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Dynamic of Morphological and Physiological Parameters and Variation of Soil Characteristics during *Miscanthus* × *giganteus* Cultivation in the Diesel-Contaminated Land

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Abstract: *Miscanthus* × *giganteus* (*M.* × *giganteus*) is a perspective plant produced on marginal and contaminated lands with biomass used for energy or bioproducts. In the current study, *M.* × *giganteus* development was tested in the diesel-contaminated soils (ranged from 250 mg kg^{-1} to 5000 mg kg^{-1}) and the growth dynamic, leaves quantity, plants total area, number of harvested stems and leaves, SPAD and NPQt parameters were evaluated. Results showed a remarkable *M.* × *giganteus* growth in a selected interval of diesel-contaminated soil with sufficient harvested biomass. The amendment of soil by biochar 1 (produced from wastewater sludge) and biochar 2 (produced from a mixture of wood waste and biohumus) improved the crop's morphological and physiological parameters. Biochar 1 stimulated the increase of the stems' biomass, while biochar 2 increased the leaves biomass. The plants growing in the uncontaminated soil decreased the content of NO_3, pH (KCl), P_2O_5 and increased the content of NH_4. Photosynthesis parameters showed that incorporating biochar 1 and biochar 2 to the diesel-contaminated soil prolonged the plants' vegetation, which was more potent for biochar 1. *M.* × *giganteus* utilization united with biochar amendment can be recommended to remediate diesel-contaminated land in concentration range 250–5000 mg kg^{-1}.

Keywords: *Miscanthus* × *giganteus*; diesel-contaminated soils; biochar; morphological and physiological parameters

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1. Introduction

The contamination of soil by hydrocarbons is a severe environmental problem caused mainly by anthropogenic activities, particularly by processing and transportation of fossil fuels or military activities [1]. Hydrocarbon's release deteriorated the land, surface and underground waters [2], which sometimes excluded the contaminated localities from the land bank [3]. Such deteriorated places possess a tremendous hazardous risk to human health and living organisms with potential carcinogenic and mutagenic effects [4,5]. Compared with traditional physical–chemical techniques, bioremediation combined with phytoremediation can offer a sustainable and less expensive alternative to contaminated land restoration even if the time required to reach the target endpoints is often a severe drawback [6]. A plant-assisted remediation technology can be implemented in situ when treating large surface areas of soils contaminated by organics [7]. However, considerable effort is needed to transition technology from the laboratory to the field conditions. In this regard, proper plant selection, the choice of agricultural practices and elimination of the plant's stress are essential components [8,9].

Miscanthus × *giganteus* (*M.* × *giganteus*) is a promising second-generation energy crop [10,11] that showed the ability to grow in marginal and contaminated lands [12–15]. It is a rhizomatous, lignocellulose-rich perennial grass grown worldwide as a source of energy or bio-based products [16,17]. This plant shows rapid growth and high yields in soils of various anthropogenic origins and is among recommended biofuel crops for commercial production in countries with limited energy resources [18].

A growing number of studies have described the crop's successful application for phytoremediation of the trace elements (TEs) contaminated soil [19] and soil health improvement during vegetation [20]. However, few publications introduced the plant's successful application to remediate organic's contaminated lands [12], including the petroleum hydrocarbons contaminated soils [6,21,22]. Nevertheless, such lands are among the most prevalent polluted areas in the environment [23,24].

During the *M.* × *giganteus* growing process, the soil's parameters changed [12]. Incorporating an amendment to the soil generally improves the plant's production during vegetation and increases the harvested biomass [25–28]. It was reported that adding sludge increased the produced *M.* × *giganteus* biomass during crop cultivation in the post-mining TEs contaminated soil [25–27], and the effect was more substantial with years of cultivation. Adding activated carbon to the soil contaminated by pesticides decreased the uptake of contaminants to the aboveground crop's biomass and made it available for further energy processing [28]. *M.* × *giganteus* demonstrated sufficient development during two vegetation seasons in the TEs contaminated former military soil [29] and three vegetation seasons in the TEs-contaminated post-mining land [19].

Minimal data are available on applying *M.* × *giganteus* to the hydrocarbons contaminated soils [6,22], counting data about plant growth supported by carbon contented amendment-biochar, i.e., porous, the polyaromatic product of an incomplete thermochemical conversion of organic biomass or wastes. This amendment may generally increase crop yields [30]. However, depending on the biochars' properties, plant productivity's impact remains unpredictable mainly due to complex interactions between soil and the environment [31,32]. Remediation of hydrocarbon contaminated soil is still a growing technology that often uses co-bioremediation of plants and microbes [6,7,12].

The simultaneous effect of soil amendment to *M.* × *giganteus* morphological state, growth parameters and soil changes while the plant was cultivated in the diesel-contaminated land was not much under experimental investigation. To overcome this gap, the current research was designed, which had a goal to examine the production of *M.* × *giganteus* in the differently artificially diesel-contaminated soils, to assess the role of two different biochars when the plant was developed in contaminated soils, to research the change of soil parameters during vegetation, and to evaluate the non-photochemical quenching (NPQt) and relative chlorophyll (SPAD) values as a response to plant's stress.

In the current study, the following hypotheses were under research:

(a) Impact of amending soil by biochars to the soil parameters, specifically: pH, NO_3, NH_4, P_2O_5, and K during *M.* × *giganteus* vegetation versus changes of parameters in soil without crops;
(b) Impact of varied diesel concentrations in the soil and its biochar amending to *M.* × *giganteus* development verifying by plant's morphological and physiological parameters;
(c) Ensuring *M.* × *giganteus* adaptive potential while the crop was cultivated in the diesel-contaminated soils, including with biochars via determination of NPQt and SPAD values.

2. Materials and Methods

2.1. Design of the Pot Experiment

One vegetation season experiment was established in the greenhouse condition using artificially diesel-contaminated soil. The initial soil was taken at the agricultural research field of Volodymyr Hnatiuk National Pedagogical University in Ternopil, Ukraine; the site's GPS coordinates are 49.5418397 N, 25.568175 E. Following World Reference Base for Soil

Resources classification [33], soil belongs to chernozem (phaeozems). The agrochemical parameters of the soil are presented in Table 1. Following standards [34], the initial soil had a neutral reaction of salt solution, low content of organic matter, low content of mineral nitrogen, high content of phosphorous and slightly high content of potassium.

