (f_d) divided by the sum of the obtained weights in the dispersing solution and distilled water ($f_{nd} + f_d$; Eq. (1)).

$$MaAS = \frac{f_d}{f_{nd} + f_d} \cdot 100. \quad (1)$$

Microaggregate stability (*MaAS*, %) was calculated according to Vageler's structure factor (Eq. (2)) from the rate of clay fractions determined with dispersion (c_d) and without any dispersion (c_{nd}) (Vageler, 1932):

$$MaAS = \frac{c_d - c_{nd}}{c_d} \cdot 100. \quad (2)$$

The value of the clay fraction was measured by LDM using a Malvern Mastersizer 2000 device with a HydroG dispersion unit. Soil samples were sieved through a 2 mm sieve prior to LDM analysis. Dry soil samples (without the prior removal of organic matter and calcium carbonate) were moistened by adding 1-1.5 ml of Calgon solution (33 g l^{-1} of sodium-hexametaphosphate and 7 g l^{-1} sodium-bicarbonate), the soil paste was washed into a $\sim 800 \text{ cm}^3$ volume tank of the dispersion unit and a further 25 cm^3 of Calgon solution was also added. The soil suspension was treated with ultrasound at 75% of maximum power (0.75 \times 35 W or 0.75 \times 40 kHz) for 240 s before the measurements were taken (Bieganowski *et al.*, 2010). The obscuration values were between 10 and 20%. The Mie theory was applied (refraction index: 1.33 for water and

1.52 for soil, absorption index: 0.1 for the dispersed phase; general purpose analysis; irregular shape ratio) (Ryżak and Bieganski, 2011). The clay-silt fraction boundary was set at 6.6 μm based on Makó *et al.* (2017), while 52.5 μm was chosen as the silt-sand fraction boundary to make the results more comparable with the sieve-pipette method. In the case of the non-dispersing technique, neither ultrasound application, nor chemical compounds were used. During laser analysis continuous stirring and pumping was applied, which, along with premoistening prevented biochar from floating on the water surface, and hence overcame the limitations of the pipette method.

Based on the PSD curves of both the dispersed and non-dispersed particles, the geometric mean diameter (GMD) was calculated. In addition, the aggregate stability index (SI_{GMD}) which is the ratio of the GMD of the non-dispersed to the dispersed particles was calculated.

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 and time on the measured soil physical parameters. The combined effects of treatment and time on the investigated soil physical properties were also tested with the analysis of variance, ANOVA (Univariate General Linear Model; SPSS 13.0). The relationships between the selected soil properties were further investigated with the linear regression method (Linear regression, SPSS 13.0).

RESULTS

In general, macroaggregate stability (*MaAS*) increased to some extent with increasing biochar addition as compared with the control (Fig. 2a). An increase in *MaAS* was also detectable in the control treatment over time, where a 49.5% higher stable aggregate ratio was found in W6 samples compared with W0 (Fig. 2a). Similar, but more pronounced results were observed at W6 when biochar was present in the soil. 64.7, 131.2, and 174.7% higher *MaAS* values were found for BC0.5, BC2.5, and BC5.0, respectively, compared with the W0 controls (Fig. 2a). Later in the experiment, *MaAS* ratios were still higher for the different treatments compared with the controls; however, due to environmental factors, such as regular irrigation, some decreases in *MaAS* values were also observed towards the end of the experiment (Fig. 2a). These changes were still not significant among treatments over time, except for the control (W0 compared with W6) and the BC5.0 treatment (between W6 and W12).

During microaggregate stability measurements, the LDM PSD of differently treated soil samples from different stages of the experiment were measured with and without dispersion pretreatment. Figure 3a illustrates the PSD curves of the soils using both methods during the plant maturing period, while Fig. 3b illustrates the BC5.0 dosages at different stages of plant growth. The PSD curves of the dispersed samples (which in the present study are

considered the true PSD) did not change significantly either due to different biochar (BC) doses, nor during the different phenological periods. On the contrary, the non-dispersed PSD curves of the samples, which reflect the structure and aggregate stability of the soil, showed substantial differences depending on BC treatments and plant growth stages. A greater similarity between the dispersed and non-dispersed PSD curves of a given sample indicates the reduced

