



Article

Assessing the Ecotoxicity of Soil Affected by Wildfire

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Abstract: This study was carried out to assess the ecotoxicity of soil affected by wildfire using two laboratory toxicity tests, and to investigate the possibility of application of selected soil amendment into the burnt soil in order to improve its properties for faster post-fire reclamation. A phytotoxicity test is a fast-indicative test for revealing acute toxicity and was performed on quickly growing plant species *Sinapis alba* L. and *Lepidium sativum* L., while a pot experiment is a standardized toxicity test with a longer experiment duration and was carried out with *Lolium perenne* L., *Festuca rubra* L., *Brassica juncea* L. Diatomite, bentonite, compost, and biochar were supplemented to the soil. Regarding the phytotoxicity test only 3% w/w of biochar stimulated the growth of *Lepidium sativum* L. Pot experiment confirmed that effect of soil application amendments on biomass yield is more significant than the plant species. The average highest biomass yields were achieved in treatments with bentonite and diatomite. Subsequent research should focus on investigating possible combinations of soil amendments for burnt soil reclamation and complementing the experiments with chemical analysis.

Keywords: wildfire; environment; soil amendments; phytotoxicity; pot experiment



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1. Introduction

Wildfires are a natural phenomenon with an important role in shaping and influencing environment and triggering their renewal and change. Wildfires have also their seamy side, they destroy homes, wildlife habitat and wood, and pollute the air with emissions that can harm human health. Recently, the increased frequency of wildfires has started to be linked to climate change [1,2]; however, several studies indicate that the increased frequency of wildfires in the past has also been caused by other factors, by the policy of fire suppression, by intentional afforestation, and by abandonment and depopulation of rural areas. The fire suppression policy and the abandonment of natural areas have led to natural revitalization and accumulation of biomass in these areas. The accumulated biomass serves as a supply of fuel for potential fires and promotes its spread [3,4]. Fire-affected areas can be impacted to a different extent depending on fire parameters and the pre-fire history of each area [5,6]. Fire impacts can also strike adjacent areas which have not been directly hit by the fire. One of the components of the environment that is brought into immediate contact with fire is the soil. Soil is a valuable non-renewable resource and essential component of ecosystems, and can undergo substantial modifications because of the direct (heating) or indirect (ash) effects of fire [7]. Soil is deemed to be a non-renewable resource because its creation can take hundreds to millions of years [3]. Soil properties can be altered by fire reversibly or irreversibly depending on many factors pertaining to fire. It has been well documented that the impact of fire on soil properties can be short term, long term, or even permanent. However, long-term studies are essential to gain a good understanding of the ecological role of fire [7]. Thanks to the fact that some effects of fire on soil can be permanent, the fire

has come to be considered a seventh soil-forming factor by some authors [3–6] alongside with parent material, time, humans, climate, organisms, and topography.

In the course of time ecosystems sequester potentially toxic elements (PTEs) which originate from anthropogenic sources, transport, industry, overuse of agrochemicals, improper waste disposal [8]. Some parent materials (metal-rich minerals) contain PTE naturally and due to pedogenic processes and weathering, these PTEs become a natural part of the soil and can be found in the soil in trace amounts. Depending on the amount of PTE in the soil, these elements can have different effect on plants, ecosystems, and human health. Certain PTEs are fundamental for plant growth (essential micronutrients (Cu, Fe, Zn)) when occurring in trace amounts, but when the amount exceeds a certain level and these same elements accumulate to high concentrations they become toxic for the environment and affect the ecosystems detrimentally [9]. Other PTEs (Cd, Pb) are toxic by nature. The highest amount of PTEs is adsorbed by reactive soil fractions such as the soil organic matter (SOM), clays, carbonates, and Fe and Mn oxides [10], and subsequently by plants which take them in from the soil. After the PTEs are bonded in reactive soil fractions and in plants, they get immobilized in the system [8]. As mentioned above, the soil properties can be altered after being affected by fire. The alteration of soil properties can result in releasing the bonded PTEs from the soil reactive fractions and plants. Therefore, PTEs can change their state from immobilized to mobilized and can spread through ash and smoke jeopardizing other components of the environment, water resources and fauna. Wildfires are also an important natural source of polycyclic aromatic hydrocarbons (PAHs) [11], which are mobilized into the terrestrial compartment and become a potential source of contamination for aquatic ecosystems [12].

The impact of wildfires on the environment can be observed in the fire-affected area. Terrestrial plant phytotoxicity tests are a very useful tool for assessing the state and conditions of samples acquired from a potentially contaminated area. The tests are able to demonstrate the potential of chemicals and other pollutants to harm organisms and ecosystem functions and are often used as a part of an ecological risk assessment [13]. Small-scale toxicity tests (Phytotoxkit™) are commonly used because they are rapid and when paired with chemical analysis, they can quickly provide a toxicology profile of the studied [14] and indicate if there is an urgent need to investigate the affected area more intensely. Seed germination stage and radicle elongation stage tests are commonly used to evaluate the phytotoxicity of pollutants and polluted environmental compartments. Phytotoxicity is a term used to describe the toxic effect of a compound on plant growth. According to Titah et al. [15], damage can be caused by a variety of compounds. Toxicity is a state when a plant is incapable of sequestering or removing excessive concentrations of organic or inorganic pollutant.

