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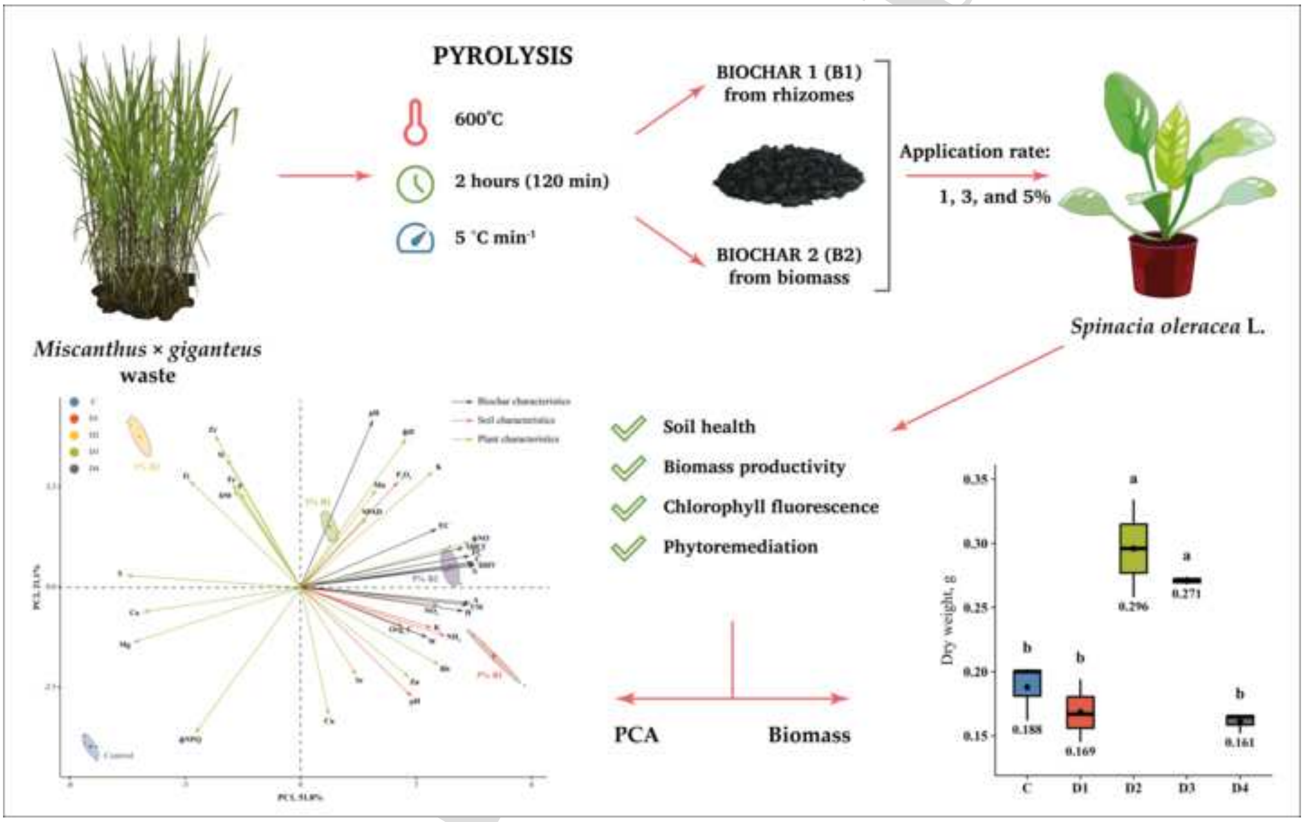
Graphical Abstracts



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Evaluation of the impact of varied biochars produced from *M.* × *giganteus* waste and application rate on the soil properties and physiological parameters of *Spinacia oleracea* L.

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Abstract: The use of *M.* × *giganteus* in phytoremediation requires treatment of the contaminated biomass, which can be done by pyrolysis to produce biochar. Due to its potentially detrimental properties, the application of biochar in soil remediation must first be evaluated on a test plant to infer how the growth process was affected by the impact on soil parameters. The main goal of the current research was to investigate the effects of waste-derived *Miscanthus* biochars (from contaminated rhizomes (B1) and aboveground biomass (B2)) on soil properties and evaluate the impact of biochar doses and properties on *Spinacia oleracea* L. growth. It was revealed that incorporation of B1 at a dose of 5% and B2 at doses of 1, 3, and 5% increased soil organic carbon, pH, K (at 3 and 5%), and P_2O_5 (at 5% B2). Cultivation of *S. oleracea* reduced organic carbon, soil pH as a function of biochar dosage, and K, P_2O_5, NH_4, and NO_3 content in all treatments tested. The highest biomass yield was recorded at 3% B2. The photosynthetic parameters indicated that the doses of 3 and 5% B2 led to dissociation of light-harvesting complexes. Increasing the biochar dose did not necessarily increase yield or improve photosynthetic parameters. *S. oleracea* adapted to the initial stress by incorporating biochar and managed to establish a balance between nutrients, water supply, and light. It is recommended that the effects of biochar on the development of the target crop be evaluated through preliminary trials before biochar is applied at field scale.

Keywords: *Miscanthus* biochar, *Spinacia oleracea* L., antagonistic element interactions, soil nutrients, chlorophyll content

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1. Introduction

Biochar is a solid carbon-rich fraction produced by the thermal decomposition of biomass under limited or absent oxygen supply (Lehmann and Joseph, 2015; Shackley et al., 2013). This material is proposed as a promising option for enhancing the soil carbon sink having ability to resist abiotic and biotic degradation and decrease CO_2 emission from organic compounds in the soil (Herath et al., 2015; Smith et al., 2014; Zhang and Ok, 2014). Pyrolysis and gasification are the main physicochemical thermal processes for the production of biochar, and the type of initial raw materials, temperature, and treatment time are the main factors affecting the properties of the resulting biochar (Tan et al., 2017; Tomczyk et al., 2020). The raw materials used for biochar production are varied and endowed biochars with a broad structure and properties (Alghamdi et al., 2021; Tomczyk et al., 2020; Zhao et al., 2021). When biochar serves as a soil amendment, it can optimise soil structure and composition (Alghamdi et al., 2021), increase water retention capacity, stimulate nutrient availability (Enaime and Lübken, 2021) and cycling (DeLuca et al., 2015), reduce nutrient loss from leaching (Liang et al., 2006; Liu et al., 2018), and affect the soil biota by altering the composition and enzyme activities of the microbial community (Lehmann et al., 2011).

The incorporation of highly aromatic biochar into the soil during barley field production was found to affect soil functions (carbon sequestration, water content, greenhouse gas emissions, nutrient cycling, soil food web functioning, and food production) (Llovet et al., 2021). After 6 years of the experiment, carbon sequestration increased. Depending on the biochar dose (12 and 50 t ha^{-1}), the increases were 23 and 68% higher compared to control; a higher rate of biochar treatment led to enhancement of the soil water content. Biochar addition neither abated nor increased emission of CO_2 equivalents (carbon dioxide plus nitrous oxide and methane), and the system shifted from being a methane sink (-0.017 $\pm$ 0.01 mg CH_4-C m^{-2} h^{-1} at a smaller dose of 12 t ha^{-1}) to a net source (0.025 $\pm$ 0.02 mg CH_4-C m^{-2} h^{-1} at a higher dose of 50 t ha^{-1}). However, biochar amendment did not stimulate any enhancements in yield during the 6-year experiment.

The growth, physiology, and yield of wheat were positively affected by biochar amendment of saline soil in one study, particularly under high salinity levels (Akhtar et al., 2015). Biochar addition reduced plant sodium uptake by transient Na^+ binding due to its high adsorption capacity, decreasing osmotic stress by enhancing soil moisture content and releasing mineral nutrients (particularly K^+, Ca^{2+}, Mg^{2+}) into the soil solution.

Increases in pH, N, P, K, Ca, and Mg concentrations in a soil with low organic carbon and fertility were observed after the addition of peanut hull biochar (Gaskin et al., 2010); a significant simultaneous response of corn yield following biochar application was recorded during 2 years of monitoring. The root depth and the presence of biochar in the root zone played a primordial role stimulating in plant growth.

Adding biochar increased biomass and seed yields of soybean genotypes by 67 and 54% on average, respectively; when applications of biochar and NPK fertiliser were combined, the increases were 391 and 367%, respectively, compared to control (Mete et al., 2015). A correlation was found between leaf chlorophyll content (single-photon avalanche diode value) and nodule number. The increase in yield was due to a decrease in soil pH caused by biochar and NPK fertiliser applications, thereby increasing P availability in this alkaline soil.

When plants grow in contaminated soil, the incorporation of biochar often assists in improving the development and decreasing the trace elements (TEs) extractability (Radziemska et al., 2022); the effect was enhanced with increases in the application rate (Houben et al., 2013). Amendment of the highly TE-contaminated soil with biochar (in mg kg^{-1} soil: Cu (780 ± 144), Cd (25.9 ± 2.5), Pb (13 540 ± 669), and Zn (8 433 ± 1 376)) increased the effectiveness of biochar-assisted phytostabilisation in *Dactylis glomerata* L., soil pH, and plant biomass. In the case of organochlorine pesticide-contaminated soil, the addition of carbon-rich substances improved the development of *Miscanthus sinensis* And. and the yield of harvested biomass (Mamirova et al., 2021) by decreasing the translocation of pesticides to aboveground biomass. Amendment of diesel-contaminated soil with biochars produced from wastewater sludge or a mixture of wood waste and biohumus improved the morphological and physiological parameters of *M. × giganteus* production, with enhanced biomass and prolonged vegetation period (Pidlisnyuk et al., 2021a).

However, recently published observations (Brtnicky et al., 2021; Mukherjee et al., 2014) have illustrated that the application of biochar must be selective: before utilisation, the pros and cons in effects must be considered, which is particularly important during field-scale application. Therefore, the necessity of preliminary biochar testing is evident. This will ensure the rationality of biochar utilisation, allowing the appropriate variety and dose of biochar and defining the conditions for its application.

Based on a literature analysis of *Miscanthus* biochar production and application, considering the impact on phytoremediation parameters, soil properties, microbial community, and fauna, a theoretical zero-waste approach was proposed (Pidlisnyuk et al., 2021b) on utilisation of biochar obtained from *Miscanthus* biomass wastes after utilization in Miscanthus phytomanagement (Alasmary et al., 2021; Bilandžija et al., 2022). The approach is in line with the circular economy requests (Casarejos et al., 2018; Donia et al., 2018; FAO, 2016; Maaß and Grundmann, 2018; Wiesmeth, 2021). This theoretical assumption has to be proven by investigation the process of converting the contaminated Miscanthus waste into biochar, testing Miscanthus biochars as impacted soil parameters: organic C, NO_3, NH_4, and P_2O_5 contents and pH during the growing process of testing plant *Spinacia oleracea* L. as assessed by plant's physiological and morphological parameters, which were the main goals of the current study.

