Received: 21 October 2022 | Revised: 8 June 2023 | Accepted: 16 June 2023

DOI: 10.1111/sum.12936

RESEARCH ARTICLE

Soil Use and Management BRITISH SOCIETY OF SOIL SCIENCE WILEY

Manure application followed by biochar application increases plant production regardless of soil dehydrogenase activity

H. Dvořáčková[1] | **J. Dvořáček**[2]

[1]Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of AgriSciences, Mendel University in Brno, Brno, Czech Republic

[2]Pedologie Dvořáčkovi, Brno, Czech Republic

Correspondence
H. Dvořáčková, Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1, 60200 Brno, Czech Republic.
Email: helenadvorackovaa@gmail.com

Abstract

Biochar is obtained by the pyrolysis of biomass, and contains abundant carbon and minerals. Biochar supplementation of soils can greatly improve soil health and quality, but these beneficial effects typically develop slowly over time. Depending on the quality of the biochar and the soil to which it is applied, it may take years before positive effects are apparent. This is because organic substances are slowly sorbed onto the biochar over time, and the biochar eventually becomes part of the sorption complex of the soil. It is therefore advisable to apply biochar together with some organic material. We examined the effect of co-application of different doses of biochar with manure on soil dehydrogenase activity (DHA), soil oxidizable carbon (COX), cumulative soil respiration, soil buffering capacity, the soil exchange reaction (pH/KCl) and the production yield of winter rape seeds. We also determined seed production when artificial granular fertilizers were added to biochar and manure. The results showed that the application of biochar and manure significantly increased grain yield, DHA, the soil exchange reaction and cumulative respiration. Thus, application of biochar with organic material can increase seed yield and some properties of agricultural soils. However, the positive effect of biochar on seed yield was not directly proportional to biochar dose, in that the seed yield was lower for a biochar dose of 45 t/ha than 30 t/ha.

KEYWORDS

biochar, buffering capacities, carbon, manure, soil, soil respiration

1 | INTRODUCTION

Biochar is carbonized organic matter, and its addition to nutrient-poor soils can increase plant production (Dai et al., 2020), improve soil quality (Joseph et al., 2021) and promote carbon sequestration (Nan et al., 2020). However, it is difficult to predict the specific effects of biochar supplements in different types of soils (Lehmann & Joseph, 2015). Different methods are used to produce biochar from various types of biomass, especially waste materials such as shrimp shells, bones, spoiled food, sewage sludge, farmyard manure and agricultural intermediate products. Use of these waste materials for biochar production is more environmentally friendly than pyrolysis of other materials, for example, cattle manure or wood chip (Kalus et al., 2019; Sekaran et al., 2020). However, uncertainties regarding the benefits of biochar on different soils have prevented its more widespread use as a soil supplement in actual agricultural practice (Lehmann & Joseph, 2015; Dvořáčková et al., 2022). Biochar, in addition to its potentially long residence time in the soil environment, has the added benefit of increasing soil fertility (Glaser et al., 2002) and stimulating key rhizobial microorganisms (Warnock et al., 2007), which improves plant growth conditions and contributes to sustainable land management (Glaser, 2007).

The effect of biochar on soil quality and plant production depends on the raw materials used for its production (Ippolito et al., 2020). Some studies also reported that biochar had negative effects on certain soils and plant production (Gonzaga et al., 2018; Liu et al., 2020; Xiang et al., 2021), but these studies usually applied high doses of biochar or applied biochar to soils that already had sufficient organic matter and nutrients. Biochar is especially beneficial when used in poor-quality soils, such as soils with low levels of nutrients, poor structure or low soil reaction (Egamberdieva et al., 2022; Semida et al., 2019; Sheng & Zhu, 2018). There is a high potential for a positive effect of biochar on plant growth.

The addition of biochar to soil can simultaneously improve soil yields (Asai et al., 2009) especially in soils with poor fertility, which is very attractive given the rapid increase in world population and the reduction of productive land area. Uzoma et al. (2011) investigated the effect of cow manure biochar on maize production, nutrient uptake and physicochemical properties in a sandy soil. The results showed that maize yield and nutrient uptake were significantly improved after biochar application. Biochar application rates of 15–20 t/ha significantly increased corn grain yield by 150% and 98%, respectively, compared to the control sample. Improved growth is believed to be related to improved soil properties after biochar addition. Noguera et al. (2012) investigated the mechanism of rice biomass increase in amended soils at the cellular level. The results showed that, on the one hand, biochar increases protein catabolism by increasing proteolytic activities in the leaf, on the other hand, it also increases protein anabolism. Thus, biochar increased rice biomass production through increased leaf protein turnover (both catabolism and anabolism). In this study, the expression of genes related to both processes is shown to be increased. Plants regenerated their leaves more quickly to provide photosynthesis leading to high rates of protein degradation and synthesis in amended soils with added biochar.