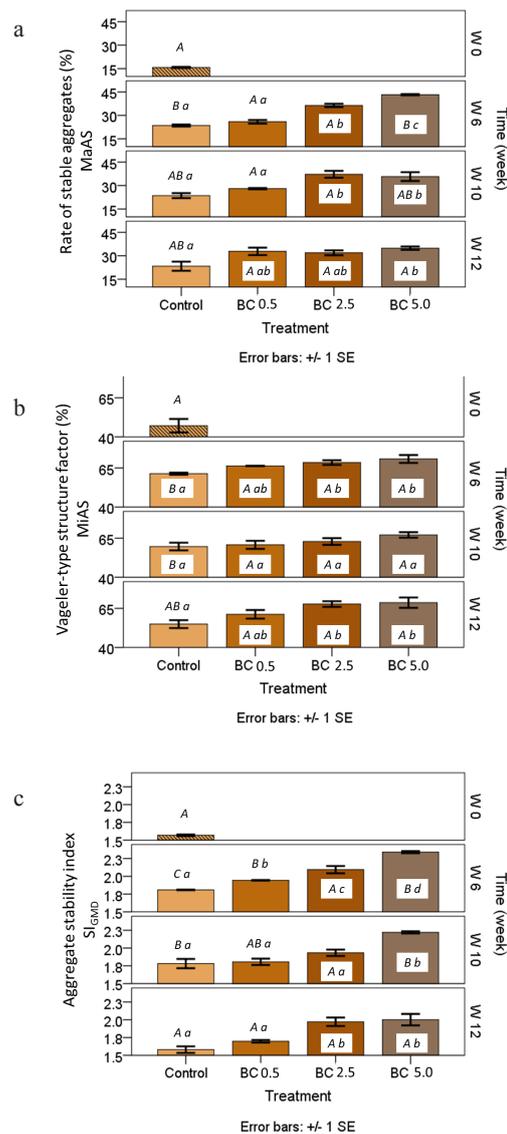


Fig. 2. Differences in (a) macroaggregate stability data; (b) microaggregate stability data; and (c) aggregate stability indices. W represents the number of weeks in the experiment, while C, BC0.5, BC2.5, and BC5.0 represent the control, 0.5, 2.5, and 5.0% biochar additions to soils, respectively. *MaAS* – macroaggregates stability; *MiAS* – microaggregates stability; SI_{GMD} denotes the aggregate stability index based on the geometric mean diameter of particles. Means denoted by the same index did not significantly differ at $p < 0.05$ according to the multiple range test; lowercase letters denote the comparison within biochar treatments, while uppercase letters compare the means of time periods. Error bars indicate ± 1 SE.

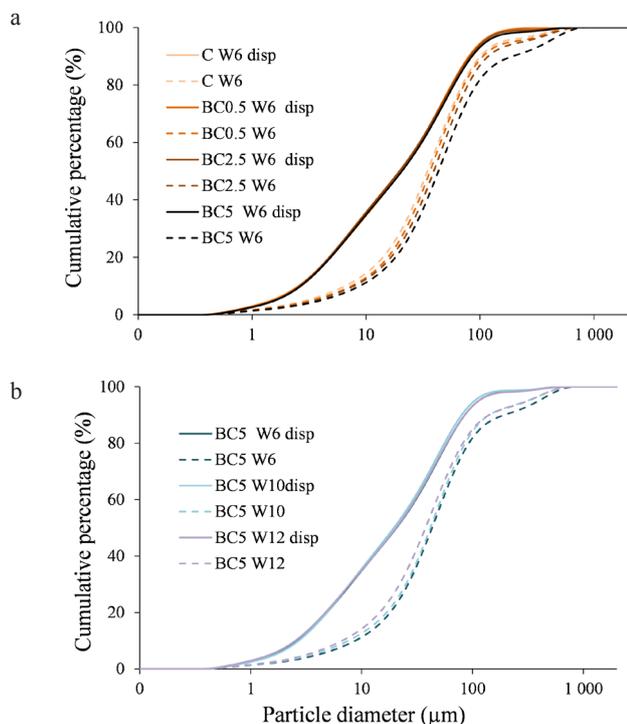


Fig. 3. Comparison between particle size distributions obtained by (a) different biochar treatment doses measured after dispersion (disp) and without dispersion pretreatment (W6); (b) different phenological stages measured after dispersion (disp) and without dispersion pretreatment (BC5.0 biochar dose). W represents the number of weeks in the experiment, while C, BC0.5, BC2.5, and BC5.0 represent the control, and 0.5, 2.5, and 5.0% biochar additions to soils, respectively.

impact of the soil aggregates caused by dispersion processes as well as reduced aggregate stability. The control samples showed the least stable aggregates at W6 (Fig. 3a) and BC5 at W12 (Fig. 3b), which implies that BC can significantly affect the *MiAS* values. The differently measured PSD data were compared in a USDA soil texture triangle (Fig. 4). Figure 4 demonstrates that the PSD of the dispersed soil is less dependent on the experimental conditions than on the PSD of the non-dispersed soils. This means that the farther apart the measured pairs of points are on the triangle in