2. Materials and methods

2.1. Soil collection

The research soil was collected at the agricultural field of Volodymyr Hnatiuk National Pedagogical University, Ternopil, Ukraine; the GPS coordinates are 49.5418397 N, 25.568175 E. The soil sampling was carried out according to the approach described in the standard DSTU

4287:2004 (2005), which recommends use of a 5 × 5 m testing square; five soil samples were taken at a depth of 0-30 cm and mixed using the envelope method. The collected soil was dried to constant weight and passed through a sieve with a pore diameter of 5 mm to remove the plant materials and stones (this diameter was selected to avoid damaging the soil structure). In accordance with the World Reference Base for Soil Resources classification (FAO, 2014), the research soil was identified as chernozem (phaeozems).

2.2. Analysis of the soil parameters

Different soil parameters were monitored while testing the impact of biochars of different origins and their application rates on the biological and physiological parameters of *Spinacia oleracea* L. (*S. oleracea*) using standard methods. Total organic C (Org_C) was determined using the Tyurin method (DSTU 4289:2004, 2005); the nitrate nitrogen (NO_3) content was determined following DSTU 4725:2007 (2008), the ammonium nitrogen (NH_4) content was determined following DSTU 4725:2007 (2008); a mobile form of potassium (K) was determined following DSTU 4725:2007 (2008); a mobile form of phosphorus (P_2O_5) was determined using Chirikov method (DSTU 4115-2002, 2003); soil pH (KCl) was measured following DSTU ISO 10390:2001 (2002). Determination of K, NH_4, and NO_3 was performed on a laboratory ionomer AI-123 (Ukraine) using ELIS electrodes (Russian Federation). The phosphorus content was detected using a UIT SFU-0172 spectrophotometer (PRC).

The agrochemical parameters of the initial soil are presented in Table 1.

Table 1.

Agrochemical parameters of the initial soil.

Agrochemical parameter	**Unit**	**Mean ± SD**	**Measuring standard**	**Method**
pH (KCl)	-	6.66 ± 0.05	(DSTU ISO 10390:2001, 2002)	pH (KCl)
Org_C	%	1.12 ± 0.02	(DSTU 4289:2004, 2005)	Tyurin
NO_3	mg kg^{-1}	151.3 ± 4.50	(DSTU 4725:2007, 2008)	Ion selective
NH_4	mg kg^{-1}	0.18 ± 0.04	(DSTU 4725:2007, 2008)	Ion selective
P_2O_5	mg kg^{-1}	79.6 ± 1.00	(DSTU 4115-2002, 2003)	Chirikov
K	mg kg^{-1}	0.50 ± 0.12	(DSTU 4725:2007, 2008)	Ion selective

In accordance with the DSTU 4362:2004 (2005), the research soil had a neutral reaction of salt solution, average contents of organic matter and phosphorus, and high contents of mineral nitrogen and potassium.

The element contents in the soil were determined at the beginning and end of the experiment using X-ray fluorescence analysis. The analysis was described in detail in Pidlisnyuk et al. (2020); briefly, estimation of the element content was carried out using an Elvax Light SDD Analyzer (Elvatech, Kyiv, Ukraine), following the United States Environmental Protection Agency standard

(USEPA, 2007). The element contents in the soil prior to the experiment are presented in Table 2.

The same X-ray fluorescence analysis was applied to measure the contents of the elements in the plant tissues during the growing process and at harvest; the procedure has been previously described (Pidlisnyuk et al., 2018).

Table 2.

Contents of the elements in the initial soil.

Element concentration, mg kg^{-1}					
Mg	**Al**	**Si**	**P**	**S**	**K**
9 817 ± 146	60 389 ± 474	367 915 ± 77.2	801 ± 67.0	30.2 ± 4.66	22 424 ± 828
Ca	**Ti**	**Cr**	**Mn**	**Fe**	**Ni**
8 523 ± 135	5 443 ± 63.5	109 ± 0.93	592 ± 19.2	21 662 ± 306	24.5 ± 0.92
Cu	**Zn**	**Rb**	**Sr**	**Zr**	**Pb**
16.5 ± 1.88	55.5 ± 4.04	104 ± 1.89	114 ± 0.89	658 ± 10.7	34.2 ± 1.82

2.3. Biochar origin and characteristics

There were two sorts of biochars tested: biochar produced from waste - *M.* × *giganteus* contaminated rhizomes produced in Všebořice TE-contaminated soil (B1) (Pidlisnyuk et al., 2022), and biochar derived from the aboveground waste biomass (AWB) produced in the field condition in Chomutov (B2) on soil slightly contaminated by TEs (Ustyak and Petrikova, 1996).

B1 and B2 were produced in a laboratory unit of the Technical University in Ostrava, Institute of Environmental Research (IET), using an externally heated fixed bed reactor (with a length of 30 cm and inner diameter of 5.5 cm) (Grycova et al., 2017) placed into an LT 50/300/13 tube furnace (LAC, Czech Republic). The pyrolysis conditions were as follows: temperature 600 °C, residence time 2 hours, heating rate 5 °C min^{-1}, and the unit was rendered inert by flushing with nitrogen at the beginning of pyrolysis process.

A LECO TGA701 analyser was used for the determination of moisture (W), volatile matter (VM), fixed carbon (FC), and ash (A) contents in accordance with ASTM D1762-84 (2021). Carbon (C), nitrogen (N), hydrogen (H), and sulphur (S) contents were measured by a LECO CHSN628 elemental analyser in accordance with ASTM D5373-21 (2021). The mass of oxygen (O, %) was calculated by difference (O = 100-C-H-N-S-A). The high heating value (HHV) was determined using a LECO AC600 bomb semi-automatic calorimeter following ASTM E711-87 (2012). Mass balance was evaluated by weighing the individual products. Referring to the initial raw material, the yield of B1 was 34 wt.%, and the yield of B2 was 30 wt.%.

The conductivity and pH of the initial materials were determined using an Accumet XL 600 instrument (Fisher Scientific Com, NH); the aqueous extract was prepared by mixing input material with deionised water at a ratio of 1:20. The elemental analyses of the initial materials are presented in Table S1.

The quality of produced biochars was tested by measuring physical (particle size, moisture, EC, SBET, and HHV) and chemical (elements content, A, EC, FC, VM, and pH) properties. Biochar was processed for soil toxicity assessment according to the requirements of the International Biochar Initiative (IBI, 2015).

The biochar porosity was evaluated by sorption measurements using the 3Flex instrument (Micromeritics, USA). Surface area analysis was carried out in accordance with the ASTM D6556-21 (2021) Standard Test Method for Carbon Black–Total and External Surface Area by Nitrogen Adsorption. The surface area was measured following the BET procedure.

2.4. Design of the experiment

In the spring of 2021, the Lab experiment was established at Ternopil Volodymyr Hnatiuk National Pedagogical University, Ukraine. The timeline of experimental stages is illustrated in Fig. 1.

The preliminary prepared soil (as described in Section 2.1) was carefully mixed with a certain dose of a specific biochar, and the receiving substrate was transferred to the vegetation pot (volume of 1 dm^3); at the bottom of each pot were placed the loaded agronomy fibre and 50 g of gravel. Variations of the experiment are presented in Table 3.

Table 3.

Experimental treatments.

Treatment	Biochar dose, % w.w	Soil mass, g	Biochar mass, g	Total mass in a pot, g
C	0	800	0	800
D2	1	792	8	800
D3	3	776	24	800
D1*, D4	5	760	40	800

**Variation D1 was tested only in one dose because of the very limited amount of initial raw material (contaminated rhizomes) that was processed to biochar.*

Fig. 1. Timeline of experiment stages.

The pots were placed in the trays for watering. There were four replicates in each set of the treatment. Pots with the substrate were stored in the laboratory from 27 April to 19 May 2021; thereafter, the planting of the crop was accomplished.

The plant selected for testing was *S. oleracea,* recommended for short-term evaluation of amendments (Pavlíková et al., 2017). The cultivar of *S. oleracea* used in the current study was hybrid Corvair F1 produced by Enza Zaden Bikhir B.V. (Haling, 1E, 1602 DB, ENKHUIZEN, The Netherlands). This crop has a high resistance to cucumber mosaic virus and downy mildew, anthracnose, white rust, and leaf spot diseases ("Spinach Corvair F1," 1999).

The seeds of *S. Oleracea* were sown to a depth of 1 cm using 4 seeds per pot filled with the substrate on 19 May 2021. Seedlings were detected 7 days after sowing (on 26 May 2021). On this day, one plant per pot was retained for evaluation, and the surface of the soil was covered with black opaque paper to prevent evaporation; an illustration is presented in Fig. S1. Subsequent plant care involved indoor temperature control (in a range of 24-26 °C), ventilation, artificial lighting equal to 150 μmol photons m^{-2} s^{-1} for 16 hours per day (Osram Fluora T8 36 W, Germany), and watering to maintain the moisture level at 60%. The pots were moved every 2 days within the array of the vessels, both in the middle of each option and between the options themselves "to minimise differences due to positional effects".

The experiment finished on 7 July 2021 (Fig. 1) at the stage when four true leaves had unfolded (BBCH 14) and the formation of the fifth true leaf began (Meier, 1997).

2.5. Plant development parameters

2.5.1. Plant photosynthetic efficiency

Plant development was evaluated by measuring photosynthetic parameters.

Chlorophyll *a* fluorescence was measured following the Photosynthesis RIDES 2.0 protocol using a MultispeQ v1.0 (PhotosynQ LLC, USA). Other parameters were consecutively determined under light acclimation, i.e., the relative chlorophyll content (SPAD), the fraction of PSII open centres (qL), the quantum yield of PSII (ΦII), the maximal quantum efficiency of PSII (Fv′/Fm′), the total nonphotochemical quenching (NPQt), the fraction of light dedicated to nonphotochemical quenching (ΦNPQ), and the fraction of light lost via nonregulated photosynthesis inhibitor processes (ΦNO) (Ben-Jabeur et al., 2020).

The measurements were conducted using 4 replicates per treatment of one leaf, which corresponds to 16 measurements for one treated variant (4 replicates × 4 leaves). Intact, fully expanded leaves were evaluated using the MultispeQ v1.0 linked to the PhotosynQ platform. The SPAD and NPQt values were estimated following Kuhlgert et al. (2016).

2.5.2. Harvested parameters

The morphological parameters of *S. oleracea* were measured at the end of the experiment (Fig. 1). Total leaf area (cm^2) was estimated using the mobile app Petiole Pro ("Petiole Pro," 2015). The cut aboveground biomass of *S. oleracea* was dried on an open surface until reaching constant weight, and the value of plant fresh weight was determined using an electronic balance. For the determination of biomass dry weight (DW), a sample of biomass was dried in a thermostatic chamber at 100–105 °C until constant weight, i.e., when the difference between two consecutively measured weights was within 0.0001 g.

