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The effect of biochar application on soil properties and growth of the model plant *Zea mays*

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Abstract – The classic way of land cultivation means the use of inorganic fertilizers that are salts that dissolve rapidly in a short time and improve soil fertility. This process negatively affects soil salinity and the life of microorganisms. The use of biochar as a soil conditioner is a promising solution. The aim of the work is to enrich the properties of less fertile soils and to enhance the growth of the model plant Zea mays (corn) by biochar application. We used four different soil types commonly spread in the Czech Republic – regosol, chernozem, cambisol and fluvisol representing a broad range of organic matter content. Also, we applied two different EBC (The European Biochar Certificate) certified biochars for use in agriculture. Corn seeds were germinated and cultivated for 3 months in repeated plant life cycles. Soils and biochar samples were characterized before and after cultivation by TGA, EA, BET, SEM, extraction of organic matter. The effect of biochar application was observed continuously through the measurement of plant height, the number of leaves and cobs. After the finalization of cultivation experiments, the dry mass of individual plants was measured, and root image analysis of every plant was performed. Fluvisol and cambisol have much higher organic matter content than regosol and chernozem. The application of biochar had the most significant impact on regosol regardless of the application dose; these results are in good agreement with the root image analysis. Furthermore, plants in soils treated with biochar had more corn cobs. The analysis on biochar samples showed the continual leaching of both organic and inorganic molecules from biochar to surrounding soil, which is crucial for its possible use as a soil conditioner and confirms the long-timescale positive effect on soil properties.

Keywords – Cultivation, dry mass, image analysis, organic matter, organic carbon, plant growth analysis

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INTRODUCTION

Soil condition has deteriorated over the last decades. Although the land is a source of food for over 7.8 billion people living on our planet, people do not care that much about it. Today’s way of land cultivation involves the application of inorganic fertilizers, mostly salts. These salts contain nutrients, i.e. nitrogen, phosphorus, potassium or sulphur (Fang et al., 2021, Huang et al., 2021), and as they dissolve quickly, they are transferred from soils to the underground layers and waters. This results in a necessity to fertilize the soil several times during the growing season. This process has a negative effect on soil salinity (Jacobs and Timmer, 2005), soil respiration (Subedi et al., 2021) and microorganisms (de Souza Silva and Fay, 2012). Biochar appears to be a potential substitute for the above-mentioned fertilizers.

Biochar is a carbon-rich product of biomass thermochemical conversion (pyrolysis) in the absence of oxygen (Daful and R Chandraratne, 2020). Properties of biochar are highly dependent on pyrolysis conditions, such as temperature (Bagreev et al., 2001), duration of combustion (Phan et al., 2008) or feedstock. Various materials can serve as feedstock (wood residues (Nirmaladevi et al., 2021), organic waste (Liu et al., 2021), etc.). The use of biochar as a soil conditioner improves soil fertility (Brewer and Brown, 2012), water retention (Werdin et al., 2021) and wastewater treatment (Kamali et al., 2021) and affects microorganism’s life (Shi et al., 2021). Additionally, the particle size of biochar must be taken into consideration, e.g., fine particles increase water retention (Gluba et al., 2021), on the other hand, coarse particles increase plant available water (Werdin et al., 2021).

Generally, biochar is not a standardized product so there is a disadvantage of rather variable properties (Nemati et al., 2015). However, some biochars meet The European biochar certificate but it is only a voluntary industry standard in Europe (The European Biochar Certificate (EBC), 2021). This fact makes biochar research a bit complicated because some researchers reported no effect of biochar on soil physical properties (Streubel et al., 2011) or water availability (Hardie et al., 2014).

The positive effect of biochar on plant growth (Yan et al., 2021) and soil condition (Zhang et al., 2021) has been studied by many researchers. Medyńska-Juraszek et al. (2021) performed a three-year experiment in which the application of pinewood biochar increased biomass (shoots and roots) yield and improved soil physical properties and water retention. Biochar treatment also significantly affects microbial abundance and activity in the soil (Abujabhah et al., 2016). As mentioned above, the problem of the application of conventional fertilizers containing nitrogen (N) is soil acidification and salinization. The study of Xie et al. (2020) shows stimulation of NH^{4+} turnover, decrease in NO3- production and N_2O emissions in soils by biochar application during 10-year vegetable cultivation experiment. Xiu et al. (2021) monitored the root growth of soybean in Albic soil, which is a low-yielding acidic, poorly ventilated soil with poor available nutrients. In their research, biochar had a positive effect on a shoot and root growth. Moreover, biological nitrogen fixation ability and enhanced nitrogen content on soybean plants and soil were observed. The effect of biochar produced from sewage sludge on tomato growth was studied by Velli et al (2021). They did not observe a significant increase in tomato yield but the dry weight of aboveground and belowground plant tissues increased. Also, the noticed that biomass type should be considered for each type of biochar application.