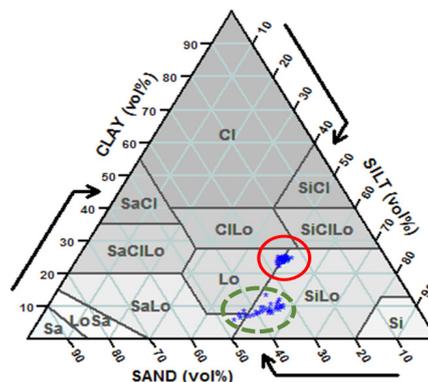


Fig. 4. Distribution of soil textures in the USDA textural triangles, where the circle represents the LDM PSD of soil samples originating from different plant phenological phases and biochar treatments measured after dispersion and the dotted area signifies the LDM for aggregate stability measurements, without chemical dispersion and sonication.

both directions, the greater the microaggregate stability of a given soil. The above-mentioned PSD results were used to calculate the indices of microaggregate stability such as the *MiAS* and the SI_{GMD} values.

The measured *MiAS* data of the differently treated samples are shown in Fig. 2b. The freshly tilled soil at W0 had an average *MiAS* value of 47.11%. Significant increases in the *MiAS* values were observed during W6 when the plants reached maturity, and stability rose with values 30.2 and 49.6% larger (for C and BC5.0, respectively) compared with the W0 data (Fig. 2b). However, these changes slowly decreased during the fruit harvesting phase, resulting in overall 8.9, 18.7, 36.5, and 33.6% higher *MiAS* values in the case of C, BC0.5, BC2.5, and BC5.0, respectively, compared with the W0 data. In general, a small amount of biochar addition increased the *MiAS* value of the soils, but BC5.0 and BC2.5 showed similar *MiAS* data. These differences were statistically detectable in the W6 and W12 samples (Fig. 2b). Similar changes were found in the SI_{GMD} values over time and different BC doses (Fig. 2c). The calculated stability indexes (*MaAS*, *MiAS* and SI_{GMD}) are presented in Fig. 5, where a very good correlation is shown

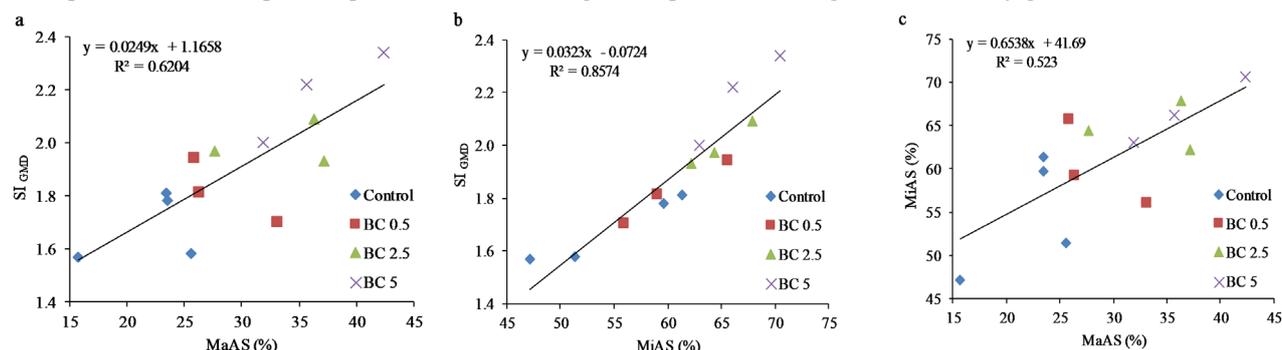


Fig. 5. Correlations between a – SI_{GMD} and *MaAS*, b – SI_{GMD} and *MiAS*, and c – *MiAS* and *MaAS* aggregate stability indexes. SI_{GMD} denotes the aggregate stability index based on the geometric mean diameter of particles; *MaAS* – macroaggregates stability; *MiAS* – microaggregates stability (n = 13).

Table 2. Effect of biochar treatment and time on the examined soil physical parameters. Summary results of ANOVA tests (Univariate General Linear Model), for macroaggregate stability (*MaAS*, %), microaggregate stability (*MiAS*, %), aggregate stability index (SI_{GMD}). *p* represents the probability (significance level from ANOVA). Represents significant difference at **p* < 0.05 and **at *p* < 0.01

Factors	Investigated soil parameters		
	<i>MaAS</i>	<i>MiAS</i>	SI_{GMD}
	<i>p</i>		
Treatment	<0.001**	0.002**	<0.001**
Time	0.111	0.012*	<0.001**
Treatment × Time	0.057	0.736	0.117

between SI_{GMD} and *MiAS* ($R^2=0.86$), a good correlation was noted between SI_{GMD} and *MaAS* ($R^2=0.62$) and between *MiAS* and *MaAS* ($R^2=0.53$). In summary, the results of ANOVA tests analysing the effect of biochar treatment and time on the examined soil physical parameters are listed in Table 2.