One of the possible methods of tackling the toxicity of the fire-affected area is the application of soil amendments. There are two basic groups of soil amendments. One used to enhance soil fertility and the other one to stabilize soil conditions. Soil amendments are substances with the ability to improve physical and biochemical soil properties [16]. Thanks to the change of the biochemical functioning of the soil induced by soil amendments, the solubility of PTEs is reduced, and so their mobility which leads to stabilizing the affected area and preventing further damage of the environment by the movement of PTEs.

The main aims of this study were: (i) to conduct: phytotoxicity tests of the fire-affected soil supplemented with increased proportions of selected soil amendments with a standard and a prolonged period of testing; (ii) to determine the efficacy of increased doses of selected soil amendments; (iii) to perform a pot experiment with fire-affected soil supplemented with 3% *w/w* of selected soil amendments.

2. Materials and Methods

2.1. Site Description and Collection of Samples

Samples of burnt soil were gathered from a forest locality called Boriky situated in the border area of Brno, Pod Hády, Maloměřice (49°12′47.1″ N and 16°39′52.0″ E), Southern

Moravia, Czech Republic (CR). The forest locality mainly comprises pine vegetation aged up to 140 years and deciduous species, white oak (*Quercus petraea*), sycamore maple (*Acer pseudoplatanus*) and black locust (*Robinia pseudoacacia*), and a hot water piping system runs through it (Figure 1).



Figure 1. Site hit by fire immediately, 6 months, and 12 months after the fire. The location has been monitored under a long-term research project.

On 11 June 2019, a forest area of approximately $5 \times 10 \text{ m}^2$ was hit by an accidental fire. The fire burnt shrubs, a few tree trunks and their lower branches. It can be considered as a surface fire because it was put out by fire brigade before reaching the tree crowns. Surface fires usually cause the least damage to the forest. A gridded sampling design was used for sample collection. Ten different random points within the set location were chosen and samples were collected from the topsoil (0–20 cm) 6 months after the fire occurrence. Fifteen kilograms of collected burnt soil was used for a preparation of a composite sample. This sample was left to air-dry for 2 weeks in a laboratory at $25 \text{ }^\circ\text{C}$ and homogenized in three steps. At first, coarse pieces (unburnt roots and pieces of wood, stones) were picked manually and then the dried soil was passed through a 6-mm mesh and subsequently through a 2-mm mesh. The collected soil was subjected to sieve analysis and with 88.64% sand, 10.16% silt and 1.20% clay fractions classified as a loamy sand according to triangular classification system. This soil was used for carrying out a Phytotoxkit™ test [17], a pot experiment, and for pH measurement. All the experiments and measurements were conducted under laboratory conditions at Mendel University in Brno, Department of Applied and Landscape Ecology.

2.2. Experimental Design

Experiments have been designed to follow up the findings from a pilot testing carried out by Martínez Barroso et al. [18]. Two ecotoxicity tests were chosen, namely Phytotoxkit™ test [17] and a pot experiment. A phytotoxicity test is a fast-indicative test for revealing acute toxicity while a pot experiment is a standardized toxicity test with a longer experiment duration. Both tests use the reaction of higher plants to provide the conditions for assessing ecotoxicity. The phytotoxicity test was enhanced by 2 modifications. The first modification, taking into account the criticism of the sensitivity and accuracy of the phytotoxicity test raised by several authors [14,19], involved prolonging the period of testing to 6 days, and the second modification increased the proportions of the selected soil amendment that demonstrated a stimulating effect in the pilot testing [18]. The phytotoxicity test was performed on fast germinating indicator plant species *Sinapis alba* L. and *Lepidium sativum* L.

The duration of the pot experiment was extended from a recommended 28 days to 40 days due to slow germination of the selected indicator plant species (*Lolium perenne* L., *Festuca rubra* L. and *Brassica juncea* L.). The average time needed for germination of *Lolium perenne* L. ranges between 7–10 and 15–20 days in case of *Festuca rubra* L. [20]. *Brassica juncea* L. germinates faster needing approximately 3–5 days. Both experiments observed

the reaction of plants on the application of selected soil amendments into the burnt soil in different test setups and on the basis of measured values (inhibition/stimulation percentage in the case of phytotoxicity test and the weight of dry above- and under-ground biomass in the case of pot experiment). Phytotoxicity of the treated burnt soil was determined based on the measured values.