Table 1. Soil abundance used in the pot experiment.

Agrochemical Parameters	Unit	Mean ± SD
pH (KCl)		6.46 ± 0.03
Organic matter	%	1.13 ± 0.04
NO_3	mg kg^{-1}	126 ± 9
NH_4	mg kg^{-1}	1.7 ± 0.2
P_2O_5	mg kg^{-1}	176 ± 12
Exchangeable K	mg kg^{-1}	116.9 ± 13.2

The content of TEs in the initial soil was determined using the X-ray fluorescence analysis, and the layout was described in detail in Pidlisnyuk et al. [19]. The results are presented in Table 2. The content of TEs in the initial soil was typical for such sort of soil and did not violate the standards of EC and Ukraine.

Table 2. TEs concentrations in the soil used in the pot experiment; depth of soil sampling: 0–30 cm.

TEs	MPC, mg kg^{-1}		TEs Concentration in the Soil, mg kg^{-1}
	EC [35]	Ukraine [34]	
Mn	NA	1500	628.3 ± 44.4
Fe	NA	NA	22,073 ± 129.6
Cu	100	55	17.0 ± 7.5
Zn	200	300	49.4 ± 5.7
Pb	60	30	25.1 ± 3.5

The soil sampling was carried out using the standard approach DSTU 4287:2004 [34] from one 5 × 5 m testing square. Five samples were taken at the depth 0–0.3 m using quartering and mixed. That mixed soil was used in the pot experiment. Before the experiment, the soil was dried at air-dried conditions until the constant weight. Dried soil was passed through a sieve with a pore diameter of 2 mm as requested by DSTU ISO 11464:2007 [36] to remove plant materials and stones, followed by thorough mixing. The soil was stored in hermetic glass containers until use.

The greenhouse experiment was carried out in the pots. After the bottom of the pots was filled with a draining material weighing 1.0 kg, drainage material was covered with gauze and river sand weighing 1.0 kg and again covered with gauze; thereafter, each pot was filled in with the soil weighing 8.0 kg. To prevent drying of the soil and the diesel release, each pot was covered with a 1 kg layer of sand. The individual pot with the research soil was 15 L in volume and weighed 10 kg. The pots were watered, while necessary during vegetation season using the pot water.

The plant studied was *M.* × *giganteus* J.M. Greef and Deuter ex Hodkinson and Renvoize (Angiospermae: Poaceae) [18]. Rhizomes were three-year-old taken from the *M.* × *giganteus* plantation in Zagreb, Croatia; the plantation was established using rhizomes of varieties "Osinnii zoretsvit" cultivated by the Institute of Energy Crops and Sugar Beets, National Academy of Agrarian Science, Ukraine [37].

Two rhizomes of *M.* × *giganteus* were planted in each pot; planting rhizomes had an average size of 20 cm. All pot experiments were established on the same day.

Each of the various experiments was carried out in three replicates. In parallel, one experiment was established with soil without planted rhizomes.

There were three variations of the experiment with soil amendments:

(a) Experiment without amendments (marked as Ctr);

(b) Experiment with biochar 1 (marked as B1);
(c) Experiment with biochar 2 (marked as B2).

2.2. Soil Artificial Contamination and Amending

The collected and prepared soil was artificially contaminated by diesel DSTU 7688:2015 [38], produced from oil per GOST 9965-76 [39] with the mass part of the polycyclic aromatic hydrocarbons equals to 11%. The soil treatment was done in separate batches; each was equal to 30 kg. The mixture was accomplished in the cement router. In the beginning, the soil was mixed with the targeted amount of selected biochar for 30 min, then the calculated amount of diesel was added, and this soil was remixed for another 30 min.

The following concentration of soil contamination by diesel was selected: 0; 250 mg kg^{-1}; 1000 mg kg^{-1}; 3000 mg kg^{-1}; 5000 mg kg^{-1} based on the published literature on the concentration of diesel products in the aged sites [6,21] and diapason of diesel concentrations in the artificially contaminated soil when a crop (oats) demonstrated satisfactory development [40]. A range of diesel concentrations reported for locations where the accidental release occurred during transportation was also considered [3].

Two sorts of soil amendments were used, both organic origins: B1 and B2; amendments were added to the soil in amount 5% related to the soil weight. That proportion between biochar and treated soil was reported as optimal in phytoremediation processes [41]. B1 was produced by firm Almeco (Czech Republic) from municipal wastewater treatment plant sludge from Brno, Czech Republic. The biochar is referred to the Czech Ministry of Agriculture requests (2000) [42].

B2 was produced by firm F.O.P. Osypenko (Ukraine) as an experimental amendment DSTU EN ISO/EC 17065 [43] and consisted of the mixture of wood biochar—25%, biohumus—50%, and sand—25%. The characteristics of B1 and B2 are presented in Table 3.

Table 3. Elemental analysis of the research amendments (B1 and B2).

TEs	B1, mg kg^{-1}	B2, mg kg^{-1}
Mg	13,139.6 ± 1622.4	14,224.3 ± 2122.1
Al	38,762.9 ± 767.3	22,223.8 ± 977.8
Si	141,728.1 ± 726.8	272,941.6 ± 1692.0
P	106,458.1 ± 919.8	46,767.8 ± 1002.0
S	15,711.4 ± 116.1	7510.8 ± 133.4
K	10,677.8 ± 1552.9	60,664.0 ± 5726.2
Ca	94,085.4 ± 1143.1	71,290.6 ± 3621.5
Ti	4811.5 ± 359.5	4906.0 ± 927.5
Cr	453.0 ± 94.5	-
Mn	896.5 ± 72.4	821.4 ± 208.3
Fe	123,923.4 ± 452.1	35,621.2 ± 411.8
Ni	137.4 ± 28.1	78.9 ± 48.9
Cu	627.2 ± 24.2	152.1 ± 36.0
Zn	6847.5 ± 54.9	727.2 ± 36.4
Sr	485.0 ± 6.3	554.2 ± 12.9
Zr	260.6 ± 5.7	662.3 ± 14.8
Sn	57.4 ± 19.6	-
Pb	100.0 ± 8.4	81.9 ± 15.4

There were five artificially contaminated by diesel soils, marked as:

A = 0 mg kg^{-1}; B = 250 mg kg^{-1}; C = 1000 mg kg^{-1}; D = 3000 mg kg^{-1}; E = 5000 mg kg^{-1}.