Many recent studies found that a promising new approach is the application of biochar as a carrier of mineral or organic fertilizers. The reason is primarily the essential share of stable organic carbon, the presence of calcium and magnesium, as well as the liming effect and an increase in the buffering capacity of the soil. All mentioned parameters have a fundamental influence on plant production and thus determine the potential of introducing biochar into agricultural practice. Biochar is also useful as an additive in the composting process and as an admixture when fertilizing with mineral or organic substances (Bello et al., 2020; Joseph et al., 2021; Robb et al., 2020). Mixing biochar with manure is also considered an effective method for improving soil quality, whereas manure brings nutrients and microorganisms into the system and biochar a area for sorption and subsequent slow release of nutrients and life area for microorganisms. In relation to biochar, however, manure is mostly tested as a raw material and not as a component (Banik et al., 2021; Lehmann et al., 2015; Qiu et al., 2019).

The positive that biochar brings to the soil, if it is applied at the same time and manure is a life area for microorganisms. According to some studies, raw biochar represents an inhospitable environment for microorganisms to which a significant part of microbes may not be able to adapt and can thus have a fumigation effect. An exception may be biochars produced from metabolites, that is, material rich in nutrients, in which it is then preserved, they gradually transform its surface for the life of other microorganisms.

In the experiments described here, we added manure with different amounts of biochar to soil in the Bohemian-Moravian Highlands region, and then measured the effect on: (a) crop yield after 4.5 years; (b) soil reaction, soil buffering capacity and organic carbon; and (c) microbial indicators (dehydrogenase activity and cumulative soil respiration) to determine the effects of biochar on selected soil parameters and plant production.

2 | METHODOLOGY

2.1 | Experimental plots

Experiments were conducted in the spring of 2016 at the Field Forage Research Station in Vatín (49.52°N; 15.97°E) in the Bohemian-Moravian Highlands (Czech Republic)—see Figure 1. The total area of the experiment is 24 research areas, each measuring 3 × 4 m. The altitude was 540 m.a.s.l., this region has a mild-warm climate and the average annual precipitation was 736 mm from 1971 to 2000. The soil (eutric cambisol) at the experimental site was classified as sandy clay-loam. The basic characteristics of the soil on the research plots are shown in Table 1.

FIGURE 1 Location of the research station.

TABLE 1 Basic characteristics of the soil (sandy clay-loam) in the experimental plots before the experiments.

							Texture		
Available P (mg/kg)	Available K (mg/kg)	Available Ca (mg/kg)	Available Mg (mg/kg)	Organic carbon (%)	pH/KCl	Nt (%)	<0.002 mm (%)	0.05–0.002 mm (%)	2.00–0.25 mm (%)
24.1	164.0	881.5	182.1	1.52	4.69	0.13	33.8	47.5	18.7

2.2 | Characteristics of the experimental plots and the biochar

There were a total of 24 research plots in the experimental area (1–24 in Figure 2). Half of these plots (1–22 were intended for determining the effect of NPK fertilization + biochar) and the other half (13–24 were intended for determining the effect of manure and biochar). For the purposes of this article, plots where manure and biochar were used were considered, in which the effect on yield and on selected soil properties was examined, in the case of NPK and biochar, only the effect on production was determined and this was compared with the production when manure and biochar were applied.

There were four different biochar treatments, each with three replicates (12 plots). Size of individual plots was 12 m^2. Each treatment had the same amount of manure (30 t/ha) but different amounts of biochar: (i) no biochar + manure (B0 control), (ii) 15 t/ha biochar + manure (B15), (iii) 30 t/ha biochar + manure (B30) and (iv) 45 t/ha biochar + manure (B45). Plots with different biochar doses were distributed randomly at the study site. The biochar was incorporated to a depth of 20 cm, and each plot had an area of 12 m^2 (3 × 4 m^2).

For some studies of grain production, granular mineral fertilizer (NPK) was added. This 15 N/15 P/15 K fertilizer was initially added at a dose of 50 kg/ha N; 30 kg/ha P fertilizer added at later time. The different treatments were: B0N (NPK + no biochar), B15N (NPK + 15 t biochar), B30N (NPK + 30 t biochar) and B45N (NPK + 45 t biochar).

In 2015, manure was applied. Before this application, the soil was mulched, disked and rolled. The field trial was divided into two rows: farming with only plant production or with only animal production. In 2015, manure was applied. Before this application, the soil was mulched, disked and rolled. Manure was applied with a dose of 30 t/ha, which corresponded to 165 kg/ha N, 30 kg/ha P, 48 kg/ha Ca and 27 kg/ha Mg. Following the application of manure, the entire study area was ploughed during the winter at a depth of 25 cm.

