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Tiered Approach for Assessing the Effective and Safe Applicability of Beech Wood Biochar for Soil Improvement

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Abstract

The soil degradation processes may cause long-term serious problems in various areas of life on Earth, thus mitigation of these processes by environmental-friendly, innovative soil protection methods is necessary. Biochar produced from organic wastes or by-products by pyrolysis may have several positive properties as additive in soil improvement technologies. In our complex research applying a tiered approach we assessed the main properties of a beech-wood biochar produced from a high volume by-product of a food industry technology. Then we studied in 4-months microcosm experiments the applicability of this biochar as amendment mixed into an acidic, degraded sandy soil. In addition, to forecast the long-term effects of the biochar, artificial physical and biological aging experiments were conducted.

Based on the results, the beech-wood biochar was able to shift the acidic pH of the soil to the alkaline range and the electric conductivity of the soil increased with incremental biochar doses. Biochar addition significantly increased the microbial activity, as well. The results had clearly shown that during the mid-term experiment not even the highest biochar dose (15%) had inhibitive effect, but it rather improved several soil parameters. Furthermore, the biochar had positive effect on the soil water holding capacity, and the available soil nutrient and organic matter content. The results of the aging experiments generally showed also favourable effects and demonstrated that the aging-mediated changes differed according to the soil types. Therefore, we have concluded that biochar application requires a char by char and soil by soil testing prior to field application.

Keywords

acidic sandy soil, beech wood biochar, integrated monitoring, multiparameter approach, soil improvement

1 Introduction

Biochar, a form of pyrogenic carbon, is a solid, carbon-rich and highly recalcitrant product (or by-product) of biomass combustion under oxygen limited condition, known as pyrolysis [1, 2]. Due to its favourable physical and chemical properties, biochar has several ways of application such as heat and power production, flue gas cleaning, metallurgical applications, building material, medical use and additive in remediation [3–6]. According to numerous studies, biochar, due to its specific physical and chemical nature, proved to be useful as soil amendment [1, 7–10]. However, the physico-chemical properties of biochars depend upon the type of the feedstock, the biochar production conditions [11–14], and also on a variety of temporal processes in the environment, called "aging" [15], including for example abiotic and biotic processes, interactions with soil microbes, organic matter (OM) and minerals in the soil environment [16, 17].

As soil amendment, biochar may increase agricultural and environmental sustainability by improving soil physical, chemical and biological properties such as nutrient- and water holding capacity, pH buffering capacity as well as soil fertility and plant productivity [7, 9, 10, 18, 19]. However, it must be considered that biochar may contain organic and inorganic contaminants such as heavy metals and polycyclic aromatic hydrocarbons (PAHs) depending on the used feedstock and pyrolysis method [20]. Therefore, when applied in agriculture or to arable land, biochar could pose a potential environmental and human health risk. The existing studies on the use of biochar in soil revealed, on the one hand, the lack of a systematic and tiered approach, as well as of adequate methods to predict its long-term applicability, which would be prerequisites for the safe field use of biochar products [9, 10, 19, 21–23].

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For the safe and beneficial application of biochar in soil amendment, the various biochar products (from different feedstocks and pyrolysis methods) need to be investigated on a "*char by char basis*"; besides, when applying the biochar for a certain low-quality soil, the effect of biochar depends also on the soil type and soil structure [22].

The pyrolysis conditions and feedstock determine the quality of biochars. Shen and Gu [24] examined the effect of temperature (from 430 to 730 °C) on the pyrolysis products, and they found that higher temperature improved the yield of the gas and oil but decreased the yield of the char; above 570 °C, the yield was less than 5% in all cases. Li et al. [21] conducted a meta-analysis to link pyrolysis temperature to biochar properties, and they found that higher pyrolysis temperature linearly increased the biochar pH for almost every feedstock type, which was due to the cleavage of weak bonds (e.g., hydroxyl bond) in the biochar structure. This observation indicated that biochars produced at higher temperature would be a better solution for acidic soil treatment than low (~400–450 °C) heat-produced ones. Li et al. [21] and Fryda and Visser [25] also reported that the morphological properties, such as porosity and the specific surface area (SSA) increased with heat expansion. Especially the SSA increased exponentially with heat increase up to 800 °C. These properties are also influenced by the feedstock type. Some studies demonstrated that wood-based biochars have a good impact on several soil types, including soil functions and fertility, but most of the studies are short-term investigations [26, 27]. Blok et al. [26] in a lab-scale research found that the chemical properties of a wood-based biochar had better effect in horticulture than the plant based ones. This is explained with the low electric conductivity (EC) values and the salt content of wood-based biochars as opposed to plant-based biochars.

Despite the increasing number of studies on the use of biochar as soil ameliorant, the aging induced changes in the properties of various biochars and their functionality in soil from the perspective of the physicochemical, biological, ecotoxicological properties and their interaction with various soil types under field aged conditions is still an area with knowledge gaps. To predict the physical and chemical properties of aged biochars and their performance in the soil, there are several physical, chemical or biological artificial aging methods such as wet-dry or hot-cold temperature cycling, chemical oxidation and microbial oxidation [28]. Li et al. [21], in a meta-analysis, compared artificial biochar aging with natural aging and found that the current artificial aging methods were only partially able to simulate biochar aging in the soil.

Our main objective was to investigate the reliable and risk-based applicability of a beech wood biochar pyrolyzed at low temperature applying a complex tiered approach. One of our hypotheses was that low temperature pyrolyzed biochars, despite the low SSA and porosity, could be beneficially applied in degraded sandy soils, to improve fertility, water holding capacity as well as biological activity of the soil. In the first phase, a comprehensive characterization of the biochar product was carried out using physicochemical, biological, and ecotoxicological methodologies. The microcosm experiments, in the next phase, aimed to measure and evaluate the biochar-mediated effects on an acidic sandy soil, to determine the influences on soil physicochemical and biological properties and to characterize the dependence on time and biochar dose. Besides, the effect of artificial biological and physical aging on the applied biochar was monitored, in order to predict its long-term, safe and effective applicability for soil improvement.