Altogether there were 45 pots with two rhizomes of *M.* × *giganteus* planted in each pot; additionally, 15 pots were established with soil without plants. The total number of monitored experimental pots was 60 units, in which 90 *M.* × *giganteus* plants were under research.

2.3. Analysis of Soil Parameters

Different soil parameters were monitored during *M.* × *giganteus* growth, which was determined using the standard methods. Total organic matter was determined using the method of Tyurin DSTU 4289:2004 [44]; the content of nitrate's nitrogen was determined following DSTU 4725:2007 [45], the content of ammonium's nitrogen was determined following DSTU 4725:2007 [45]; mobile forms of phosphorus and potassium were determined using Chirikov DSTU 4115:2002 [46,47], the pH of the soil (salt extracted) was measured following DSTU ISO 10390:2001 [48].

2.4. Measuring of Plant's Morphological and Physiological Parameters

Two sorts of parameters were under monitoring:

(a) Changes of plants' bioparameters: height, the number of stems; the number of leaves per one stem; leaves width and length, from which the leaves surface area (LSA) was calculated using the following equation:

$$LSA = Leaf\ length \times Leaf\ width \times 0.67 \quad (1)$$

(b) Changes in plant state during vegetation by measuring chlorophyll fluorescence [49,50]. The measurement was made on intact, fully expanded leaves using the MultispeQ v1.0 device [51] linked to the PhotosynQ platform (http://www.photosynq.com/technology, accessed on 17 February 2021).

The measurements of morphological and physiological parameters were provided for all plants monitored in the pots' experiments. The plant height was determined for the highest and longest leaf, i.e., the stem's height and the leaf's length (Table S1). The measuring was done separately for each designed experiment, including six parallel plants (two plants per pot and three replications). To determine the leaf surface area's assimilation, the sum area of all leaves of six parallel plants in one series was determined. Using these measurements, the morphological parameters (the plant's height and leaf surface area) were statistically calculated.

Each time measuring the physiological parameters, the second fully developed leaf from the plant's top was investigated. There were three repetitions of the same variant of the experiment and two plants in each pot.

The relative chlorophyll (SPAD) content and the non-photochemical quenching (NPQt) were estimated followed the approach described at [51–54].

The pot experiment started on 12 April 2019 and finished on 26 November 2019, when the harvest of *M.* × *giganteus* was done. The experiment's duration was 228 days, and from those measurements of bioparameters were provided from the beginning of vegetation to the appearance of the first yellow leaves. At harvest, the plant's aboveground biomass was cut and weighed (separately leaves and stems) after drying to the constant weight. The drying of biomass was first provided about 6–8 h in the oven at temperature 100–105 °C, then dried biomass was weighted and again put to the oven; the procedure continued to the constant weight of biomass when the differences between the last two weightings were less than 0.0001 g.

2.5. Statistical Analysis

The statistical data processing was conducted using the RStudio software (version 1.3.959, R Studio PBC, 2020). The multivariate analysis of variance (MANOVA) was carried out to detect a statistically significant difference between changes in soil parameters depending on the presence of amendments and *M.* × *giganteus* plants and the level of diesel contamination. MANOVA was also used to estimate *M.* × *giganteus* physiological parameters depending on three factors: soil treatment, diesel contamination levels, and time (changes during one vegetation season) (Tables S2 and S3). When MANOVA proved a significant difference, Tukey's HSD test was performed to compare. Based on Tukey's HSD test, treatments were categorized (by letters in descending gradation), and boxplots/graphs were created.

3. Results and Discussion

3.1. Impact of M. × giganteus Growing to the Parameters of Uncontaminated Soil

During growth, *M. × giganteus* is characterized by high nutrient uptake efficiency because of the extensive root system [55,56]. Biochar is considered an amendment that improves soil conditions and stimulates plant development, bioparameters [41], and dry harvested biomass [57]. Generally, adding biochar to the soil results in an increase in soil pH, the total carbon and soil nutrients content [58].

At the first stage, the impact of *M. × giganteus* growing to the control uncontaminated soil parameters was evaluated when B1 and B2 amended the soil. The changing of soil parameters, i.e., pH, NO_3, NH_4, P_2O_5, and K during the vegetation period when *M. × giganteus* grew in the pots (labelled as M) and in pots without crops (labelled as W), is presented in Figures 1 and 2a–d (for each parameter separately).

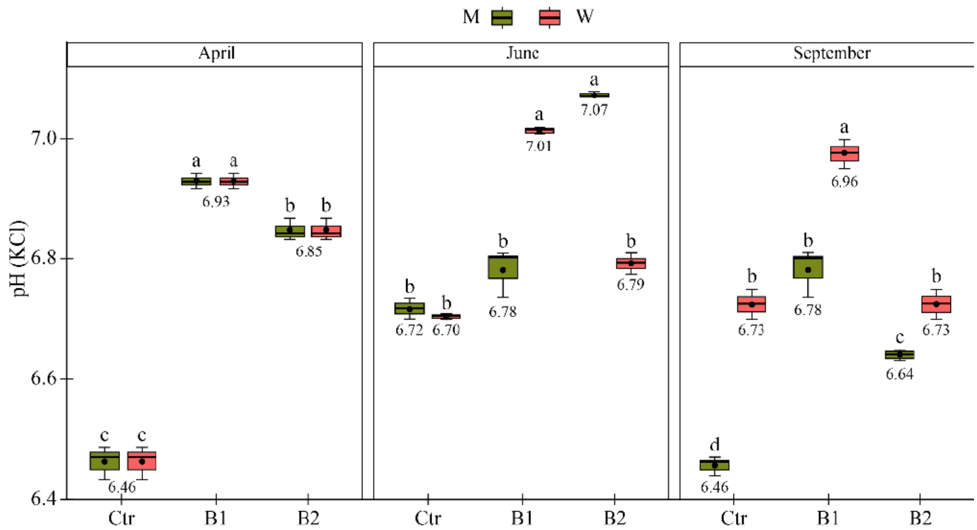

Figure 1. pH (KCl) changes in the soil during the vegetation season; treatments not sharing one letter are significantly different ($p < 0.001$).

