Original Research

Effects of Biochar Combined with N-fertilization on Soil CO₂ Emissions, Crop Yields and Relationships with Soil Properties

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> Received: 30 September 2019 Accepted: 29 January 2020

Abstract

Biochar is considered a valuable tool for improving soil fertility with a lower carbon footprint remaining in the background of sufficient and healthy production, particularly in tropical and subtropical conditions and especially on sandy soils. The aim of many scientists is to confirm this fact in other climatic regions of the world and on soils with different textures. Therefore, the aim of this study was to determine the effect of biochar applied separately or in combination with N-fertilizer (first to eliminate the wide C:N ratio after biochar application, which can have a priming effect on the soil organic matter and, secondly, to follow the standard N fertilization practice in Slovakia, where the amount of fertilizer applied is calculated according to the crop) to silty loam (the dominant soil texture in Slovakia) Luvisol (the most intensively used soil type in Slovakian agriculture) at Dolná Malanta, Slovakia, on (i) CO₂ emissions, and (ii) grain yields in 2014-2016. The cumulative CO₂ emissions from the soil for the treatments with biochar (applied with or without N-fertilizers) were from 2% to 27% lower compared to control. In 2014, grain yield was from 5% to 42% higher for the treatments with the lower biochar rate at all fertilization levels. On the contrary, a lower increase in grain yield was observed in the second year at the treatments with the higher biochar on CO₂ flux reduction and grain yield increase was observed.

Keywords: biochar, CO, emission, crop yield, Luvisol, nitrogen fertilization

Introduction

FAO forecasts confirm that food obtained by soil management (only 11% of the planet's area is suitable)

will continue to be the main source of nutrition for the Earth's population in the third millennium. However, in developed countries there has been a noticeable decline in agricultural soils area and growth of forested and built-up areas over the past 60 years. In less developed and developing countries the trend is reversed as there is an increasing effort to spread tilled agricultural areas

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and to intensify these soils [1]. Due to these reasons, sustainable concepts combining increased food production and soil sustainability are urgently needed in order to reduce the pressure on soils and prevent the negative effects of intensive agriculture on the environment.

Management practices in agriculture can affect concentrations of the three main greenhouse gases (GHGs) in the atmosphere, namely carbon dioxide (CO_2) , nitrous oxide (N_2O) and methane (CH_4) . Concentrations of CO₂ in the atmosphere have been increasing at the rate of 3.2×10^{15} g C year⁻¹, where the contribution of agriculture and land use change is about 20% [2]. Soil is a dynamic component of the global C cycle and can be a source or a sink of CO₂ depending on management practices. Although fossil fuel combustion is the major cause of the increase in atmospheric CO₂ concentration, agricultural activities have also been a significant contributor. Historically, soils have lost about 40 to 90 Pg C (estimated value) to the atmosphere. Conventional plowing has been regarded as a major cause of this C loss in cropping soils. Tillage-induced SOC losses have been well documented [3]. The rate of soil CO₂ emission is strongly related to the amount and type of organic materials present or added to soil, and the complex interaction between soil physical, chemical, and biological processes as well as environmental conditions such as temperature, precipitation, etc. [4, 5].

It was shown that more CO₂ was produced in fertilized soils compared to non-fertilized. It was also shown that dry soils after a subsequent increase of soil moisture (after rain) release more CO₂ than soils that never were too dry and that more productive soils release less CO, as compared to less productive soils [6-8]. As a result of the projected increase in atmospheric CO, concentration, the interest of the environmentalists is rising to reduce CO₂ emissions from soils and increase soil carbon (C) reserves [9], because soil organic carbon (SOC) is one of the most important factors affecting soil fertility. The content of SOC in arable soils is limited not only by soil genesis but also by the intensity and depth of plowing. The average values of SOC content in arable soils in Slovakia range from 1% to 2.5% [10]. In Luvisols in Slovakia (also the soil type at our experimental site), SOC content ranges from 0.88% to 2.17% [11], with the lower value being too low and not satisfactory for sustainable agricultural practice. The Luvisols are the most intensively used soils in Slovak agriculture [12], and one of the ways to increase their productivity could be enrichment of the soils with SOC as its beneficial effect on soil production capacity is widely known [13, 14].

One of the possible and at the same time innovative solutions to increase SOC content can be the application of biochar to the soil. A number of scientific studies have shown that biochar was a material that as a soil ameliorant had the potential to increase SOC content and improve soil quality, contributing to a higher yield from a smaller area [15, 16]. Application of biochars to soils has been reported to improve a soil's chemical [17, 18], physical [19, 20] and biological properties [21]. It was also demonstrated that biochar application to soils resulted in increased crop yields, reduced GHGs emissions, and increased soil carbon sequestration [22-24].

The objective of this study was to evaluate the effect of biochar application alone and in combination with an N-fertilizer during 2014-2016 on (1) CO_2 emissions from soil, (2) the relationship between selected soil physical and chemical properties with CO_2 emissions and (3) crop development and grain yields. In particular, our intention was to examine the hypotheses that (H1) the addition of biochar reduces CO_2 emissions from arable soils and (H2) biochar application has a positive effect on yields of agricultural crops.

Material and Methods

Field Site

The field experiment was established on the experimental site in Dolná Malanta of the Slovak University of Agriculture in the Nitra region of Slovakia (48°19'N, 18°09'W). The site is located in a temperate zone with average annual air temperature of 9.8°C and average annual rainfall of 539 mm. Prior to the establishment of the field experiment, agricultural crop production at the experimental site followed management practices conventional for several decades. The soil was classified as the silt loam Haplic Luvisol according to the soil taxonomy [25] with sand, silt and clay contents of 15.2%, 59.9% and 24.9%, respectively. On average, the soil contained 9.13 g kg⁻¹ of SOC, while the average soil pH was 5.71.