2.5.3. Bioconcentration factor

For evaluation *S. oleracea* potential to accumulate different elements present in soil, the value of bioconcentration factor (BCF) was calculated based on the following equation (Greger, 2004):

$$BCF = \frac{element\ concentration\ in\ plant\ tissues\ (mg\ kg^{-1})}{element\ concentration\ in\ soil\ (mg\ kg^{-1})}$$

2.6. Statistical Analysis

Statistical data processing was conducted using RStudio software (version 1.3.959, RStudio PBC, 2020). Two-way repeated measures analysis of variance (RM ANOVA) was carried out to detect a statistically significant differences in the growth dynamics, chlorophyll fluorescence values, agrochemical profile changes, and soil TE concentrations between different treatments. One-way ANOVA was used to evaluate the significance of differences in input material characteristics, biochar characteristics, and DW of plant parts between plants grown in the presence of different biochars and doses. In cases where a significant difference was demonstrated by ANOVA, Tukey's HSD test was performed for pairwise comparison. Treatments were categorized (by letters in descending gradation) according to the results of this test, and box plots/graphs were created.

3. Results and Discussion

3.1. Impact of initial waste materials on the properties of produced biochars

The initial *Miscanthus* wastes used to produce biochars (B1 and B2) were tested using proximate and ultimate analyses, and the results are presented in Table 4.

Table 4.
Proximate and ultimate analyses of initial *Miscanthus* wastes used for biochar production. Different letters within one parameter indicate a significant difference between the values of the input materials.

Parameter	Unit	Contaminated rhizomes	AWB	***p*-value**
W	wt.%	15.3 ± 0.02 **a**	5.05 ± 0.02 **b**	< 0.001
VM^{d}	wt.%	74.5 ± 0.51 **b**	77.2 ± 0.08 **a**	< 0.001
FC^{d}	wt.%	20.6 ± 0.40 **a**	18.8 ± 0.23 **b**	< 0.01
A^{d}	wt.%	4.84 ± 0.07 **a**	3.99 ± 0.10 **b**	< 0.001
HHV^{d}	MJ kg^{-1}	21.0 ± 0.02 **a**	19.2 ± 0.50 **b**	< 0.01
C^{d}	wt.%	47.8 ± 0.09 **a**	47.4 ± 0.13 **b**	< 0.01
H^{d}	wt.%	8.03 ± 0.10 **a**	6.67 ± 0.02 **b**	< 0.001
N^{d}	wt.%	0.81 ± 0.04 **a**	0.45 ± 0.05 **b**	< 0.001
S^{d}	wt.%	0.12 ± 0.02 **a**	0.05 ± 0.0 **b**	< 0.01
O^{d}	wt.%	38.4 ± 0.02 **b**	41.5 ± 0.02 **a**	< 0.001
pH	-	5.44 ±0.12 **b**	7.04 ±0.15 **a**	< 0.001
EC	mS cm^{-1}	0.93 ±0.0 **b**	1.26 ±0.01 **a**	< 0.001

Note: AWB—aboveground waste biomass; W—moisture; VM—volatile matter; FC—fixed carbon; A—ash; HHV—higher heating value; EC—electrical conductivity.

As seen in Table 4, the moisture content was about three times higher for contaminated rhizomes than for AWB; also, the ash content was higher for the contaminated rhizomes. Both initial materials had similar HHV, FC, and oxygen content, however, nitrogen and sulphur contents were higher for contaminated rhizomes. The pH value was slightly acidic for contaminated rhizomes compared to neutral pH for AWB; AWB had a higher EC than contaminated rhizomes (Table 4).

The same proximate and ultimate procedures were utilised for biochars B1 and B2 produced from these *Miscanthus* waste, additionally surface characteristics (SBET) and EC values were evaluated (Table 5).

Table 5.

Characteristics of biochars produced from Miscanthus wastes. Different letters within one parameter indicate a significant difference between the values of the different biochars.

Biochar	W, wt.%	VM^d, wt.%	FC^d, wt.%	A^d, wt.%	HHV^d, MJ kg^{-1}
B1	5.59 ± 0.07 **a**	25.43 ± 0.37 **a**	55.27 ± 0.70 **b**	19.30 ± 0.27 **a**	27.63 ± 0.14 **b**
B2	0.93 ± 0.05 **b**	14.97 ± 1.52 **b**	73.08 ± 1.28 **a**	11.95 ± 0.67 **b**	31.74 ± 0.03 **a**
***p*-value**	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	C^d, wt.%	**H^d, wt.%**	**N^d, wt.%**	**S^d, wt.%**	**O^d, wt.%**
B1	68.45 ± 2.60 **b**	3.51 ± 0.42 **a**	1.39 ± 0.02 **b**	0.02 ± 0 **a**	7.34 ± 0.02 **a**
B2	81.10 ± 0.47 **a**	1.83 ± 0.31 **b**	1.58 ± 0.05 **a**	0.01 ± 0 **b**	3.52 ± 0.02 **b**
***p*-value**	< 0.01	< 0.01	< 0.01	< 0.001	< 0.001
	pH		**SBET, m^2 g^{-1}**		**EC, mS cm^{-1}**
B1	5.51 ±0.05 **b**		71.0 ± 3.74 **b**		1.71 ±0 **b**
B2	9.52 ±0.20 **a**		109 ± 6.06 **a**		2.78 ±0.02 **a**
***p*-value**	< 0.001		< 0.001		< 0.001

Note: W—moisture, VM—volatile matter, FC—fixed carbon, A—ash, HHV—higher heating value; EC—electrical conductivity.

B2 exhibited more favourable characteristics than B1; having lower moisture content, VM, and ash content and higher FC, HHV, carbon molecule content, SBET, and EC. B1 had a significantly lower pH and EC values than B2 (Table 5). It must be mentioned that the pH of B2 had an alkali value (9.52) that had increased compared to the input material (7.04), while for B1, the pH value of the input material (5.44) and received material (5.51) remained almost the same (Tables 4 and 5).

The produced biochars were tested for the element contents (Table 6).

Table 6.

Element contents of biochars produced from Miscanthus wastes. Different letters within one element indicate a significant difference between the values of the different biochars.

Element	MAT, mg kg^{-1}	B1, mg kg^{-1}	B2, mg kg^{-1}	*p*-value
P	-	23 095 ± 417 **b**	36 638 ± 850 **a**	< 0.001
S	-	1 259 ± 8.88 **b**	2 449 ± 88.0 **a**	< 0.001
K	-	486 395 ± 2 347 **a**	417 808 ± 2 608 **b**	< 0.001
Ca	-	91 870 ± 1 203 **b**	249 797 ± 1 431 **a**	< 0.001
Ti	-	23 774 ± 111 **a**	4 241 ± 314 **b**	< 0.001
Mn	-	16 213 ± 267 **a**	14 638 ± 81.9 **b**	< 0.001
Fe	-	106 775 ± 3 176 **a**	16 603 ± 119 **b**	< 0.001
Ni	47 – 420	528.0 ± 15.3 **a**	229.3 ± 18.6 **b**	< 0.001
Cu	143 – 6 000	610.6 ± 2.49 **a**	443.9 ± 3.16 **b**	< 0.001

Zn	416 – 7 400	2 691 ± 52.6 **b**	4 021 ± 11.4 **a**	< 0.001
Rb	-	1 085 ± 22.2 **a**	555.0 ± 12.6 **b**	< 0.001
Sr	-	890.7 ± 22.9 **b**	1 513 ± 28.1 **a**	< 0.001
Pb	121 – 300	104.9 ± 3.68	< **LOD**	-

Note: TEs are marked in bold; MAT—maximum allowable thresholds (IBI, 2015).

B1 had higher TEs concentrations than B2, indeed, B2 was rich in nutrient (P, S, and Ca) contents (Table 6). It was notable that Mg, Al, Si, and Zr were detected in the input wastes, however, were not present in biochars. In contrast, Ni was not detected in the input wastes but found in biochars (Tables S1 and 6). A linear dependence was observed for the majority of elements, except S and Zn; i.e., the higher the element concentration was in the input wastes, the higher the concentration detected in the biochar (Tables S1 and 6).

According to values of the maximum allowable thresholds (MAT), the produced biochar products could be considered safe; the only exception was the presence of Ni in B1, which was above the upper level of MAT (IBI, 2015).

3.2. Changing the soil parameters as influenced by biochar's incorporation

3.2.1. Soil agrochemical profile

The plant growth and development depend on the combination and concentration of nutrients in the soil (Fageria and Baligar, 2005), so, it was initially necessary to evaluate changes in the nutrients in biochar-enriched soil. The concentrations of Org_C, P_2O_5, K, NH_4, NO_3, and pH were investigated in the soil on the 30th day after mixing with amendments (Fig. 2), when the seedlings of *S. oleracea* appeared in the pots (Fig. 1).

Fig. 2. Agrochemical characteristics of soils amended by different doses of biochars on the 30th day after mixing: a) organic carbon; b) soil pH; c) K; d) P_2O_5; e) NO_3; f) NH_4. Different letters on the boxplots within one agrochemical parameter indicate a significant difference between the values of the different treatments at (at least) $p < 0.05$.

The results illustrate that incorporation of B1 at a dose of 5% and B2 at three different doses (1, 3, and 5%) significantly increased the organic carbon content in the substrate and its pH; the value was increased proportionally to the incorporated dose of biochar (Fig. 2a, b). The K content in the control soil was low and increased after the incorporation of biochars with doses of 3 and 5% (Fig. 2c), while a dose of 1% did not significantly affect K content (D3) (Fig. 2c). This may be explained by the high K content of biochars (Table 6), which improves the element availability by increasing the soil pH (Ding et al., 2016). The content of P_2O_5 in the soil at the highest dose of B2 (5%) increased to 112.6 ± 1.68 mg kg^{-1}, which was probably associated with the high concentration of this element in biochar (Table 6). At the same time, when B2 was utilised at smaller doses (1 and 3%) the content of P_2O_5 did not increase after biochar incorporation; moreover, the P_2O_5 content decreased (Fig. 2d). The decrease in D2 and D3 can be explained by biochar's high specific surface area and the existence in its content of polar or nonpolar substances, which have a strong affinity for inorganic ions such as trace element ions, P_2O_5, and NO_3 (Ding et al., 2016; Kammann et al., 2015; Schmidt et al., 2014).

For the D4 treatment, the effect of the chemical content of D4 the substrate was becoming more important than its sorption properties (Table 5), which led to a decrease in the potential buffer ability of the substrate related to phosphate ions (Tikhonenko et al., 2005). The experimental soil had a high NO_3 content and a low NH_4 content (Fig. 2e, f), which indicates the high level of nitrification of the ammonium nitrogen (Gospodarenko, 2013). When biochars were incorporated into the soil, the concentration of NH_4 essentially increased (at doses of 3 and 5% B2); however, the concentration of NO_3 significantly decreased, which illustrates that nitrogen is present in biochar in the form of ammonium. The impact of biochar on the soil is strongly connected with the conditions of biochar production, i.e., the temperature and duration of the process, in addition to soil properties, plant variety, and applied biochar dose (Ding et al., 2016).