2.3. Phytotoxicity Test

The phytotoxicity test is a rapid bioassay which evaluates the toxicity of a chosen contaminated area based on seed germination and root growth of higher plants exposed to contaminated soil for at least 72 h and incubated in darkness at 25 °C using a unique test setup [17]. The testing method is based on calculating a number of germinated seeds and measuring the length of roots of the germinated seeds in contaminated soil. These values are then compared to the values achieved in the controls grown in the reference—soil (artificial soil widely used substrate in soil toxicity tests prepared according to OECD guideline 207 (1984)) and inhibition or stimulation of the seed germination (SG) and of the root growth (RI) is calculated based on the following Formula (1) [17]:

$$SG/RI = (A_{OECD} - B_{test})/A_{OECD} \times 100 \quad (1)$$

where:

A_{OECD} —represents a mean of germinated seeds or a mean of root length in the control OECD soil

B_{test} —represents a mean of germinated seeds or a mean of root length in the test soil (amended burnt soil)

Comment: negative values represent stimulation effect while positive values shows inhibition effect.

For assessing the effectiveness of the soil amendments on reducing the soil phytotoxicity the following Formula (2) is used [18]:

$$SG/RI = (A_{burnt} - B_{test})/A_{burnt} \times 100 \quad (2)$$

where:

A_{burnt} —represents a mean of germinated seeds or a mean of root length in the unamended burnt soil

B_{test} —represents a mean of germinated seeds or a mean of root length in the test soil (amended burnt soil)

Comment: negative values represent stimulation effect while positive values shows inhibition effect.

The OECD soil is supplied in the Phytotoxkit™ and it is a medium (artificial soil) that should provide optimum growth conditions for higher plants. It comprises of 75% air-dried sand, 15% kaoline, and 5% sphagnum peat, and it is recommended as a substrate for ecotoxicological tests. The soil amendments that demonstrated a stimulating effect in the pilot testing [18] were chosen and tested. These were diatomite, biochar, and compost. The tested plant species were *Sinapis alba* L. (white mustard) and *Lepidium sativum* L. (garden cress). Each amendment was added to the burnt soil in the following proportions: 3% w/w, 6% w/w, 9% w/w, 15% w/w and the soil mixture was saturated till 70% of water holding capacity and left for 2 weeks in darkness in order to provide time for equilibration of the soil mixture. The phytotoxicity test is made of a transparent plastic test plate with a flat cover. The test plate is divided into two compartments. Ninety milliliters of the previously prepared soil mixture (measured in a 100 mL beaker) was placed to the lower compartment, flattened with a spatula and saturated till reaching the water holding capacity. Afterwards, a filter paper was laid on the saturated soil and 10 seeds of a same test plant were positioned on the top of the filter paper in one line with equidistance between each other. The test plate was closed with the cover and labelled with specifications (soil amendment type,

proportion, type of plant, number of replicate) [17]. This procedure was repeated for each combination of soil amendment and the plant species. Three replicates were prepared for each combination. The organization of the phytotoxicity test is in the Table 1. Prepared test plates were put vertically into the holder and incubated in darkness at 25 °C for 72 h.

Table 1. Organization of phytotoxicity test. BS—burnt soil.

Treatment	Tested Plant Species	Model Soil
100% BS	<i>Sinapis alba</i> L., <i>Lepidium sativum</i> L.	OECD
BS + 3% w/w biochar	<i>Sinapis alba</i> L., <i>Lepidium sativum</i> L.	OECD/BS
BS + 6% w/w biochar	<i>Sinapis alba</i> L., <i>Lepidium sativum</i> L.	OECD/BS
BS + 9% w/w biochar	<i>Sinapis alba</i> L., <i>Lepidium sativum</i> L.	OECD/BS
BS + 15% w/w biochar	<i>Sinapis alba</i> L., <i>Lepidium sativum</i> L.	OECD/BS
BS + 3% w/w compost	<i>Lepidium sativum</i> L.	OECD/BS
BS + 6% w/w compost	<i>Lepidium sativum</i> L.	OECD/BS
BS + 9% w/w compost	<i>Lepidium sativum</i> L.	OECD/BS
BS + 15% w/w compost	<i>Lepidium sativum</i> L.	OECD/BS
BS + 3% w/w diatomite	<i>Sinapis alba</i> L.	OECD/BS
BS + 6% w/w diatomite	<i>Sinapis alba</i> L.	OECD/BS
BS + 9% w/w diatomite	<i>Sinapis alba</i> L.	OECD/BS
BS + 15% w/w diatomite	<i>Sinapis alba</i> L.	OECD/burn soil

After 72 h the germinated seeds were counted, the length of roots measured and on the basis of formulas for counting percentage of inhibition of seed germination (SG) and root growth inhibition (RI) from standard operational procedure (SOP), the toxicity of burnt soil and of burnt soil supplemented with each tested proportion and type of soil amendment, was calculated. After this procedure all the testing plates were carefully opened, and the roots were moistened with distilled water. Subsequently, the plates were closed again and left 72 h vertically positioned in daylight. The setting of the test was modified because growing plants need light in contrast to germinating seeds where the presence or absence of light is not essential [21].

