THE EFFECTS OF BIOCHAR SURFACE CHEMISTRY AND PHYSICAL PROPERTIES ON SOIL BIOTA IN DIFFERENT BIOCHAR-SOIL SYSTEMS

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1. Introduction

Soil degradation caused by acidification, salinity, compaction, loss of water holding capacity, and decreased cation exchange capacity as a result of soil organic matter depletion requires global efforts in soil restoration and protection. Biochar, the solid product of biomass pyrolysis applied as soil amendment may improve the physical, chemical and biological properties of the soil^{1,2}, enhance nutrient availability to plants³ and improve soil fertility on the long term, mainly by indirect effects, such as increase of cation exchange capacity, surface area and water retention in soil pores, which decreases nutrient leaching^{4,5}. The effectiveness of biochar amendment is highly dependent on the physico-chemical properties of the biochar on the soil type, the biochar ageing and the climatic conditions^{6,7,8,9}.

2. Background and main objectives

Biochar products have presented positive effects on soil properties and crop yields in most cases, but it is uncertain whether the same positive effects can be obtained in all soil types. The mechanism of the direct biochar-mediated biological and ecological effects is poorly understood, particularly those on soil biota. There are only few studies describing the possible connections between biochar properties and the soil biota, and their implications for soil processes in different soil-biochar systems and the influence of biochar aging^{10,11}.

The main objective of our work was to get a better understanding of biochar-mediated effects on soil physico-chemical and biological properties as well as to assess and analyse the direct and indirect interactions between biochar surface chemistry, physical properties and soil biota. There are very few studies which provide quantitative data and descriptions of fundamentals concerning biochar effect on soil biota or on changes in soil physico-chemical properties. Moreover, these studies with conflicting results determined the biocharmediated physico-chemical changes and biological effects separately not correlated with each other, and heterogeneity was found with regard to the soil type and its characteristics used in different research.

¹ Glaser, B., Lehmann, J., Zech, W. 2002. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal—a review, Biology and Fertility of Soils 35(4):219–230.

² Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday WC, Crowley D. 2011. Biochar effects on soil biota—a review. Soil Biol Biochem. 43:1812–1836.

³ Major, J., Rondon, M., Molina, D., Riha, S.J., Lehmann, J., 2012. Nutrient Leaching in a Colombian Savanna Oxisol Amended with Biochar. J. Environ. Qual. 41, 1076.

⁴ Fischer, B.M.C., Manzoni, S., Morillas, L., Garcia, M., Johnson, M.S., Lyon, S.W., 2019. Improving agricultural water use efficiency with biochar – A synthesis of biochar effects on water storage and fluxes across scales. Sci. Total Environ. 657, 853–862.

⁵ Lehmann, J., Gaunt, J., Rondon, M., 2006. Bio-char sequestration in terrestrial ecosystems - A review. Mitig. Adapt. Strateg. Glob. Chang. 11.

⁶ de la Rosa, J.M., Rosado, M., Paneque, M., Miller, A.Z., Knicker, H., 2018. Effects of aging under field conditions on biochar structure and composition: Implications for biochar stability in soils. Sci. Total Environ. 613–614, 969–976

⁷ van Zwieten, L., Kimber, S., Morris, S., Chan, K.Y., Downie, A., Rust, J., Joseph, S., Cowie, A., 2010a. Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. Plant Soil 327, 235–246.

⁸ Jeffery, S., Verheijen, F.G.A., van der Velde, M., Bastos, A.C., 2011. A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. Agric. Ecosyst. Environ. 144, 175–187.

⁹ Zhao, R., Coles, N., Kong, Z., Wu, J., 2015. Effects of aged and fresh biochars on soil acidity under different incubation conditions. Soil Tillage Res. 146, 133–138.

¹⁰ Domene, X., Enders, A., Hanley, K., Lehmann, J., 2015. Ecotoxicological characterization of biochars: Role of feedstock and pyrolysis temperature. Sci. Total Environ. 512–513, 552–561.

¹¹ Sohi, S.P., Krull, E., Lopez-Capel, E., Bol, R., 2010. A Review of Biochar and Its Use and Function in Soil. Adv. Agron. 105, 47–82.

3. Results

In this project, according to our aims, we studied applying a tiered approach how the surface chemistry and physical properties of biochar affect soil biota in numerous biochar-soil systems. Our research work started with laboratory screening of various biochar products for selection of the most effective biochar types and establishment a scoring-ranking system for biochars, followed by a short term and a mid-term microcosm experiment with selected biochar products aiming to assess the influences of biochar surface chemistry and physical properties on soil biota in different biochar-soil systems.