3.2.2. Content of elements

The individual influences of the biochars on TEs contents in the research soils are presented in Table S2. The incorporation of research biochars did not significantly affect the contents of Al, Cr, Ni, Cu, and Pb in amended soils compared to the control; the concentrations ranged from 59,620 to 60,289 mg kg^{-1}, 104 to 117 mg kg^{-1}, 23.7 to 30.9 mg kg^{-1}, 16.5 to 20.9 mg kg^{-1}, and 33.5 to 36.4 mg kg^{-1}, respectively. Considering that the tested biochars did not contain Mg, Al, Si, Cr, and Zr, the absence of influence on the contents of Al and Cr in the soils is reasonable. In contrast, the changes in Mg, Si, and Zr concentrations with the incorporation of biochars were unexpected. Mg concentration significantly decreased with application of increasing biochar doses, with the highest decrease observed in D1 (5% B1) and D3 (3% B2); Si content decreased (D1 and D4), while Zr content significantly increased in all research treatments compared to the control (Table S2).

Based on these results, it can be concluded that the tested biochars did not release Ni, Cu, and Pb (D1) into the soil and sequestrated Mg and Si, decreasing their concentrations in the soil. The reason for the increased Zr content in the research treatments must be further investigated.

The P, S, Mn, and Zn concentrations increased respective to the increasing dose of applied biochar, i.e., the highest increase was observed in treatments with addition of 5% biochar (D1 and D4). Addition of 1% B2 did not significantly increase P and S concentrations in soil, while even 3% of B2 did not significantly affect Zn. The Ca, Fe, and Sr concentrations increased in all treatments compared to control, whereas the highest increase was observed in D4 (5% of B2), the contents of the elements in D1-D3 were statistically at the same levels (Table S2). Potassium (K) concentration in the soil increased only in the presence of 5% B1 (D1). Ti and Rb concentrations in the soil increased equally in all tested treatments, regardless of the doses of applied biochars (Table S2).

3.3. Changes in soil parameters in the "biochar–soil–plant" system

3.3.1. Changing of the soil agrochemical profile

The second step in research was the evaluation of nutrient's changes in the research soil amended by biochar in the presence of *S. oleracea* (Fig. 3), which was evaluated three times: on 26 May, 16 June, and 7 July.

Fig. 3. Changes in the agrochemical characteristics of soils amended by different doses of biochars: a) organic carbon; b) soil pH; c) K; d) P_2O_5; e) NO_3; f) NH_4. Different letters on the boxplots within one agrochemical parameter indicate a significant difference between the values of the different treatments at (at least) $p < 0.05$.

From the 21st day (16 June) until the end of the experiment (7 July), the Org_C content decreased for all variations of the experiment; consequently, for variants with different doses of B2, the Org_C content decreased to the level of the control, whereas for B2, the decrease was higher than in the control experiment. The observed decrease may be explained by intensified mineralisation of the compounds with Org_C caused by the high pH value (Fig. 3a, b) (Curtin et al., 1998), increasing the porosity and water-holding capacity of the soil, activation of certain microbial groups (Ding et al., 2016), and possible peptisation of soil organomineral colloids in an alkaline environment (Fig. 3b) accompanied by their destruction (Tikhonenko et al., 2005). The Org_C content in the control treatment remained almost the same throughout the experiment (1.23-1.24%), tending to slightly increase at the end, which may be because of bacterial biosynthesis in the substrate.

For the control experiment, soil pH was stable and neutral with a slight increase at the end (Fig. 3b). For the soil with biochar, the soil environment was at its most alkaline at the beginning of the experiment and varied depending on the biochar dose (Fig. 3b). Closer to the end of the experiment, the alkalisation effect was reduced; in particular, it was visible for smaller doses of biochar (1 and 3%), which may be linked to assimilation of a proportion of the alkaline cations by plants, microbial soil activity, and soil buffering (Gospodarenko, 2013; Tikhonenko et al., 2005).

With time, the K content in variants D1, D3, and D4 (Fig. 3c) decreased because of immobilisation by plants, binding by colloids present in the soil fraction, and transformation to less available forms.

With time, the content of P_2O_5 decreased to middle (51-100) and low ($<$50 mg kg^{-1}) levels (DSTU 4362:2004, 2005). A similar trend in the soil phosphorus concentration was detected for B1 at 5% dose and control treatments (Fig. 3d). The observed change may be explained by the fast transformation of the mineralised mobile form of phosphorus into hard soluble salt and its immobilisation by plants and microorganisms (Gospodarenko, 2013; Tikhonenko et al., 2005), decreasing in pH (EC et al., 2011).

With time, the nitrate and ammonium nitrogen contents in the soil naturally decreased (Fig. 3e, f) under the influence of nitrification and denitrification, nitrogen immobilisation by plants and microorganisms (Gospodarenko, 2013), increased NH_3 evaporation with increasing soil pH (EC et al., 2011), and possible adsorption by biochar (Ding et al., 2016).

3.3.2. Changes in soil element concentrations in the presence of *S. oleracea* plants

This section describes the changes in element concentrations that occurred in the presence of *S. oleracea*. At the end of the experiment, the soil Ti, Cr, Cu, Zn, and Pb contents were not significantly different compared to their initial concentrations (Table S3).

The Mg content significantly decreased in the control and D2 treatments, which was higher at the beginning of the experiment. Al content decreased only in D1, which can be explained by the higher sorption capacity of B1, i.e., its sequestration by B1 over time. P content significantly decreased in all treatments because the plant utilised this element during development. S content decreased in control, D1, and D4, evidencing the release of this element by both biochars at a dose of 5%. K, Ca, Sr, and Zr contents decreased only in D4. Thus, at the beginning of the experiment, D4 contained the highest concentrations of Ca, Sr, and Zr; therefore, 5% B2 prompted release of a bioavailable form of these elements. Si and Rb contents significantly increased in D4, evidencing their continuous release. Mn and Ni contents changed inversely: Mn content decreased in D1 and increased in D4, while Ni content increased in D1 and decreased in D4. Fe content increased in control and D3 and decreased in D4 (Table S3).

3.4. Development of Spinacia oleracea L. in the soil amended by biochars

The current study tested how the biochars derived from different *Miscanthus* waste materials and their doses influenced *S. oleracea* development by assessing physiological parameters, i.e.: plant total area and harvested biomass DW at the end of the experiment on 7 July. The results are illustrated in Fig. 4.

Fig. 4. Physiological parameters of *Spinacia oleracea* L. at the end of the experiment: a) biomass DW, and b) plant leaf total area. Different letters on the boxplots within one parameter indicate a significant difference between the values of the different treatments at (at least) $p < 0.05$.

The values of *S. oleracea* biomass according to the biochar origin and doses are presented in Fig. 4a. For B2, the highest increase in biomass was for a dose of 1%, which not significantly different from the biomass following a dose of 3%; the plant biomass decreased for a dose of 5%.

The total plant leaf area at harvest depended on the biochar varieties and doses, as presented in Fig. 4b. The peculiarities are similar to the variation in biomass: the largest leaf total area was recorded when B2 was incorporated at a dose of 3%, which was not significantly different from the result for a dose of 1%. The incorporation of B1 and B2 at a dose of 5% did not improve crop development despite improving the state of soil nutrition (Fig. 3).

Our observation shows that an increase in biochar dose does not necessarily lead to enhancement of the plant biomass value at harvest. A similar tendency was shown by Khan et al. (2017). Obviously, before application of biochar at the field scale, preliminary testing of its impact on development of the target crop must be conducted.

The changes in plant state during vegetation can be interpreted based on measuring the value of chlorophyll fluorescence (Krause and Weis, 1984; Malinská et al., 2020). The changes in photosynthesis parameters (ϕII, SPAD, ϕNO, and ϕNPQ) of *S. oleracea* are presented in Fig. 5.

Fig. 5. Changes in the photosynthesis parameters of *S. oleracea* during the experiment: a) ϕII; b) SPAD; c) ϕNO; and d) ϕNPQ. Different letters on the boxplots within one stress parameter indicate a significant difference between the values of the different treatments at (at least) $p < 0.05$.

On the twelfth day of vegetation (when the second true leaf unfolded), ϕNPQ in the D4 treatment increased significantly, and the share of PSII open reaction centres (qL) decreased accordingly, a decrease in SPAD was observed for treatments D1 and D3.

Thus, during moderate stress, which was caused by the presence of B2 at a dose of 5% (D4), plant seedlings prefer light-dependent dissipative processes (ϕNPQ) for mitigation of the effects of reactive oxygen species (ROS) (Gómez et al., 2018).

Starting from the twenty-first day of vegetation, an essential change in the photosynthesis parameters of *S. oleracea* was observed (Fig. 5) when plant adapted to the earlier stress from the incorporation of biochar and managed to achieve a balance between nutrients, water supply, and light. Such adaptation might reduce the harmful effects of different exogenous factors (Basu et al., 2016). At the end of the experiment, the SPAD values for D1-D3 were higher than for the control, and the SPAD for D4 was almost equal to control.

In the presence of biochar, the production of chlorophyll in treatments D1-D3 was more intensive than for the control; for treatment D4, the plant development was similar to that of the C (Fig. 5b). In general, SPAD is negatively correlated with the nitrogen content, which was confirmed by our experiment (Figs. 3e, f, 5b), particularly for D1-D3.

3.5. Phytoremediation potential of Spinacia oleracea L.

The BCF value gives more insight into plant development under the different conditions (Alexander, 1999), which in our study were the impacts of biochar varieties and doses. The value of this parameter for the evaluated elements is presented in Fig. 6.

Fig. 6. The BCFs for elements noticeably accumulated by *S. oleracea* biomass: a) Mg; b) P; c) S; d) K; e) Ca; f) Zn. Different letters on the boxplots within the BCFs of one element indicate a significant difference between the values of the different treatments at $p < 0.001$.

The results show that plants do not have the potential to uptake Al, Cr, Ni, and Pb from the soil; at the same time, three gradations in BCF were observed – 1st group of elements, BCF < 0.1 (Si, Ti, Fe, Rb, Zr); 2nd group, BCF < 1 (Mn, Cu, and Sr); 3rd group, BCF > 1 (Mg, P, S, K, Ca, Zn) (Fig. 6).

The BCF values for the 1st group of elements (BCF < 0.1) differed within treatments ($p < 0.001$) ranging from 0.009 to 0.022 for Si; from 0.014 to 0.0036 for Ti; from 0.021 to 0.045 for

Fe; from 0.101 to 0.253 for Rb; from 0 to 0.014 for Zr (Table S5). For all elements except Rb, the highest BCF was observed in D2, which decreased with the increasing dose of applied biochars; moreover, the BCF values for D4 were significantly lower than for control. This tendency was observed for Zr, its uptake was observed only in treatments D2 and D3. Rb showed the opposite tendency: its uptake increased with the increasing biochar dose, and the highest BCF was calculated in D1 (Table S5).