DISCUSSION

The present paper reports on soil structural and physical parameter changes caused by biochar addition under natural environmental conditions and over the course of plant growth and development. In summary, both biochar dosage and time combined had positive effects on *MaAS* values. 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 changes in soil organic carbon content (Liu *et al.*, 2014), and with the support of a binding agent present in the soils, microaggregates can develop into macroaggregates. The growth of plant roots may also act as a binding agent in the development of more resilient aggregates via polysaccharides or organic residues (Amézqueta, 1999). During plant growth both *MiAS* and *MaAS* values were substantially higher compared with the W0 data, especially in the case of biochar amended soils, which also indicates improved soil conditions for crop growth. The high *MiAS* value produced by BC5.0 shows that more coagulated colloids are present in BC5.0 treatment; therefore, more water stable aggregates may be formed. Several publications reported increased aggregate stability after biochar addition (Ouyang *et al.*, 2013; Šimanský *et al.*, 2016), which was also confirmed by the present experiment. Macroaggregate stability is also affected by plant root development over time, without the addition of any soil enhancer materials (Angers and Caron, 1998; Vergani and Graf, 2016). Microbial synthetic prod-

ucts may serve as binding agents for aggregate formation while water may function as the main agent for aggregate breakdown (Bossuyt *et al.*, 2001; Lynch and Bragg, 1985). *Capsicum annuum* L. has a high probability of mycorrhizal colonization (Sensoy *et al.*, 2007), which means that fungal hyphae growth may be enabled. The relationship between biochar and mycorrhiza in the root and soil system, however, may be dependent on the types of biochar or soil, among many other environmental conditions. The plant growth, which is enhanced by biochar amendment may result in a different crop yield as well, especially when the soil is not nutrient limited (Albuquerque *et al.*, 2013). An increase in fruit growth may also increase nutrient uptake by the plant from the soil, and consequently less nitrogen may be available for mycorrhizal and microbial growth, which in turn influences aggregate stability. In the present study, the best fruit yield was observed in the case of BC5.0, when only mature (> 25 g) fruit were measured at harvest. When all mature and undeveloped fruit were accounted for in the total fruit yield, then the BC2.5 treatment showed the highest fruit biomass (8% higher compared to BC5.0). Even though these changes were pronounced, they were statistically insignificant (*p* > 0.05); therefore, the crop yields were not considered as a major factor contributing to soil physical changes.

Soil moisture content may influence the physical characteristics of a given soil (*e.g.* compaction), such as the particle size distribution (PSD) by splash detachment, however, over the course of the experiment this was unlikely to occur. In the present study, the same soil was used for all treatments; therefore, the expected result was to find zero or minimal changes over time in PSD (Fig. 3). It was found in previous studies that biochar may help to retain more water in amended soils resulting in improved soil water holding capacities (Ulyett *et al.*, 2014). On the other hand, biochar amended soils may experience a faster drying period after rainfall events compared with the control or amended soils with lower concentrations of biochar (Horel *et al.*, 2018b), resulting in similar overall water contents in the investigated soils. The concentration of biochar however, may reach a limit when soil moisture increases or retention does not result in significantly higher moisture contents (Dugan *et al.*, 2010). Our study showed similar results, the highest overall soil moisture content was observed in the case of the amended treatments with the most biochar, where the studied soil had a relatively good infiltration capability, which may in turn facilitate more suitable environmental conditions for plant growth and development.

CONCLUSIONS

1. The present study emphasizes the strong connections between soil structure changes and plant development phases, and also emphasizes the importance of soil and site specific analyses prior to amendment with biochar.

2. There were changes in aggregate stability without the addition of biochar to silt loam soil; however, these changes were less pronounced when compared to the biochar amended treatments, indicating better soil structural strength in the presence of biochar.

3. The amount of biochar addition may influence the rate of aggregate stability increase, such that too much biochar addition may not provide optimal results.

4. There were distinct connections between the physical and structural properties of the investigated soil.

5. Good correlations were found between the investigated soil structural parameters and soil changes due to biochar addition, including changes, which occurred during different plant phenological stages.

6. Laser diffraction method was used to analyse soil particle size distribution instead of the sieve-pipette method, and shown to be a very useful tool with which to analyse highly organic soils, and soils amended with ash, charcoal, or biochar for particle size distribution.

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