The aim of our work is to investigate the biochar impact on physico-chemical properties of selected representative soil samples, to correlate these effects with the crucial parameters of selected soils (mainly soil organic matter content) as well as with the observed effects of biochar application on growth and vitality of a model plant (in our experiments used Zea mays) in the laboratory pot cultivation experiments.

MATERIALS AND METHODS

Soil

Four different soil samples were collected from a depth of 30 cm in the fields at a different selected location in Czech Republic. These sampling sites provided us the creation of a soil database containing common soil types in Czech Republic. The attention was also paid to have soil samples with a broad range of organic matter. The first type is regosol (arid sandy soil) located in the area of Hodonín–Pánov, CZ (48.878150°N, 17.132275°E), the second type is chernozem (arenic soil) located in the area of Žabčice u Brna, CZ (49.006433°N, 16.591367°E), the third type is cambisol (modal soil) located near Náměšť nad Oslavou, CZ (49.213263°N, 16.162481°E), and the last one is fluvisol (modal brown soil) located in the area of Iváň, CZ (49.921686°N, 16.561494°E). Collected soils were air-dried at room temperature before use.

Biochar

Two different biochars were purchased from Sonnenerde GmbH (Bio Pflanzenkohle, Austria) and NovoCarbo GmbH (NovoTerra, Germany) and used without further pre-cultivation treatments. These two biochar samples possess a European Biochar Certificate (EBC) for application in agriculture. The doses of biochar used during the cultivation experiments were 0, 10 and 20 g of biochar per 1 kg of dry weight soil.

Table 1: Overview of soils and biochar samples in pots.

number of pots	**soil type**	**biochar type**	**biochar dose (g per 1 kg of soil)**
1	regosol	blank	0
1	chernozem	blank	0
1	cambisol	blank	0
1	fluvisol	blank	0
2	regosol	NovoTerra	10
2	chernozem	NovoTerra	10
2	cambisol	NovoTerra	10
2	fluvisol	NovoTerra	10
2	regosol	NovoTerra	20
2	chernozem	NovoTerra	20
2	cambisol	NovoTerra	20
2	fluvisol	NovoTerra	20
2	regosol	Bio Pflanzenkohle	10
2	chernozem	Bio Pflanzenkohle	10
2	cambisol	Bio Pflanzenkohle	10
2	fluvisol	Bio Pflanzenkohle	10
2	regosol	Bio Pflanzenkohle	20
2	chernozem	Bio Pflanzenkohle	20
2	cambisol	Bio Pflanzenkohle	20
2	fluvisol	Bio Pflanzenkohle	20

Demineralized water (MiliQ, ELGA Purelab Classic system) was used in all experiments. Calcium carbonate dihydrate ($CaCl_2 \cdot 2\ H_2O$) powder of purity 99.9 % (CAS 10035-04-8) was purchased from Penta s.r.o. and used without further purification.

Pot preparation for the cultivation experiments

Firstly, bags (4×4 cm) were prepared from polypropylene textile by impulse bag sealer and filled with 5.0 g of biochar (48 bags with Bio Pflanzenkohle and 48 bags with NovoTerra biochar samples in total). 1 kg of the dry weight of individual soil was mixed with perlite (4:1 in volume). Perlite is used to aerate the soil. Half of the volume was placed into a pot and bags with biochar (either 2 for a dose of 10 g or 4 for 20 g of biochar per 1 kg of the dry weight of individual soil) were placed around the perimeter of the pot, the rest of the soil was sprinkled evenly. Pot content (type of soil and biochar) is summarized in Table 1. Pots were placed into a grow box with controlled light exposure based on an average time of sunrise and sunset in the Czech Republic (6:15 – 18:30) The pots with different soil-biochar systems were watered before planting of germinated corn seed.

Cultivation study with Zea mays (long-term)
Zea mays (corn) was chosen as a model plant because it is a common plant on the Czech fields. It grows relatively fast and has measurable properties, such as height, number of leaves and number of cobs. Corn seeds were germinated on a moist paper towel at room temperature for five days. The germinated seed was planted into a hole 3 cm deep and lightly covered with soil, one seed per pot. Plants were cultivated under controlled conditions for 3 months (long-term). Cyclic irrigation (100 ml per pot) took place 3 times a week using tap water (pH and conductivity were measured). The plant height, number of leaves and corn cobs were measured 3 times a week. After three months of cultivation, the watering of the plants was stopped, plants were harvested and air-dried at room temperature for 1 month. Dry plants were divided into underground (roots) and aboveground (stem, leaves and corn cobs) parts and weighed.