In 2016, biochar was applied. The plot was ploughed during the fall and spring, and the experimental areas were established as described above (four treatments B0, B15, B30 and B45), each with three replicates. In 2016, corn was sown by hand with the use of stakes in a 15 × 40 cm and at a depth of ca. 8 cm. One month later, the Titus 60WG (60 g/ha) herbicide was applied with the Trend wetting agent (0.1%) and 200 L/ha of water. The plots were subsequently

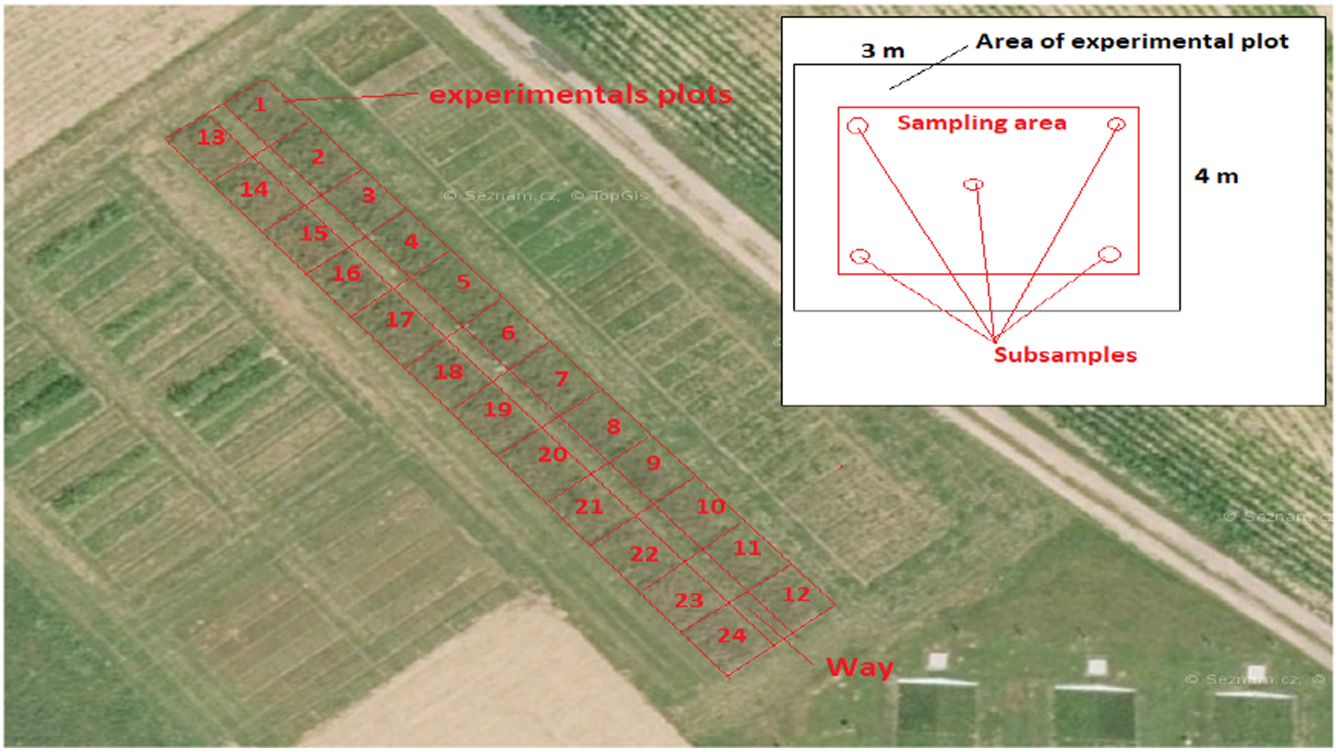

FIGURE 2 Scheme of sampling and trial plots.

fertilized because of the high acidity of the soil, the low level of P and the purple colour of the plants (indicating P deficiency). The entire experimental area was manually fertilized with 200 kg/ha of superphosphate (90 kg/ha P). Harvest was in October, and the land was ploughed at a depth of 25 cm later in the autumn.

In 2017, the plots were first harrowed and the topsoil was then levelled. Then, the plots were marked out the same as previously. A Saxonia seeder was used to plant spring barley at 250 kg/ha, and meadow clover was applied (as underseeding) using an Oyjord precision seeder at 20 kg/ha. One month later, the plots were treated with an herbicide (Dicopur, 0.3 L/ha, with 300 L/ha water) that has low toxicity to clover. In August, the harvest was performed. Samples of barley from 1 m^2 of each plot were cut by hand with a sickle and placed in a dryer. The remaining biomass was completely harvested, and the straw was dug up and removed.

In 2018, the land was not treated in any way. The first cut of clover took place in May, the second in June and the third in October, after which the land was ploughed to a depth of 25 cm.

In the spring of 2019, the plots were first harrowed and the topsoil was then levelled in preparation for the sowing of wheat. The plots were marked out as previously. In April, the entire area was sown with spring wheat (Epos variety) using an Oyjord precision seeding machine at a rate of 250 kg/ha. In May, Mustang insecticide was applied (0.7 L/ha with 200 L/ha water). In August, the immature spring wheat (just after flowering) was cut with a Solo mower. Yields were determined for all plots, and samples were taken for drying and chemical analysis. It was necessary to harvest the spring wheat when it was unripe so that the rapeseed could be sown in time.