The researched soil had a neutral reaction at the beginning of the experiment: the pH (KCl) value was 6.46 (Figure 1). When soil amendments were added to the soil, pH increased to 6.93 for B1 and 6.85 for B2. This trend is following literature data [58] when adding biochar led to alkalization of the soil.

With vegetation, the soil's pH slightly decreased. In particular, it was in September for system B2. For system W (without crop), the pH did not change (within the statistical difference). The decreasing of pH can be due to taking alkaline elements (Ca and Mg) by the plant during its development.

The incorporation of biochar into the soil enriched it for nitrogen; specifically, B1 enriched the soil for ammonium nitrogen, while B2 mainly enriched the content of nitrate nitrogen. During crop's growing, the nitrogen content in both forms decreased in the soil; in particular, this decreasing was observed for nitrogen nitrates. This trend is typical for different plants' vegetation due to nitrogen's good availability to the plant during the growing period [59,60]. In our case, the availability of nitrogen was higher, while B2 was presented in the system. The observed increase of NO_3 concentration in July compared to April ensured the active elaboration of nitrogen from both amendments and nitrification of ammonium nitrogen thanks to intensive microbiological activities [59]. The prevalence of using NO_3 nitrogen during the plant's development was also pointed because the content of ammonium nitrogen did not change significantly during the experiment: this can be explained by less ammonium nitrogen in the plant [60]. Moreover, the higher content of

NH_4 in the soil with plants compared to the soil without plants showed its less active role in plant's development and soil microbes in the rhizosphere, promoting the accumulation of NH_4 in the soil [59].

The phosphorus concentration was higher at the initial soil amended with B2 compared with control because B2 had phosphorus in the content. However, amending the initial soil by B1 did not increase this element (Figure 2c). With vegetation, the phosphorus transformed to a more available form, and its content increased for the soil amended by B1 caused by intensive usage of phosphorus by the plant for the development. As a result, in September, the soil's phosphorus content decreased for the system with plant and B1; in the system without plant, phosphorus was still available in the soil. The phosphorus availability was higher for the soil with B2 than for B1, and its content decreased started from June to September.

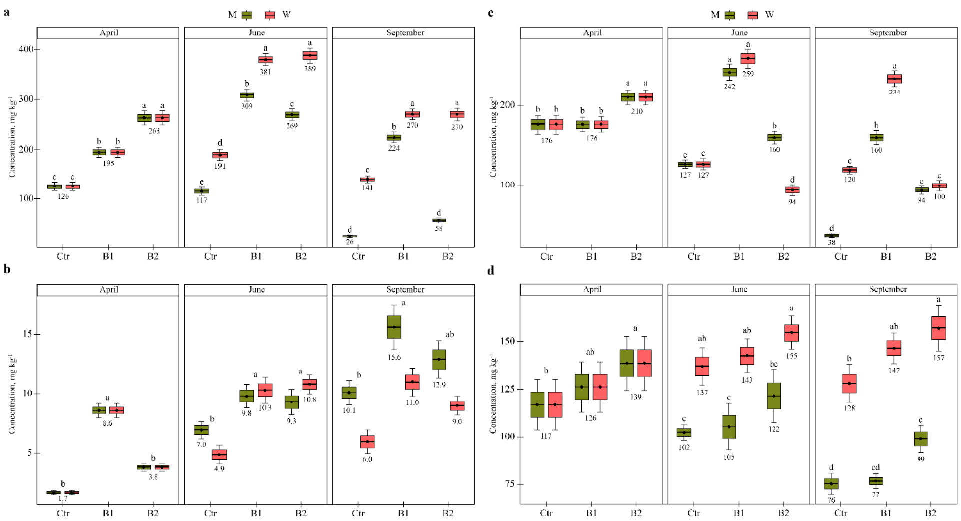

Figure 2. Soil parameters changes during M. × giganteus growing in uncontaminated soil (A = 0 mg kg^{-1}): (**a**) NO_3 ($p < 0.001$); (**b**) NH_4 ($p < 0.001$ for difference between statistical groups, namely between Ctr, B1 and B2); (**c**) P_2O_5 ($p < 0.001$); (**d**) K (April: $p < 0.05$; June: $p < 0.001$; September: $p < 0.001$); treatments not sharing one letter are significantly different.

Adding amendments to the initial soil increased the potassium's content in both cases; however, the effect was higher for B2 (Figure 2d). Results showed that *M. × giganteus* used this element during growing because potassium's content decreased starting from June, then the effect was evident for September due to a fewer utilization of this element by the plant.

During growth, *M. × giganteus* intensively uptakes nutrients having broad roots' structure [55,56]. The data received in the current study confirmed that fact because the concentration of NO_3, P_2O_5 and K decreased in the soil with growing *M. × giganteus* compared to the soil without the plant. Indeed, the content of NH_4 increased consistently to the end of vegetation, which showed its less active role in *M. × giganteus* development. The increase may be additionally caused by soil microbes' activities in the plant's rhizosphere promoting the accumulation of NH_4 in the soil [6]. That is why it is recommended to compensate for the nutrient uptake by fertilizers when the crop was cultivated multiyear in the field conditions; nevertheless, the nutrient requirements of *M. × giganteus* are low than other crops [61].

3.2. Impact of Soil Contamination by Diesel to M. × giganteus Bioparameters

The soil contamination by hydrocarbons leads to profound changes in soil properties, i.e., deterioration of water–air and physicochemical properties, absorption capacity, and reduced reserves of mineral nutrients, which overall violated soil fertility [62]. The hydrocarbons can hinder the uptakes of nutrients and water from the soil by plants [63], cause their bio-membrane injury by leading to accumulation of reactive oxygen species [64], and inhibit the photosynthesis and transpiration [65].

The phytotoxicity of the diesel-contaminated soil increases with the level of contamination, and extreme contamination levels can finally lead to plant death and the degradation of plant communities [22,66].

The remediation of hydrocarbon contaminated soil is still a growing technology that is rather often used co-bioremediation of plants and microbes [67,68].