Treatments of Soil with Biochar

The experiment was set up in March 2014. The crop rotation for three growing seasons included spring barley (*Hordeum vulgare* L.) in 2014, corn (*Zea mays* L.) in 2015 and spring wheat (*Triticum aestivum* L.) in 2016. Biochar (0, 10 and 20 t ha⁻¹) was applied in March 2014, and that was followed by the application of a nitrogen fertilizer (calcium ammonium nitrate LAD 27) at three application levels (N0, N1 and N2). The N0 fertilization level had no N-fertilizer. The N1 fertilization level was calculated according to each crop requirement using the balance method, and the N2 fertilization level included 100% more N-fertilizer in 2014 and 50% more in 2015 and 2016 than the N1 fertilization level. The field experiment included the following 9 treatments in three replicates:

- B0N0: 0 t ha⁻¹ biochar; 0 kg N ha⁻¹
- B10N0: 10 t ha⁻¹ biochar (applied in 2014 only); 0 kg N ha⁻¹
- B20N0: 20 t ha⁻¹ biochar (applied in 2014 only); 0 kg N ha⁻¹

- B0N1: 0 t ha⁻¹ biochar; 40, 160 and 100 kg N ha⁻¹ in 2014, 2015 and 2016, respectively
- B10N1: 10 t ha⁻¹ biochar (applied in 2014 only);
 40, 160 and 100 kg N ha⁻¹ in 2014, 2015 and 2016, respectively
- B20N1: 20 t ha⁻¹ biochar (applied in 2014 only);
 40, 160 and 100 kg N ha⁻¹ in 2014, 2015 and 2016, respectively
- B0N2: 0 t ha⁻¹ biochar; 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively
- B10N2: 10 t ha⁻¹ biochar (applied in 2014 only); 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively
- B20N2: 20 t ha⁻¹ biochar (applied in 2014 only); 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively

The 27 test plots (4 m x 6 m) were arranged in a random design, separated by 0.5 m-wide protective strips and in the intermediate rows by 1.2 m wide access pathway. The entire experimental field was plowed prior to the start of the experiment, followed by localization of the plots with different treatments and biochar application with immediate cultivator incorporation into the soil (0-10 cm). The N-fertilizer was applied to the soil surface before growing season in 2014 and split N-fertilizer application (before and during the growing season) was used in 2015 and 2016. The subsequent soil management procedures carried out in individual years of the experiment are shown in detail in Fig. 1.

The biochar used in the experiment was produced from paper fiber sludge and grain husks (1:1, Sonnenerde,

Austria) by pyrolysis at 550°C for 30 minutes in a Pyreg reactor (Pyreg GmbH, Dörth, Germany). The basic physical and chemical parameters of the biochar: C content 53.1%, N content 1.4%, pH 8.8, bulk density 0.21 g cm⁻³, surface area 21.7 m² g⁻¹ and ash content 38.3%.

Measurement of CO₂ Emissions from Soil

Air samples were collected between March and November in 2014, 2015 and 2016 using the closed chamber method [26]. A metal collar frame was inserted 10 cm deep into the soil in every treatment plot and left undisturbed between the soil management operations. For management operations the collars were removed and then placed back again to their original positions. Gas samples were collected at weekly intervals, the chambers (30 cm in diameter and 25 cm in height) were water-sealed onto the bottom collars at every sampling event and gas samples were collected through tube fittings (20 ml, sealed with septum) at 0, 30 and 60 minutes after chamber deployment using an air-tight syringe (Hamilton) and transferred to pre-evacuated 12 ml glass vials (Labco Exetainer). Gas samples were analyzed for CO₂ using a gas chromatograph (GC-2010 Plus Shimadzu) equipped with a thermal conductivity detector (TCD). Average daily CO₂ emissions are reported in kg ha-1 day-1. Cumulative CO, fluxes (March - November) were calculated by interpolating the emissions between each sampling day and are reported in t ha⁻¹.

Year	Crop	January February March April May June July August September October November December
2014	spring barley	B10; B20; N40; N80 H D D 10. 3. 22. 5. 24. 7. 25. 9. 11. 3. 14. 7.
2015	corn	D N80, N120 H N80, N120 D 24. 3. 24. 4. 15. 5. 5. 8. 12. 11. 27. 4. 29. 9.
2016	spring wheat	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
Crop	sowing ha	→ arwest
B10, B20	 application of bio 	ochar (10 and 20 t.ha ⁻¹)
10. 3.	- date	
N (x,y)	- application of N f	fertilizer at rates x,y (kg.N.ha ^{.1})

- H application of herbicide
 - disking

D

Fig. 1. Scheme of crop management practices during 2014-2016.