A week later, the plots were ploughed to a depth of 25 cm, harrowed and then rapeseed (*Brassica napus var. napus*) was sown using the Oyjord precision machine at a rate of 4 kg/ha (24 lines) After sowing, the plots were rolled and measured, and stakes were used to mark out the plots as previously before sowing canola. Two weeks later, a tractor sprayer was used to apply the herbicide Butisan S (2 L/ha) and the insecticide Proteus (0.6 L/ha) with 240 L/ha water. In September, the rape was fertilized (15 N/15 K/15P, 50 kg/ha N) (three replicates) on half of the plots (B0N, B15N, B30N and B45N; the other half of the plots remained without fertilizer, B0, B15, B30 and B45). In August, the rape plants were purple (indicating P deficiency), so P was added in the form of superphosphate 45% at 30 kg/ha. Rapeseed was pulled from the footpaths between the parcels so that mulching was not necessary during the spring.

In May 2020, the rapeseed was treated with an antifungal agent (Pictor, 0.5 L/ha with 200 L/ha water). In August, the plants were harvested (without pod-shatter) and soil samples were collected for analysis. From each plots, 10 samples were taken and were prepared mixing samples.

2.2.1 | Properties of biochar and manure

Biochar was applied in April 2016. Owing to the low pH of the soil and its low level of phosphorus, superphosphate was applied 2 months later at a rate of 200 kg/ha. This study focused on the characteristics of soils in the experimental plots 4.5 years after the application of biochar (2020).

In a field experiment in Vatín, biochar produced from digestate (80%) and cellulose fibre in the process of thermochemical reduction (pyrolysis) was used. The continuous pyrolysis temperature was between 450 and 470°C. The carbon content is around 65%.

The biochar was produced by Biouhel (Czech Republic, biouhel.cz, accessed on 20 February 2022), using waste from woody biomass (wood chips) as the primary starting material (Table 2).

TABLE 2 Basic properties of the biochar[a].

BET sorption analysis	
Specific surface	584 (m^2/g)
Optical emission inductively coupled plasma spectroscopy	
N	1.17 hm%
C	96.3 hm%
H	0.1 hm%
S	0.00 hm%
O	2.2 hm%
Combustion analysis with GC detection	
Ca	64.4 mg/g
K	16.4 mg/g
Na	4.4 mg/g
P	15.80 mg/g
Al	3.4 mg/g
Mg	6,7 mg/g
Mn	3.4 mg/g
Pb	0.0 mg/g
Zn	0.1 mg/g
Cd	0.0 mg/g
Ash	10.7 hm%
Gas chromatography/mass spectrometry	
Naphthalene	0.034 μg/g
Acenaphthylene	0.245 μg/g
Acenaphthene	BLD
Anthracene	0.346 μg/g
Fluorene	0.216 μg/g
Phenanthrene	0.247 μg/g
Benz (a) anthracene	0.636 μg/g
Chrysene	0.259 μg/g
Benzo (b) fluoranthene	0.668 μg/g
Benzo (k) fluoranthene	BLD
Benzo (a) pyrene	BLD
Indene (1,2,3-cd) pyrene	0.807 μg/g
Dibenz (a, h) anthracene	BLD
Benzo (ghi) perylene	0.535 μg/g
Fluoranthene	0.267 μg/g
Pyrene	0.299 μg/g
ISO 10390:2005	
pH	10.2
C/N ratio	29

Abbreviations: BLD, below level of detection; hm%, relative content.

[a]Results were obtained by BET sorption analysis, optical emission inductively coupled plasma spectroscopy and gas chromatography/mass spectrometry. Measurements were made in 2020.

2.3 | Sampling and analysis of soil properties

Commonly used and recognized procedures and methods were used for sample analysis—see below. All analyses were performed at the Mendel University in Brno.

The following methods and procedures were used:

Rapeseed biomass was determined: the seeds were subsequently dried at 105°C to a constant weight, based on measurements with an analytical scale (model AEJ 100-4NM, KERN, Berlin, Germany). Qualitative analysis of the biomass was not performed. The collection took place immediately before the collection of soil samples.

2.4 | Soil sampling

Five sub-samples were taken from each experimental plot, from which one mixed sample was created. One mixed sample was taken from each experimental area, which was made up of five sub-samples. The sampling scheme is shown in Figure 2. On each experimental area, protected zones were defined with a size of 0.5 m from the edge, so as to limit the influence of the surroundings of these per sampling area. The samples were taken during autumn 2020. The samples were taken from a depth of 0–10 cm.

Soil samples were collected in resealable LDPE (low-density polyethylene) bags and transported to the laboratory in a portable refrigerator (at a temperature of 7°C). In the laboratory, they were stored for a maximum of 1 week in a refrigerator at a temperature of 7°C before processing.