3.1. Screening-Scoring-Ranking methodology for biochars

Based on the results of the complex methodology we developed a screening and ranking approach applied to 14 selected biochars (*Table 1* and *Table 2*) aimed at soil improvement to assess the possible applicability of biochars to soil and to select the main influencing properties of the tested biochars as soil additive. The focus was on improving soil functions as habitat for soil biota.

Table 1 Properties of the selected biochars

Biochar code	Biomass	Pyrolysis temperature (°C)	Pyrolysis residence time (min)
BC1-PFS	Grain husk and paper fiber sludge	500 °C	20
BC2-PFSA	Grain husk and paper fiber sludge, N-enriched biochar, post treated with stone powder and compost	500 °C	20
BC3-BCM	Grain husk and paper fiber sludge, post treated with digastate, minerals	450 °C	20
BC4-BCMO	Grain husk and paper fiber sludge, post treated with digastate, minerals and organic liquid	450°C	20
BC5-W	Woodscreenings	600-700 °C	20
BC6-V	Vine	600-700 °C	15
BC7-BC	Black cherry	600-700 °C	15
BC8-S	Straw	600-700 °C	15
BC9-MP	Meadow plants	600-700 °C	15
BC10-NB	Natural biomass	700 °C	15
BC11-WSD	Wood sawdust	600-700 °C	20
BC12-SP	Spelts mixed with paper (2:1)	600-700 °C	15
BC13-CM	Cow manure	650-750 °C	n.a.
BC14-M	Miscanthus	600-700 °C	15

For ranking of the biochar products and to select the best biochar(s) for soil improvement a complex multicriteria evaluation system was developed (*Table 2*) based on scores (from -5 as the least appropriate to 5 as the most appropriate).