Root image analysis (short-term)
For root image analysis, a short-term cultivation study was performed. 500g of the dry weight of individual soil was thoroughly mixed with perlite (4:1 in volume) and biochar (same dose) directly in a pot. Corn seeds were germinated and planted in the same way as for long-term cultivation study. Also, watering and light exposure time was regulated as was already described for long-term cultivation study. After a short-term cultivation study, plants were harvested, the soil was washed from the roots under running water to keep the structure of the fine roots intact. The plants were then air-dried for two weeks. After drying, the plants were separated into underground and aboveground parts, moreover, corn seed was separated from the root. The roots were scanned on glossy black paper and converted to digital form for further processing (root image analysis). All images were processed first in the ImageJ software and then in the HarFa software.

The original image in ImageJ software was first converted to an 8-bit grayscale image using the Invert function. Furthermore, thresholding occurs using the Threshold function. Thresholding is based on different levels of object intensity and background. Finally, the image was cleaned of small fragments and impurities using the BoneJ plugin. The pre-processed image was transferred to the HarFa program, where 2D wavelet analysis was used. An area of 2048 x 2048 pixels was selected for analysis. In the HarFa software, fractal image parameters were obtained and the obtained graphic data were saved to a text file, which converted the pixels into coordinates, then the data were exported to MS Excel.

Thermogravimetry
The content of moisture, organic matter and ash (inorganic matter) of the samples of biochar and soil was determined by the method of thermogravimetry (TGA) using a Q5000 thermogravimetric analyser (TA Instruments, New Castel, USA). Approximately 5 mg of a sample was weighed into a platinum pan. During the analysis, the residual weight was continuously measured during a heating programme with a heating rate 10° C/min from ambient to 800 °C under air atmosphere. The content of sample moisture was determined as the weight difference at 105 °C, the weight difference between 105 °C and 800 °C corresponds to the content of organic matter, while the residual weight at 800 °C was assigned to the content of ash.

Elemental analysis
The relative content of organic elements in the samples of soil and biochar was characterized using a CHNS/O analyser EA 3000 (Euro Vector, Pavia, Italy). For purposes of the analysis, 0.5–1.0 mg of the sample was weighed into a tin capsule and packed. The analysis was performed through the combustion of prepared tin capsules with the samples at 980 °C in the analyser using oxygen as the combustion gas and helium as the carrier gas. Calibration of the determination of relative contents of carbon (C), hydrogen (H), nitrogen (N), and sulphur (S) was performed using a sulphanilamide as a standard sample. The relative oxygen content was calculated from the residual combustible mass, and the data obtained were corrected for moisture and ash content determined using TGA analysis. The individual measurements were performed in triplicates.

BET analysis
The specific surface area of both used biochar samples has been measured using a specific surface analyser (Nova 2200, Quantachrome Instruments) concerning Brunauer-Emmett-Teller analysis. The samples have been degassed for 24 hours at a constant temperature 200 °C. The measurement of the specific surface area of biochar samples was performed in an inert atmosphere (nitrogen was used as analysis absorption gas) at constant temperature (273 K). The values of the specific surface area presented in the manuscript are the average values of at least two individual repeated measurements.

Conductivity and pH measurement
The individual biochar and soil samples (both original and after the pot cultivation experiments) were characterized using pH and conductivity of water extracts. For these purposes, 1 g of individual dried biochar samples was dispersed in 10 ml of demineralised water. After 1 hour shaking, pH was measured directly in the suspension. For the conductivity measurement, the individual samples were filtered through 0.45 μm syringe filters (nylon membrane).

Physical soil characterization
Fundamental physical soil properties (porosity, maximal capillary water capacity and water retention capacity) have been measured and compared before/after application of biochar into the soil. The porosity of soil samples before/after

application of biochar was calculated via *Eq. 1*, were ρ_s represent the specific weight of soil, ρ_d is reduced volume weight.

$$\boldsymbol{P = \frac{\rho_s - \rho_d}{\rho_s} \cdot 100} \qquad \text{(Eq. 1)}$$

Maximal capillary water capacity (MCWC, *Eq. 2*) and water retention capacity (WRC, *Eq. 3*) were determined via pouring water through the soil sample. For these purposes, 20 g (w_0) of dried soil was poured by water and then placed on the filter paper. After two hours from pouring, the weight of the sample was recorded (w_{2h}). Then the samples were placed on dry filter paper again and the weight of the sample was recorded after 24 hours from pouring (w_{24h}).