Measurement of Soil Physical and Chemical Properties

Soil samples were collected to determine soil pH (potentiometrically in 1 M KCl at KCl:soil ratio of 1:2.5) and content of inorganic nitrogen forms (N-NH₄ ⁺ and N-NO₃⁻). The amount of soil N-NH₄ ⁺ and N-NO₃⁻ in filtrates were determined with a spectrometer (WTW SPECTROFLEX 6100, Weilheim, Germany) using the calorimetric method. At every soil sampling occasion three disturbed randomly spaced soil samples

were collected at each plot from a depth of 0-10 cm and, after mixing, representative soil samples were formed for all the plots. At each gas sampling occasion, disturbed soil samples from a depth of 0-10 cm were also collected to determine the soil water content (gravimetric method) while the soil temperature was measured at a depth of 5 cm (Volcraft DET3R thermometer). The soil bulk density (BD) was measured twice a year (in March and May 2014; in March and November 2015; and in May and October 2016) in undisturbed soil samples collected from a depth of



Fig. 2. Daily CO₂ emissions for individual treatments in 2014-2016. Error bars represent±standard error. B - application of biochar (10 and 20 t ha⁻¹); N - nitrogen fertilization (kg N ha⁻¹); S - sowing of crop; Z - crop harvest; D - disking. B0N0: 0 t ha⁻¹ biochar; 0 kg N ha⁻¹, B10N0: 10 t ha⁻¹ biochar (applied in 2014 only); 0 kg N ha⁻¹, B20N0: 20 t ha⁻¹ biochar (applied in 2014 only); 0 kg N ha⁻¹, B20N0: 20 t ha⁻¹ biochar (applied in 2014 only); 0 kg N ha⁻¹, B0N1: 0 t ha⁻¹ biochar; 40, 160 and 100 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B10N1: 10 t ha⁻¹ biochar (applied in 2014 only); 40, 160 and 100 kg N ha⁻¹ in 2014, control tha⁻¹ biochar; 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B10N1: 20 t ha⁻¹ biochar (applied in 2014 only); 40, 160 and 100 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B10N1: 20 t ha⁻¹ biochar (applied in 2014 only); 40, 160 and 100 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B10N1: 20 t ha⁻¹ biochar (applied in 2014 only); 40, 160 and 100 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B0N2: 0 t ha⁻¹ biochar; 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B10N2: 10 t ha⁻¹ biochar (applied in 2014 only); 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N2: 20 t ha⁻¹ biochar (applied in 2014 only); 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N2: 20 t ha⁻¹ biochar (applied in 2014 only); 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N2: 20 t ha⁻¹ biochar (applied in 2014 only); 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N2: 20 t ha⁻¹ biochar (applied in 2014 only); 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively.

2-7 cm with steel cores 100 cm³ in volume. The volumetric soil water content was calculated using gravimetric water content and soil BD data. The soil water-filled pore space (WFPS) for the soil of each plot was calculated from the volumetric soil water content and soil bulk density data, assuming that the soil specific bulk density was 2.65 g cm⁻³.

Crop Development and Grain Yield Measurements

To assess the effect of biochar application on the crop biomass development, a non-destructive method was used: canopy images were taken by a digital camera (SONY NEX3 in 2014 and 2016 and Canon EOS 60D in 2015) from a height of 1.7 m above the plant canopy and focusing toward the center. The images were taken after the beginning of crop emergence until approximately the milk maturity stage (the peak of green biomass development) [27] in two replicates per plot, giving a total sum of six photographs per treatment per sampling date. In total, the canopy was photographed 8, 5 and 5 times in 2014, 2015 and 2016, respectively. Using Breedpix software [28], the vegetation indices were calculated as the ratio of the green pixels (plant biomass) over all pixels in the photograph (Fig. 2). More detailed information on the principle of calculating vegetation indices derived from red-greenblue (RGB) images in the above-mentioned software are presented in a paper by Vergara-Díaz et al. [29]. When weeds were present among the crop canopy, the specific photo had to be modified accordingly to prior processing with the GIMP 2.10.12 software in order to determine the green fraction only from the studied crops [30].

At the maturity stage, crops were harvested from randomly selected areas (0.5 m by 0.5 m) on each plot each year. Whole aboveground plant biomass was transported to the laboratory and processed to determine yield parameters (3 replicates per treatment). Total aboveground biomass was determined after drying the biomass in an oven at 60°C to constant weight. The final grain yield was calculated according to [27] as a multiplication of the total number of ears per m², number of grains per ear and average grain weight. Grains of barley and wheat were threshed in a mechanical thresher, while corn was processed manually. All grains were counted by a digital seed counter.

Statistical Analyses

The impact of biochar amendment on CO_2 emissions, selected chemical and physical soil characteristics and yield parameters was assessed by a statistical one-way analysis of variance (ANOVA) using the Statgraphics Centurion software (XV v. 15.1.2) by LSD test (p<0.05). Further, regression analyses to determine the interrelationships between the CO_2 emission and the soil properties as well as between the vegetation indices, aboveground biomass and grain yields were used.

Results and Discussion

Effect of Biochar on CO₂ Emissions

Fertilization is an important factor affecting CO, emission from soils [7, 8], which was also confirmed by our findings. The treatments with the fertilizer resulted generally in higher CO₂ emissions when compared to treatments without the fertilizer (Table 1). In the first year of the field experiment (2014), the soil average daily CO₂ emissions in the treatments where biochar application was combined with N-fertilization (both levels) were lower compared to the treatments with the fertilizer but without biochar. However, due to the high variability between replicates, a significant increase in average daily CO2 emissions (P<0.05) was found only in the B20N2 treatment compared to the control (B0N2). The cumulative CO₂ emissions for 2014 for the treatments with biochar (either in combination with or without the fertilizer) were 2-27% lower compared to their respective controls without biochar. Similarly, in the second year of the experiment (2015), the soil average daily CO₂ emissions in the treatments with biochar were 1-15% lower compared to non-biochar treatments, but these differences were not statistically significant. Only in treatment B10N1 was the average daily CO₂ emission 25% higher compared to the control without biochar (B0N1), and it was statistically significant. In 2016, unlike in 2014 and 2015, no reduction in CO₂ emissions from the soil was observed in the treatments with biochar application. In contrast, the average daily CO₂ emissions were higher in the treatments with biochar compared to the treatments without biochar. Two exceptions of the positive effect of biochar on the reduction of average daily CO₂ emissions were observed: in the treatments B10N1 (12% increase, not significant compared to B0N1) and B20N2 (5% increase, not significant compared to B0N2). The same picture was reflected in the cumulative CO₂ emissions over the whole measurement period (27 months), where the treatments with biochar showed 11-16% higher CO₂ emissions compared to their controls. The spatial variability of CO₂ emissions between replicates of the same treatment could be a factor contributing to the statistical ambiguity of the results, as it was also reported in the literature [7, 31-35]. Biochar is considered to be a soil ameliorant that contributes to an increase of carbon sequestration [11, 20, 36-39] and reduction of CO₂ emissions. These findings are confirmed by our results, as the soil sample analyses have shown that biochar application - either in combination with or without N-fertilizer – led to an increase in SOC content during the period of 2014-2016 compared to control treatments [39] and, at the same time, the application of biochar showed potential to reduce (not statistically