Table 2: Main characteristics of tested biochar products

			Charact	eristics o	f biochars	- Technolo	gical effic	Characteristics of biochars - Environmental efficiency							
Biochar	рН	WHC [%]	тос [%]	Sum-N [%]	AL P	AL K [mg/kg]	Ash content	Sum pore volume	BET [m²/g]	Toxic element– Co. Cu. Cr. Ni. Zn	Viability index [CFU*10 ⁻ ³/g]	Plant growth 1.		Plant growth 2. Wheat	<i>Fc</i> lethality
		į <u> </u>		,	[6/8]	[6,6]	[%]	[%] [cm³/g]		[mg/kg]	Bacteria ± Fungi	Root	Shoot	root [%]	[%]
BC1-PFS	8.75± 0.04	169.10 ±6.13	63.20± 9.48	1.49± 0.22	5713± 857	8889± 1333	40.17± 3.09	0.145± 0.022	175.0± 26.3	116.2±11.2; 52.0±5.0; <lod; 20.8±1.4<="" <lod;="" td=""><td>1409</td><td>39.89± 0.06</td><td>-27.29± 2.56</td><td>58.39± 2.01</td><td>10.0±0.9</td></lod;>	1409	39.89± 0.06	-27.29± 2.56	58.39± 2.01	10.0±0.9
BC2-PFSA	6.78± 0.0	105.33 ±0.73	20.85± 3.13	1.37± 0.206	16871± 2531	20894± 3134	68.50± 1.56	0.021± 0.003	4.6± 0.7	55.5±6.1; 20.7±2.2; <lod; <lod;="" <lod;<="" td=""><td>7094</td><td>38.69± 2.73</td><td>-29.78± 1.47</td><td>42.97± 0.80</td><td>0.0±0.0</td></lod;>	7094	38.69± 2.73	-29.78± 1.47	42.97± 0.80	0.0±0.0
BC3-BCM	8.28± 0.01	115.82 ±0.79	21.44± 3.22	0.69± 0.10	5913± 887	9260± 1389	63.32± 1.09	0.044± 0.007	57.0± 8.6	855.3±52.3; 140.4±0.5; 77.8±18.0; 199.2±39.2; 43.9±13.1	19914	9.81± 0.28	22.19± 1.22	83.55± 9.60	25.0±0.0
вс4-всмо	8.03± 0.00	113.67 ±0.51	22.63± 3.39	0.83± 0.12	9343± 1401	9768± 1465	60.07± 3.23	0.029± 0.004	19.0± 2.9	863.4±77.1; 132.7±9.1; <lod; <lod; 23.6±4.3<="" td=""><td>12132</td><td>87.27± 6.43</td><td>60.10± 7.54</td><td>91.94± 18.05</td><td>30.0±0.</td></lod;></lod; 	12132	87.27± 6.43	60.10± 7.54	91.94± 18.05	30.0±0.
BC5-W	9.29± 0.03	150.88 ±2.22	74.1± 11.12	1.15± 0.17	1610± 242	16871± 2531	19.60± 1.41	0.053± 0.008	71.0± 10.7	334.0±9.5; 41.1±3.5; <lod; <lod;="" <lod<="" td=""><td>1048</td><td>84.17± 6.63</td><td>61.92± 4.15</td><td>67.74± 11.34</td><td>22.5±0.0</td></lod;>	1048	84.17± 6.63	61.92± 4.15	67.74± 11.34	22.5±0.0
BC6-V	9.76± 0.07	178.77 ±2.06	52.32± 7.85	0.35± 0.05	4757± 714	16262± 2439	16.05± 1.69	0.148± 0.022	257.0± 38.6	219.7±27.6; 111.8±3.0; <lod; <lod; 29.1±1.4<="" td=""><td>12</td><td>94.25± 24.20</td><td>97.70± 41.16</td><td>88.22± 19.27</td><td>35.0±5.8</td></lod;></lod; 	12	94.25± 24.20	97.70± 41.16	88.22± 19.27	35.0±5.8
вс7-вс	8.54± 0.01	168.54 ±3.38	38.64± 5.80	0.21± 0.03	403± 60	2887± 433	4.61± 1.48	0.099± 0.015	183.6± 27.5	46.2±0.8; 16.4±2.3; <lod; <lod;="" <lod<="" td=""><td>4937</td><td>20.67± 1.58</td><td>49.65± 7.11</td><td>78.75± 10.06</td><td>17.5±2.6</td></lod;>	4937	20.67± 1.58	49.65± 7.11	78.75± 10.06	17.5±2.6
BC8-S	10.01 ±0.03	312.11 ±1.36	30.31± 4.55	0.27± 0.04	1837± 276	35570± 5335	18.36± 0.75	0.011± 0.002	9.9± 1.5	274.3±37.2; 23.2±1.7; <lod; <lod;<br="">33.3±13.6</lod;>	21	93.14± 15.41	95.71± 10.41	100.00± 0.00	52.5±7.9
вс9-мр	9.03± 0.03	196.86 ±1.22	41.11± 6.11	0.14± 0.02	703± 105	1622± 243	6.80± 0.44	0.145± 0.022	260.0± 39.0	48.6±9.1; 42.7±3.8; <lod; <lod;<br="">70.2±18.9</lod;>	625	57.04± 8.79	19.14± 2.57	75.85± 5.18	37.5±4.8
BC10-NB	9.85± 0.01	135.16 ±2.31	26.96± 4.04	0.24± 0.04	4300± 645	14518± 2178	14.30± 0.12	0.038± 0.006	35.8± 5.4	30.3±4.6; <lod; <lod;="" <lod<="" td=""><td>1371</td><td>100.00± 0.00</td><td>100.00± 0.00</td><td>77.54± 12.70</td><td>50.0±7.5</td></lod;>	1371	100.00± 0.00	100.00± 0.00	77.54± 12.70	50.0±7.5
BC11-WSD	8.42± 0.03	153.44 ±4.11	32.5± 4.88	0.39± 0.06	373± 56	3084± 463	17.82± 0.83	0.090± 0.014	185.0± 27.8	343.8±12.6; 23.1±3.5; <lod; <lod;="" <lod;<="" td=""><td>493</td><td>39.83± 0.97</td><td>50.94± 9.74</td><td>60.32± 5.03</td><td>55.0±8.6</td></lod;>	493	39.83± 0.97	50.94± 9.74	60.32± 5.03	55.0±8.6
BC12-SP	8.98± 0.02	107.28 ±11	38.0± 5.70	0.84± 0.13	3664± 550	10403± 1561	30.64± 0.33	0.036± 0.005	22.0± 3.3	224.5±7.5;72.1±1.7; <lod; <lod;="" <lod<="" td=""><td>3040</td><td>61.72± 5.37</td><td>23.75± 1.12</td><td>72.90± 11.77</td><td>67.5±10. 6</td></lod;>	3040	61.72± 5.37	23.75± 1.12	72.90± 11.77	67.5±10. 6
BC13-CM	8.54± 0.07	265.11 ±0.57	26.2± 3.93	0.24± 0.04	4527± 679	16667± 2500	25.59± 0.40	0.015± 0.002	8.7± 1.3	893.6±55.3; 250.8±14.8; <lod; <lod; <lod<="" td=""><td>1443</td><td>97.50± 21.21</td><td>97.86± 40.99</td><td>66.42± 2.28</td><td>15.0±1.2</td></lod;></lod; 	1443	97.50± 21.21	97.86± 40.99	66.42± 2.28	15.0±1.2
BC14-M	9.2±0 .06	267.55 ±9.20	80.0± 12.00	0.60± 0.09	1100± 165	7500± 1125	12.26± 1.81	0.234± 0.035	440.0± 66.0	160.6±17.8; 48.6±0.2; <lod; 46.7±7.3<="" <lod;="" td=""><td>833</td><td>100.00± 0.00</td><td>100.00± 0.00</td><td>91.94± 16.38</td><td>40.0±7.3</td></lod;>	833	100.00± 0.00	100.00± 0.00	91.94± 16.38	40.0±7.3