$$\boldsymbol{MCWC = \frac{w_{2h} - w_0}{w_{2h}}} \qquad \text{(Eq. 2)}$$

$$\boldsymbol{WRC = \frac{w_{24h} - w_0}{w_{24h}}} \qquad \text{(Eq. 3)}$$

RESULTS AND DISCUSSION

The main idea of our work was to investigate the biochar impact on physico-chemical properties of selected representative soil samples, to correlate these effects with the crucial parameters of selected soils (mainly soil organic matter content) as well as with the observed effects of biochar application on a growth and vitality model plants used in the laboratory pot cultivation experiments. For these purposes, we combined the cultivation experiments with continual controlling of plant and roots growth and their vitality with the in-depth investigation of biochar effects on soils properties (total organic content, content of organic carbon, soils physical characteristics) as well as with the investigation of changes in biochar itself caused by its application in soil (total organic content, content of organic carbon, pH and conductivity of water extract, morphology and specific area). These individual aspects will be discussed in the following chapters.

The effect of biochar on growth of Zea mays

In a long-term cultivation study, the effect of two different EBC certified biochars, Bio Pflanzenkohle and NovoTerra, of different application doses (0, 10 and 20 g per 1 kg of dry weight soil) on the growth of the model plant, Zea mays, in four different soils, commonly spread in the Czech Republic, was studied. Both selected samples of biochar) represent standard materials possessing the European Biochar Certificate for use in agriculture as a soil conditioner. These materials have highly developed porous internal structure as was verified by the BET analysis (168±4 m^2/g for Bio Pflanzenkohle, respectively 191±5 m^2/g for NovoTerra), which is desirable for the application in agriculture and predetermine biochar positive effect on soil physico-chemical properties and the microbial activity of soil microorganisms. Quantitative characterization during the long-term cultivation study is based on growth parameters measurement, that is, according to the literature (Rafique et al., 2017), plant height, number of leaves and overall plant condition, and after harvesting, the weight of the plant parts (aboveground and underground). Corn cobs, if grown, was separated from the aboveground part. The individual (aboveground, corn cob, underground) parts were weighed on the analytical balances.

During the long-term cultivation study, the temperature and relative humidity of the air in the growing box were measured three times a week using a Thermo-Hydro meter (Fig. S1, Supplementary). Additionally, the pH and conductivity of tap water used for watering were measured. The average pH of tap water was 7.7 ± 0.3 and the average conductivity value was calculated to be 65 ± 5 mS/m. During normal corn growing in the fields, the plants emerge under optimal conditions (soil temperature and humidity) 5-7 days after planting the seeds in the soil (about 1-2 inches deep). After the emergence of the plants, the plants enter the vegetative phase of growth. In this phase, the young plant develops first, followed by elongated growth and finally throwing that ends the vegetative phase and begins the reproductive phase. At the end of the vegetative phase, the plant reaches almost its maximum height (approximately 53 days after planting) (Corn Growth Stages | Integrated Crop Management, 2021). The measured heights of the individual plants show that the most significant growth was observed in the plants in the first weeks (2-3 weeks from the beginning of the cultivation experiment) when the plants grew up to 32 cm in one week - specifically a plant planted in chernozem soil with application dose 10 g of biochar per 1 kg of soil. All soil types show significant growth retardation from day 51 of the cultivation experiment, which is in line with the maize vegetation cycle described above (Corn Growth Stages | Integrated Crop Management, 2021). The average height of plants is shown in Figure 1. The application of biochar has a negligible effect on plant growth in soils with higher organic matter content (fluvisol, cambisol), regardless of application dose. Generally, these plants have a lower height than those grown in soils with lower organic matter content (regosol, chernozem). At the end of the cultivation experiment, the highest plants were in soils treated with Phlanzenkohle biochar. In the case of fluvisol, chernozem and regosol soil, the application dose of biochar was 20 g of biochar per 1 kg of soil and in the case of cambisol with the application dose of 10 g biochar per 1 kg of soil.

In a short-term experiment after cultivation study, roots were carefully washed and air-dried at room temperature for two weeks. Dry roots were converted into a digital form (procedure described above) and processed by ImageJ software and HarFa software for quantitative root image analysis. Figure 2 shows representative root image analysis of root growth in fluvisol (a), regosol (b), cambisol (c), chernozem (d) and application dose of NovoTerra biochar 20 g per 1 kg of soil. The plant has the longest roots in sandy soil due to the soil low organic matter content, the root in this soil tends to look for nutrients and water and are therefore they are very long.