2.4. Pot Experiment

The pot experiment allows the observation of plants and their growth in controlled conditions [22]. The experiment was set up in pots filled with burnt soil with the aim of assessing the effect of added amendments on the burnt soil properties. A pot filled with 100% burnt soil was used as a control sample. Samples of burnt soil (each time weighing 194 g) were supplemented with 6 g of different amendments and 100 seeds of each selected plant species (*Lolium perenne* L., *Festuca rubra* L., *Brassica juncea* L., respectively) were sowed in the pots and allowed to grow for a set time period, in this case 40 days (Figure 2 and Table 2). Triplicates were prepared for each combination of a plant species and a soil amendment. The pots were arranged randomly in the laboratory and were provided with natural daylight. The temperature was maintained at 16–17 °C with the use of laboratory air-conditioning to provide favorable conditions for germinating of *Lolium perenne* L. and *Festuca rubra* L. seeds. After 40 days aboveground biomass and roots were separated. Aboveground biomass, roots and soil were left to air dry and were weighed (Figure 2). This experiment was carried out under greenhouse conditions in Mendel University in Brno laboratory, Department of Landscape and Applied Ecology.

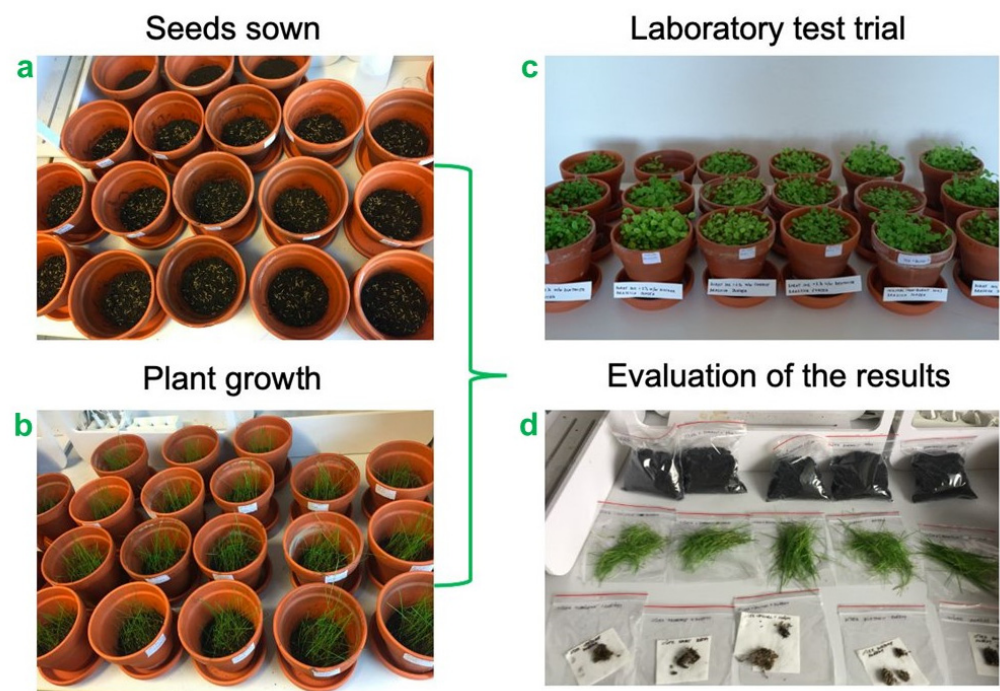


Figure 2. Design of laboratory experiment. (a) Beginning of the pot experiment—seeds sown into prepared amended burnt soil; (b) Growth of *Festuca rubra* L.; (c) Pot experiment after 40 days (*Brassica juncea* L.); (d) Separated aboveground and underground biomass. Dimensions of the used experimental pots: height: 13.1 cm, diameter: 13.1 cm, depth: 11.3 cm.

Table 2. Organization of laboratory experiment.

Treatment	Description	Total No. of Repetition	Indicator Plant
100% unburnt soil	Soil collected from a place adjacent to the fire-affected area	3 for each indicator plant	<i>Lolium perenne</i> L., <i>Festuca rubra</i> L., <i>Brassica juncea</i> L.
100% burnt soil	Control	3 for each indicator plant	<i>Lolium perenne</i> L., <i>Festuca rubra</i> L., <i>Brassica juncea</i> L.
Burnt soil amended with 3% w/w bentonite	6 g of bentonite per pot	3 for each indicator plant	<i>Lolium perenne</i> L., <i>Festuca rubra</i> L., <i>Brassica juncea</i> L.
Burnt soil amended with 3% w/w diatomite	6 g of diatomite per pot	3 for each indicator plant	<i>Lolium perenne</i> L., <i>Festuca rubra</i> L., <i>Brassica juncea</i> L.
Burnt soil amended with 3% w/w biochar	6 g of biochar per pot	3 for each indicator plant	<i>Lolium perenne</i> L., <i>Festuca rubra</i> L., <i>Brassica juncea</i> L.