Table 3: The complex multi-criteria evaluation system for screening and scoring of biochars

	Characteristics of biochars - Technological efficiency											Characteristics of biochars - Environmental efficiency										
	WHC TOC Sum-N pH1 pH2 [%] [%] [%]			m-N ALP ALK co		Ash content	content volume	BET [m²/g]	Toxic element	Toxic element Cu	Toxic element Cr	Toxic element Ni	Toxic element– Zn	Viability index [CFU*10 ⁻³ /g]	U	rowth 1. stard	Plant growth 2. Wheat root [%]	Fc lethality				
															[mg/kg]	Bacteria + Fungi	Root	Shoot				
BC1-PFS	5	-3	3	1	1	1	1	3	3	5	5	5	5	5	5	1	0	5	-3	3		
BC2-PFSA	0	0	3	-3	1	5	5	3	-3	-5	5	5	5	5	5	5	0	5	-1	5		
BC3-BCM	5	-3	3	-3	0	1	1	3	-1	0	-5	5	5	-3	-5	5	3	1	-5	0		
BC4-BCMO	5	0	3	-3	0	1	1	3	-3	-3	5	5	5	5	-5	5	-5	-3	-5	0		
BC5-W	5	-5	3	3	1	-3	5	0	-1	0	5	5	5	5	5	0	-5	-3	-3	0		
BC6-V	5	-5	3	0	0	0	5	0	3	5	5	5	5	5	5	-3	-5	-5	-5	-1		
вс7-вс	5	-3	3	-1	-1	-5	-1	-3	0	5	5	5	5	5	5	5	1	-1	-3	3		
BC8-S	5	-5	5	-3	0	-3	5	0	-3	-5	5	5	5	5	5	-3	-5	-5	-5	-3		
BC9-MP	5	-3	3	-1	-1	-5	-3	-1	3	5	5	5	5	5	5	-1	-3	3	-3	-1		
BC10-NB	5	-5	3	-3	-1	0	3	-1	-3	-1	5	5	5	5	5	1	-5	-5	-3	-1		
BC11-WSD	5	-3	3	-1	0	-5	0	0	0	5	5	5	5	5	5	-1	0	-3	-3	-3		
BC12-SP	5	-3	3	-1	0	0	3	1	-3	-3	5	5	5	5	5	5	-3	1	-3	-3		
BC13-CM	5	-3	5	-3	-1	0	5	1	-3	-5	5	-3	5	5	-5	1	-5	-5	-3	3		
BC14-M	5	-5	5	3	0	-3	1	-1	5	5	5	5	5	5	5	0	-5	-5	-5	-1		

This system takes into consideration the ideal properties and effects of a soil amendment (eg. biochar) for low quality degraded sandy soils, such as good water holding capacity, high specific surface area, alkaline pH, low ash content, support for microbes, low toxic metal content and no toxic effect. The greatest potential benefits and risks of biochars were evaluated. Biochar Quality Indices (BQI) were created by choosing appropriate indicators, indicator scores were determined; the indicator scores were weighted, combined and integrated into the Biochar Quality Index.

The developed screening-scoring-ranking methodology provided a decision support tool for selection of biochar products in terms of their suitability for risk-based soil amelioration/amendment. From the point of view of further reliable and efficient application for the improvement of degraded low quality acidic and neural soils without risk the best biochars were in this phase of the research: biochar (BC) from grain husks and paper fibre sludge, post-treated grain husks and paper fibre sludge, wood screenings, miscanthus and vine (Figure 1).

Degree of correlation factor between investigated parameters of biochars and final score was also determined. In terms of final score, it has been confirmed that BET surface area, the sum pore volume and the TOC content represent the most critical properties of biochars. In addition to the BET surface area, sum pore volume and TOC, it has been found that the pH, water holding capacity and toxicity tests are of outstanding importance in the characterization and classification of products.

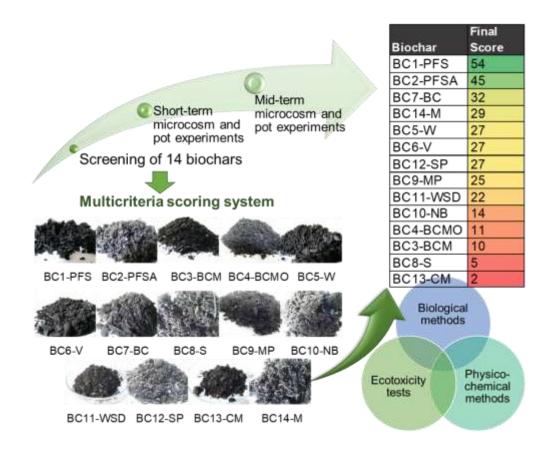


Figure 1. Main concept of Screening-Scoring-Ranking of biochars

Most of the biochars have good water holding capacity as they are able to hold more than 100% water compared to their dry mass. The results of specific surface area (BET) varied from 4.6 m²/g to 440 m²/g.