Dehydrogenase activity (DHA) of soil was determined as described by Casida et al. (1964) and Šimek et al. (2011), using an incubator (FC/FC 222, BMT Medical Technology, Brno-Zábrdovice, Czech Republic) and a spectrophotometer (model DR 3900, HACH Company, Düsseldorf, Germany).

Cumulative soil respiration was measured as described by Keith and Wong (2006). Alkaline absorbent natrocalcite (soda lime) was added to soil samples once per week for 48 h. Before the measurements, granules (50 g of undried granules per 0.08 m^2) were dried at 105°C to a constant weight, which was then recorded. During the measurements, the soil was incubated with natrocalcite granules for 48 h. The formation of carbonates was accompanied by a weight gain, which was measured after incubation and drying of the calcite. The difference in weight before and after incubation was then used to determine CO_2 production (g of C per m^2 in 24 h) using the scale described above. Measurements were repeated in 4 weeks in mature rape. These results were multiplied by 3.5 to calculate respiration per week, and each result indicated 'cumulative respiration'.

The soil exchange reaction (pH/KCl) was determined according to ISO 10390: 2005 m 1996 using a pH metre (model MS 22, Laboratory Instruments, Prague, Czech Republic).

The buffering capacity of soil was determined using a two-step procedure. First increasing amounts of 0.1 mol HCl/dm^3 and 0.1 mol NaOH/dm^3 were added to a soil sample, and pH was measured after 24 h. Then, buffering capacity was calculated by plotting the pH values on a graph, and determining the area (cm^2) between the buffering curve and a standard curve (Ostrowska et al., 1991).

COX: oxidizable carbon in the soil. The soil organic carbon content was determined by oxidation in a chromium-sulphur mixture, with final cyclooxygenase determination using spectrophotometry according to ISO/DIN 14235: 1998.

Total nitrogen (Nt) was determined using a LECO analyser (LECO TruSpec CN, Vancouver, Canada).

Particle size distribution was determined according to ISO 11277: 2009, in which a pipetting method was used to determine sedimentation times of soil particles that had different sizes, and evaluation was performed using the USDA Soil Texture Triangle.

Nutrients were measured using the Mehlich III soil test (Thermo Scientific iCAP 7400 Duo, Thermo Fisher Scientific, Cambridge, UK). The levels of P, K, Ca and Mg were determined using a colorimetric assay with measurement of absorbance ($P = 690$ nm; $K = 760$ nm; Mg = 285, 2 nm; Ca = 422, 7 nm).

TABLE 3 ANOVA of the relationship of dose of biochar (without fertilizer) to seed yield and soil properties ($\alpha = 0.05$).

	***p*-value**
Seed yield	**.005**
DHA	**.001**
Cumulative respiration	**6.774×10^{-9}**
COX	.572
Buffering capacity	.108
pH	**.001**

Abbreviation: COX, oxidizable carbon in the soil.

2.5 | Statistical analysis

Software Statistica 12 was used for the implementation of the analyses and for the graphical data processing. All parameters of the experiment were measured at least in three repetitions. The level of significance selected for all analyses was set at $p < .05$. One-way ANOVA was carried out on the effects of level of biochar supply on rapeseed yield and on soil properties, and pairwise <i>t</i>-tests were carried between plus and minus inorganic fertilizer at each of the four rates of biochar supply.

3 | RESULTS

Statistical analysis using ANOVA showed that biochar dose had a statistically significant effect on seed yield ($p = .005$, Table 3). In particular, the average seed yield was 0.78 t/ha (±0.25) in the B0 (control) group, 1.35 t/ha (±0.09) in the B15 group, 1.39 t/ha (±0.43) in the B30 group and 1.20 t/ha (±0.17) in the B45 group (Table 4).

We also measured seed yield with nitrogen with the biochar (Table 4). In this experiment, the average amount of biomass was 0.85 t/ha (±0.15) in the BON group, 0.85 t/ha (±0.09) in the B15N group, 0.81 t/ha (±0.18) in the B30N group and 0.72 t/ha (±0.19) in the B45N group.

We used the *t*-test to perform pairwise comparisons of these different groups (Table 5). The B0 and BON groups (p = 0.733329) and the B30 and B30N groups ($p = .117421$) had no significant differences in seed yield. However, the seed yield was significantly greater in the B15 group than in the B15N group ($p = .002466$) and was greater in the B45 group than the B45N group ($p = .0316$).

Our measurements of soil DHA indicated that it decreased as the dose of biochar increased (Table 6). In particular, the DHA was 1.93 g TPF/g soil/h (±0.06) in the BO (control) group, 1.81 g TPF/g soil/h (±0.03) in the B15 group, 1.66 g TPF/g soil/h (±0.10) in the B30 group and 1.49 g TPF/g soil/h (±0.11) in the B45 group. ANOVA

TABLE 4 Seed yield in the different groups (three replicates per group).