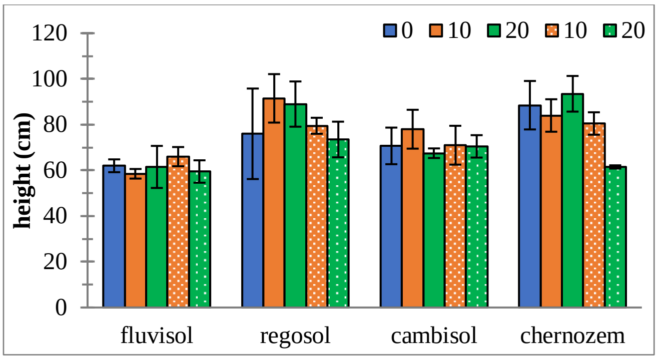

Figure 1: Plant height after three months of cultivation, biochar dose: 0 (blue), 10 g per kg of soil (orange), 20 g per kg of soil (green), Bio Pflanzenkohle (plain) and NovoTerra (dots).

On the other hand, roots in soils with a higher organic matter content (fluvisol, cambisol), richer branching of roots can be observed, but these roots do not reach such lengths as in the case of sandy soil. On the other hand, the roots in regosol were the least branched. In chernozem, the roots are well-branched, but they are very short. Generally, roots grown in soils treated with biochar are more branched which is a desirable effect for plant growth because they can better absorb water and nutrients from the soil. In comparison among soils, the greatest root branching occurs in soils with a higher organic matter content (fluvisol and cambisol). According to the results of the root image analysis, the roots of plants in regosol had the largest volume, which is in agreement with the results of a long-term cultivation study, where the roots of plants from regosol were the heaviest in comparison with the roots of other soils.

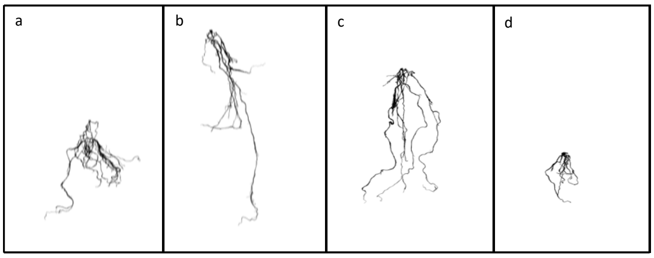

Figure 2: Root image analysis – the effect of NovoTerra biochar (20 g of biochar per 1 kg of soil) on root size and branching after short-term cultivation study in fluvisol (a), regosol (b), cambisol (c), chernozem (d) soils.

Analysis on cultivation effects on biochar properties

The elemental composition, organic matter content and ash content of individual biochars (before and after cultivation) are presented in Table 2. The organic matter content of biochars ranged from 57.2 % to 71.7 % varying with the soil type selected for the cultivation procedure. The highest organic matter contents (72.7 % and 78.7, respectively) were obtained for the original biochars. The ash content of original biochars can depend both on the origin of the source material as well as on the pyrolysis procedure. Overall, the inorganic ash content of all incubated biochars in the individual soil samples was higher as compared to the original biochars. Among the individually incubated biochars, the highest ash (non-combustible) content was observed for soils that were characterized by lower content of the organic matter (see Table 2). Besides, this may be attributed to the higher extraction of organic matter during the incubation process.

Table 2: TGA and EA analysis of the organic matter, inorganic ash and organic elements content
in the biochar samples (both original and after the cultivation in the individual soil samples).

sample	**cultivation in soil**	w_{org} **(wt.%)**	w_{inorg} **(wt.%)**	**elemental composition (wt.%)**			
				C	**H**	**O**	**N**
Biochar_S	original	72.7	21.4	65.22 ± 2.68	3.71 ± 0.22	0.97 ± 0.16	2.92 ± 0.16
	A	71.7	22.2	64.08 ± 1.69	3.64 ± 0.05	0.63 ± 0.05	3.02 ± 0.11
	B	67.6	26.5	59.85 ± 0.33	3.64 ± 0.04	0.54 ± 0.09	2.94 ± 0.09
	C	59.1	37.3	52.06 ± 0.28	3.23 ± 0.13	0.70 ± 0.12	3.01 ± 0.06
	D	69.0	27.3	61.40 ± 2.18	3.96 ± 0.07	0.77 ± 0.16	3.03 ± 0.02
Biochar_N	original	78.7	16.5	70.48 ± 0.64	4.31 ± 0.14	0.85 ± 0.42	2.89 ± 0.18
	A	70.8	23.3	61.15 ± 0.51	4.22 ± 0.02	1.00 ± 0.62	2.77 ± 0.08
	B	62.3	32.9	55.43 ± 2.49	3.60 ± 0.11	1.18 ± 0.16	2.68 ± 0.07
	C	61.3	34.8	54.34 ± 0.72	3.54 ± 0.03	0.79 ± 0.15	2.68 ± 0.21
	D	57.2	38.9	50.37 ± 1.56	3.12 ± 0.24	0.63 ± 0.21	2.89 ± 0.07