The BCF values for the 2nd group elements (BCF < 1) also differed within biochar treatments, ranging from 0.23 to 0.44 for Mn ($p < 0.001$); from 0.57 to 0.77 for Cu ($p < 0.05$); and from 0.64 to 0.78 for Sr ($p < 0.001$). The uptake and accumulation of Mn increased with incorporation of the research biochars without a clear dependence on applied doses; the highest BCF was observed in D3 (3% of B2). The uptake of Sr was not changed in the presence of B1 at 5% dose, increased in the presence of B2 at 3%, while decreased at doses of 1 and 5%. The BCF for Cu significantly decreased only in D3 (1% of B2).

For the 3rd group elements (BCF > 1), only Zn belonged to the TEs. Mg, S, and Ca accumulation significantly (with the exception of S accumulation in D2) decreased with increasing the applied biochar dose (Fig. 6a, c, e). P accumulation significantly increased only in the presence of B2 at 3% dose (Fig. 6b). K uptake significantly increased in all research treatments without any dependence on the biochar application rate (Fig. 6d). In the case of Zn, BCF significantly decreased only in D2 (1% of B2) (Fig. 6f).

When the doses of B2 increased from 1 to 3 and 5%, consequent accumulation of K was observed (Fig. 6d) with a simultaneous decrease in Mg (Fig. 6a). It can be hypothesised that the high K concentration inhibits Mg uptake from the soil, which led to a Mg deficiency in plants (Heenan and Campbell, 1981; Salmon, 1963).

For high efficiency of plant photosynthesis, the optimal delivery of nutrients has to be ensured (Kirizii et al., 2014). K and Mg are important during development of plants playing a valuable role during photosynthesis and enhancing the transport of photoassimilates. When these elements are lacking, the absorption level of photosynthetic carbon decreases. As a result, excessive production of reactive oxygen species (ROS) inevitably causes photooxidation of the photosynthetic apparatus and activation of photoprotective mechanisms (Tränkner et al., 2018).

The critical concentration of Mg in plants must be in diapason 1.5–3.5 mg DM g^{-1}; nevertheless, it is species-specific (Hauer-Jákli and Tränkner, 2019). In the case of *S. oleracea,* the critical concentrations of Mg in vegetation were higher than is common and must be in diapason 3.5-8.0 mg g^{-1} for fully developed leaves (Bergmann, 1993). In the conditions of Mg deficit and limited light photosynthesis, when light absorption exceeds the capacity of the photosynthetic transport of electrons, the excessive absorbed light energy leads to overexcitation of chlorophyll molecules (*Chl*). This will accordingly increase the probability of *Chl* triplet formation and, hence, the formation of ROS (Bhatla and Lal, 2018).

As it is following from Figs. 4 and 5, the incorporation of biochars had a specific impact on the morphophysiological parameters of *S. oleracea*. In the presence of biochars, the content of Ca and Mg in the plant tissues decreased while the K content increased, being the highest for D3

(Fig. 6d). A similar tendency for the impact of a 5% dose of biochar on Ca, Mg, and K content during the vegetation period of *S. oleracea* and mustard was described in Pavlíková et al. (2017). The authors emphasised that the K content in plant tissues indicates its high consumption, while Mg content can be reduced by the antagonistic interaction of these two elements (Ohno and Grunes, 1985). Nevertheless, in the long term, biochar must have a positive effect on the accumulation of K and P in plant tissues.

Even with K and Mn concentrations in plant tissues being statistically different, they did not differ within the two biochars in contrast to other nutrient elements (P, S, Ca, and Fe). There was a noticeable distinction in Ca concentrations (Table 6), which in B2 was approximately three times higher, which may explain the higher rate of plant growth: a constant supply of Ca contributes to vigorous leaf and root development and regulates plant responses to numerous environmental stresses (Amor and Marcelis, 2003; Naeem et al., 2018).

At low concentrations, Ti is beneficial for plants (Lyu et al., 2017), however, it has an antagonistic relationship with Fe, and high Ti concentrations may therefore cause phytotoxicity under conditions of high Fe abundance. The strong Ti contamination of B1 was detected, so this biochar can be considered a less suitable amendment than B2. Ti and Fe concentrations were significantly lower in B2 (by 5.6 and 6.4 times, respectively), creating an ideal condition for plant growth: the beneficial effects of Ti appeared when plants experienced a deficient Fe supply.

Mg and K deficiency in plants can cause photoinhibition of photosynthesis processes, which are evaluated through the parameter Fv'/Fm' (Levine and Mattson, 2021; Tang et al., 2012). The changes in Fv'/Fm' values are associated with damage to the PSII complex that releases oxygen or with an increase in the number of restored forms of QA (Yang et al., 2012). One of the photoprotective mechanisms is the nonphotochemical quenching of excess absorbed light energy in the form of NPQ heat (Niyogi, 1999; Ruban, 2016). Plants increase heat dissipation in response to Mg and K deficiency to protect the photosynthetic apparatus (PSA) from damage and maintain photosynthetic function. In these conditions, plants have a limited ability to convert light energy into chemical energy; high light intensity enhances the formation of NPQ heat. It was shown the Fv'/Fm' value was decreased during the development of citrus and sugar beets (Hermans et al., 2004; Tang et al., 2012; Yang et al., 2012); however, for sunflower, Mg deficiency does not influence the Fv'/Fm' value ((Farhat et al., 2015; Lasa et al., 2000). In the case of Mg deficiency, an increasing NPQ value was detected for *Pinus radiata* (Laing et al., 2000). Increases in NPQ value were observed in the case of K deficiency for three varieties of citrus cultivars (Tang et al., 2012; Yang et al., 2012), two varieties of rice (Jia et al., 2008), and sunflower (Jákli et al., 2017). In contrast, during *S. carnosa* growth, NPQ was not affected, even if Mg was excluded from the nutrient solution.

From the data presented in Fig. 6, it follows that the BCF values were statistically different for Ti, Mn, and Fe, while for the other TEs were not. In the case of B2, the BCF values were higher for the limiting concentrations of Ti and Fe than for the same elements in other treatments. This may be explained by the lower concentrations of these elements in B2, which ensured optimal conditions for plant development (Lyu et al., 2017).

For Cu, Zn, and Sr, the differences in BCF values for biochars were not so visible, which may be related to the less effective sorption of these elements during plant development. This was confirmed by an almost equal concentration of these elements in the soil at the beginning and end of the experiment (Tables S2 and S3). In the case of the nutrients, the BCF values were significantly different compared to TEs for all elements (Fig. 6). The BCF values of Mg, S, and Ca behaved similarly: the accumulation decreased with increases in the biochar dose. Accumulation of P and K was higher at a dose of 3% B2. The higher uptake of these elements stimulated improved plant development, which was confirmed by the chlorophyll parameters (Fig. 5) and increasing biomass DW.

3.6 Influence of biochar characteristics on soil properties

In order to examine the influence of biochar properties on the soil agrochemical profile, Pearson correlation was performed (Fig. 7).

Fig. 7. Heatmap of the Pearson correlation between biochar properties and soil agrochemical characteristics. Abbreviations: A—ash, EC—electrical conductivity, FC—fixed carbon, Org_C—organic carbon, VM—volatile matter, and W—moisture.

The correlation matrix shows that the soil phosphate content was not influenced by the physical properties of the biochar. Furthermore, only the nitrate content in the soil was negatively correlated with biochar pH value. As expected, the NH_4 content was positively correlated with the contents of biochar volatiles, ash, hydrogen, and soil pH. Soil Org_C and K contents increased with the increase in FC, nitrogen, and SBET of biochar. Soil pH was positively correlated with biochar parameters such as VM, ash, carbon, hydrogen, nitrogen, and HHV (Fig. 7).

3.7. Impact of soil agrochemical profile and biochar on the physiological parameters of S. oleracea L.

Statistical evaluation of the data confirmed that all monitored parameters were defined by biochar characteristics (Fig. 8). The first two principal components (PCs) captured 72.9% of the variance in the analysed data.

Fig. 8. Biplot of PC1 and PC2 for biochars, soil, and plant data. PCA of biochar properties, soil agrochemical parameters, plant productivity, and BCF values. Abbreviations: A—ash, DW—dry weight, EC—electrical conductivity, FC—fixed carbon, Org_C—organic carbon, VM—volatile matter, W—moisture.

PC1 mainly comprised biochar properties such as nitrogen, carbon, hydrogen, ash, fixed carbon, volatile matter, HHV content, electrical conductivity, and SBET. However, the BCF values of S, Mg, Ca, and K, the ammonium and nitrate contents in the soil, and the ϕNO parameter also contributed to PC1. PC1 distinguished control and D2 treatments from the other treatments in the PC1 and PC2 biplot, demonstrating the insignificant influence of 1% B2 application rate (Fig. 8). The main contributors to PC2 were the morphological and physiological parameters and the phytoremediation potential of the plant. The biochar pH, soil pH, and soil K and P_2O_5 contents strongly contributed to PC2. PC2 separated control and D1 treatments from B2 treatment at all doses (D2-D4). This distinction was based on the better representation of plant productivity and phytoremediation potential in the soil amended with B2 (Fig. 8).

The following statements described the correlation between the analysed parameters: a) biochar parameters were positively correlated with each other, with the exceptions of pH and moisture values, which did not show any correlation; b) agrochemical soil properties were positively correlated within the group, with the exceptions of soil pH and phosphate content, which did not show any correlation; c) stress indicators such as ϕII, SPAD, and ϕNO were positively correlated with each other but negatively correlated with ϕNPQ; d) ϕII and SPAD showed a strong positive correlation with the soil P_2O_5 content; e) soil NO_3 content had a strong positive correlation with such biochar parameters as volatile matter, ash, and hydrogen content; f) plant biomass DW showed a strong positive correlation with Fe and P accumulation.

The control treatment was characterised by higher Mg and Ca accumulation and higher ϕNPQ values (Fig. 8). The addition of 1% B2 (D2) influenced the uptake of Ti, Fe, P, Si, and Sr and, more importantly, led to increased biomass yield. Incorporation of 3% B2 (D3) improved chlorophyll fluorescence parameters, especially ϕII, and increased soil phosphate content, while an increase in ϕNO was observed with 5% B2. Incorporation of 5% B1 significantly improved the soil agrochemical parameters and increased the accumulation of Zn, Sr, and Rb.

The results suggest that the behaviour of two varied biochars and three different doses of B2 affected the soil agrochemical properties and plant parameters differently; only incorporation of B1 (D1) significantly changed the soil properties, while smaller doses of B2 (D2 and D3) did not have this effect.