The number of heterotrophic aerobic colony forming bacteria and fungi presented that biochars may have been colonized by microorganisms during storage. This results indicated that biochars may ensure a habitat for a variety of microorganisms in soil too. The toxicity tests demonstrated that most biochars represent a favourable environment for plants and soil living animals, however, four biochars have strongly inhibited (more than 75% inhibition) plant growth both in their native and extract forms. These results are not correlated with either the specific surface area (BET) results or the toxic element content data. It proves the importance of ecotoxicity testing with different organisms and test systems.

The first results of the development of this *Screening-Scoring-Ranking system* were presented at the ICEEM Conference in Bologna in the framework of an oral presentation in 2017¹².

The establishment of this methodology based on multicriteria analysis had been completed, the manuscript is prepared for submission in the Agronomy (open access journal)¹³.

¹² Molnár, M., Feigl, V., Vaszita, E., Gruiz, K., Farkas, É. (2017) Screening and ranking methodology applied to biochars aimed at soil improvement. In: Buburuzan A.M., Teodosiu, C., Fava, F., Corvini Ph. X., Gavrilescu D. (Ed.), 9th International Conference on Environmental Engineering and management (ICEEM09): Circular Economy and Environmental Sustainability: Conference Abstracts Book, pp. 249-250.

¹³ Gruiz, K., Molnár, M., Feigl, V., Vaszita, E., Farkas, É. (2020) Complex screening-scoring methodology for ranking of the biochars aiming at soil improvement. Agronomy, Prepared for submission

3.2. Interactions between biochar physico-chemical properties and biochar-mediated effects on soil biota – microcosm experiments

Based on the screening-scoring methodology biochar products were selected and tested in different biochar-soil systems in short- and mid(long)-term laboratory microcosm experiments.

According to the results of the short-term long microcosm experiments with selected biochars (paper fibre sludge-grain husk, post-treated paper fibre sludge-grain husk, woodscreening, mischantus and sawdust) advantageous effects on the monitored properties; increase in the soil organic carbon content, water holding capacity, pH, EC and aerobic heterotrophic fungi concentration was observed. To assess and evaluate the short-term effects of the biochar treatments, we summarized the significant positive effects on the examined soil characteristics. It was found that the biochar from paper fibre sludge-grain husk had the most promising short-term effects on acidic sandy soil.¹⁴

Short-term ageing experiment series were also carried out to evaluate the effects of simulated conditions of artificial biological aging on the physicochemical, biological, ecotoxicological properties of five biochar types compared to their properties before aging¹⁵. The aim was to support efficient long-term utilization of biochar in soil focusing also on the potential environmental risks posed to soil biota. Five biochar (BC) types were "aged" by biological ageing method. The biochar types were the following: grain husk and paper fibre sludge BC (A1), wood screenings BC (B1), woodchips BC (F1), herbal pomace BC (H1), miscanthus BC (M2). Each biochar underwent changes through the aging processes, especially regarding nutrients and biological activity. The accelerated biological aging resulted in higher microbial activity in case of all biochars depending on their surface areas. The highest increase (more than two-fold) was found for M2 (mischantus) and F1 (woodchips) biochars with the largest specific surface area (BET). BET values of biochars correlated with the aerobic heterotrophic cell counts (CFU) of weathered biochars. The total N-content of each biochar decreased - except A1 with the highest initial NO3-N content - while there was an increase in the available nitrate due to the increased bacterial activity. Most of biochars exhibited significant decrease (~40-60%) in the pH, organic matter and POX (labile) carbon content (POXC) as well as in available potassium and phosphorous upon aging. These changes were accompanied by decreases in biochar CEC. We assume that enhanced bacterial activity triggers microbial OM production and sorption of OM, resulting in a range of new organic functional groups affecting CEC.

Microcosm studies on the mid-term effects (12 months) of 3 biochar types, applied individually and combined with compost and fertilizer at various rates were also carried out, to study the effects on the physico-chemical, biological properties and ecotoxicity of an acidic sandy soil in Hungary.¹⁶

¹⁴ Molnár, M., Kordisz, V., Vaszita, E., Uzinger, N., Rékási, M., Kutasi, J., Feigl, V., Tolner, M., Gruiz, K., Farkas, É. (2017) The effect of biochar combined with PGPR and compost on an acidic sandy soil – a pot experiment. In: Buburuzan A.M., Teodosiu, C., Fava, F., Corvini Ph. X., Gavrilescu D. (Ed.), 9th International Conference on Environmental Engineering and management (ICEEM09): Circular Economy and Environmental Sustainability: Abstracts Book, (2017) pp. 199-200.