Detailed characterization of soil amendments is presented in the Table 3.

Table 3. Characterization of soil amendments.

Soil Amendment	Chemical Composition
Bentonite	SiO ₂ —61.28% w/w; Fe ₂ O ₃ —17.79% w/w; Al ₂ O ₅ —13.01% w/w; CaO—4.54% w/w; Na ₂ O—2.70% w/w; MgO—2.10% w/w; K ₂ O—1.24% w/w [23]
Biochar	Carbon—80.97% d.m.; Nitrogen—0.61% d.m.; Cd—0.5 mg·kg ⁻¹ d.m.; Cr—0.1 mg·kg ⁻¹ d.m.; Cu—6.8 mg·kg ⁻¹ d.m. [24]
Compost	C:N max 30; Cd—2 mg·kg ⁻¹ ; Pb—100 mg·kg ⁻¹ ; Hg—1 mg·kg ⁻¹ ; As—20 mg·kg ⁻¹ ; Cr—100 mg·kg ⁻¹ ; Mo—20 mg·kg ⁻¹ ; Ni—50 mg·kg ⁻¹ ; Cu—150 mg·kg ⁻¹ ; Zn—600 mg·kg ⁻¹
Diatomite	SiO ₂ —54.72% w/w; Fe ₂ O ₃ —25.50% w/w; Al ₂ O ₅ —14.82% w/w; C ₂ O—4.18% w/w; MgO—0.79% w/w [25]

2.5. Data Treatment

Measured values were evaluated by descriptive statistics, exploratory data analysis in the programme Statistica 12 (Dell Software, Round Rock, TX, USA) to ascertain the suitability of the data analysis for the purpose of identifying statistically significant differences. Input analysis was complemented with one-way and two-way analysis of variance (ANOVA) in combination with post hoc Tukey's Honest Significant Difference test (HSD) and F-test for identification of significant differences. All statistical analyses were performed on the level of significance $p < 0.05$.

3. Results and Discussion

The modified setup of the Phytotoxkit™ allowed to assess the importance of the time factor on the results of this bioassay. The graphs clearly show that the results after 3 and 6 days of testing do not match. Differing results obtained from the same treatment after 3 and 6 days (testing the same combination of a plant species and the same proportion of soil amendment) can be caused by many factors. The phytotoxicity test has not been designed for long-term testing [17]. Testing plates contain only a limited amount of air in the upper compartment and the air becomes exhausted, the conditions for the growth and development of a plant can deteriorate. Even though after 72 h the exchange of the air in the upper compartment was facilitated (during opening the cover of the plate to moisten the roots), the soil air could not be exchanged because the soil was saturated up to reaching the maximum water holding capacity at the beginning of the test. A moistened filter paper can be another factor affecting plant growth because it can start degrading with the time placed in the permanently wet conditions. The initial seed germination is sustained by accumulated reserves in the seed coat which suggests that testing that lasts longer may correspond more accurately with a real state of a contaminated area because plants already need to take up substances and nutrients present in the soil. Increased proportions of soil amendments do not display significantly better effect on seed germination or the root growth than the recommended proportion 3% *w/w* [25]. A widely held assumption that a recovery action is necessary immediately after a fire is erroneous and a given action can even worsen the ecological stress experienced by the fire-affected area [26]. The main post-fire management priority is to boost the ability of the fire-affected area to recover naturally and primarily to abandon such activities that can further damage or slow down the recovery of original plant species [5,26].

3.1. Results of the Phytotoxicity Test

Figure 3 shows the effect of diatomite application in increasing proportions. Diatomite is a naturally occurring sedimentary rock consisting of fossilized remains of diatoms which are single-celled golden-brown algae. Diatomite is mainly made of silicon dioxide and is highly absorbent thanks to a high porosity and a big surface area [27]. It was demonstrated in studies by Angin et al. [28] and by Aksakal et al. [27] that it enhances nutrient and moisture retention in light textured soils and prevents crust and large aggregate formation [28]. Figure 3 indicates that the less inhibitory effect on root growth was shown by the burnt soil with no added amendment in 3-day testing. This finding applies to all tested amendments (diatomite, biochar, compost). Increasing proportions of diatomite in 3 days of testing had an inhibitory effect on root growth of *Sinapis alba* L. A proportion of 15% *w/w* of diatomite stimulated the root growth of *Sinapis alba* L. in the 6 days of testing. Unamended burnt soil had the highest stimulation effect on the root growth of *Sinapis alba* L. and it even exceeded the conditions provided by OECD soil in the 6-day testing.