4. Conclusion

According to basic physical (particle size, moisture, EC, SBET, and HHV) and chemical (elements content, A, EC, FC, VM, and pH) characteristics, the biochar produced by pyrolysis from aboveground Miscanthus biomass waste (B2) exhibited more favourable properties than biochar produced from Miscanthus TEs-contaminated rhizomes (B1).

The incorporation of biochar changed the properties of the initial soil (Org_C, K, P, NH_4, NO_3 contents, and pH); specifically, the Org_C content proportionally increased with incorporated biochar doses (1, 3, and 5%); the K content increased for the 3 and 5% doses only, which was rationalised by overall high content of this element in biochar, which improves its availability; the P content increased for 5% dose and decreased for the other doses likely due to the high SBET of the biochar; the NH_4 content essentially increased at doses 3 and 5%, however, the NO_3 concentration significantly decreased illustrating that biochar's nitrogen was in the form of ammonium; soil pH changed to an alkaline environment for all biochars and doses.

The changes in the nutrient elements of the soil after planting *S. oleracea* was evaluated. It was established that Org_C content decreased for all experimental variants, which may be explained by intensified mineralisation of Org_C compounds caused by high pH value which increased the soil porosity and water-holding capacity, triggered the activation of certain microbial groups, peptised the soil organomineral colloids led to their destruction. The soil pH became less alkaline toward the end of vegetation and effect was more visible for smaller doses of biochar (1 and 3% B2), associating with assimilation of a proportion of the alkaline cations, soil microbial activity and buffering. With continued vegetation, K content decreased by its immobilisation in plants, bounded by colloids and transformed into less available forms; P content decreased, caused by the fast transformation of mobile form into hard soluble salts and immobilisation by plants and microorganisms. The impact of Miscanthus biochars and doses on development, physiological parameters, and bioconcentration factors of testing plant *S. oleracea* was revealed.

Among three biochar doses (1, 3, and 5%) the dose of 3% was the most effective for the leaf surface area, DW, and monitored photosynthesis parameters (ϕII, SPAD, ϕNO, and ϕNPQ). With continuous vegetation, the action of the biochar was manifested in the dissociation of light-harvesting complexes of the photosynthetic reaction centres, showing that *S. oleracea* adapted to the earlier stress and achieved a balance between nutrients, water supply, and light.

The potential of *S. oleracea* to accumulate different elements from the soil was estimated and it was found that the plant did not uptake Al, Cr, Ni, and Pb; while for other elements three levels of BCFs were detected: less than 0.1 (Si, Ti, Fe, Rb, and Zr); less than 1 (Mn, Cu, and Sr); and more than 1 (Mg, P, S, K, Ca, and Zn). The detected peculiarities indicated the existence of antagonistic relationships in element pairs, which were enhanced in the presence of biochars.

The obtained results show that increasing the dose of biochars did not necessarily lead to a proportional improvement of plant's photosynthesis, development and biomass, and ensured the necessity of preliminary stage of biochar evaluation by testing plant before application at the field scale.

Author contribution statement

OK: Methodology, Validation, Investigation, Writing – Original Draft, Writing - Review & Editing. **VP:** Conceptualization, Investigation, Writing – Original Draft, Writing - Review & Editing, Supervision, Project administration, Funding acquisition. **AM:** Methodology, Software, Formal Analysis, Writing – Original Draft, Writing - Review & Editing, Visualization. **VK:** Methodology, Validation, Investigation, Writing - Review & Editing. **AH:** Methodology, Validation, Investigation, Resources, Data curation, Writing – Original Draft, Writing - Review & Editing. **BG:** Methodology, Resources, Writing - Review & Editing. **KK:** Investigation. **PL:** Methodology, Resources, Investigation. **PS:** Formal Analysis, Writing - Review & Editing, Resource.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Highlights:

- Biochar derived from waste is important for *Spinacia oleracea* L. development
- Biochar from aboveground waste biomass showed better characteristics
- 3% dose of biochar from waste biomass was the most effective compared to 1 and 5%
- Biochar enhanced antagonistic interactions between elements' pairs

Figure 1

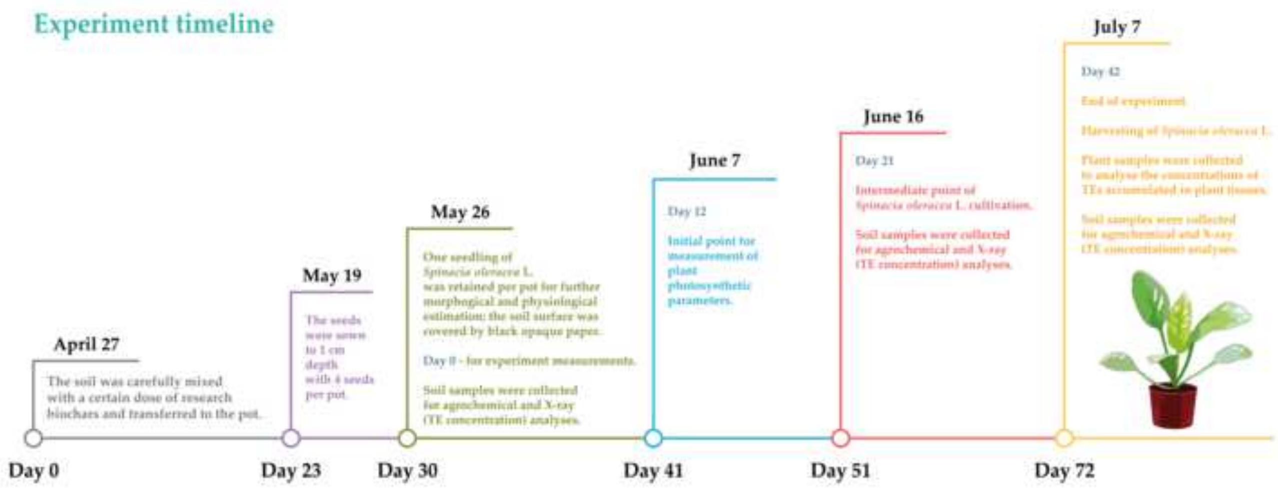

Experiment timeline
April 27
The soil was carefully mixed with a certain dose of research biochars and transferred to the pot.
May 19
The seeds were sown to 1 cm depth with 4 seeds per pot.
May 26
One seedling of Spinacia oleracea L. was retained per pot for further morphological and physiological estimation: the soil surface was covered by black opaque paper.
Day 0 - for experiment measurements.
Soil samples were collected for agrochemical and X-ray (TE concentration) analyses.
June 7
Day 12
Initial point for measurement of plant photosynthetic parameters.
June 16
Day 21
Intermediate point of Spinacia oleracea L. cultivation.
Soil samples were collected for agrochemical and X-ray (TE concentration) analyses.
July 7
Day 42
End of experiment
Harvesting of Spinacia oleracea L.
Plant samples were collected to analyse the concentrations of TEs accumulated in plant tissues.
Soil samples were collected for agrochemical and X-ray (TE concentration) analyses.
Day 0
Day 23
Day 30
Day 41
Day 51
Day 72