Earlier, *M.* × *giganteus* showed proper development in the organics contaminated soils [28] supported by soil amendments. In the current study, applying this crop to the diesel-contaminated soils was explored, including the cases when biochars amended the soil.

The dynamic of bioparameters during *M.* × *giganteus* growing in the diesel-contaminated soils with biochar's amendments is presented in Figure 3 (for the height of plant), Figure S1 (for the leaves quantity) and Figure 4 (for leaf blade area).

As expected, with increasing the level of soil contamination by diesel, the plant's parameter (height) decreased (Figure 3), and the most significant deterioration in the plant's development was determined for the highest level of diesel contamination (marked as E). Same time at the small concentrations of soil contamination (experiment B and C), the parameters of *M.* × *giganteus* were similar to those observed for the control soil (A). It may be concluded that *M.* × *giganteus* may successfully be applied to the diesel-contented soil in a range of contamination concentrations 0–1000 mg kg^{-1} because those doses of contamination disturb the plant's development insignificantly. The soil contamination's negative impact was neglected when biochar was incorporated into the system; the plant height in September for cases B and C was similar to A in the presence of B1 and B2.

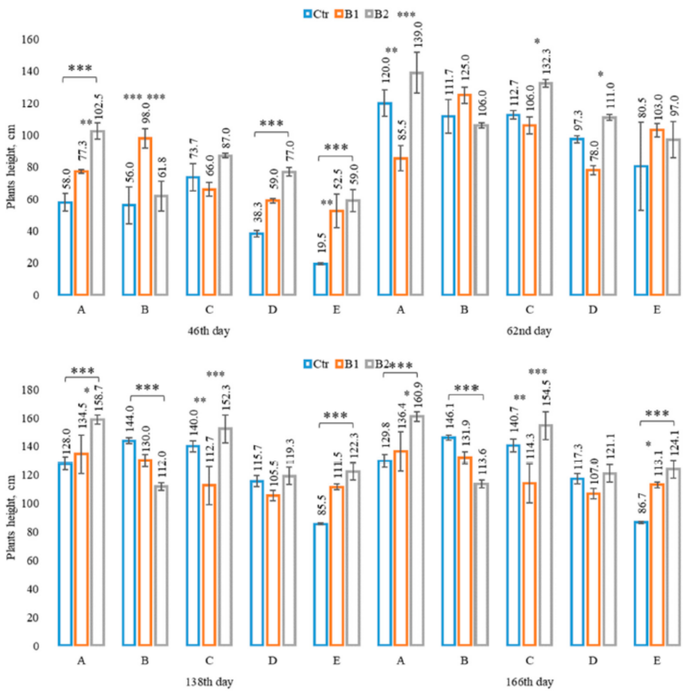

Figure 3. Growth dynamic of M. × giganteus during vegetation season. Asterisks denote the significant difference between compared pairs (*—$p < 0.05$; **—$p < 0.01$; ***—$p < 0.001$). (**A**) Diesel concentration in soil was 0 mg kg^{-1}; (**B**) diesel concentration in soil was 250 mg kg^{-1}; (**C**) diesel concentration in soil was 1000 mg kg^{-1}; (**D**) diesel concentration in soil was 3000 mg kg^{-1}; (**E**) diesel concentration in soil was 5000 mg kg^{-1}.

The morphological characteristics of *M.* × *giganteus* when the plant was cultivated in the traditional agricultural land showed a close correlation between height and weight of the shoots, numbers of leaves in each shoot and surface of the leaves [69]. Current research confirmed this correlation (Figure 3, Figure 4 and Figure S1) when an essential impact of the soil contamination on the morphological parameters was detected. In the experiment without contamination (A), the increasing number of leaves was observed during vegetation; at the highest diesel concentration in the soil (E), the number of leaves decreased at the end of vegetation (September), the same trend was seen for the plant's height (Figure 3). In summary, the presence of B1 and B2 in the soil protected the plant's development.

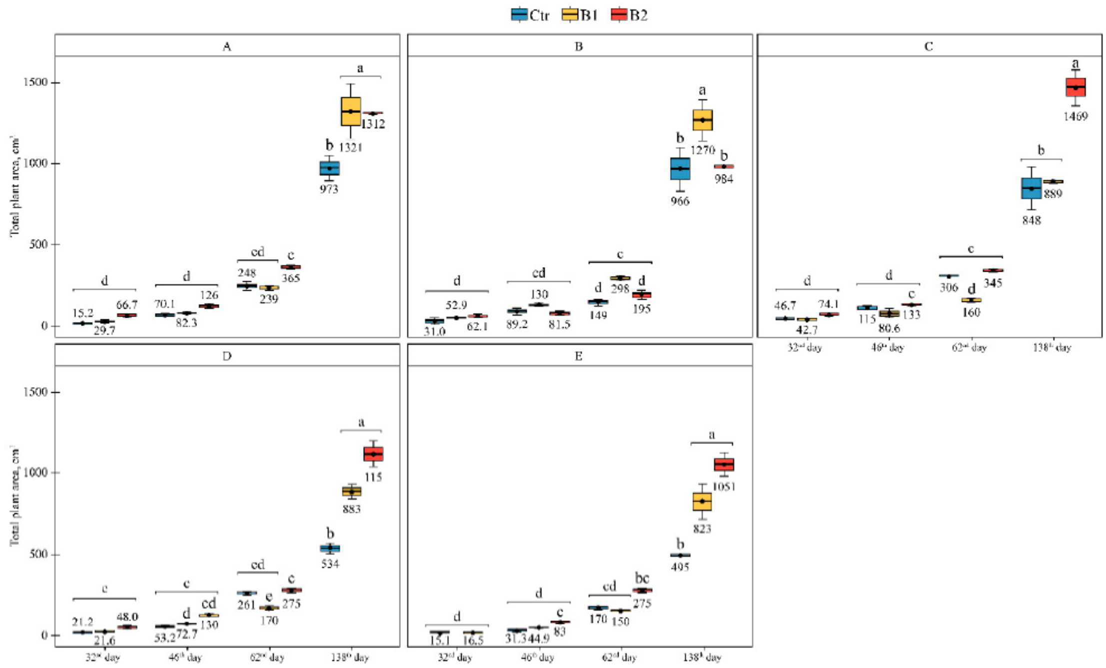

Figure 4. Total plant's surface area as changed during vegetation season. (**A**) Diesel concentration in the soil was 0 mg kg^{-1}; (**B**) diesel concentration in the soil was 250 mg kg^{-1}; (**C**) diesel concentration in the soil was 1000 mg kg^{-1}; (**D**) diesel concentration in the soil was 3000 mg kg^{-1}; (**E**) diesel concentration in the soil was 5000 mg kg^{-1}. Treatments not sharing one letter are significantly different ($p < 0.001$).