Treatments	Avera;	ge daily emissions of kg CO ₂ -C ha ⁻¹ day ⁻¹	of CO ₂)	Cumulative emissions CO_2 (t CO_2 -C ha ⁻¹)					
	2014	2015	2016	2014	2015	2016			
B0N0	145.7±19.6ª	47.8±9.1ª	135.9±21.2ª	36.2±5.0ª	10.7±2.0ª	30.4±5.0ª			
B10N0	116.8±36.4ª	44.4±6.7ª	155.2±18.7ª	29.2±5.4ª	10.1±1.5ª	34.4±4.3ª			
B20N0	126.9±24.4ª	46.9±7.1ª	152.9±27.0ª	31.6±3.7ª	10.6±1.6ª	34.1±6.0ª			
B0N1	146.5±32.8ª	45.9±8.3ª	163.8±28.5ª	36.6±8.4ª	10.3±1.8ª	36.4±6.4ª			
B10N1	133.0±21.0ª	55.5±15.3ª	142.5±17.9ª	33.5±5.8ª	12.8±3.8ª	32.0±4.2ª			
B20N1	126.3±15.5ª	44.0±5.9ª	193.8±31.6ª	31.6±4.1ª	9.9±1.3ª	42.2±6.7ª			
B0N2	179.5±23.7 ^b	55.6±17.0ª	191.4±30.8ª	44.8±6.2 ^b	12.2±3.5ª	42.2±6.8ª			
B10N2	174.9±17.1 ^b	45.8±7.3ª	212.5±25.9ª	43.8±4.5 ^b	10.4±1.7ª	46.7±5.5ª			
B20N2	132.1±12.9ª	46.3±5.9ª	181.4±31.3ª	32.8±3.4ª	10.5±1.3ª	40.2±6.9ª			

Table 1. Average daily and cumulative CO_2 emissions (±standard error) from the silt loam Haplic Luvisol during the studied period of 2014-2016.

Note: The different letters in the columns indicate that the mean values of the individual treatments are significantly different at P<0.05 in accordance with the LSD test. B0N0: 0 t ha⁻¹ biochar; 0 kg N ha⁻¹, B10N0: 10 t ha⁻¹ biochar (applied in 2014 only); 0 kg N ha⁻¹, B20N0: 20 t ha⁻¹ biochar (applied in 2014 only); 0 kg N ha⁻¹, B0N1: 0 t ha⁻¹ biochar; 40, 160 and 100 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B10N1: 10 t ha⁻¹ biochar (applied in 2014 only); 40, 160 and 100 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N1: 20 t ha⁻¹ biochar (applied in 2014 only); 40, 160 and 100 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N1: 20 t ha⁻¹ biochar (applied in 2014 only); 40, 160 and 100 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B10N2: 0 t ha⁻¹ biochar; 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N2: 20 t ha⁻¹ biochar (applied in 2014, 2015 and 2016, respectively, B20N2: 0 t ha⁻¹ biochar; 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N2: 20 t ha⁻¹ biochar (applied in 2014, 2015 and 2016, respectively, B20N2: 20 t ha⁻¹ biochar; 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N2: 20 t ha⁻¹ biochar (applied in 2014, 2015 and 2016, respectively, B20N2: 20 t ha⁻¹ biochar (applied in 2014, 2015 and 2016, respectively, B20N2: 20 t ha⁻¹ biochar (applied in 2014, 2015 and 2016, respectively, B20N2: 20 t ha⁻¹ biochar (applied in 2014 only); 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N2: 20 t ha⁻¹ biochar (applied in 2014 only); 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N2: 20 t ha⁻¹ biochar (applied in 2014 only); 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively.

significantly) daily CO_2 emissions compared to the treatments without biochar (Fig. 2).