The elemental compositions are shown in Table 2 in weight percent (wt.%). The highest content of carbon was found for original biochars such as NovoTerra (78.7 %) and Bio Pflanzenkohle (72.7 %). Oxygen and nitrogen represent a minor component of all biochar samples (before and after the cultivation in the individual soil samples) and their contents of oxygen and nitrogen ranged from 0.54 % to 1.18 % for the oxygen and ranged from 2.68 % to 3.03 % for the nitrogen. The content of organic carbon and hydrogen decreased during the cultivation in the individual soil type, while the oxygen content oscillated around its original value (0.85–0.97%). A greater decrease in major elements such as the content of carbon and hydrogen was observed for the biochar NovoTerra incubated in the soil C classified to the FAO (FAOSTAT, 2021) soil classification system as a regosol. The elemental composition of original biochars was comparable to other biochars produced from different European producers possessing the EBC certification for use in agriculture (del Bubba et al., 2020, Wiedner et al., 2013).

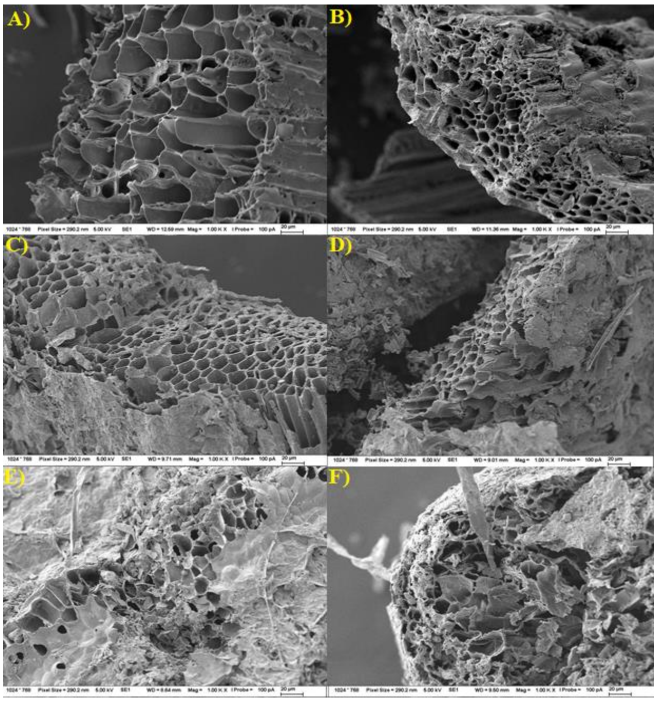

Figure 3: SEM analysis of original biochar samples A) NovoTerra, B) Bio Pflanzenkohle and biochar sample of Bio Pflanzenkohle after the pot cultivation experiments in C) fluvisol – Soil A; D) cambisol – Soil B; E) regosol – Soil C; F) chernozem – Soil D.

Figure 3 indicates the comparison of internal morphology of both the original biochar samples (Figure 3A – NovoTerra; Figure 3B-Bio Pflanzenkohle) used for the cultivation experiments and the samples biochars obtained after finalization of the cultivation in different used soils (Figure 3C, D, E and F) as was obtained by SEM. The SEM comparison of both original biochar samples confirms the results obtained from the BET determination of specific surface area. Both these biochar samples possess a highly developed internal structure with high content and broad distribution of internal pores, which is desirable for biochar soil applications. Moreover, Figures 3C, D, E and F show a comparison of SEM visualisation of internal morphology of biochar samples obtained after the application in soils. From the comparison with the original biochar sample (Figure 3A), some minor differences can be distinguished. The porous structure with highly developed specific surface area is maintained, but several impurities and artefacts caused probably by the cultivation in soils can be observed mainly in Figure 3C and D, which corresponds to the cultivation in regosol (Figure 3C) and chernozem (Figure 3D) – soils with lower content of organic matter. The differences correlate with more significant changes in the content of organic matter and organic carbon, as was shown in Table 2. But the basic structural motives were maintained. These results are in good agreement with the published high stability of biochars in soils (Bagreev et al., 2001, Hardie et al., 2014, Yan et al., 2021).

Figure 4 indicates the effect of the cultivation of biochars in different soils on the leaching of inorganic ions.