Nevertheless, the number of leaves decreased with the increase of soil contamination. The decrease itself was slower when amendments were added (Figure S1). In September, the leaves' number of experiment B was 25.0% higher with B1 and 21.9% higher with B2 than Ctr. For experiment C, these numbers were highest at 15.9% and 21.7%, respectively, and for experiment D, these numbers were highest at 43.0% and 27.1%, respectively.

The number of *M.* × *giganteus* leaves was connected with their surface having a correlation coefficient equal to 7.76 ± 0.07 [69]. The *M.* × *giganteus* leaves' surface was calculated using the approach described at Kvak et al. [70] and presented in Figure 4. The leaves' surface was less affected by the small diesel concentrations in the soil (B, C), and the effect was more evident with the higher concentrations (D and E). Conversely, in the presence of B1 and B2, this decrease was less significant.

Incorporating B1 and B2 into the soil without contamination increased the value of the harvested aboveground biomass compared to the Ctr (Figure S2), which is following published results when biochar was used in combination with fertilizes during cultivation of *M.* × *giganteus* in the agricultural land [32].

The results of harvested dry biomass (leaves and stems separately, as well as their sum) as impacted by biochars are presented in Figure S2, and as impacted by different concentrations of diesel in the soil amended by B1 and B2 are presented in Figure 5.

Results illustrated that level of contamination affected the dry biomass of stems more essential compared with leaves. This trend was evident at the high concentration of diesel (experiment D and E). At the small concentration, the harvested biomass value was similar to Ctr. For almost all experiments, the biochars' incorporation increased the harvest value, as demonstrated in [32].

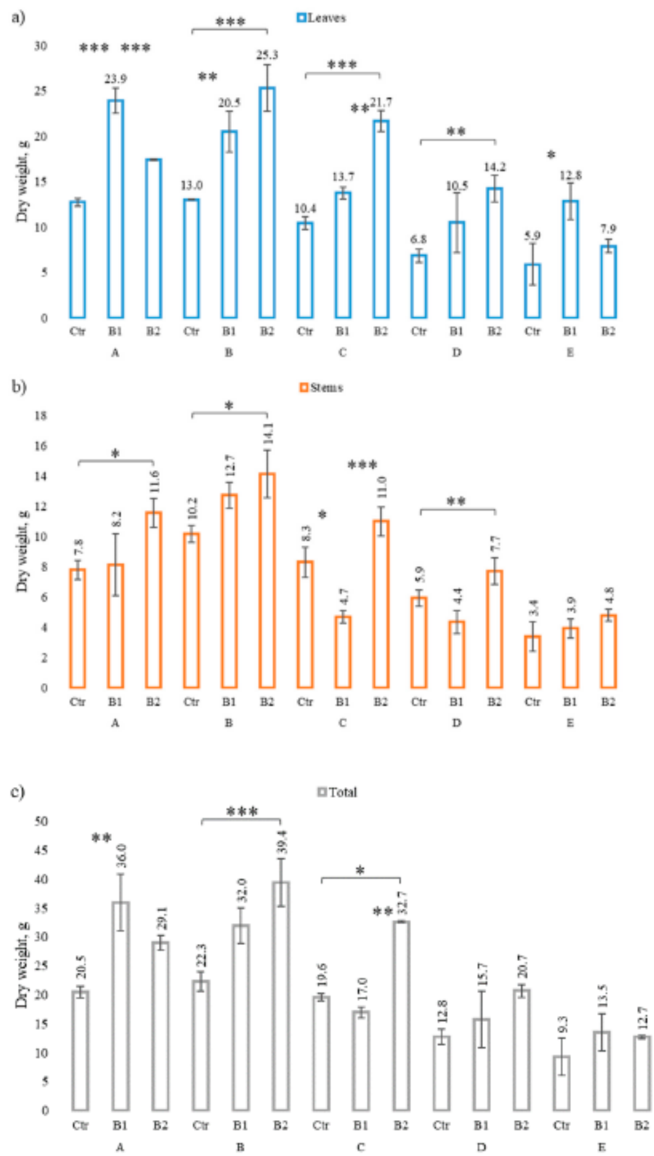

Figure 5. Dry weight of *M.* × *giganteus* aboveground biomass at harvest: (**a**) leaves; (**b**) stems; (**c**) total aboveground biomass. Asterisks denote the significant difference between compared pairs (*—$p < 0.05$; **—$p < 0.01$; ***—$p < 0.001$). (A) Diesel concentration in the soil was 0 mg kg^{-1}; (B) diesel concentration in the soil was 250 mg kg^{-1}; (C) diesel concentration in the soil was 1000 mg kg^{-1}; (D) diesel concentration in the soil was 3000 mg kg^{-1}; (E) diesel concentration in the soil was 5000 mg kg^{-1}.

While comparing the impact of soil amending by biochar to the plant height at the same diesel concentration (Figure 6, Table S1), B2 showed a different influence than B1 and Ctr. For the earlier development stage (May and June), the amendment by B1 did not show any effect.

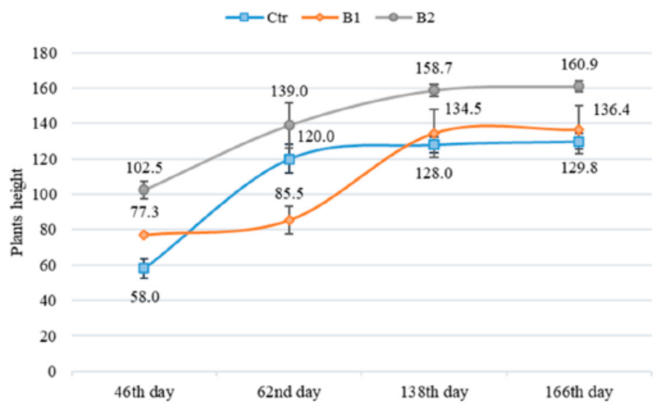

Figure 6. Growth dynamic of *M.* × *giganteus* in uncontaminated soil amended by biochars.