2 Materials and methods

2.1 Soil properties

All of the studied soils were collected from two different places in Hungary (Nagyhörcsök, Nyírlugos). For the microcosm study, acidic sand from Nyírlugos (47°43'N, 22°00'E), the sandy Alföld region of Hungary was applied. The acidic sandy soil (brown forest soil with alternating thin layers of clay, Lamellic Arenosol) from Nyírlugos and a loamy soil (calcareous chernozem, Calcaric Phaeosem) from Nagyhörcsök, Hungary were used for the artificial biological aging. The properties of the soils used for the study are listed in Table 1.

Table 1 Properties of the soils applied in the technological microcosms and aging experiments

Soil properties	Loamy soil, Nagyhörcsök	Acidic sandy soil, Nyírlugos
pH [H_2O]	7.6	4.9
$CaCO_3$ [%]	1.8	0
Mechanical composition - sand:silt:clay [w/w%]	17:60:23	80:10:5
Cation exchange capacity (CEC) [Na mg eq./100 g]	32	5
Total N, P, K [mg/kg]*	N: 2000; P: 903; K: 4496	N: 400; P: 173; K: 300
Organic matter (OM) content [w/w%]	3.1	0.5

* Methodology explained in the *Integrated methodology* section

2.2 Biochar properties

The feedstock of the tested biochar was beech tree (*Fagus sylvatica*) (Rauchergold® wood chips, 100% wood fibers from untreated natural hardwood, average particle size 0.5 mm–1 mm). The pyrolysis temperature was 450 °C with 10 min residence time. The main physical, chemical and biological properties of the biochar are shown in Table 2.

Regarding its elemental content determined with NITON XRF XL3t 600 we found that the limit values set by the International Biochar Initiative [29] recommendation were not exceeded (data not shown).

Table 2 Physicochemical, biological and ecotoxicological parameters* of the applied beech tree biochar

Physical/chemical parameter	Measured value - average and deviation	
BET specific surface area [m^2/g]	1.05 (± 0.21)	
Total pore volume (SUM V_{pore}) measured with N_2 [cm^3/g]	1.92E-03 (± 4.81E-05)	
pH [-]	7.26 (± 0.21)	
EC [mS/cm]	109.9 (± 6.6)	
Water holding capacity (WHC) [%]	52.08 (± 2.68)	
Organic C [m/m%]	46.49 (± 0.4)	
Loss on ignition (LoI) [%]	97.98 (± 0.07)	
Total N [m/m%]	0.147 (± 0.004)	
NO_3+ NO_2-N [mg/kg]	1.88 (± 0.11)	
NH_4-N [mg/kg]	1.81 (± 0.01)	
$CaCO_3$ [m/m%]	0.20 (± 22)	
AL - K_2O [mg/kg]	2417 (± 755)	
AL - P_2O_5 [mg/kg]	349.2 (± 11.7)	
CEC [Na mg eq./100 g]	56.9 (± 4.9)	
Biological parameter		
Aerobic heterotrophic bacteria [CFU, cell/g biochar]	3.77E+05 (±5.75E+04)	
Aerobic heterotrophic fungi [CFU, cell/g biochar]	2.09E+05 (±2.05E+04)	
Ecotoxicological parameters	Inhibition [%]	Characterization
Aliivibrio fischeri – bioluminescence inhibition [%]	<10	non-toxic
Germination inhibition – *Sinapis alba* [%]	<5	no inhibition
Germination inhibition – *Triticum aestivum* [%]	<5	no inhibition
Shoot length inhibition – *Sinapis alba* [%]	23	slight inhibition
Shoot length inhibition – *Triticum aestivum* [%]	33	slight inhibition
Folsomia candida – lethality [%]	<10	no mortality

* Methodologies explained in *Integrated methodology* section

2.3 Microcosm incubation study for soil improvement

After testing the main biochar properties, soil microcosms were set up in 15 × 30 cm bioreactors to study the effect of beech wood biochar on degraded soil. The Nyírlugos sandy soil was mixed with biochar at 0.5w/w%, 1w/w%, 5w/w%, 15w/w% concentrations in each reactor reaching 2 kg total weight/reactor. The control microcosm contained only the sandy soil (without biochar), and it was kept under the same conditions as the biochar treated reactors. Water was added to the soils to reach 60w/w% of their water holding capacity during the complete study period. The duration of the microcosm study was four months and the reactors were incubated at room temperature (22±1 °C). Homogenous samples were collected after one and four months and analysed with a complex integrated methodology, detailed in Section 2.5.

2.4 Lab-scale experiments for modelling biochar aging

Biological and physical artificial aging was modelled separately, and the effects of both aging processes were monitored with an integrated methodology. The physical artificial aging experiment was performed according to Wang et al. [28]. During the experiment 100 g biochar in three parallels was exposed periodically to two highly different temperatures. The biochar was contained in plastic bags, and was stored periodically at 30 °C for 24 hours then in a −18 °C freezer for 24 hours. The physical artificial aging experiment lasted for five weeks. The biological artificial aging experiment aimed to determine the changes in beech wood-based biochar in different soil types. To model high microbiological activity and to shorten the natural biological aging effect a microbial inoculant was added at high concentration. To assess the influence of various soils on the aging process, two different soil types were used: loamy soil (Nagyhörcsök) and acidic sandy soil (Nyírlugos). The experiment was set up in 1500 ml glass bioreactors. Each reactor consisted of five layers, of which the first, the third and the fifth layer was made up of 200 g of one soil type and the second and fourth layer of 80 g of beech wood-based biochar. The layers were separated with gauze cloth, plastic tubes were inserted for ventilation and to enable introduction of the inoculum into all layers. 90 mL nitrogen and phosphorus solution (50.0 g NH_4NO_3 + 16.6 g Na_2HPO_4 dissolved in 1000 ml water) was added equally to the bioreactor. The bioreactor was incubated for 48 hours, then 100 ml of microbial inoculum (from Biofil Ltd.) was added. The inoculum contained phyla from the following genera: *Azospirillum*, *Pseudomonas*, *Bacillus* and *Kocuria*. The inoculum was prepared according to the recommendations of Biofil Ltd. The bioreactors were incubated for five weeks in the dark and at room temperature.

2.5 Integrated methodology

An integrated methodology including physicochemical analysis, biological methods, and ecotoxicity tests [9] was applied for the assessment of biochar characteristics and in the subsequent microcosm and biochar aging experiments.