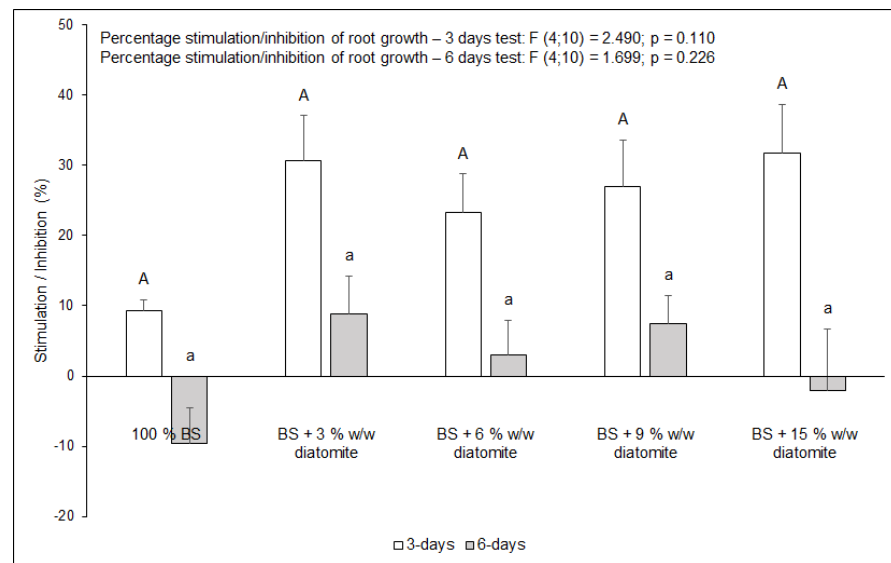


Figure 3. Comparison of inhibition/stimulation of root growth of *Sinapis Alba* L. after 3 and 6 days of exposure to burnt soil supplemented with diatomite in increasing proportions (applied to OECD soil). Average values ($n = 3$) + SE are presented, different uppercase letters indicate significant differences between individual variants after 3 days of testing and small letters after 6 days (one-way ANOVA with post hoc Tukey’s HSD test). The results of the F test are shown at the top of the graph.

Above mentioned differences in phytotoxicity have not been statistically significant. The effect of individual tested amendments on reducing the phytotoxicity of fire-affected burnt soil or in OECD soil (Figures 3–6) was not proven. This was confirmed by the results of Tukey’s HSD test and F-test ($p < 0.05$). The reciprocal comparison of biochar and diatomite (Table 4) shows no mutual significant difference in the effect that biochar and diatomite had on the soil phytotoxicity during the testing with OECD soil (Figures 3 and 5).

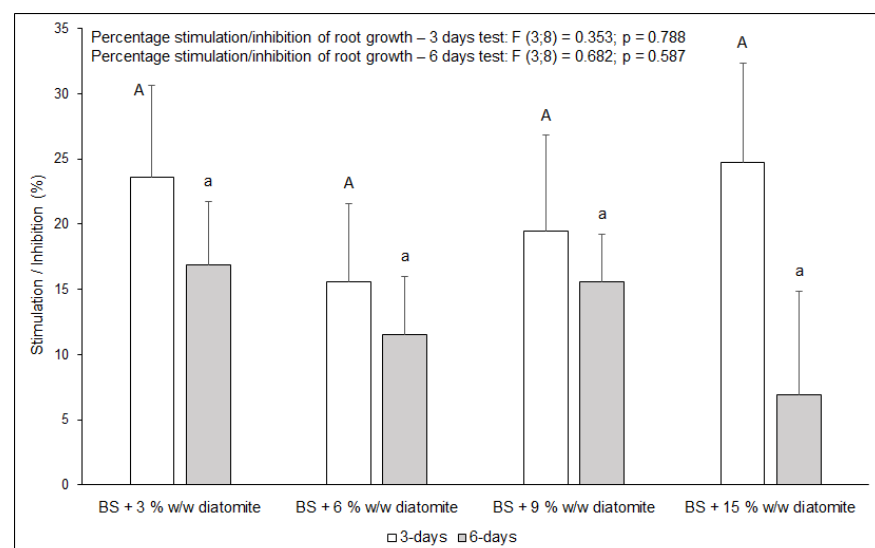


Figure 4. Comparison of inhibition of root growth of *Sinapis Alba* L. after 3 and 6 days of exposure to burnt soil supplemented with diatomite in increasing proportions (compared to burnt soil). Average values ($n = 3$) + SE are presented, different uppercase letters indicate significant differences between individual variants after 3 days of testing and small letters after 6 days (one-way ANOVA with post hoc Tukey’s HSD test). The results of the F test are shown at the top of the graph.