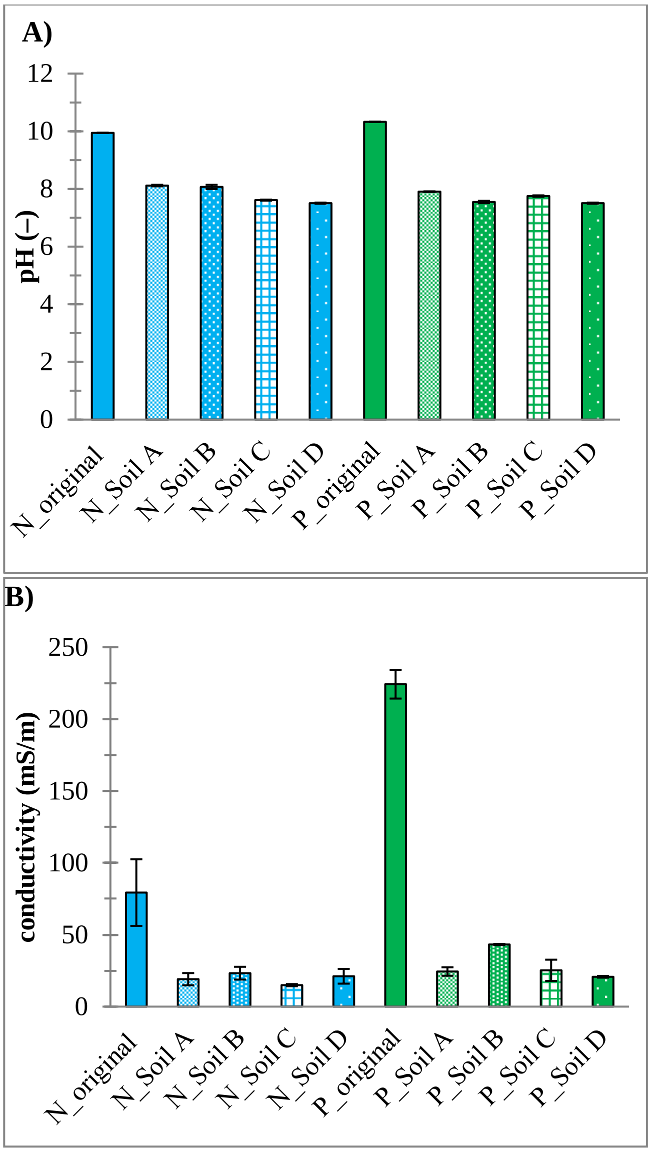

Figure 4: A) pH and B) conductivity measurement of water leachate from the biochar samples (N = Novoterra, P = Bio Pflanzenkohle) before and after the cultivation experiments in soils.

For these purposes, simple extraction with distilled water with pH and conductivity detection was used. As can be seen from Figure 4, the cultivation in different soils significantly decreased the pH and conductivity of water extracts. These can be assigned to the leaching of inorganic salts (mainly salts of alkaline metals) during the long-term cultivations into the soils. These salts are causing the alkaline response of biochars. The results of Figure 3 indicate that the leaching of alkaline salts into the soils can be expected only at the initial step of biochar application and continuously will decrease.

Analysis on cultivation effects on soil properties

The organic elemental compositions, organic matter content and non-combustible residue such as inorganic content of all used soils are summarized in Table 3. The organic matter content of studied soil samples ranged from 1.9% to 6.8% depending on the type of soil. The highest organic matter contents (6.7% and 5.5%) were obtained for soil samples, which were classified as Fluvisol and Cambisol using the FAO soil classification system. In contrast, the lowest contents of organic matter were determined for soils marked as C and D. Likewise, the ash content of soils is directly related to the above-mentioned organic matter content and method of its determination using thermogravimetric analysis. Despite their different soil origins and cultivation with the individually added biochar, the elemental compositions of the soil samples are comparable: C, 0.6–2.6 wt.%; H, 0.1–1.5 wt.%; O, 0.2–2.7 wt.%; and N, 0.1–0.3 wt.%. In other words, the differences among the soils before and after the cultivation process are not significant.

Table 3: TGA and EA analysis of the organic matter, inorganic ash and organic elements content in the soil samples (both original and after the cultivation with the individual added biochar samples).

sample	sample description	w_{org} (wt.%)	w_{inorg} (wt.%)	elemental composition (wt.%)			
				C	H	O	N
Soil A (fluvisol)	original sample	5.493	92.85	2.616 ± 0.243	1.024 ± 0.089	1.547 ± 0.039	0.305 ± 0.020
	+ Novoterra	4.535	93.55	2.461 ± 0.104	1.147 ± 0.142	0.997 ± 0.078	0.231 ± 0.014
	+ Pflanzenkohle	5.040	93.02	2.328 ± 0.101	1.245 ± 0.053	1.233 ± 0.030	0.234 ± 0.008
Soil B (cambisol)	original sample	6.751	91.46	2.605 ± 0.180	1.151 ± 0.080	2.689 ± 0.031	0.306 ± 0.020
	+ Novoterra	6.764	90.87	2.607 ± 0.011	1.450 ± 0.099	2.455 ± 0.050	0.251 ± 0.002
	+ Pflanzenkohle	6.329	91.51	2.360 ± 0.062	1.380 ± 0.017	2.355 ± 0.009	0.234 ± 0.001
Soil C (regosol)	original sample	1.935	97.57	1.088 ± 0.017	0.206 ± 0.009	0.195 ± 0.004	0.193 ± 0.009
	+ Novoterra	2.857	96.36	0.903 ± 0.038	0.141 ± 0.029	1.705 ± 0.019	0.108 ± 0.010
	+ Pflanzenkohle	3.233	96.00	1.053 ± 0.078	0.241 ± 0.020	1.867 ± 0.017	0.072 ± 0.015
Soil D (chernozem)	original sample	2.460	96.53	0.644 ± 0.023	0.264 ± 0.005	1.424 ± 0.003	0.127 ± 0.003
	+ Novoterra	2.290	96.83	0.746 ± 0.114	0.315 ± 0.014	1.143 ± 0.011	0.086 ± 0.008
	+ Pflanzenkohle	2.654	96.26	0.639 ± 0.016	0.237 ± 0.027	1.641 ± 0.015	0.136 ± 0.002