2.5.1 Physicochemical methodology

The EC, pH, water holding capacity (WHC), loss on ignition, permanganate oxidizable organic carbon (POXC) content, inorganic nutrient-, organic matter- and toxic element content were determined in the different phases of the research. For the biochar product the SSA and total pore volume was also determined. BET specific surface area [30] was measured based on low temperature (−196 °C) nitrogen vapour adsorption. EC and pH were measured in a 1:2.5 soil suspension according to the Hungarian Standard MSZ 21470-2:1981 [31]. The pH was determined with a WTW (Wissenschaftlich Technische Werkstätten GmbH, Germany) instrument using a pH 330 Meter including a precision Sentix 81 pH Electrode. For the EC a Consort C535 instrument (Turnhout, Belgium) was used.

The WHC was determined, as described by Öhlinger [32]. Loss on ignition was measured according to Sluiter et al. [33] to estimate soil/biochar organic matter based on gravimetric weight change associated with high-temperature oxidation of organic matter. POXC method was performed according to Weil et al. [34] to determine labile carbon fractions. In case of biochar samples, 0.2 g biochar, in case of soil samples, 2.5 g soil were used. The mineral nitrogen (i.e., NH_4-N and NO_3-N concentration) was determined from 1 M KCl extract according to the Hungarian Standard MSZ 20135:1999 [35] and the CEC values with the modified method of Meglich [36]. The plant available ammonium lactate soluble P_2O_5 and K_2O content of the soils (AL-P_2O_5 and AL-K_2O, respectively) were determined according to the Hungarian Standard MSZ 20135:1999 [35]. The soil organic matter content was determined according to MSZ-08-0452:1980 [37]. Kjeldahl method [38] was applied for the measurement of the total N content. The toxic element content was measured with X-Ray Fluorescence spectroscopy using NITON XL3t instrument by Thermo Scientific™.

2.5.2 Biological methods

To assess the biological activity, cell concentrations of aerobic heterotrophic bacteria and fungi were determined by aerobic plate count technique based on the method originally described by Benedetti and Dilly [39]. For the cultivation of bacteria, meat agar, for fungi, malt agar was used, as described by Ujaczki et al. [40]. The number of the developed colonies (Colony Forming Units – CFU) was counted after 72 hours incubation and the results were given in CFU/g soil.

Metabolic activity and functional diversity were characterized by the BIOLOG EcoPlate™ method, carried out according to Feigl et al. [41]. This method was used only in the microcosm incubation study. The area under the curve (AUC) for characterisation of the microbial activity and the Shannon diversity index were determined. The AUC gives information about the utilization of the carbon sources in the plates and the Shannon diversity index (H) about the physiological diversity of the bacterial communities in the samples [42].

2.5.3 Ecotoxicological methods

To assess the potential toxic effects of biochar and the influence on soil habitat function, plant and animal ecotoxicity tests were carried out. For both the microcosm study and the artificial aging study the following ecotoxicological methods were applied: plant root and shoot growth inhibition tests with *Sinapis alba* (white mustard) and *Triticum aestivum* (common wheat), plus animal mortality test applying *Folsomia candida*.

The plant tests were performed, according to the Hungarian Standards MSZ 22902-4:1990 [43] and MSZ 21976-17-1993 [44]. The standards were modified to direct contact with soil as described by Leitgib et al. [45] and the test was carried out as described by Molnár et al. [9]. The animal, *Folsomia candida* (Collembola) mortality test was carried out according to the modified version of ISO [46] and OECD [47] standards as described by Molnár et al. [9].

2.6 Statistical analysis

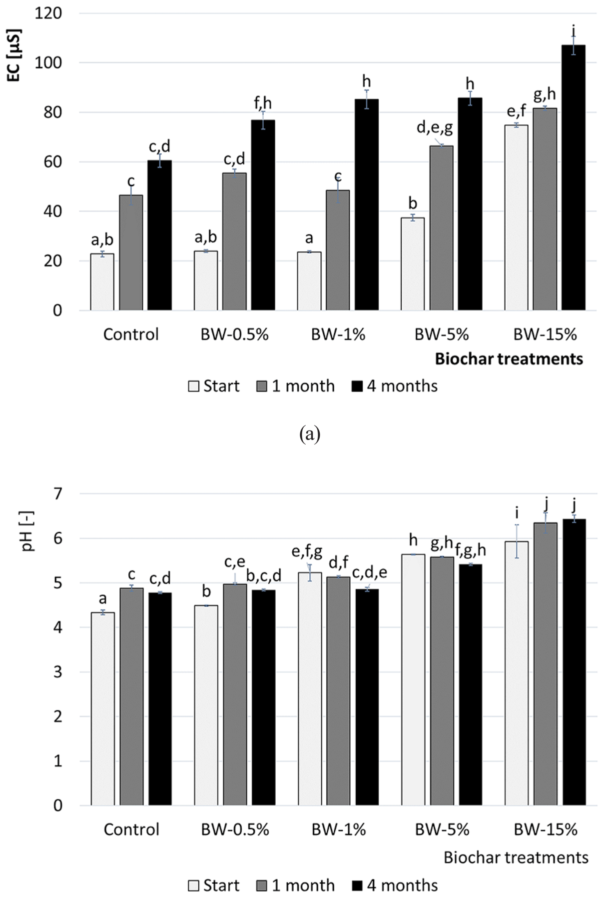
The statistical evaluation of the results was carried out with TIBCO Statistica™ 13.3 software [48]. Repeated measures analysis of variance (RM ANOVA) was performed to investigate whether the biochar treatments, the incubation time and their interactions had an effect on the given soil parameter during the experiment. All treatments were performed in three replicates. In the aging studies, one-way analysis of variance (ANOVA) was performed. Both RM ANOVA and one-way ANOVA analyses were performed at the $p < 0.05$ significance level. In case of the microcosm experiments and in the aging experiments, Fisher least significant difference test or Newman-Keuls test was carried out to compare the effects. The significant effects are marked with letters in the figures and tables in alphabetical order, where "a" is the smallest value. Values signed with the same letter indicate that there was no significant difference between them.

3 Results

3.1 Microcosm study with beech wood biochar for soil improvement

3.1.1 Impact of biochar treatments on the soil physicochemical parameters in the microcosm study

As illustrated, both the EC and pH was enhanced by biochar addition (Fig. 1).