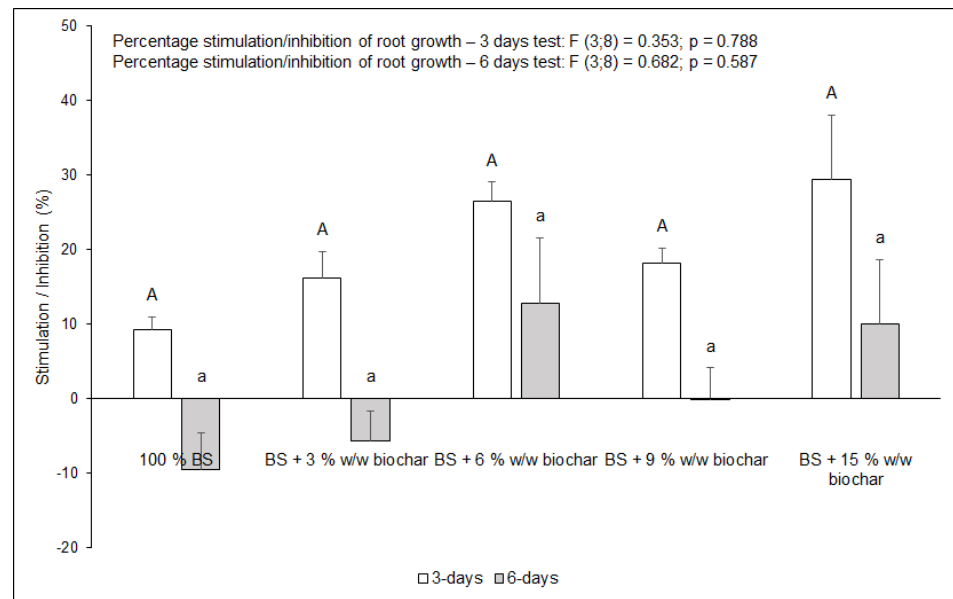


Figure 5. Comparison of inhibition/stimulation of root growth of *Sinapis Alba* L. after 3 and 6 days of exposure to burnt soil supplemented with biochar in increasing proportions (applied to OECD soil). Average values ($n = 3$) + SE are presented, different uppercase letters indicate significant differences between individual variants after 3 days of testing and and small letters after 6 days (one-way ANOVA with post hoc Tukey’s HSD test). The results of the F test are shown at the top of the graph.

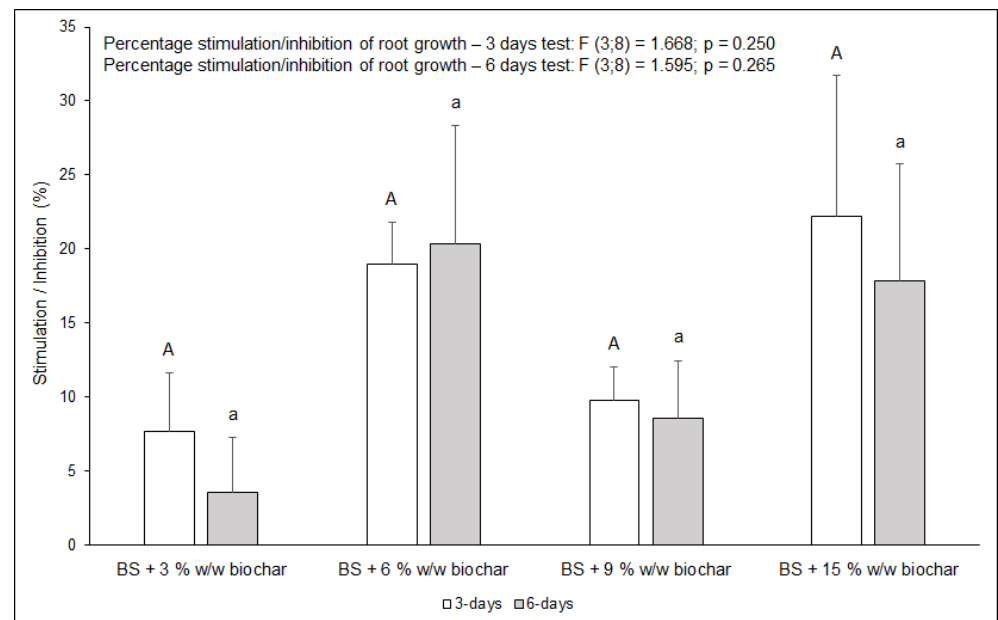


Figure 6. Comparison of inhibition/stimulation of root growth of *Sinapis Alba* L. after 3 and 6 days of exposure to burnt soil supplemented with biochar in increasing proportions (applied to burnt soil). Average values ($n = 3$) + SE are presented, different uppercase letters indicate significant differences between individual variants after 3 days of testing and small letters after 6 days (one-way ANOVA with post hoc Tukey’s HSD test). The results of the F test are shown at the top of the graph.

Table 4. Comparison of all average of stimulation/inhibition of root growth of *Sinapis alba* L. after 3 and 6 days of exposure to burnt soil supplemented with diatomite or biochar in increasing proportions (compared to OECD soil).