Relations between Daily CO₂ Flux and Soil Physical and Chemical Properties

Daily CO₂ emissions from the soil for the individual treatments in 2014 - 2016 are shown in Fig. 2, and the assessed soil physical and chemical properties for the same period of time have already been published [18, 39-41]. Generally, in all three evaluated years a similar temporal CO, emissions dynamic was observed. It correlated with the average daily air and soil temperatures with emissions increasing in spring and summer and decreasing in autumn and winter. The maximum average daily CO₂ fluxes were always recorded in summer. However, in the first year (2014), these maximum CO₂ fluxes were influenced by a significant increase in soil moisture after precipitation at a time when the soil temperatures ranged from 25 to 30°C, providing optimum conditions for high CO₂ fluxes [42-44]. Due to the increase in soil moisture, there was an immediate increase in CO, emissions related most likely to an increase in microbial activity under favorable soil temperature conditions [45]. For 2015 and 2016, the maximum CO₂ fluxes were only related to a substantial increase in soil temperature but not to a combined increase of soil temperature and soil moisture. In assessing the impact of the observed physical (soil temperature, soil moisture, saturation degree) and soil chemical properties (soil pH, N-NO₃⁻, N-NH₄⁺) on CO₂ emissions over the whole period of measurements, the physical properties of the soil were the most important factors affecting CO₂ emissions (P<0.05-0.001). The effect of the studied chemical properties on CO₂ emissions was observed only sporadically in some treatments (Table 2). The Pearson correlation coefficient identified the most significant effect of temperature (r = 0.34 - 0.50; P < 0.001) as well as the water-filled pore space (WFPS) (not in B20N1, B0N2, B10N2 and B20N2 treatments) (r = 0.20 - 0, 34; P < 0.05 - 0.001) on CO₂ emissions. Correlation analyses revealed a significant relationship between average daily CO₂ emissions and observed soil chemical properties (soil pH, N-NO₃⁻, N-NH₄⁺): for soil pH only in the B0N0 treatment (r = 0.42; P<0.05) and for N-NO₂⁻ content in the B0N0 and B20N0 treatments (r = 0.41; P < 0.05). The results showed that soil temperature and WFPS are the key factors influencing CO₂ emissions from soil to the atmosphere in the studied region.

Effect of Biochar on Crop Development and Grain Yields

An overview of mean values of vegetation indices for spring barley, corn and spring wheat is shown in Table 3, and examples of the input and output images

Treatments	Mass water content - w (% mass)	Water-filled pore space - WFPS (% vol.)	Soil temperature $-t_p$ (°C)	pH (KCl)	NO ₃ - (mg kg ⁻¹)	NH4 ⁺ (mg kg ⁻¹)
B0N0	0.09	0.31**	0.50***	0,42*	0,41*	0,08
B10N0	0.22*	0.27**	0.49***	0.3	0.32	0.04
B20N0	0.15	0.20*	0.48***	0.15	0.41*	0.11
B0N1	0.15	0.28**	0.42***	0	0.23	0.1
B10N1	0.18	0.34***	0.48***	0.05	0.02	0.07
B20N1	0.11	0.1	0.36***	0.08	0.3	0.07
B0N2	0.09	0.18	0.37***	0.13	0.21	0.18
B10N2	0.12	0.15	0.34***	0.07	0.1	0.15
B20N2	0.11	0.15	0.37***	0.21	0.1	0.17

Table 2. Pearson correlation dependencies between CO_2 emissions and selected physical and chemical properties of the silt loam Haplic Luvisol for different treatments (averages for all years together; * P<0.05; ** P<0.01; *** P<0.001).

Note: B0N0: 0 t ha⁻¹ biochar; 0 kg N ha⁻¹, B10N0: 10 t ha⁻¹ bioachar (applied in 2014 only); 0 kg N ha⁻¹, B20N0: 20 t ha⁻¹ bioachar (applied in 2014 only); 0 kg N ha⁻¹, B0N1: 0 t ha⁻¹ biochar; 40, 160 and 100 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B10N1: 10 t ha⁻¹ biochar (applied in 2014 only); 40, 160 and 100 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N1: 20 t ha⁻¹ biochar (applied in 2014 only); 40, 160 and 100 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N1: 20 t ha⁻¹ biochar (applied in 2014 only); 40, 160 and 100 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B0N2: 0 t ha⁻¹ biochar; 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B10N2: 10 t ha⁻¹ biochar (applied in 2014 only); 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N2:20 t ha⁻¹ biochar (applied in 2014 only); 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N2:20 t ha⁻¹ biochar (applied in 2014 only); 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N2:20 t ha⁻¹ biochar (applied in 2014 only); 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N2:20 t ha⁻¹ biochar (applied in 2014 only); 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively.



Fig. 3. Examples of the input (left) and output (right) images taken from the same plot (No. 8 - replicate of B10N1 treatment) at different dates of the photo sampling in 2014, 2015 and 2016. VI stands for vegetation index as calculated with Breedpix software. Ocassional differences in the outputs are due to applied preprocessing in order to remove weeds.