(a)

(b)

Fig. 1 Beech wood biochar induced changes in the EC (a) and pH (b) in the microcosm study with acidic sandy soil. Letters on the columns indicate significant differences ($p < 0.05$)

Fig. 1 (a) illustrates the time- and biochar dose-dependent increase of the EC. With incremental biochar concentrations, the EC increased, except in one case. This means that higher biochar doses result higher EC and this effect lasts at least up to four months. However, for the two higher concentrations the picture is different as if there would be a threshold for immediate reaction. The highest difference (51 µS) between the EC of the control and the 15% BC treatment was found at the start of the experiment. The highest EC achieved with biochar treatment was in case of 15% BC treatment (~107 µS), which means a ~77% increase.

Based on the results of the RM ANOVA (Table 3) analyses, both the biochar treatments and the time have significant effects on the EC, and the effect of treatments differs at the different sampling times.

In our research, among the tested soil parameters, the pH is of paramount importance, due to the acidic character of the sandy soil. According to the pH results (Fig. 1 (b)), the beech wood biochar treatment in the microcosm study had significant positive effect on the pH of the acidic sandy soil. This was demonstrated by the RM ANOVA analysis (Table 3) too. At the lowest, 0.5 and 1.0% concentrations, the biochar treatment induced significant positive changes in the pH only on the short term. While 5% biochar treatment increased the pH by 0.6 unit, from 4.8 to 5.4 after four months. 15% biochar treatment

Table 3 RM ANOVA results over time to evaluate effects of beech wood biochar treatment on the EC, pH, loss on ignition and POXC. Bold p-values indicate significant differences at $p < 0.05$

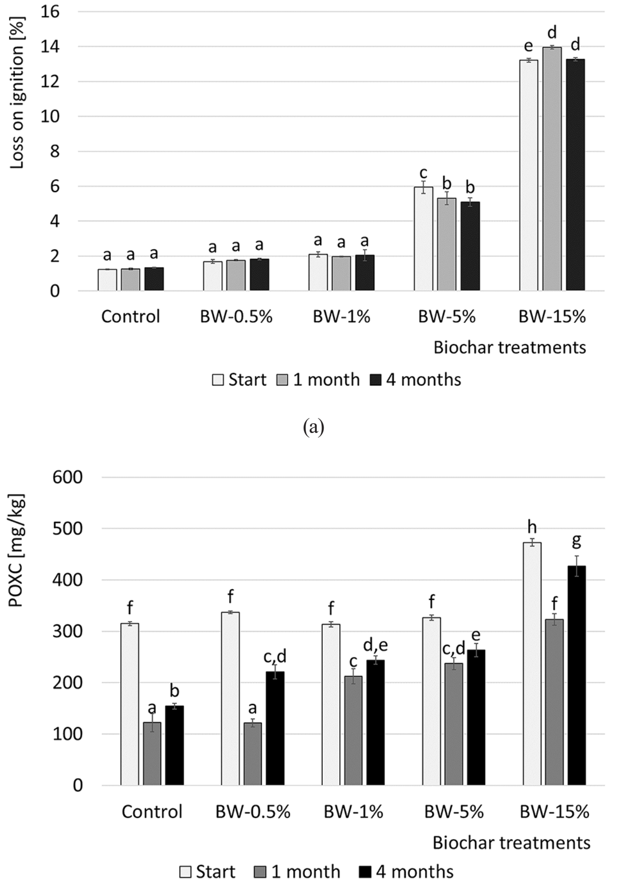
Effect	d.f	Mean square	F-ratio	p-value
EC				
Treatment	4	1481.15	102.99	**0.0015**
TIME	2	360.86	469.53	**0.0000**
TIME × Treatment	8	99.74	12.98	**0.0029**
pH				
Treatment	4	34.43	116.9	**0.0002**
TIME	1	4.32	1.4	0.3016
TIME × Treatment	4	2.53	0.82	0.5729
Loss on ignition				
Treatment	4	113.72	900.74	**0.0000**
TIME	2	1.57	18.23	**0.0001**
TIME × Treatment	8	0.35	4.03	**0.0112**
POXC				
Treatment	4	41522	246.21	**0.0000**
TIME	2	53578	533.12	**0.0000**
TIME × Treatment	8	2454	24.42	**0.0001**

had the best results increasing the pH with almost 2 pH units compared to the control, resulting pH 6.4. This positive effect lasts at least up to four months (from pH 4.9 to 6.4 at the end of the experiment) (Fig. 1 (b)). However, as shown in Table 3 the time and the time × treatment did not affect significantly the pH.

Fig. 2 shows the beech wood biochar induced changes in the loss on ignition (Fig. 2 (a)) and POXC (Fig. 2 (b)) in soils.

Loss on ignition was remarkably and significantly increased at the highest biochar doses (5 and 15%). As expected, the highest increase (~870%) compared to control was exhibited at 15% biochar application. RM ANOVA analysis of our results also demonstrated the advantageous effect of biochar, both the biochar treatment, time and treatment × time had significant positive effects (Table 3).

With biochar addition POXC is expected to be higher than in the control acidic sandy soil. Fig. 2 (b) shows that at the start of the experiment, only the 15% biochar treatment increased significantly the POXC. However, after one month the biochar addition resulted significant enhancement compared to control already at 1% biochar dose. After four months, the POXC increased significantly already at 0.5% biochar concentration in all treatments. RM ANOVA results in Table 3 also supported our findings that the changes were significant both in terms of treatment and time, plus the effect of treatments differed at the different sampling times.



(a)

(b)

Fig. 2 Beech wood biochar induced changes on the loss on ignition (a) and POXC (b) in the microcosm study with acidic sandy soil. Letters on the columns indicate significant differences ($p < 0.05$)

Table 4 shows the effect of biochar treatments on soil NPK-supply, OM content, available P_2O_5, available K_2O, CEC and WHC after four months.