Physical soil characterization

One of the benefits of biochar application should be not only the positive effect on plant growth but also the improvement of soil quality. One should expect that the soil porosity as one of the crucial parameters in quality assessment should increase. The soil porosity after the application of biochar is significantly increasing only if biochar Pflazenkohle is applied into cambisol soil. The role of biochar on soil porosity is not significant. This should be caused by the short exposure time of biochar in studied soils.

Probably the most crucial parameter in soil quality assessment is the water retention capacity (the content of water that can be retained by soil). After the application of biochar into less fertile soils, the water retention capacity slightly increased which is a promising finding considering the application of biochar into the soil. It could be expected that if the biochar will be applied repeatedly and/or at a longer exposure time, the water retention capacity should significantly increase. This could be beneficial (and one of the promising solutions) in solving a problem with the retention of water in the landscape.

Table 4: The effect of biochar application on physical characteristics of soils – soil porosity, maximal capillary water capacity (MCWC) and water retention capacity (WRC).

sample	sample description	*Porosity (%)*	*MCWC (%)*	WRC (%)
Soil A	original sample	53.7 ± 1.1	14.9 ± 0.9	12.7 ± 0.6
	+ Novoterra	52.1 ± 2.0	15.7 ± 1.4	12.9 ± 0.7
	+ Pflanzenkohle	55.2 ± 1.9	15.2 ± 1.0	13.5 ± 0.6
Soil B	original sample	58.2 ± 1.9	17.9 ± 0.9	15.7 ± 0.6
	+ Novoterra	60.1 ± 1.7	23.4 ± 0.4	20.1 ± 1.9
	+ Pflanzenkohle	64.8 ± 0.5	19.3 ± 0.9	18.4 ± 1.2
Soil C	original sample	40.0 ± 2.0	13.1 ± 0.8	10.0 ± 0.7
	+ Novoterra	41.1 ± 0.8	18.8 ± 1.3	18.0 ± 1.3
	+ Pflanzenkohle	41.2 ± 0.9	15.2 ± 1.3	15.2 ± 1.2
Soil D	original sample	52.2 ± 1.3	19.3 ± 0.3	17.7 ± 0.3
	+ Novoterra	48.8 ± 1.0	19.4 ± 0.7	17.6 ± 0.7
	+ Pflanzenkohle	51.0 ± 2.5	18.9 ± 1.8	17.1 ± 0.4

CONCLUSION

The main idea of our work was to investigate the biochar effect on physico-chemical properties of selected representative soil samples, to correlate these effects with the crucial parameters of selected soils (mainly soil organic matter content) as well as with the observed effects of biochar application on a growth and vitality model plants used in the laboratory pot cultivation experiments. The results of our work indicated that even at a short time scale of biochar application in soils some minor effects were observed. The increase in plant heights and total dry mass was statistically significant for the soils with low content of organic matter (chernozem, regosol). In these soils, the individual Zea mays plants had longer and less branched roots. The application of biochar helped to increase the roots branching. At the observed time scale, the effects of biochar application on soils physicochemical properties were negligible. On the other side, the changes in observed biochar properties were statistically significant. Both the biochar sample, possessing the EBC certification for use in agriculture, showed a minor decrease in the content of organic matter and organic carbon as well as some changes in the leaching potential of inorganic alkaline salt into the water environment. SEM morphological visualization confirmed the beginning changes in the internal porous structure of biochar. To summarize, our results are confirming the continual leaching of both organic and inorganic matter from biochar to surrounding soil. These findings seem to be crucial for helping to understand biochar role and effect in soils. The ongoing research could provide also important findings helping to shed new light on the possible use as a soil conditioner and confirming the long-timescale effect of biochar on the improvement of the properties of soils.

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