¹⁵ Molnár, M., Kőszegi, M., Vaszita, E., Gruiz, K., Farkas, É. (2019) Influence of artificial biological aging on physicochemical, biological and ecotoxicological properties of five biochars - a laboratory incubation study, ECCE12, The 12th European Congress of Chemical Engineering, ECAB5, The 5th European Congress of Applied Biotechnology, Florence 15-19 September 2019, Book of Abstracts, pp. 1832-1833, ISBN: 978-88-95608-75-4, DOI: 10.3303/BOA1901

¹⁶ Farkas, É., Feigl, V., Gruiz, K., Vaszita, E., Ujaczki, É., Fekete-Kertész, I., Tolner, M., Horváth, C.M., Berkl, Z., Uzinger, N., Rékási, M., Molnár, M. (2018) Microcosm incubation study for monitoring the mid-term effects of different biochars on acidic sandy soil applying a multiparameter approach. *Process Safety and Environmental Protection*, 120, 24–36. ISSN 0957-5820, https://doi.org/10.1016/j.psep.2018.08.027.

In the mid-term long microcosm experiments (12 month) with biochars from different feedstocks (grain husks and paper fibre sludge (A1), post-treated grain husks and paper fibre sludge (A2), woodscreenings (B1)) the applied multiparameter approach made possible the selection of the most optimal treatment. All the three biochars had favourable influence on the soil, but the effects were different in terms of extent and time. Although the biochar from woodscreenings had not the most promising short-term effects, but it was highly favourable treatment after 12 months.

The results of the combined assessment applying a multiparameter approach demonstrated that the application of 0.5% biochar from woodscreening combined with fertilizer (0.5% B1+NPK) was the most favourable treatment. Both the induced physico-chemical and the biological changes confirmed the added value and the positive influence of this treatment on acidic sandy soil parameters. The biochar from grain husks and paper fibre sludge (A1) had also beneficial effects on the physico-chemical characteristics of the tested acidic sandy soil and improved the soil functions for plants. The effects of this biochar were manifested to the greatest extent at the applied 1% concentration. The significance of this work is the multiparameter approach and the comparative evaluation of the mid-term (12-month) effects of individual and combined biochar treatments with compost and fertilizer on an acidic sandy soil in microcosms with focus not only on the physico-chemical properties, but also on the soil microbes and the ecotoxicological effects. This study contributes to the required level of scientific understanding of biochars in sandy soils, which is a particularly appealing target for sustainable soil improvement, adding conclusions to the weaker represented areas of biochar research in non-field conditions.

In selected microcosm experiments as part of the ecotoxicological methodology, soil preference behavioural test was conducted using a modified *Enchytraeus albidus* soil preference tests (Figure 2). This method was developed for the testing the effects of biochar products on soil habitat function. In the *Enchytraeus albidus* (potworm) soil preference test applied in the microcosms besides the common methodology, we offered not only control and treated soil pairs to the animals, but we also paired all the biochar concentrations with each other.

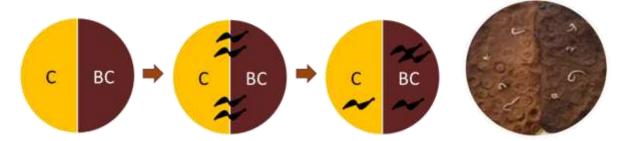


Figure 2. Main concept of the soil preference test applying potworm for testing biochar habitat function

The soil preference test in all cases showed that the tested biochars had no toxic effect on the test organisms and biochars applied at 2 w/w% rate can supply a more liveable habitat for soil living animals than the acidic sandy soil without treatment. *E. albidus* chose the higher biochar (A1 and F1) doses in all cases and clearly preferred the biochar produced from paper fibre sludge and grain husk (A1) (Figure 3 and Figure 4).

The soil preference response in the applied behavioural ecotoxicity test enabled the assessment of both the negative and positive effects of this soil amendment providing a more detailed picture on potworm behaviour in contact with biochar-amended soils.

This developed method was also applied in field studies to study the biochar effect on soil habitat function.¹⁷

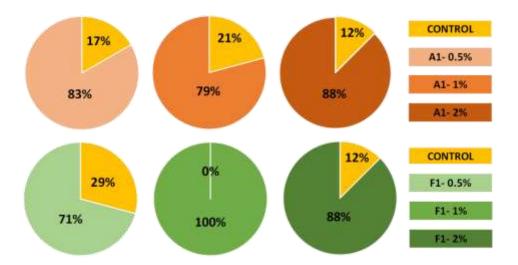


Figure 3. Soil preference - *Enchytraeus albidus*. Percentage selection activity between control and biochar treated soils

The first results of the development of this *soil preference tests* were presented at the *XIV. Kárpát-medencei Környezettudományi* Conference and ISTA2019^{18,19}.