The biochar treatment had slight positive significant effect on total nitrogen content in case of 5% and

Table 4 Effect of biochar treatments on soil NPK-supply, OM content, available P_2O_5, available K_2O, CEC and WHC. Letters next to the data indicate significant differences (level of significance: $p < 0.05$)

	Total-N [m/m%]		NH$_4$-N [mg/kg]	
Cont. Start	0.043^{a}	±0.004	14.297^{b}	±0.239
4 months				
Control	0.041^{a}	±0.002	3.558^{a}	±0.268
BW-0.5%	0.043^{a}	±0.004	3.500^{a}	±0.292
BW-1%	0.043^{a}	±0.004	3.403^{a}	±0.268
BW-5%	0.047^{a,b}	±0.000	3.523^{a}	±0.268
BW-15%	0.052^{b}	±0.003	3.478^{a}	±0.246
	NO$_3$-N [mg/kg]		Organic matter [m/m%]	
Cont. Start	3.65^{b}	±0.05	0.53^{a}	±0.01
4 months				
Control	22.74^{d}	±0.9	0.51^{a}	±0.02
BW-0.5%	20.68^{c}	±0.93	0.76^{a,b}	±0.09
BW-1%	20.73^{c}	±1.63	0.98^{b}	±0.05
BW-5%	16.92^{b}	±0.35	1.83^{c}	±0.29
BW-15%	10.24^{a}	±0.58	3.88^{d}	±0.26
	AL-P$_2$O$_5$ [mg/kg]		AL-K$_2$O [mg/kg]	
Cont. Start	109.4^{a}	±1.2	55.2^{a}	±0.7
4 months				
Control	115.3^{a}	±2.7	50.6^{a}	±2.7
BW-0.5%	115.5^{a}	±6.8	68.6^{b}	±0.8
BW-1%	113.3^{a}	±5.4	71.6^{b}	±1.2
BW-5%	117.2^{a}	±5.6	151.1^{c}	±1.7
BW-15%	136.0^{b}	±6.5	335.9^{d}	±9.8
	CEC [Na mg eq./100g soil]		WHC [%]	
Cont. Start	2.15^{a}	±0.09	23.12	±0.03
4 months				
Control	2.18^{a}	±0.07	21.78^{a}	±1.24
BW-0.5%	2.36^{a,b}	±0.09	21.52^{a}	±0.30
BW-1%	2.65^{b,c}	±0.07	23.00^{a}	±0.99
BW-5%	2.91^{c}	±0.05	25.64^{b}	±0.88
BW-15%	4.38^{d}	±0.30	33.38^{c}	±0.69

15% treatment (15 and 27% increase, respectively), but at lower concentrations the total nitrogen content was not affected by biochar. The NH_3-N content exhibited decreasing tendency with increasing incremental biochar doses. Compared to the four-month control soil, biochar significantly decreased the NH_3-N content from 22.7 mg/kg to 10 mg/kg. According to the results, biochar treatment clearly increased the organic matter (OM) content with the increasing doses of the biochar. The highest OM content was 3.9w/w% at 15% biochar concentration; which was a 7 fold increase compared to the control soil.

The changes in plant available K_2O and P_2O_5 exhibited increasing tendency with biochar treatment, however not to the same extent. The plant available K_2O in the soils increased proportionally with the amount of the biochar used. The highest plant available K_2O (~336 mg/kg) was detected at 15% biochar treatment, which means that it contains 6.6 times more K_2O than the acidic sandy control soil. The second-best result (~151 mg/kg K_2O) was achieved at 5% biochar concentration. The P_2O_5 concentration was significantly higher compared to the control only at the highest biochar dose.

The cation exchange capacity (CEC) increased slightly at lower biochar concentrations. There was a significant increase in case of 1% treatment, but it was only by 0.47 units. 5% treatment resulted in 0.73-unit increase and the most significant result was the 2.20-unit increase (twice the control) at 15w/w% treatment.

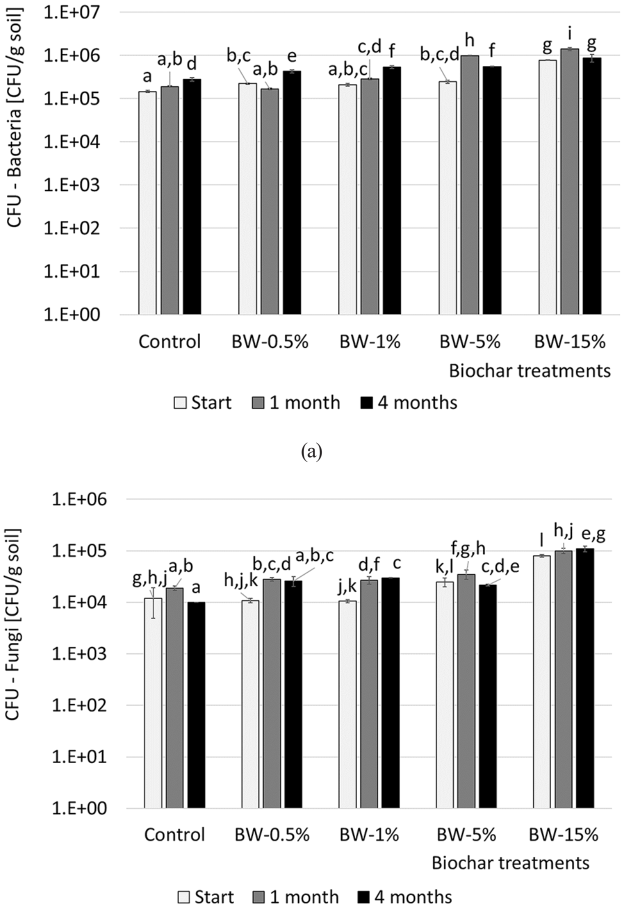
Further positive biochar-mediated effect is the increase of the water holding capacity upon biochar treatment. Our results show that the lower concentrations (0.5%, 1%) did not change the water holding capacity values compared to the control soil. 5% biochar treatment resulted in a ~18% increase, while 15% biochar treatment resulted in ~53% increase in water holding capacity, which was definitely a significant result.

3.1.2 Impact of biochar treatments on the soil biological parameters

The aerobic heterotrophic bacterial and fungal cell concentrations (Fig. 3) exhibited increase upon biochar treatment both on the short and mid-term (one and four months).

Fig. 3 (a) shows that the number of bacteria significantly increased in the soil from 1% biochar treatment.

The highest increase could be detected after one month at 5% and 15% biochar treatment where the increase was 5 and 7.4 times compared to the control sample. The results of the fungal CFU in Fig. 3 (b) show some similarities to the bacterial CFU, the highest significant difference was at 5 and 15w/w% biochar treatment compared to the untreated control, while the lower biochar concentrations resulted only a slight increase.