Figure 2

Click here to access/download;Figure;Fig. 2.jpg

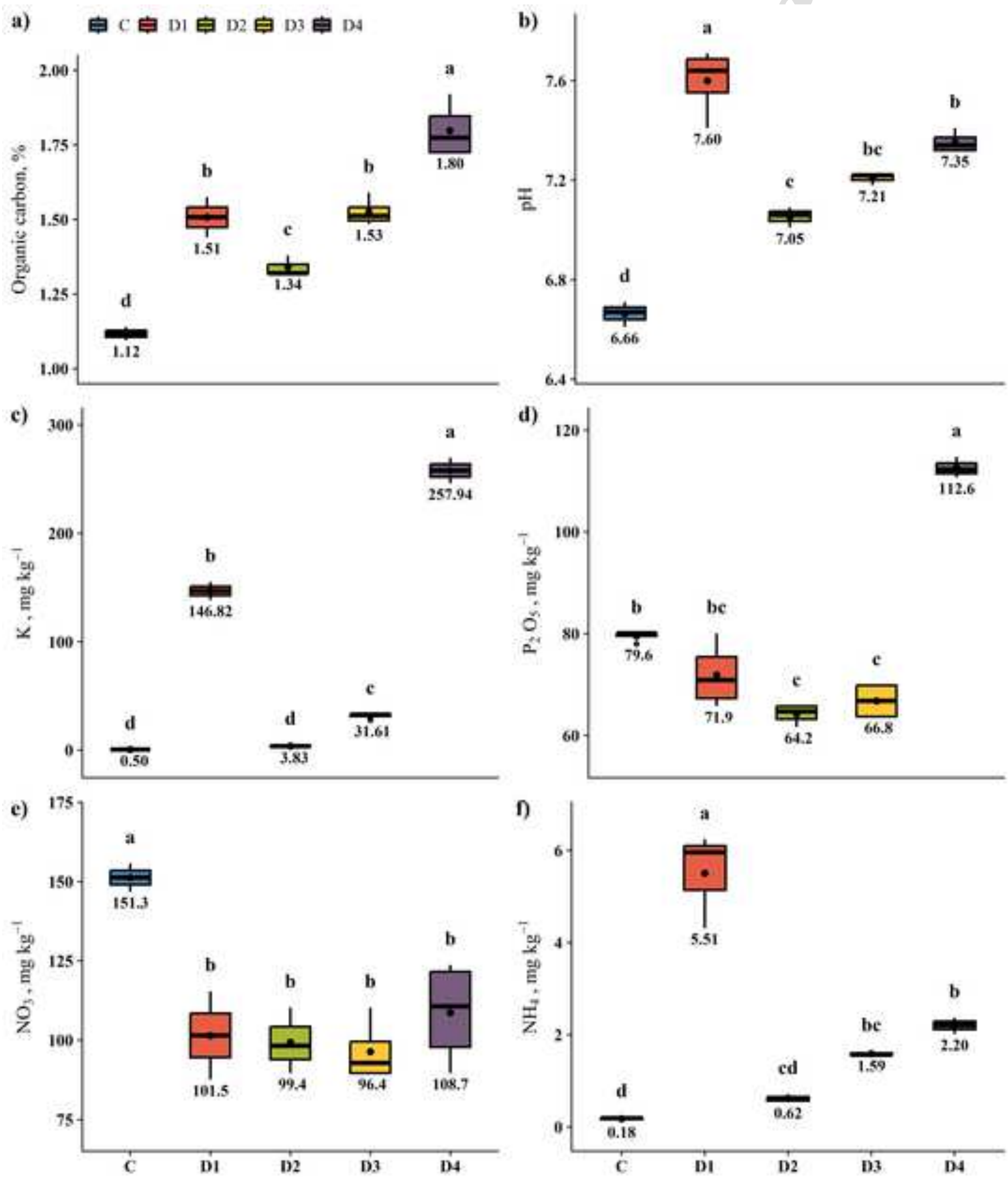


Figure 3

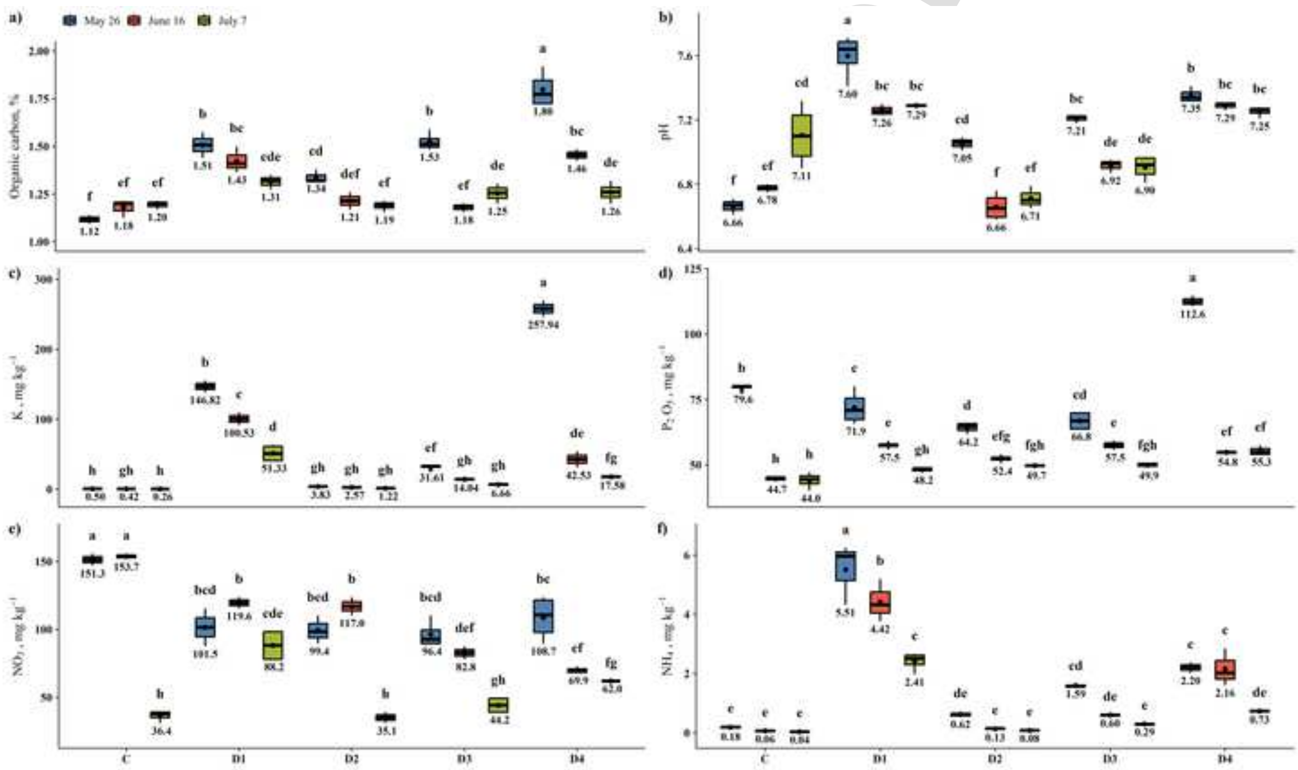


Figure 4

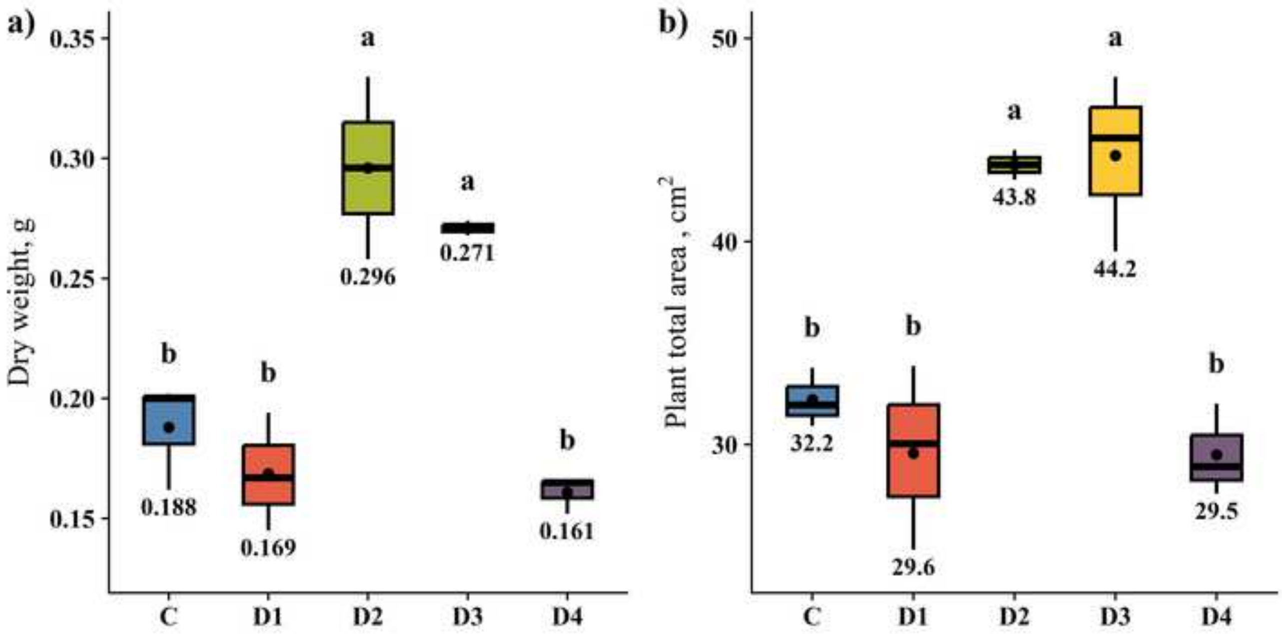

a)
Dry weight, g
0.35
0.30
0.25
0.20
0.15
a
a
b
b
b
0.296
0.271
0.188
0.169
0.161
C
D1
D2
D3
D4
b)
Plant total area , cm2
50
40
30
a
a
b
b
b
43.8
44.2
32.2
29.6
29.5
C
D1
D2
D3
D4

Figure 5

Click here to access/download;Figure;Fig. 5.jpg

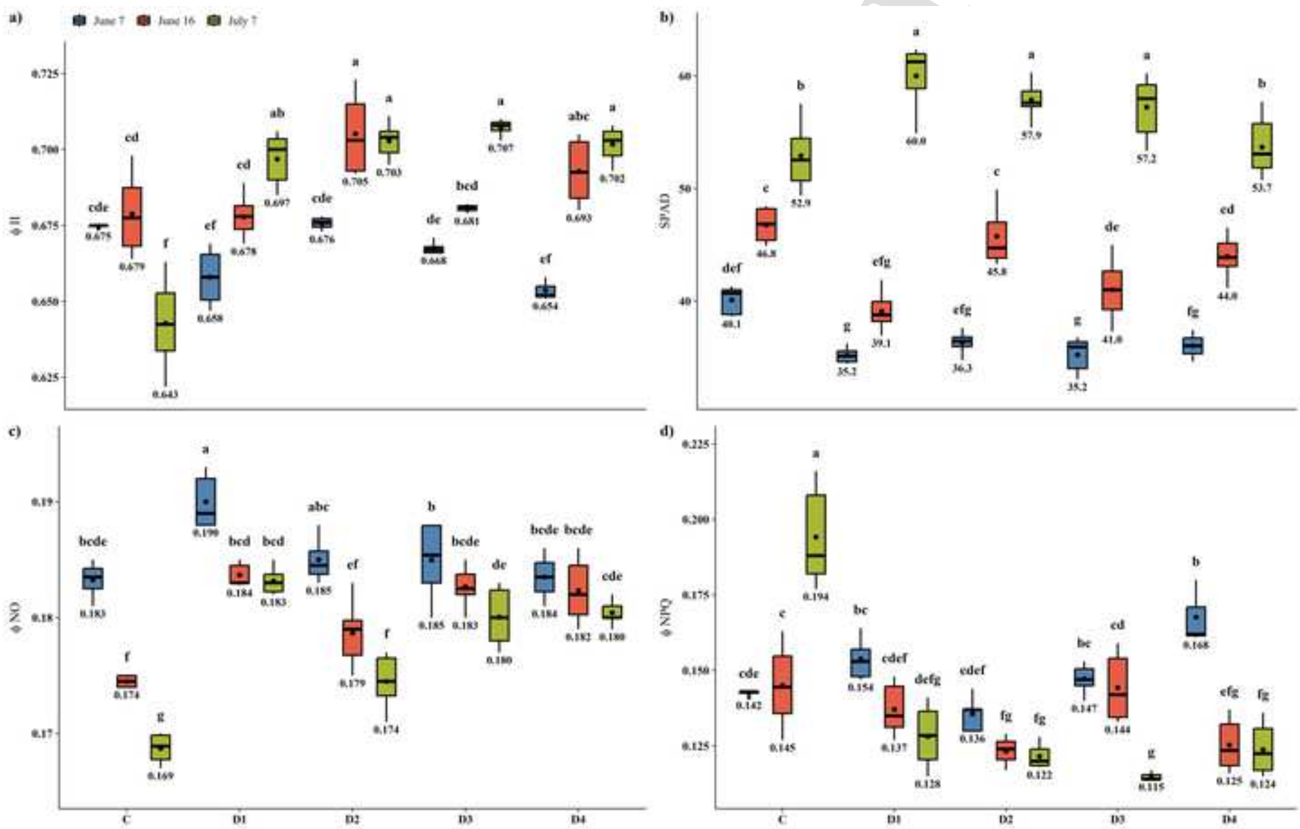
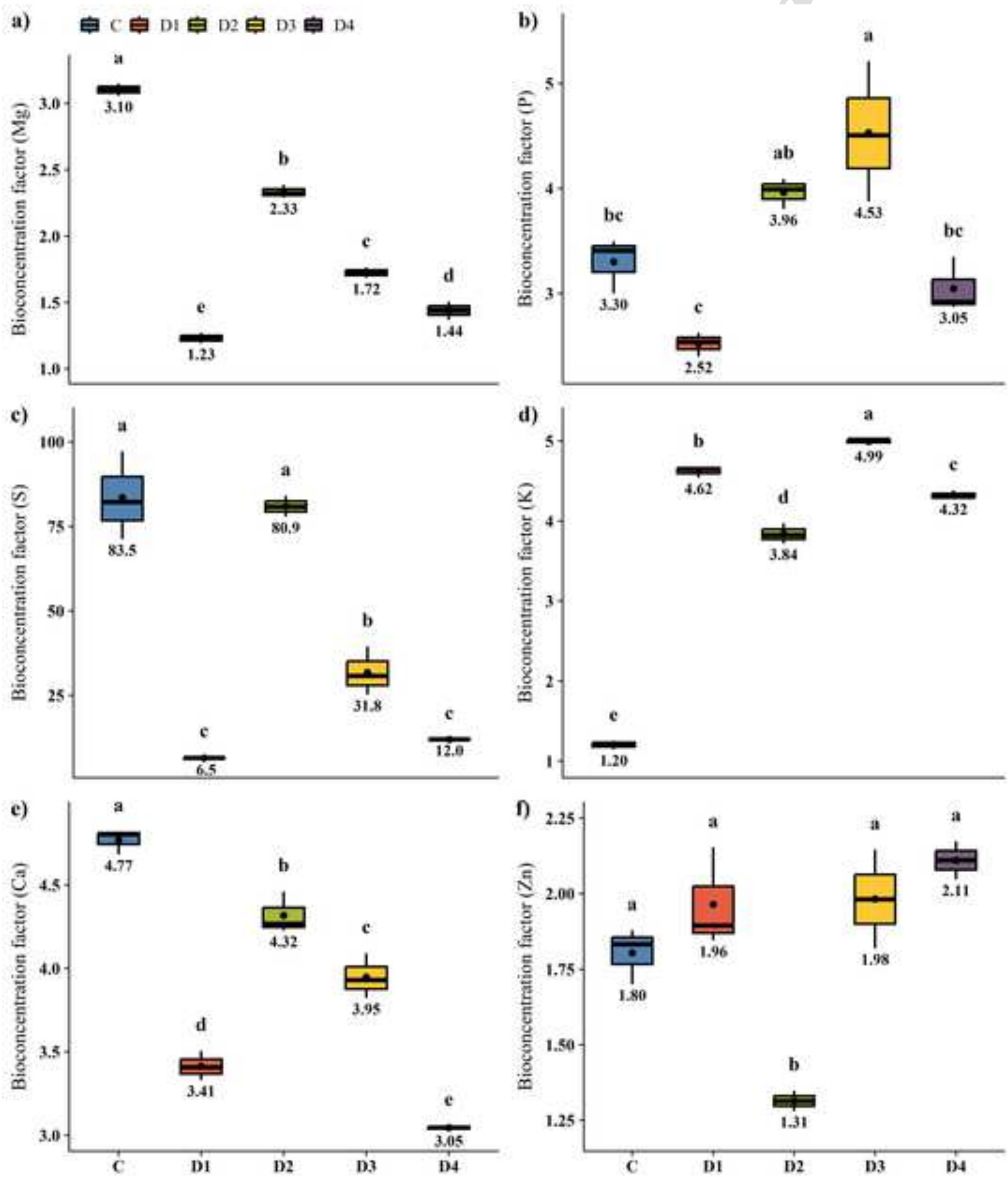