Yield (t/ha)	B0	B15	B30	B45	B0N	B15N	B30N	B45N
Mean	0.78	1.35	1.39	1.20	0.85	0.85	0.81	0.72
Median	0.77	1.33	1.21	1.18	0.79	0.88	0.81	0.68
Standard deviation	0.25	0.09	0.43	0.17	0.15	0.09	0.18	0.19
Variance	0.06	0.01	0.18	0.03	0.02	0.01	0.03	0.04
Normality	0.11	0.11	0.01	0.03	0.01	0.03	0.08	0.11
n	3	3	3	3	3	3	3	3

TABLE 5 Pairwise comparisons of seed yield in the different groups (*t*-test, $\alpha = 0.05$).

Comparison groups		*p*-value
B0	B0N	.733329
B15	B15N	**.002466**
B30	B30N	.117421
B45	B45N	**.0316**

showed that the dose of biochar had a statistically significant effect on soil DHA ($p = .001$, Table 3).

Our measurements of the effect of biochar dose on cumulative soil respiration had a similar trend, in that cumulative respiration decreased as the biochar dose increased (Table 6). Thus, the average cumulative soil respiration was 47.19 mg CO_2/g soil/24 h (±0.60) in the BO (control) group, 41.90 mg CO_2/g soil/24 h (±0.17) in the B15 group, 41.25 mg CO_2/g soil/24 h (±0.38), in the B30 group and 36.43 mg CO_2/g soil/24 h (±0.32) in the B45 group. As above, ANOVA showed that the dose of biochar had a statistically significant effect on cumulative soil respiration ($p = 6.774 \times 10^{-9}$, Table 3).

Our ANOVA indicated that biochar dose had no significant effect on the level of soil COX ($p = .572$, Table 3). However, there was a trend for an increase of COX from the B0 (control) group (1.56% ± 0.11), to the B15 group (1.68% ± 0.31) and to the B30 group (1.80% ± 0.27), but there was a lower level of COX in the B45 group (1.59% ± 0.12) (Table 6).

Our ANOVA also indicated that biochar had no significant effect on soil buffering capacity ($p = .108$, Table 3). However, there was a slight trend for increasing buffering capacity from the B0 (control) group (32.05 ± 0.66), to the B15 group 33.33 (±1.17), to the B30 group (33.34 ± 0.02) and the B45 group (33.71 ± 0.66) (Table 6).

The lowest pH was in the B0 (control) group (4.37 ± 0.01), and pH increased as the dose of biochar increased (B15: 4.51 ± 0.06; B30: 4.56 ± 0.04; B45: 4.65 ± 0.03) (Table 6). ANOVA indicated that biochar had a statistically significant effect on soil pH/KCl ($p = .001$, Table 3).

4 | DISCUSSION

The experiments presented here are part of a series of long-term experiments examining the effect of biochar on agricultural soils. The second part of this study consists of experiments that focus on the effects of biochar in combination with mineral fertilizer (instead of manure), and are presented elsewhere (Dvořáčková et al., 2022). These results were not published together because of differences in the sowing procedures during 2018, in that clover was not sown when mineral fertilizer was used. This study reports the results of rapeseed yield in 2020, and the results were very different when manure was used instead of mineral fertilization. Thus, it is necessary to consider the importance of co-application of biochar with organic matter, in our case manure. In spite of that, the previous sowing of clover may have slightly affected our results.

There are two basic mechanisms by which biochar can affect crop yield: changing the soil reaction and adding nutrients. Analysis of the nutrient content of our biochar (Table 2) indicated the levels of different nutrients were typical for biochar that is produced by rapid pyrolysis of mainly woody matter (Lehmann et al., 2015). At almost 5 years after the application of biochar, there was a significant increase in soil pH as the dose of biochar increased from 0 t/ha to 45 t/ha. In contrast, seed yield only increased with biochar dose up to 30 t/ha (B30 group), and seed yield was 13.6% lower when the biochar dose was 45 t/ha (B45 group). When no biochar was added (B0 group), mineral and organic fertilizer had similar effects on the soil. Font-Palma (2019) found the beneficial effects of manure application declined over time, so we assumed that its effect at 4.5 years after our application was less than during earlier years. Yagüe et al. (2016) reported similar results. On the other hand, if biochar is applied with manure, it is likely that the beneficial effects of manure last longer. This is probably because biochar is a very porous material, in that its cation-exchange capacity (CAC) is greater than that of most soils, and the CAC also increases over time (Lehmann et al., 2015). The reason for this is the presence of carbonyl (C=O) groups in biochar that can bind

TABLE 6 Descriptive statistics.