(a)

(b)

Fig. 3 Beech wood biochar induced changes in the microbiological activity (bacterial cell concentration (a) and fungal cell concentration (b)) in the microcosm study with acidic sandy soil. Letters on the columns indicate significant differences ($p < 0.05$)

Table 5 also presents the significant positive effect of beech wood biochar on soil microbiological activity. The biochar treatment, the time and the treatment connected to time had a significant effect on the concentration of bacteria. However, in the case of fungal numbers, only the time and treatment had a significant effect, the time connected with treatment had not.

Fig. 4 shows the beech wood biochar induced changes in the microbiological activity and diversity determined by Biolog Ecoplate™ technique.

The results demonstrated that the biochar treatment had positive significant effect on the microbial activity represented by AUC (Fig. 4 (a)) which means that the biochar facilitated the microbes to metabolise the Biolog Ecoplate

Table 5 RM ANOVA results over time to evaluate effects of beech wood biochar treatment on the microbiological activity. Bold p-values indicate significant differences at $p < 0.05$.

Effect	d.f	Mean square	F-ratio	p-value
Bacteria				
Treatment	4	2.29E+11	597.58	**0.0017**
TIME	2	6.86E+11	1721.99	**0.0000**
TIME × Treatment	8	1.15E+11	289.38	**0.0000**
Fungi				
Treatment	4	7.11E+09	1558.36	**0.0000**
TIME	2	4.79E+08	8.047	**0.0121**
TIME × Treatment	8	1.47E+08	2.482	0.1100
Shannon index				
Treatment	4	0.0440	27.4	**0.0001**
TIME	1	0.1290	95.1	**0.0000**
TIME × Treatment	4	0.0156	11.5	**0.0021**
AUC				
Treatment	4	0.60599	34.605	**0.0076**
TIME	1	0.49124	20.186	**0.0206**
TIME × Treatment	1	0.26079	10.716	**0.0402**

carbon sources. This mechanism appeared from the beginning of the experiment and the smallest significant change could be detected from 1% biochar treatment.

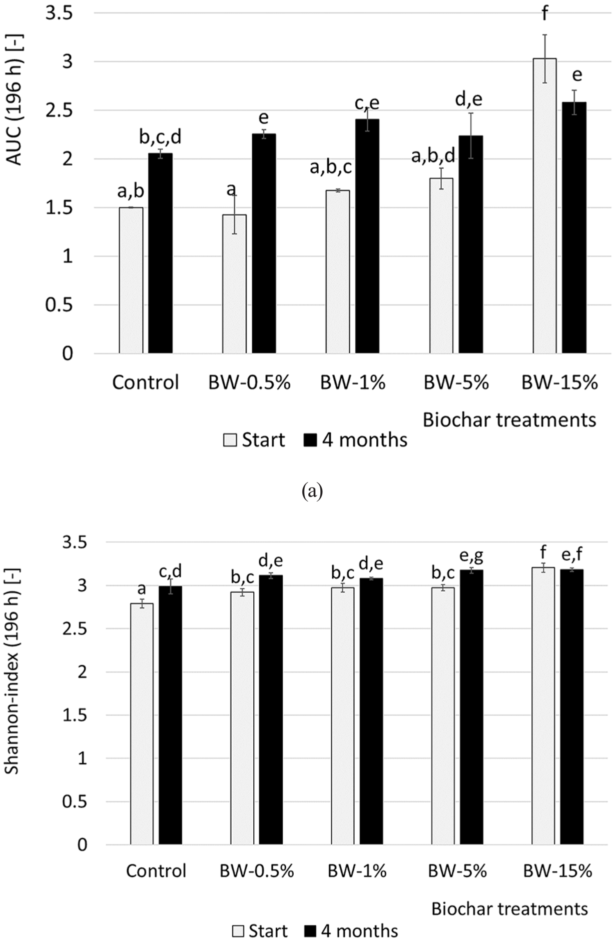
Addition of 15% biochar to the soil resulted an increase of AUC from 1.5 to 3.03, which was the highest increase. According to the results the AUC increased from the 1st to the 4th month except for the 15% BC treatment.

Fig. 4 (b) presents the physiological (functional) diversity of the bacterial communities after biochar treatment. The Shannon diversity significantly increased from 0.5% biochar treatment at the start point. The 0.5%, 1% and 5% biochar treatments show similarities in diversity, but the 15% treatment shows a higher increase compared to the lower concentrations both at the beginning and the end of the experiment.

Results of RM ANOVA analysis (Table 5) demonstrated that these changes were significant both in treatment and time, plus the effect of treatments differed at the different sampling times.

3.1.3 Impact of biochar treatments on the ecotoxic effects of soil

During our study we found that beech wood based biochar had no toxic effect on *Sinapis alba* root and shoot elongation or on the lethality of *Folsomia candida (*data not shown*)*. After one month the 1% and 15% biochar treatments induced increase in *S. alba* root elongation. After four months 0.5%, 5% and 15% biochar treatment significantly increased the *S. alba* root elongation, the 15% treatment resulted the highest increase, where the roots grew 2.14 times more. There were no significant differences in *S. alba* shoot growth neither after one nor four months. The *Triticum aestivum* was more sensitive to the biochar treatment and a slight inhibition was detected at 5% and 15% treatment after four months in both root and shoot growth.



(a)

(b)

Fig. 4 Beech wood biochar induced changes in the microbiological activity (a) and diversity (b) in the microcosm study with acidic sandy soil (Biolog Ecoplate™). Letters on the columns indicate significant differences ($p < 0.05$)

However, an increase was detected after one month. In the *Folsomia candida* mortality test the living number of Collembolas was higher in the biochar treated soil compared to the control acidic sandy soil.

3.2 Results of lab-scale experiments for modelling biochar aging

This research evaluated the effects of simulated conditions of artificial physical and biological aging on the properties of the beech wood biochar compared to its properties before aging. The biochars before and after accelerated aging were examined by an integrated methodology, including physicochemical, biological and ecotoxicological methods. Table 6 shows the physicochemical and biological results of the artificial aging experiments. Significant differences between biochars before and after aging determined by one-way ANOVA are indicated by different letters.