Date/JD	8.4.2014	15.4.2014	24.4.2014	1.5.2014	7.5.2014	21.5.2014	31.5.2014	17.6.2014
Treatment	98	105	114	120	126	141	151	168
B0N0	0.083 ª	0.156ª	0.285 ª	0.498 ª	0.519ª	0.563 ª	0.541 ª	0.208 ª
B10N0	0.084 ª	0.150 ª	0.272 ª	0.479ª	0.512ª	0.541 ª	0.562 ª	0.194ª
B20N0	0.108 ª	0.185 ª	0.333 ª	0.522 ª	0.560 ª	0.576ª	0.576ª	0.159ª
B0N1	0.005 a	0.176ª	0.305 a	0.535 a	0.545 a	0.624 a	0.584 a	0 110 a
B10N1	0.093	0.170	0.346ª	0.535 a	0.545	0.639a	0.504	0.119
B20N1	0.070	0.175	0.352ª	0.555	0.507 a	0.661 a	0.598 a	0.137ª
DZ0111	0.117	0.207	0.332	0.500	0.377	0.001	0.570	0.137
B0N2	0.089ª	0.190ª	0.353 ª	0.553 ª	0.574 ª	0.710 ^a	0.700 ª	0.185 ª
B10N2	0.107ª	0.202 ª	0.383 ª	0.608 ª	0.645 ª	0.718ª	0.716ª	0.144 ª
B20N2	0.115 ª	0.202 ª	0.401 ª	0.626 ª	0.673 ª	0.741 ª	0.726ª	0.123 ª
Date/JD	18.5.2015	28.5.2015	4.6.2015	25.6.2015	2.7.2015			
Treatment	138	148	155	176	183			
B0N0	0.021 ª	0.052 ª	0.100 ª	0.425 ª	0.331 ª			
B10N0	0.021 ª	0.036 ª	0.096 ª	0.385 ª	0.399ª			
B20N0	0.022 ª	0.044 ª	0.121 ª	0.426 ª	0.388 ª			
DON1	0.017a	0.051 a	0.119.8	0.291 a	0.221 a			
DUNI D10N1	0.017	0.031	0.150%	0.526%	0.321			
B20N1	0.023	0.040	0.130	0.320	0.429			
B20N1	0.023	0.037	0.102 a	0.475	0.403			
	0.020	0.040	0.102	0.405	0.570			
B10N2	0.025 ª	0.042 ª	0.107 ª	0.374ª	0.367ª			
B20N2	0.024ª	0.034 ª	0.146ª	0.550 ª	0.409 ª			
Date/JD	18.4.2016	25.4.2016	2.5.2016	18.5.2016	7.6.2016			
Treatment	136	143	150	156	163			
B0N0	0.037ª	0.029ª	0.075 ^b	0.332ª	0.703ª			
B10N0	0.027ª	0.022ª	0.050ª	0.303ª	0.690ª			
B20N0	0.044ª	0.037ª	0.077 ^b	0.372ª	0.731ª			
B0N1	0.026ª	0.027ª	0.079ª	0.453a	0.018ª			
BIONI	0.020	0.027	0.108ª	0.553ª	0.913			
B20N1	0.033 0.043ª	0.032	0.108ª	0.333	0.923			
	0.015	0.010	0.100	0.107	0.752			
B0N2	0.054ª	0.035ª	0.081ª	0.519ª	0.950ª			
B10N2	0.077ª	0.033ª	0.087ª	0.526ª	0.934ª			
B20N2	0.050ª	0.030ª	0.067ª	0.457ª	0.939ª			

Table 3. Vegetation index in 2014 (spring barley), 2015 (corn) and 2016 (spring wheat) as calculated with Breedpix software (JD = Julian days); the color gradient shows differences between the values (red - lowest, green - highest).

Note: The different letters in the columns indicate that the mean values of the individual treatments are significantly different at P<0.05 in accordance with the LSD test. B0N0: 0 t ha⁻¹ biochar; 0 kg N ha⁻¹, B10N0: 10 t ha⁻¹ biochar (applied in 2014 only); 0 kg N ha⁻¹, B20N0: 20 t ha⁻¹ biochar (applied in 2014 only); 0 kg N ha⁻¹, B0N1: 0 t ha⁻¹ biochar; 40, 160 and 100 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B10N1: 10 t ha⁻¹ biochar (applied in 2014 only); 40, 160 and 100 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N1: 20 t ha⁻¹ biochar (applied in 2014 only); 40, 160 and 100 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N2: 0 t ha⁻¹ biochar; 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B10N2: 10 t ha⁻¹ biochar (applied in 2014 only); 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N2: 20 t ha⁻¹ biochar (applied in 2014, colloced), respectively, B20N2: 20 t ha⁻¹ biochar; 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N2: 20 t ha⁻¹ biochar (applied in 2014, colloced), respectively, B20N2: 20 t ha⁻¹ biochar; 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N2: 20 t ha⁻¹ biochar (applied in 2014, colloced), respectively, B20N2: 20 t ha⁻¹ biochar (applied in 2014, colloced), respectively, B20N2: 20 t ha⁻¹ biochar (applied in 2014, colloced), respectively, B20N2: 20 t ha⁻¹ biochar (applied in 2014, colloced), respectively, B20N2: 20 t ha⁻¹ biochar (applied in 2014, colloced), respectively, B20N2: 20 t ha⁻¹ biochar (applied in 2014, colloced), respectively, B20N2: 20 t ha⁻¹ biochar (applied in 2014, colloced), respectively, B20N2: 20 t ha⁻¹ biochar (applied in 2014, colloced), respectively, B20N2: 20 t ha⁻¹ biochar (applied in 2014, colloced), respectively.

	Spring barley (14.7.2014)		Corn (29	.10.2015)	Spring wheat (20.7.2016)					
Treatments	Aboveground biomass (t ha ⁻¹)	Grain yield (t ha ⁻¹)	Aboveground biomass (t ha ⁻¹)	Grain yield (t ha ⁻¹)	Aboveground biomass (t ha ⁻¹)	Grain yield (t ha ⁻¹)				
Not fertilized: N0										
B0N0	8.01±1.23ª	3.6±0.8ª	30.10±3.14ª	13.6±1.5ª	8.77±1.65ª	3.5±0.7ª				
B10N0	10.83±2.12ª	5.1±0.9 ^b	28.51±1.98ª	13.9±0.9ª	5.87±1.54ª	3.1±0.3ª				
B20N0	7.14±0.81ª	3.2±0.5ª	26.33±4.40ª	12.7±2.8ª	6.79±1.48ª	3.3±0.0ª				
	Fertilization: N1									
B0N1	8.35±0.48ª	3.7±0.5ª	19.18±2.33ª	8.6±1.4ª	19.47±4.18 ^b	4.2±0.6ª				
B10N1	8.24±0.14ª	3.9±0.2ª	20.38±3.16ª	8.7±1.7ª	6.30±0.79ª	3.8±0.5ª				
B20N1	7.85±0.91ª	3.6±0.5ª	21.77±2.37ª	9.0±0.6ª	12.56±2.52 ^{ab}	4.1±0.4ª				
Fertilization: N2										
B0N2	10.83±0.69ª	5.0±0.3ª	21.59±1.4ª	8.6±0.3ª	13.03±1.96ª	4.5±0.9ª				
B10N2	11.41±2.14ª	5.4±0.9ª	22.64±3.27ª	9.9±2.5ª	16.07±2.41ª	4.5±0.8ª				
B20N2	10.33±0.73ª	4.9±0.4ª	22.96±1.38ª	10.6±0.9ª	13.80±5.77ª	5.3±0.3ª				

Table 4. Aboveground biomass and grain yield from different treatments in 2014-2016 (mean±standard error, n = 3).