3.3. Impact of Soil Amendment by Biochar and Contamination by Diesel to the Plant State

The photosynthesis of plants growing in the hydrocarbons contaminated soils was inhibited due to induced toxicities to living cells by contaminants [71]. The toxic components could be taken via the roots, stem and leaves, which may alter the integrity and permeability of plant membranes leading to disturbance of carbon metabolisms in the leaves, ion and water uptake in the roots [63]. Hydrocarbons' hydrophobic nature prevents water from spreading inhomogeneously in the contaminated soil, resulting in a water deficiency [72].

It is known [22,50,73] that changes in the photosynthetic apparatus (PSA) can be a marker for assessing the plant's overall functional state, the effectiveness of the adaptive plant's reaction and its development as a response to different growing conditions. Each quantum of the light absorbed by pigments can induce the primary charge separation in the photosystem's reaction centers (RC), be dissipated to heat, or be illuminated as a fluorescence quantum. Light energy differs depending on the PSA state, the content of chlorophyll, and the external conditions. The decreasing chlorophyll content in plant leaves due to RC's closure may cause a decrease in the quantum efficiency of photosystem II (PSII). That will inevitably slow down the rate of CO_2 assimilation and impairing the effectiveness of PSA. Under such conditions, the extra light energy causes the reactive oxygen species' appearance (ROS), which can inhibit and damage the plant's photosynthetic apparatus [73]. One of the protection mechanisms against oxidative damaging of PSA is NPQt, and increasing NPQt's level ensures the primary message of the light-protective reaction for the prevention or reduction of the ROS negative effect the primary level of photosynthesis.

In the current study, the stress response of *M.* × *giganteus* grown in the diesel-contaminated soils was evaluated with and without soil amendments. For this reason, the changes of physiological parameters, i.e., NPQt and the SPAD, were determined in the different phases of *M.* × *giganteus* vegetation followed the approach described at [51,53]. Results are presented in Figure 7 and Figure S3.

It may be concluded that SPAD changes in plant leaves during vegetation in the Ctr soil without amendments increased in the summer month (June, August) and decreased in September when the first signs of plants wilting appeared with fixed yellow leaves. These peculiarities are typical for the plant's development [74,75] and illustrated earlier when the photosynthesis of *Amorpha fruticosa* seedlings to different concentrations of petroleum-contaminated soils was measured during vegetation from April to September [76]. However, in our case, when B1 or B2 was amended to the soil, the SPAD was still high in September. The SPAD of B1 was higher in September than in the summer months, while the SPAD of B2 was equal during the summer months and September. Such observation permits us to conclude that incorporating B1 and B2 into the uncontaminated soil increased the vegetation period's duration.

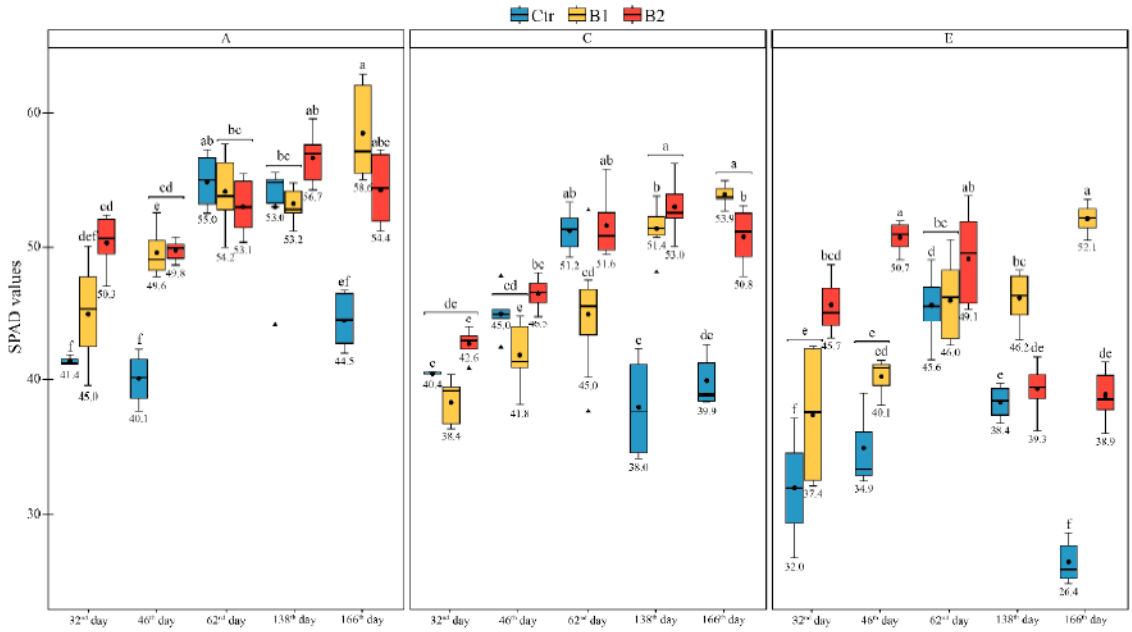

Figure 7. *M.* × *giganteus* SPAD changes during vegetation. (**A**) Diesel concentration in soil was 0 mg kg^{-1}; (**C**) diesel concentration in soil was 1000 mg kg^{-1}; (**E**) diesel concentration in soil was 5000 mg kg^{-1}. Treatments not sharing one letter are significantly different ($p < 0.001$).

In the case of middle contaminated soil C (concentration of diesel 1000 mg kg^{-1}), the SPAD parameter was much higher in the amended soil (for B1 and B2) than Ctr soil; the withering of the plant was fixed for Ctr soil even in August with decreasing of the SPAD.