The comprehensive evaluation of the results had been completed, the manuscript is prepared for submission in the *Environmental Science and Pollution Research ESPR-D-20-01242* as an invited paper.

¹⁷ Farkas, É., Feigl, V., Gruiz, K., Vaszita, E., Ujaczki, É., Fekete-Kertész, I., Tolner, M., Kerekes, I., Kari, A., Uzinger, N., Rékási, M., Kirchkeszner, Cs., Molnár, M. (2020) Long-term effects of biochar on acidic and calcareous sandy soils - success story of a scale-up field experiment. *Science of the Total Environment* (STOTEN-D-20-01768) submitted and reviewed (major revision)

¹⁸ Farkas, É., Kerekes, I., Tolner, M., Szabó, Á., Vaszita, E., Molnár, M. (2019) Biochar mediated short-term effects on acidic sandy soil and influence on soil living animal Enchytraeus albidus – preference behavioural test as a screening tool to assess soil habitat function. In: Abstract books of 19th International Symposium on Toxicity Assessment August 25-30, 2019, Thessaloniki, Greece, ISTA19 Conference Abstracts, ISTA 19, p. 39. http://ista19.civil.auth.gr/wp-content/uploads/2019/08/Abstracts ISTA-2019.pdf

¹⁹ Kerekes, I.K., Molnár, M., Feigl, I. (2018) Közönséges televényféreg elkerülési tesztek alkalmazásának talajjavító adalékok és szennyezőanyagok minősítésére. In: Füleky, György (szerk.) XIV. Kárpát-medencei Környezettudományi Konferencia. Gödöllő, Magyarország: MAG Mezőgazdaságért Alapítvány, (2018) 143–147.

4. Supporting material for the final report of the project OTKA K_16 120464

Supporting materials (publications, posters and thesis) for the final report of the project OTKA K_16 120464 are available at http://envirotox.hu/en/k 16-120464-research-project/.

4.1. Publications, oral and poster presentations related to our research project and with NKFIH acknowledgement

Molnár, M., Kőszegi, M., Vaszita, E., Gruiz, K., Farkas, É. (2019) Influence of artificial biological aging on physicochemical, biological and ecotoxicological properties of five biochars - a laboratory incubation study, ECCE12, The 12th European Congress of Chemical Engineering, ECAB5, The 5th European Congress of Applied Biotechnology, Florence 15-19 September 2019, Book of Abstracts, pp. 1832-1833, ISBN: 978-88-95608-75-4, DOI: 10.3303/BOA1901

Farkas, É., Kerekes, I., Tolner, M., Szabó, Á., Vaszita, E., Molnár, M. (2019) Biochar mediated short-term effects on acidic sandy soil and influence on soil living animal Enchytraeus albidus – preference behavioural test as a screening tool to assess soil habitat function. In: Abstract books of 19th International Symposium on Toxicity Assessment August 25-30, 2019, Thessaloniki, Greece, ISTA19 Conference Abstracts, ISTA 19, p. 39. http://ista19.civil.auth.gr/wp-content/uploads/2019/08/Abstracts_ISTA-2019.pdf

Farkas, É., Feigl, V., Gruiz, K., Vaszita, E., Ujaczki, É., Fekete-Kertész, I., Tolner, M., Horváth, C.M., Berkl, Z., Uzinger, N., Rékási, M., Molnár, M. (2018) Microcosm incubation study for monitoring the mid-term effects of different biochars on acidic sandy soil applying a multiparameter approach. *Process Safety and Environmental Protection*, 120, 24–36. ISSN 0957-5820, https://doi.org/10.1016/j.psep.2018.08.027.

This article (full-text publication) is deposited at the Repository of the Academy's Library: http://real.mtak.hu/89125/

Kerekes, I.K., Molnár, M., Feigl, I. (2018) Közönséges televényféreg elkerülési tesztek alkalmazásának talajjavító adalékok és szennyezőanyagok minősítésére. In: Füleky, György (szerk.) XIV. Kárpát-medencei Környezettudományi Konferencia. Gödöllő, Magyarország: MAG Mezőgazdaságért Alapítvány, (2018) 143–147.

Molnár, M., Kordisz, V., Vaszita, E., Uzinger, N., Rékási, M., Kutasi, J., Feigl, V., Tolner, M., Gruiz, K., Farkas, É. (2017) The effect of biochar combined with PGPR and compost on an acidic sandy soil – a pot experiment. In: Buburuzan A.M., Teodosiu, C., Fava, F., Corvini Ph. X., Gavrilescu D. (Ed.), 9th International Conference on Environmental Engineering and management (ICEEM09): Circular Economy and Environmental Sustainability: Conference Abstracts Book, (2017) pp. 199-200.