Figure 6



a)
C D1 D2 D3 D4
Bioconcentration factor (Mg)
a
3.10
e
1.23
b
2.33
c
1.72
d
1.44
b)
Bioconcentration factor (P)
bc
3.30
c
2.52
ab
3.96
a
4.53
bc
3.05
c)
Bioconcentration factor (S)
a
83.5
c
6.5
a
80.9
b
31.8
c
12.0
d)
Bioconcentration factor (K)
e
1.20
b
4.62
d
3.84
a
4.99
c
4.32
e)
Bioconcentration factor (Ca)
a
4.77
d
3.41
b
4.32
c
3.95
e
3.05
f)
Bioconcentration factor (Zn)
a
1.80
a
1.96
b
1.31
a
1.98
a
2.11
C
D1
D2
D3
D4

Figure 7

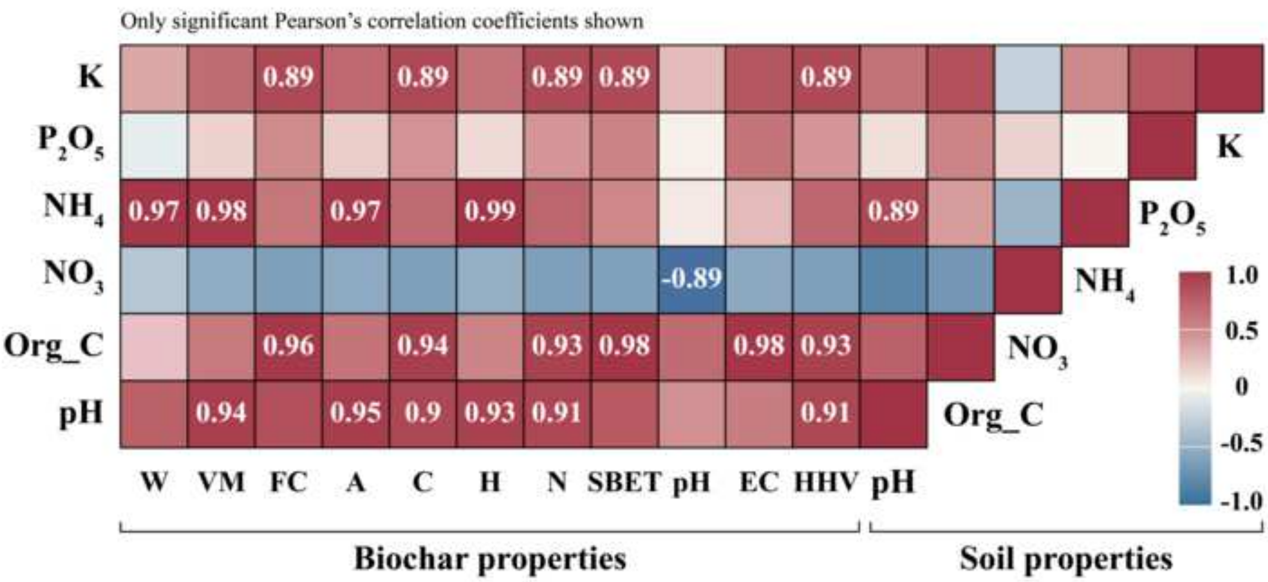
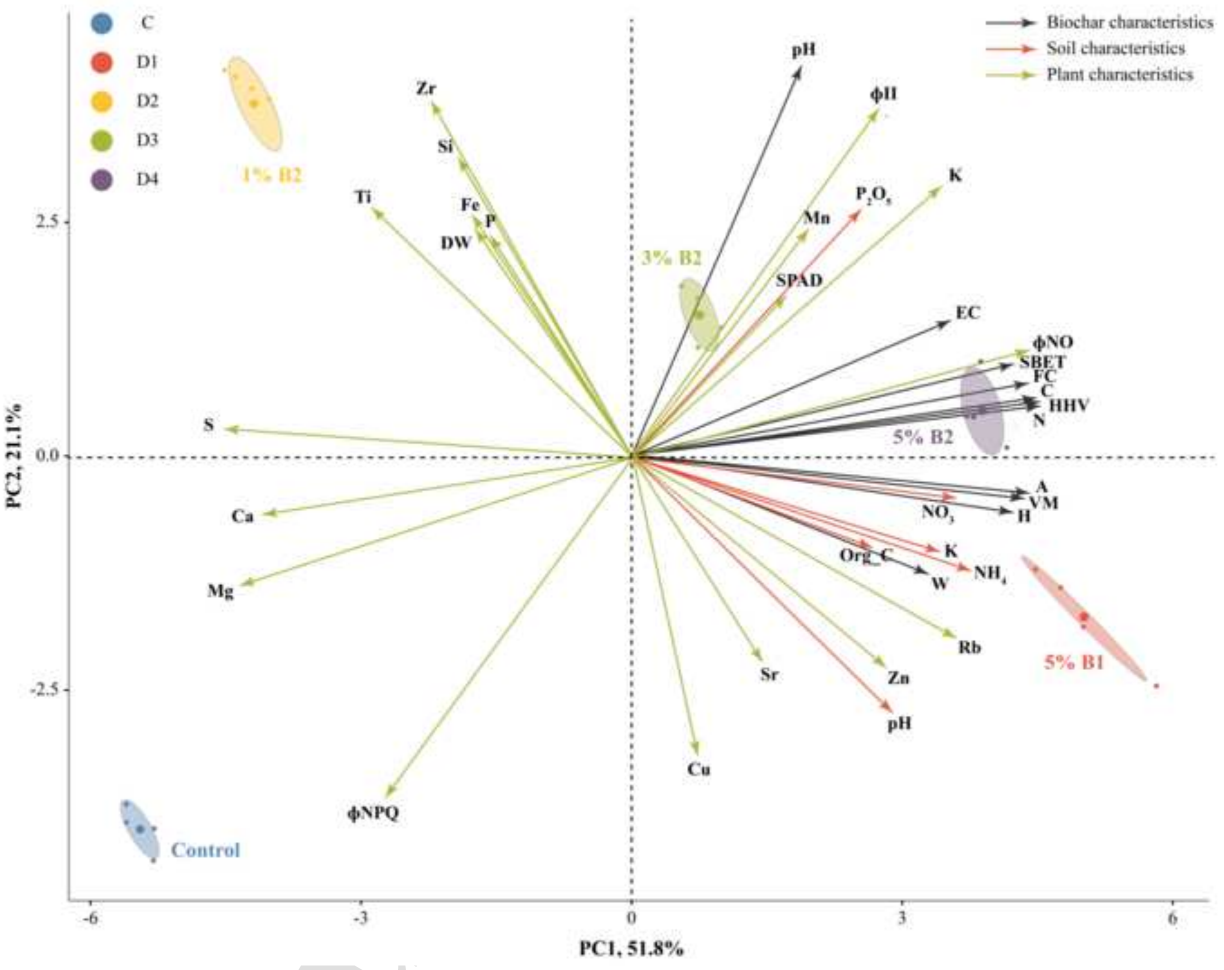

Only significant Pearson's correlation coefficients shown
K
P_2O_5
NH_4
NO_3
Org_C
pH
0.89 0.89 0.89 0.89 0.89
0.97 0.98 0.97 0.99 0.89
-0.89
0.96 0.94 0.93 0.98 0.98 0.93
0.94 0.95 0.9 0.93 0.91 0.91
W VM FC A C H N SBET pH EC HHV pH
Biochar properties
Soil properties
1.0
0.5
0
-0.5
-1.0

Figure 8


C
D1
D2
D3
D4
1% B2
3% B2
5% B2
5% B1
Control
Biochar characteristics
Soil characteristics
Plant characteristics
pH
ϕII
K
P_2O_5
Mn
SPAD
EC
ϕNO
SBET
FC
C
HHV
N
A
VM
H
NO_3
K
NH_4
Org_C
W
Rb
Zn
Sr
pH
Cu
ϕNPQ
Zr
Si
Ti
Fe
P
DW
S
Ca
Mg
PC1, 51.8%
PC2, 21.1%

Conflict-of-interest statement

All of the authors have read and approved the paper which has not been published previously nor it is being considered by any other peer-reviewed journal.
The authors have no conflicts of interest to declare.