As demonstrated (Table 6), each treatment resulted in significant aging-mediated changes, especially regarding pH, EC, carbon content (LoI and POXC) and biological activity.

The results of the preliminary artificial aging experiments showed generally favourable effects, especially in the case of biological aging. Depending on the soil used, we experienced both an increase (loamy soil) and a decrease (acidic sandy soil) in the pH as a result of artificial aging, but the pH was still around the neutral range. Although the water-holding capacity decreased slightly, it was still significantly higher than that of the untreated acidic sandy soil. At the same time, we did not measure a decrease in the SSA as a result of aging.

Table 6 Effects of physical and biological aging on the characteristics of biochar. Letters indicate significant differences ($p < 0.05$).

	Characteristics of biochar			
	Before aging		After physical aging	
	Average	SD	Average	SD
pH [-]	7.26^{b}	0.21	6.95^{a}	0.05
EC [mS/cm]	109.9^{a}	6.6	95.8^{a}	3.1
SSA [m^2/g]	1.0533^{a}	0.0252	1.1125^{a}	0.1447
SUM V_{pore} [cm^3/g]	0.0019^{b}	0.0001	0.0021^{b}	0.0002
WHC [%]	52.08^{c}	2.68	41.47^{a}	0.91
LoI [%]	97.98^{c}	0.07	92.16^{b}	1.60
POXC [mg/kg]	39.60^{a}	7.38	138.56^{b}	9.99
CFU$_{Bacteria}$ [CFU/g soil]	3.77E+05^{a}	5.75E+04	4.92E+05^{a}	9.95E+03
CFU$_{Fungi}$ [CFU/g soil]	2.09E+05^{ab}	2.05E+04	1.36E+05^{a}	4.24E+03
	After biological aging			
	Acidic sandy soil		Loamy soil	
	Average	SD	Average	SD
pH [-]	7.05^{a}	0.13	7.81^{c}	0.13
EC [mS/cm]	243.25^{c}	18.1	156.4^{b}	14.4
SSA [m^2/g]	1.148^{a}	0.018	1.118^{a}	0.016
SUM V_{pore} [cm^3/g]	0.00153^{a}	0.0002	0.0032^{c}	0.0003
WHC [%]	47.62^{b}	1.56	38.45^{a}	1.21
LoI [%]	87.85^{a}	1.95	89.16^{a}	1.93
POXC [mg/kg]	239.42^{c}	17.98	898.80^{d}	52.51
CFU$_{Bacteria}$ [CFU/g soil]	1.86E+06^{b}	1.32E+04	2.21E+06^{c}	1.61E+05
CFU$_{Fungi}$ [CFU/g soil]	1.85E+05^{bc}	6.62E+04	2.53E+05^{c}	2.65E+04

The concentration of bacteria increased significantly due to biological aging in both soils. In the acidic sandy soil, the number of bacteria increased by 5 times compared to the original, while in the loamy soil it increased by approximately 6 times.

The labile available carbon content also increased significantly as a result of biological aging; 6-fold and 23-fold increase was experienced in the acidic sandy and loamy soil, respectively.

At the same time, the water-holding capacity showed a slight decrease (8% and 27%) due to biological aging in the acidic sandy soil and in the loamy soil, respectively. The BET surface area and the porosity increased slightly (not significantly) due to the simulated aging processes. Freeze-thaw cycling as a physical aging method changed not only the biochar structure but it caused significant changes in pH, loss on ignition, POXC. However, these changes were much smaller than the effects caused by biological aging. We observed a slight decrease (<20%) in the pH and EC, as well as in the water-holding capacity. Physical aging did not induce changes in the concentration of bacteria.

4 Discussion

The utilization of waste/by-products in the form of biochar, or of the by-product of an industrial technology resulting large biochar amounts, can offer solutions to many of today's environmental problems. A complex, tiered approach was applied for the assessment of the reliable and effective applicability of a wood-based biochar. After laboratory testing of biochar without soil by a complex methodology, mid-term microcosm experiments were performed aiming to assess the effects of beech wood biochar on acidic sandy soil.

Then artificial physical and biological aging pre-experiments were conducted in the laboratory to predict the long-term applicability of the tested biochar.

4.1 Impact of beech wood biochar treatments on the acidic sandy soil

Based on several soil studies, electrical conductivity has been demonstrated to be linked closely to other soil properties, such as nutrient supply, cation-exchange capacity, water holding capacity, soil mineralogy, soil structure etc. Biochar-mediated changes in EC (mainly increase in EC) were also reported by other studies [49–51]. This phenomenon can be explained with the high salt content (NO^{3-}, K^+, Ca^{2+}) of the biochars [51]. Similarly, to other studies, we also found that biochar increased the EC of the soil [49, 52, 53] in the microcosm incubation study. Especially when applying the two highest biochar doses (5 and 15%), the EC was large enough to significantly exceed the EC for the control after one month. The results can be explained by the higher levels of water-soluble soil nutrients, mainly from the biochar. This outcome is consistent with the soil nutrient supply and CEC results in our study. A threshold value can also be observed in these parameters at a biochar concentration of 5%, above which significantly higher values were obtained compared to the control.

According to previous studies in biochar literature [8, 54], biochars may have a raising effect on the pH of the soil (especially in acidic soils) due to their generally alkaline pH. The applied biochar had an initial pH of 7.29 which explained the proportional pH increase in the acidic sandy soil (pH 4.9).

Our results on the loss on ignition displayed similarities with the literature, which reported that biochars were able to increase the soils' organic matter content and consequently the loss on ignition values [55, 56]. Cen et al. [57] and other researchers also reported, that biochar increased the soil OM [57, 58]. Lehmann et al. [58] used pine and corncob biochar for vineyard soil amendment and in both biochar treatments, they found that the biochars increased the loss on ignition, but in contrast to our study their results showed no significant quantitative changes in time. At the same time, due to the carbon input from biochars, the increase in the organic matter was also clearly proven as demonstrated by the OM content (Table 4), which was significantly higher at the 1% and higher doses of biochar.

POXC represents the labile (and presumably available) carbon content of the soil.