Note: The different letters in columns indicate that the mean values of the individual treatments are significantly different at P<0.05 in accordance with the LSD test. B0N0: 0 t ha⁻¹ biochar; 0 kg N ha⁻¹, B10N0: 10 t ha⁻¹ biochar (applied in 2014 only); 0 kg N ha⁻¹, B20N0: 20 t ha⁻¹ biochar (applied in 2014 only); 0 kg N ha⁻¹, B0N1: 0 t ha⁻¹ biochar; 40, 160 and 100 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B10N1:10 t ha⁻¹ biochar (applied in 2014 only); 40, 160 and 100 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N1: 20 t ha⁻¹ biochar (applied in 2014 only); 40, 160 and 100 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N1: 20 t ha⁻¹ biochar (applied in 2014 only); 40, 160 and 100 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B10N2: 0 t ha⁻¹ biochar; 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B10N2: 10 t ha⁻¹ biochar (applied in 2014 only); 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N2:20 t ha⁻¹ biochar (applied in 2014, 2015 and 2016, respectively, B10N2: 10 t ha⁻¹ biochar (applied in 2014, 2015 and 2016, respectively, B10N2: 10 t ha⁻¹ biochar (applied in 2014, and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N2:20 t ha⁻¹ biochar (applied in 2014 only); 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N2:20 t ha⁻¹ biochar (applied in 2014 only); 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N2:20 t ha⁻¹ biochar (applied in 2014 only); 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively, B20N2:20 t ha⁻¹ biochar (applied in 2014 only); 80, 240 and 150 kg N ha⁻¹ in 2014, 2015 and 2016, respectively.

taken from the same plot at different dates in 2014, 2015 and 2016 are given in Fig. 3. The first images were taken just after the crop emergence; thus the effect of different treatments could be studied. In 2014, 2015 and 2016, the positive effect of biochar application at all fertilization levels was observed as the ratio of the green plant was generally higher in comparison to control. The only exception was B10N0 in 2016. The combinations of biochar and N-fertilizer had higher vegetation indices; with increasing rates of biochar and N-fertilizer the vegetation index also increased. This especially applies for 2014 as the vegetation index of spring barley rose until the end of May 2014. The vegetation index on 17. 06. 2014 (Table 3) was considerably lower compared to the values from previous sampling days. Due to plant senescence, the vegetation index could not be calculated accurately at that date as the green biomass did not correspond to the total aboveground biomass. Using such vegetation indices is strictly limited to green stages of vegetation growth. Due to this reason, the last sampling day in 2014 was not used in further calculations. In 2015, the best positive effect of biochar application was observed in the treatments B10N1, B20N2 and B20N1. However, these results corresponded to final corn grain yield only partially (Table 4). The vegetation indices on the last day of sampling in 2015 were slightly

lower than values from the previous sampling day. The difference was attributed to the corn canopy height, which was considerably larger than that of barley or wheat. Thus, with canopy development, at some point only a fraction of the plant canopy could be captured by the camera instead of the whole plant, as it was in the early stages (Fig. 3b). Because of this, no more images were taken in 2015 even if the plants were still green. In 2016, biochar had a positive effect on canopy development - especially in the treatment B10N1. At the end of the sampling season, the vegetation indices were the highest at the treatments with the second level of fertilization (with or without biochar). It was assumed that at that stage the fertilizer had a greater effect on plant development than biochar application. However, all reported values of vegetation indices were not statistically different (P>0.05), except for the B10N0 treatment on 2 May 2016, when the vegetation index was 33.3% lower in comparison to control. Estimating vegetation index using Breedpix software from ground images has already found its application in various areas of agricultural research. This simple remote sensing method was successfully used to evaluate grain yield losses in yellow-rusted durum due to disease [46], corn yield [29, 47] and eggplant yield [48]. This method can be considered as low-cost and fast, with a relatively

small level of sampling difficulty. Post-processing of the obtained images can in some cases considerably prolong the processing time [48]. This depends on the specific field conditions during the day of photo sampling (the presence of green weeds, crop residues, shadows in the green spectrum, etc.) [30]. Also, Gracia-Romero et al. [47] reported a lower correlation of vegetation indices of corn against its yield due to the added noise derived from the crop residue coverage of the soil.