	B0	B15	B30	B45
DHA (g TPF/g soil/h)				
n	3	3	3	3
Mean	1.93	1.81	1.66	1.49
Median	1.91	1.79	1.67	1.53
Standard deviation	0.06	0.03	0.10	0.11
Variance	0.00	0.00	0.01	0.01
Normality	0.08	0.00	0.05	0.06
Cumulative soil respiration (mg CO2/g soil/24 h)				
n	3	3	3	3
Mean	47.19	41.90	41.25	36.43
Median	47.47	41.97	41.19	36.30
Standard deviation	0.60	0.17	0.38	0.32
Variance	0.36	0.03	0.14	0.10
Normality	0.01	0.75	0.68	0.03
Soil COX (%)				
n	3	3	3	3
Mean	1.56	1.68	1.80	1.59
Median	1.58	1.57	1.75	1.65
Standard deviation	0.11	0.31	0.27	0.12
Variance	0.01	0.09	0.07	0.01
Normality	0.06	0.02	0.03	0.04
Soil buffering capacity (no unit)				
n	3	3	3	3
Mean	32.05	33.33	33.34	33.71
Median	32.15	32.99	33.34	33.75
Standard deviation	0.66	1.17	0.02	0.66
Variance	0.44	1.37	0.00	0.43
Normality	0.11	0.14	0.00	0.00
Soil pH/KCl				
n	3	3	3	3
Mean	4.37	4.51	4.56	4.65
Median	4.37	4.51	4.58	4.65
Standard deviation	0.08	0.06	0.04	0.03
Variance	0.01	0.003	0.002	0.001
Normality	0.08	0.00	0.05	0.06

Abbreviation: TFP, triphenylformazan.

to organic substances (Lonappan et al., 2018). Biochar also adsorbs nutrients and other molecules onto its surface, and then slowly releases these substances to plants over time. This is the reason for even coverage of nutrients for the plants (Haider et al., 2020; Qiao & Wu, 2022), and moreover, there are no significant losses of nutrients there as is common in conventional agriculture today (Shi et al., 2020; van Grinsven et al., 2015).

Abdullah and Wu (2009) found that about 5 years after biochar application, it was partially pulverized because of weathering and the activity of the soil fauna, and probably became part of the overall sorption complex. Archanjo et al. (2017) and Rafique et al., 2020 found that biochar particles reacted with Fe oxides, Al, Si, Ca phosphates, Fe, Al carbonates and chlorides. The resulting microformations were 1–50 nm in diameter and were attached by organic substances onto the biochar surface. Haider et al., 2020 found that old biochar particles retained nitrate and ammoniacal nitrogen. Thus, according to these previous studies, this biochar-associated nitrogen is available to plants for several years after its addition, and it provides a benefit similar to that of mineral nitrogen as $Ca(NO_3)_2$.

No previous long-term field studies have examined the co-application of farmyard manure and graded doses of biochar on soil characteristics. Our results suggest that this practice provides promising benefits. Our approach is roughly comparable to the practice of Native Americans during the pre-Columbian era, who applied charred cooking residue, excrement from animals and humans, and other organic materials to create regions with very fertile soil for growing crops that were surrounded by less fertile soils (Lehmann & Sohi, 2008; Bezerra et al., 2019). Similar soils have also been found in Germany, indicating that this soil-forming process can also take place in temperate zones (Wiedner et al., 2015). A possible reason for the lower seed yield in our B45 group relative to the B30 group may be that an excess of biochar retains soil nutrients, making them unavailable to plants. Another possible reason is the presence of excess salts in the biochar, as reported by Fernandes et al. (2019). However, we believe this second explanation is unlikely in our case because experiments were performed with periodic washing, so that salts were washed out of the soil (Wilkinson, 2009). Another possible cause of the negative effect of high doses of biochar on crops is the presence of polycyclic aromatic hydrocarbons (PAHs) (Mayer et al., 2016; Chen et al., 2019). Biochar is a pyrolysis product, and it can contain potentially dangerous hydrocarbon compounds. However, our chemical analysis of the biochar we used indicated it did not contain a significant percentage of PAHs or heavy metals (Table 2). Additionally, Fabbri et al. (2013) reported that 4.5 years after the addition of biochar (16 t/ha from orchard pruning biomass), the levels of PAHs gradually declined to near the level of untreated controls.

Similar to the effect of biochar on seed yield, our results also indicated the level of soil COX did not increase uniformly as the biochar dose increased. In particular, COX and seed yield were both lower in the B45 group than the B30 group (Table 4). We measured COX using the wet oxidation method with potassium dichromate. Calvelo Pereira et al., 2011 reported this method reliably evaluates the labile fraction of COX in biochar, but does not consider aromatic carbon. Knicker et al. (2008) described the limitations of this method for estimating the levels of labile COX in soil affected by natural fire. They concluded that the mixture of dichromate and acid affected stable biochar structures to a lesser extent, so this finding should be considered when comparing COX levels in biochar prepared from different feedstocks. We only used one type of biochar in this study, and therefore consider our COX results (determined by wet oxidation with potassium dichromate) as the purely labile fraction. It is clear that the level of COX (Table 6) paralleled the rapeseed yield (Table 4). These results are apparently unrelated to the labile fraction of fresh biochar, which is processed very quickly in the soil, in the order of months or days (Lehmann et al., 2015; Wang et al., 2020). Wang et al., 2020 reported the labile fraction of COX in biochar was 3% after 108 days. A more likely explanation for our COX results is that they were affected by the release of plant exudates from the root hairs into the soil, leading to changes in the physical–chemical properties of the soil around the roots (Panchal et al., 2022; Zhou et al., 2022). Notably, the amount of plant root exudates decreases as the volume of root hairs increases (Oburger & Jones, 2018). Thus, the lower level of COX in our B45 group may be attributable to the adverse effect of root exudates on soil microbiota.