Our study demonstrated a positive correlation between the biochar dose and POXC level in the soils. Demisie et al. [59] also found that biochar treatment (wheat straw, oak wood and bamboo biochar) highly increased the POXC rate of the soil. Furthermore, they found that microbial biomass positively correlated with labile carbon. In accordance with these studies, we also observed increasing concentration of living cells upon biochar addition, indicating that microbial activity may have caused mineralization of organic matter, resulting in a higher level of labile carbon.

Biochar may act as a slow-release fertilizer, and the nutrients are released into the soil at different rates. As expected, the beech wood biochar had different impact on NH_4-N and NH_3-N content in connection with the nitrogen cycling. The NH_4-N content of the soil significantly decreased after four months in all treatments including the control soil, which could be related to the higher nitrification activity intensifying the microbiological activity, as reported by Xu et al. [60] and Farkas et al. [10]. The available phosphorus and potassium content of the studied biochar (before aging) is relatively low similarly to other wood-based biochar products [22]. Nevertheless, the K_2O and P_2O_5 content of the treated soil significantly increased, which might be attributed to the direct release from the biochar.

The positive biochar-mediated influences on soil nutrient supply revealed that the applied biochar might be a source of organic matter, phosphorous and potassium for the low quality acidic sandy soils.

As reported by Lehmann et al. [58] during biochar application for soil improvement its large surface area may result higher CEC [58]. Even though the tested beech wood biochar had low SSA, the cation exchange capacity increased slightly at low biochar concentrations and it had doubled at 15% treatment. The increase in CEC could also be linked to the process of losing acidic surface functional groups; changing the surface charge of colloids, the pH of the soil could affect CEC. Jones et al. [61] reported that the enhancement in CEC affected the availability of nutrients and reduced their leaching.

However, some studies reported inconsistent results about the effect of biochar on WHC. For example, according to Liang et al. [56] biochar addition to calcareous soil had no significant effects on the WHC.

Similarly, to our findings, Wang et al. [62] concluded in a study that walnut shell (SSA: 57.5 m^2/g) and soft wood (SSA: 2.0 m^2/g) biochar addition to coarse-textured soils improved the water retention capacity on the short-term.

The biochar-mediated changes in the soil microbial activity have been widely studied [63–65].

Similarly, to our results, the biochar treatment increased generally the substrate utilization and the metabolic activity of microbes [64, 65]. But Xu et al. [66] found that wood-based biochar did not alter microbial functional diversity represented by the Shannon diversity index. Usually, biochar treatment increased the CFU in a similar way to our study [67] however, some studies detected a decrease or no significant difference compared to the control [68, 69].

The potential ecotoxicity of biochar was tested from the perspective of environmental safety using plant growth tests and animal test organisms.

There are conflicting results in the literature about the effect of biochar on plant growth [20, 70], however, in general, in our experiment significant plant growth inhibition or Collembola mortality has not been detected.

Based on our mid-term laboratory result, it could be stated that the applied beech wood biochar doses proved to be effective for improving sandy soil's pH, WHC and nutrient supply and their microbiological activity, even at the highest tested concentration (15w/w%). Besides, from an ecotoxicological perspective, the chosen treatments did not have any toxic effect.

4.2 Effect of artificial accelerated aging on the properties of beech wood biochar

Upon field application of biochars one must take into account several environmental factors such as temperature variation, solar radiation, precipitation and microbial activities which will affect in time the physical and chemical properties of biochars persistent in the soil determining their long-term effects and functionality in soil. This is why laboratory simulation methods that model different weathering processes are of particular importance. These techniques may be able to accelerate aging of biochar, shortening the time from years to weeks or days. However, only several researches have measured which artificial aging is able to model biochar aging under natural conditions [21].

In our preliminary aging study, the beech wood biochar underwent different changes through the physical and biological aging processes, affecting both their physicochemical and biological characteristics.

Significant, but slight decrease was exhibited in the pH, water holding capacity (WHC) and loss on Ignition (LoI) upon physical and biological aging in acidic sandy soil. At the same time, neither the decrease in pH nor WHC questions the applicability of biochar, as these were only small decreases. Biochar is a porous material with a high volume of micro- and mezopores; this structure may be formed and damaged during the aging processes affecting the WHC. In accordance with our results, Rechberger et al. [71] and Hale et al. [72] reported that the pH of wood-based biochars decreased with aging due to the formation of carboxylic and phenolic functional groups.

Accelerated biological aging resulted in higher microbial activity of biochars aged in both soils, which was accompanied by further changes, an increase in EC, LoI and POXC. Physical aging was not associated with an increase in the number of microbes, nevertheless, it did not result in a significant decrease of bacteria and fungi concentration. Current preliminary findings suggest that the biochar weathering processes did not involve changes that would have had a toxic effect on the microflora. However, the reliability of these methods still needs to be verified by field-scale aging, since the quantitative changes differ according to the aging methods and soil types, therefore biochar application requires a *char by char* and *soil by soil* investigation prior to field application.

5 Conclusion

A complex, tiered approach was applied for the assessment of the reliable and effective applicability of a beech wood-based biochar with very low specific surface area. The benefits of the beech wood biochar in the acidic sandy soil were confirmed even in the highest application dose (15w/w%) in the laboratory microcosm study. The applied biochar proved to be effective for improving the pH, WHC, nutrient supply and microbiological activity of the sandy soil. Besides, from an ecotoxicological perspective, the chosen treatments did not have any toxic effect. In spite of its low BET surface area (~1 m^2/g), the tested beech-wood biochar could be applied efficiently and reliably and without any risk in the amendment of degraded sandy soils. While natural aging takes several years or decades, it is important to find an artificial aging method to model the aging-induced changes in biochars and their long-term impact on soil. To efficiently use biochar in environmentally sustainable agriculture, aiming to enhance not only soil quality but also to prevent soil organic matter loss is needed. Besides, the complex effects of the aging processes in biochars aged alone and in soil–biochar mixtures must be understood. The results of our artificial aging experiments demonstrated that in order to predict and understand the effect of the applied biochar to a certain soil type, the effects have to be studied on a *char by char* and *soil by soil basis*.

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Author contribution

MM and KL conceptualization; MM conceived and designed research. SZM and MM planned the experiments. SZM and DSZ conducted experiments. MM, SZM and SZD analysed data, contributed to the interpretation of the results. MM, SZM, EV wrote the manuscript. EV language editing. All authors read and approved the manuscript.

Data availability

Experimental data are available within this research article. The raw datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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