In the first year (2014), only the treatments with the lower biochar rate (10 t ha-1) B10N0 (statistically significant), B10N1 and B10N2 (not statistically significant) increased the grain yield of spring barley by 5-42%. The treatments with the higher biochar rate (20 t ha⁻¹) showed (not statistically significantly) 2-11% lower grain yield when compared to their respective controls without biochar. This phenomenon may be related to the sorption ability of the biochar. Liang et al. [17] reported that the incorporation of biochar into soil increases specific surface area of the soil, which may have a positive impact on soil sorption [49] but may cause nutrient release problems as biochar as nutrient sorbent [50] can be a reason for lower grain yield. In 2015, all the biochar treatments resulted in higher yields of corn: an increase from 1% to 5 % was recorded for treatments without fertilizer, and with the first level of fertilizer compared to their controls without biochar (B0N0, B0N1). For the second level of fertilization, the grain yield increased by 15 and 23% for treatments B10N2 and B20N2, respectively, compared to control without biochar (B0N2). The only exception was B20N0 treatment, where the grain yield decreased by 7% compared to control (B0N0). In the last year (2016), a decrease (2% to 11%) in spring wheat grain yield was observed in all the treatments with biochar, except for the B20N2 treatment. The application of the higher rate of biochar in combination with the higher rate of N-fertilizer resulted in an increase of wheat grain yield in 2016 by 18% compared to the respective control (B0N2). However, none of the differences in grain yield was statistically significant. A similar trend with a few exceptions was observed for aboveground biomass yield grown during 2014-2016. In 2014, the values were higher only in B10N0 and B10N2 treatments (35% and 3.5%, respectively), in comparison to the controls (B0N0 and B0N2). In the other treatments the aboveground biomass yield was slightly lower (between 1.3% and 11%) compared to the controls (B0N0, B0N1 and B0N2). In 2015, all the fertilized treatments resulted in an increase (ranging from 4.8 to 13.5%) of the aboveground biomass yield, while in 2016 only biochar amendment at the fertilization level 2 (B10N2 and B20N2) resulted in an increase in the aboveground biomass yield (by 23.3% and 5.9%, respectively) compared to the respective control. A gradual decrease in aboveground biomass development has been observed throughout the studied period. While in 2014, the differences for the treatments with lower aboveground biomass were on average lower only by 5.7%, this difference increased to 8.9% in

2015 and to 39.7% in 2016. The aboveground biomass at B10N1 treatment was 67.7% lower, and that was a statistically significant difference at the confidence level of 95%.

The accuracy at which the future crop yield could be predicted by the vegetation index was examined by evaluating the linear relationships between calculated



Fig. 4. Linear relationships between vegetation index (last sampling date of the year), total aboveground biomass and grain yield for different crops. R^2 is determination coefficient. Note: For 2014, the vegetation indices for pictures taken on 31.5.2014 were taken into account.

vegetation indices and total aboveground biomass (Fig. 4a) or grain yield (Fig. 4b). When considering the vegetation indices, the best relationship between the data was found in 2016, when the vegetation index could explain 57.9% of the variation in the aboveground biomass (Fig. 4a). However, the same values of vegetation index could explain only 35.1% of the grain yield variation in the same year (Fig. 4b). At the same time, the lowest relationship ($R^2 = 0.71$) between the aboveground biomass and grain yield was observed in 2016. Although the most significant linear relationship for aforementioned properties was observed for corn in 2015 (Fig. 4c), they did not correlate with the values of vegetation indices determined in the earlier corn growth stages. Despite the fact that the vegetation indices from the last day of image sampling in 2015 were used, due to the longer vegetation period of corn (compared to barley and wheat), the values did not correlate well with the yield parameters evaluated after harvest, in October 2015. This result agrees with Vergara-Diaz [46], who reported that the best correlations between vegetation index and grain yield were found at anthesis stages of wheat, whereas the coefficients were lower at the jointing and post-anthesis stages. Thus, in the case of corn, a longer monitoring period than in our case was required. However, considering the height of the corn, different approaches must be taken. One of the possible solutions might be using an unmanned aerial vehicle flying over the high corn canopy [29]. Considering the observed linear relationships, it can be concluded that the vegetation index is suitable for early prediction of the crop yield, and that is in agreement with other studies [46-48].

Conclusions

The application of biochar to silty loam Luvisol showed its potential to reduce CO₂ emissions from the soil. The highest emission reduction was found in the first year after biochar application in the treatment with the higher biochar and the higher N-fertilizer application. However, this effect has weakened in the two following years. The cumulative CO₂ emissions for the treatments with applied biochar (either in combination with or without fertilizer) were 2-27% lower compared to their respective controls without biochar. In all three evaluated years a similar temporal CO₂ emission dynamics was observed, which correlated with the average daily air and soil temperatures with emissions increasing in spring and summer and decreasing in autumn and winter. The results showed that the soil temperature and water-filled pore space are the key factors influencing CO₂ emission from the soil to the atmosphere in the studied soil-climatic conditions.

The positive effect of biochar on grain yield in the first year was observed only for the treatments with the lower biochar rate. In the second year, the grain yield was positively affected by all the treatments with biochar at both N-fertilizer application levels, and CO_2 emissions were also reduced. However, in the third year, the effect of biochar application on grain yield increase was observed only for treatment with the higher rates of biochar and N-fertilizer.

It was concluded that vegetation index is a suitable tool for early prediction of crop yield. This method does not require expensive instrumentation as nowadays it can be performed with a conventional camera or smartphone. The results suggest that to get the best results the height at which the images are obtained should be proportional with crop height. Furthermore, this method is limited to early growth stages since during senescence plants generally start to lose green color, and that is when the vegetation index would not be a reliable reference of the canopy coverage.

The results of this work suggest that attention should be drawn to biochar as a more effective combination with other plant nutrients as well as to biochar application rates in relation to applications to silty-loam soils in mild climatic conditions. Further studies are required before the final recommendation of biochar for standard agronomic practice could be made with the aim to support sustainable plant production while leaving the lowest possible carbon footprint in the environment.

Acknowledgements

This study was supported by the Slovak Research and Development Agency under contract No. APVV-15-0160, the Cultural and Educational Grant Agency, KEGA (No. 019SPU-4/2020), the Slovak Grant Agency (VEGA) project Nos. 1/0747/20 and 1/0064/19 and the Ministry of Science and High Education of Russian Federation state assignment.

Conflict of Interest

The authors declare no conflict of interest.

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