Molnár, M., Feigl, V., Vaszita, E., Gruiz, K., Farkas, É. (2017) Screening and ranking methodology applied to biochars aimed at soil improvement. In: Buburuzan A.M., Teodosiu, C., Fava, F., Corvini Ph. X., Gavrilescu D. (Ed.), 9th International Conference on Environmental Engineering and management (ICEEM09): Circular Economy and Environmental Sustainability: Conference Abstracts Book, pp. 249-250.

Farkas, É., Feigl, V., Ujaczki, É., Fekete, Kertész I., Tolner, M., Vaszita, E., Gruiz, K., Molnár, M. (2017) Microcosm incubation study for monitoring the mid-term effects of biochar on acidic sandy soil. In: Buburuzan A.M., Teodosiu, C., Fava, F., Corvini Ph. X., Gavrilescu D. (Ed.), 9th International Conference on Environmental Engineering and management (ICEEM09): Circular Economy and Environmental Sustainability: Conference Abstracts Book, (2017) pp. 253-254.

Farkas, É., Feigl, V., Gruiz, K., Vaszita, E., Ujaczki, É., Fekete-Kertész, I., Tolner, M., Kerekes, I., Kari, A., Uzinger, N., Rékási, M., Kirchkeszner, Cs., Molnár, M. (2020) Long-term effects of biochar on acidic and calcareous sandy soils - success story of a scale-up field experiment. Science of the Total Environment (STOTEN-D-20-01768) submitted and reviewed (major revision)

Molnár, M., Kerekes, I., Szabó, Á., Gruiz, K., Vaszita, E., Tolner, M., Feigl, V., Farkas, É. (2020) Biochar mediated short-term effects on acidic sandy soil and influence on soil living animal Enchytraeus albidus – preference behavioural test as a screening tool to assess soil habitat function. Environmental Science and Pollution Research, ESPR-D-20-01242 Prepared for submission

Gruiz, K., Molnár, M., Feigl, V., Vaszita, E., Farkas, É. (2020) Complex screening-scoring methodology for ranking of the biochars aiming at soil improvement. Agronomy, Prepared for submission

4.2. Thesis

- Szabó Ádám: Bioszén termékek komplex vizsgálata és jellemzése talajjavítást célzó alkalmazásukhoz, MSc környezetmérnök diplomamunka, 2017 (Témavezető: Dr. Molnár Mónika, Konzulens: Farkas Éva)
- Egri Zsanett: Bioszén savanyú homoktalaj javítását célzó hosszú távú hatásának komplex vizsgálata és értékelése
 laboratóriumtól a szabadföldi alkalmazásig. 2018. MSc biomérnök diplomamunka, (Témavezető: Dr. Molnár Mónika, konzulens: Farkas Éva)
- Kiticsics Anna: Bioszén termékek hatásainak összehasonlító értékelése különböző bioszén-talaj rendszerekben komplex megközelítéssel. 2018. MSc biomérnök diplomamunka, (Témavezető: Dr. Molnár Mónika, konzulens: Farkas Éva)
- Reményi Marietta: Bioszén hatása a talaj aktivitására összefüggések a bioszén termékek fizikai-kémiai jellemzői és a talajbiológiai hatásaik között, MSc Diplomamunka, 2019 (Témavezető: Dr. Molnár Mónika, konzulens: Farkas Éva)
- Kőszegi Márta: Bioszén jellemzőinek és hatásainak vizsgálata mesterséges érlelési és talajjavítást célzó modellkísérletekben, MSc Diplomamunka, 2019 (Témavezető: Dr. Molnár Mónika, konzulens: Farkas Éva)
- Molnár Imre: Degradálódott homoktalaj javítása bioszénnel hosszú távú hatások modellezése mikrokozmosz kísérletekben komplex metodikával, MSc Diplomamunka, 2019 (Témavezető: Dr. Molnár Mónika, konzulens: Farkas Éva)
- Vágó Krisztina: Bioszén talajjavítást célzó alkalmazásának vizsgálata és a mikrobiológiai aktivitásra gyakorolt hatásának követése és értékelése mikrokozmosz kísérletekben, MSc Diplomamunka, 2019 (Témavezető: Dr. Molnár Mónika, konzulens: Farkas Éva)
- Nyamsuren Yeruult: Biochar for improvement of degraded sandy soils microcosm experiments for modelling long-term effects using complex monitoring methodology. MSc thesis, 2019 (Témavezető: Dr. Molnár Mónika, konzulens: Farkas Éva)
- Éva Farkas PhD thesis (PhD Defence in 2020)