DHA, a measure of microbial redox systems, is among the most important enzymes in the soil environment because it catalyses intracellular hydrogen transfer from organic substrates to inorganic acceptors. Measurements of DHA therefore provide a good measure of soil microbial oxidation activity and biological oxidation of soil organic matter (Bucheli et al., 2015). Importantly, this enzyme is rapidly degraded after cell death and does not accumulate in the soil (Paneque et al., 2016; Bucheli et al., 2015; Zhao et al., 2013). We found that soil DHA decreased as the dose of biochar increased (Table 6). Chintala et al., 2013 and Ameloot et al., 2014 reported similar results, and they attributed this decrease to reduced mineralization of C and N. In contrast, Park et al. (2011) and Paz-Ferreiro et al. (2012) performed container experiments and reported a significant increase in DHA after adding biochar that was derived from chicken manure and sewage sludge. These authors attributed the increased DHA to an improvement in the soil environment. In contrast, our results indicated no such benefit. Ameloot et al., 2014 concluded that biochar which was produced at high pyrolysis temperatures had high porosity and a large active surface, and that application of this biochar to soils decreased the DHA because of the presence of toxic substances, although they did not identify these molecules. Lehmann et al. (2015), Zhou et al. (2012) and Mierzwa-Hersztek et al., 2020 also recommended against the use of biochar that produced at pyrolysis temperature above 400°C when a goal is to retain soil DHA. The biochar used in our experiments was produced at 500°C, and this may explain its adverse effect on DHA. Specifically, we found that DHA was more than 40% lower in the B45 group than in the control group (Table 6). Sandhu et al. (2019) performed short-term experiments and concluded that the mixture of biochar with manure had a more positive effect on soil microbiota and soil enzymatic activity than biochar alone. However, our long-term experiments indicated that the beneficial effects of manure on soil microbiota decline over time (Hendrix et al., 2020).

We also performed direct measurements of cumulative soil respiration at the experimental sites over 4 weeks. The results showed that respiration decreased as the biochar dose increased (Table 6), similar to the effect of biochar on DHA (Table 6). Lu et al. (2014), Lu et al. (2014) and Zhang et al. (2012) also measured soil CO_2 production in field experiments in which biochar was applied 2–5 years previously, and they reported similar results. Our results and those of these previous studies suggest that biochar becomes part of the metabolic cascade of soils, in that it stabilizes soil organic matter and does not undergo mineralization. The results of Wardle et al. (2008) are consistent with this interpretation.

As demonstrated by Dvořáčková et al. (2022), organic and mineral fertilizers can increase the buffering capacity of soils. As you can see in this work, the variant, which contained charred residue after the fire showed a 30% reduction in buffering capacity compared to the control variant. In addition, Dvořáčková et al. (2022) also reported a positive correlation between buffering capacity and microbial metabolism (cumulative soil respiration and DHA). These results are similar to the results presented here, and indicated that biochar had a tendency to reduce soil buffering capacity and significantly reduce microbial metabolism. In contrast, De Villiers and Jackson (1967) found that application of biochar increased soil buffering capacity, and Xu et al. (2013) reported similar results. Buffering capacity is a very complex soil parameter that is affected by the physical, chemical and biological properties of the soil (Nelson et al., 2010, Weaver et al., 2004). Our results suggest that the buffering capacity of soils after the application of biochar mainly depended on microbial metabolism (DHA and cumulative respiration).

5 | CONCLUSION

The presented article dealt with the issue of using biochar to increase production. We were particularly interested in the clear effect of biochar on plant production after 4.5 years after application and on selected soil properties. Our results indicated that low doses of biochar (15 t/ha and 30 t/ha) prolonged the positive effect of manure on plant production, probably because it promoted the gradual release of nutrients. Measurements of soil respiration indicated that the biochar used in our experiments had a negative effect on the soil microbiota at all applied doses. More specific conclusions are: (a) biochar inhibits microbial activity (DHA and cumulative soil respiration), in doses of 15 and 30 t/ha it increases Cox, doses of 15, 30 and 45 t/ha improve soil buffering capacity and soil reaction.

ACKNOWLEDGEMENTS

We gratefully acknowledge the Research grassland station Vatín – Faculty of Agronomy, Mendel University in Brno, Czech Republic.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors

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How to cite this article: Dvořáčková, H., & Dvořáček, J. (2023). Manure application followed by biochar application increases plant production regardless of soil dehydrogenase activity. *Soil Use and Management*, *39*, 1557–1569. https://doi.org/10.1111/sum.12936