In the case of the highest contaminated soil E (concentration of diesel 5000 mg kg^{-1}), the first sight of plant's withering accompanied with the decreasing of the SPAD was evident even in June for the Ctr soil without amendment, while in the presence of biochar the first sight of withering was postponed to August for B2 and the plant started to wilt only after August. In the case of B1, the SPAD was relatively stable during summer, and in September, it was even a little higher. That fact clearly shows that incorporating B1 or B2 into the system protected the plant from the stress caused by the high diesel concentration in the soil and prevented plants from earlier withering. The incorporating biochar into the diesel-contaminated soil prolonged the vegetation period of *M.* × *giganteus,* and the effect was evident even at the high level of contamination. These results are perspective for crop utilization in diesel contaminated soil because it illustrated a profitable growth and harvest value in a range of contaminated concentration 250–1000 mg kg^{-1}. The plant's development was better in B1 and B2, which stressed the positive effect of carbon contented amendments to *M.* × *giganteus* cultivation in the diesel-contaminated soil.

During the vegetation period in the plants growing in the control or contaminated soils, the SPAD monitoring showed differences in the dynamic of green pigment's content and its concrete values [74,75].

In the current study, with applying B1 or B2, the level of NPQt in the light-harvesting complex (LHCII) of *M.* × *giganteus* significantly decreased (Figure S3). This fact indicated a decrease in sharing of the thermal energy dissipation in the LHCII of PSII and accordingly illustrated energy utilization improvement. Based on this hypothesis, we assumed an increase in the efficiency of excitation energy transfer between the pigments of LHCII and RC of PSII [77]. Ultimately, the detected positive effect of biochar on photosynthesis can be a prerequisite for increasing *M.* × *giganteus* biomass productivity, including the case when the crop was cultivated in the diesel-contaminated soils.

Based on three indexes, i.e., biomass at harvest, the SPAD and NPQt, the positive effect of biochar incorporation on the plant's development was noticed. In particular, the effect was distinguishable for B1: when the plant was grown in soil amended by B1, it was characterized by the highest SPAD value, the lowest NPQt value that logistically resulted in the highest productivity at harvest.

With B1 and B2, the negative effect of soil contamination by diesel to PSA parameter was lower for all diesel-contamination concentrations, and the effect of B2 was more potent than for B1. At the highest contamination level equal to 5000 mg kg^{-1}, adding B1 ensured the growing condition, which kept the relative level of SPAD over 40. Nevertheless, the productivity of *M.* × *giganteus* was lower compared to the control. Starting from August, the SPAD parameter was below 40, followed by decreasing *M.* × *giganteus* morphometric parameters [78]. When the plant grew in amended soil, the increasing value of the SPAD in leaves may be due to additional nitrogen contribution to the soil with biochar.

Thus, an essential character of *M.* × *giganteus* growing in the diesel contaminated soil was the low-level chlorophyll in the mesophilic cells of leaf and high dissipation of light energy in heat through the mechanism of NPQt. This feature is a good illustration of the adaptive *M.* × *giganteus* strategy, allowing good vegetation even when the crop was developed in severe soil contamination conditions. One mitigation action for proper *M.* × *giganteus* production in the diesel-contaminated soil is amending that soil by biochar.

4. Conclusions

The greenhouse experiment with the diesel-contaminated soils in a range of 250–5000 mg kg^{-1} showed sufficient *M.* × *giganteus* development during vegetation. When two biochars enriched the soil: B1 (received from wastewater sludge) and B2 (received from the mixture of wood biochar and biohumus), the plant illustrated profitable growth. Results showed that incorporating B1 and B2 enriched the soil with nutrients, prolonged the plant's vegetation period, improved the crop's morphological and physiological parameters. The surface of *M.* × *giganteus* leaves was less affected by the small diesel concentrations in the soil, and the effect was evident with the higher level of contaminations; in the presence of biochars, this leaves' decreasing was not as prominent.

Based on the SPAD and NPQt values and harvested biomass parameters, biochar's positive effect was illustrated, which was more visible for B1: plant growing in this soil had the highest SPAD and lowest NPQt values, which logistically resulted in the highest productivity at harvest. The physiological parameters analysis illustrated that incorporating carbon-contented amendments decreased the plant's stress and prolonged vegetation.

The growing of *M.* × *giganteus* in the uncontaminated soil changed its parameters: with vegetation, the content of NO_3 and K decreased, which is a typical process occurring during plants' development; the content of NH_4 increased, more evidently due to soil microbes' activity in the plant's rhizosphere; the content of P_2O_5 increased for summer months and decreased in autumn because of the phosphorus transformation into more available for plants form. The incorporation of biochars increased the soil pH; with vegetation, the value slightly decreased due to the alkaline elements' (Ca and Mg) uptake during vegetation.

An essential feature of *M.* × *giganteus* grown in the diesel-contaminated soil was the low chlorophyll level in the leaf's mesophilic cells and high dissipation of light energy into the heat of the NPQt mechanism. This is an important illustration of the *M.* × *giganteus* adaptive strategy, which ensures good vegetation, even if the crop was grown in highly diesel-contaminated soil; the effect was more essential in the presence of biochar. The received results look perspective for using *M.* × *giganteus* to the revitalization of the diesel-contaminated soil. Future research must be focused on studying the biodegradation of hydrocarbons in the diesel-contaminated soils stimulated in the vicinity of *M.* × *giganteus* roots due to the enhancement of microbial biomass activity following root exudation [79].

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11040798/s1, Figure S1. Leaves quantity changes occurring in *M.* × *giganteus* grown in soil differently contaminated by diesel with amendments. Asterisks denote the significant difference between compared pairs (*—$p < 0.05$; **—$p < 0.01$; ***—$p < 0.001$); Figure S2. Aboveground biomass dry weight of *M.* × *giganteus* grown in uncontaminated soil with the presence of different amendments; Figure S3. *M.* × *giganteus* NPQt photometric parameters changes during one vegetation;

Table S1. *M.* × *giganteus* height increasing (in percentages), while growing in soil with biochars (B1 and B2); Table S2. MANOVA test results for processing soil parameters changes (T—treatment; ID—presence or absence of *M.* × *giganteus*; M—the month of measurements; ges—generalized eta squared); Table S3. MANOVA test results for processing *M.* × *giganteus* physiological parameters changes (T—treatment; M—the month of measurements; C—contamination level; ges—generalized eta squared; DW—dry weight).

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