Treatment	After 3 Days			After 6 Days		
	Stimul. Inhibition (%)	±SE	HSD *	Stimul. Inhibition (%)	±SE	HSD *
100% BS	9.23	1.04	C	−9.57	3.17	A
BS + 3% w/w diatomite	30.66	6.41	B,C	−5.73	4.07	A
BS + 6% w/w diatomite	23.37	5.44	B,C	12.72	8.77	A
BS + 9% w/w diatomite	26.94	6.64	B,C	−0.17	4.26	A
BS + 15% w/w diatomite	31.68	6.93	B,C	9.97	8.66	A
BS + 3% w/w biochar	16.17	3.58	A,B	8.92	5.30	A
BS + 6% w/w biochar	26.48	2.52	B,C	3.02	4.90	A
BS + 9% w/w biochar	18.11	2.07	B,C	7.52	3.96	A
BS + 15% w/w biochar	29.39	8.63	A,B	−2.01	8.68	A

Comment: Different letters indicate significant differences between individual variants within 3 or 6 days of testing. Symbol * represents significant differences between 3 and 6 days of testing within one variant.

The properties of burnt soil did not improve after the addition of diatomite and on the contrary the Figure 4 shows an inhibition effect on *Sinapis alba* L. root growth. Proportion of 6% w/w of diatomite proved to be less inhibitory in 6-day testing just like the proportion of 15% w/w of diatomite than in 3-day testing. A decreasing trend in toxicity can be observed with time. This trend has not been statistically significant.

Figure 5 displays effects of addition of increasing proportions of biochar to burnt soil compared to OECD soil. Biochar is produced through thermochemical conversion of different kinds of biomass (plant- or animal-based) under oxygen-limited conditions. Recent studies promote biochar as a soil conditioner enhancing plant growth and crop yield; however, long-term effects are not known yet because available findings have been obtained only during the course of recent years [29]. It is noticeable in Figure 5 that all proportions of biochar played a negative, inhibitory effect on root growth in 3 days of testing; however, statistically significant differences have not been confirmed. Results after 6 days of testing changed and 3% w/w and 9% w/w proportions stimulated root growth slightly but less than unamended burnt soil. Similar findings of biochar application resulting in inhibition of plant growth were confirmed in several studies where the inhibition effect was explained by the fact that biochar decreases the availability of nutrients in unfertilized soils owing to stronger adsorption [30].

The efficacy of biochar addition with the aim to improve burnt soil properties was not confirmed and all added proportions of biochar affected the root growth negatively. The inhibitory effect after 6 days is lower in the case of 3% w/w and 9% w/w proportion than after 3-day testing (Figure 6). But this decrease is not statistically significant. The changes in the phytotoxicity in the treatments with increased proportion of biochar (6% w/w, 9% w/w, and 15% w/w) are noteworthy. It is generally known [31] that biochar increases phytotoxicity because it contains polycyclic aromatic hydrocarbons. By contrast, values for treatments with the proportion of 9% w/w and 15% w/w shows decrease in spite of not being statistically significant. Adsorption of phytotoxic substances by the soil is increased after biochar application [32]. This subsequently reduces biologically available concentrations of these substances and lowers the soil phytotoxicity.

Tables 4 and 5 provide a comparison of effects of diatomite and biochar on phytotoxicity in the test setup applied to the OECD soil (Table 4) and to the burnt soil (Table 5). There was a difference in the effect on soil phytotoxicity after application of diatomite and biochar regarding both soils—the OECD and the fire-affected burnt soil. Diatomite showed higher values of growth stimulation on indicator plant (*Sinapis alba* L.) than in the case of biochar. On the other hand, these differences were not significant in either of the soils. It was only proven that after the application of diatomite and biochar in proportion higher than 3% *w/w* to the burnt soil, the phytotoxicity of the amended soil was lower than the phytotoxicity of the 100% burnt soil (100% BS after 3 days (Table 5)).

Table 5. Comparison of all average of stimulation/inhibition of root growth of *Sinapis alba* L. after 3 and 6 days of exposure to burnt soil supplemented with diatomite or biochar in increasing proportions (compared to burn soil).

Treatment	After 3 Days			After 6 Days		
	Stimul. Inhibition (%)	±SE	HSD *	Stimul. Inhibition (%)	±SE	HSD *
BS + 3% <i>w/w</i> diatomite	23.61	7.06	A	16.87	4.83	A
BS + 6% <i>w/w</i> diatomite	15.57	5.99	A	11.49	4.47	A
BS + 9% <i>w/w</i> diatomite	19.51	7.32	A	15.60	3.61	A
BS + 15% <i>w/w</i> diatomite	24.73	7.64	A	6.90	7.92	A
BS + 3% <i>w/w</i> biochar	7.64	3.94	A	3.51	3.72	A
BS + 6% <i>w/w</i> biochar	19.00	2.78	A	20.34	8.00	A
BS + 9% <i>w/w</i> biochar	9.78	2.28	A	8.58	3.89	A
BS + 15% <i>w/w</i> biochar	22.20	9.51	A	17.83	7.90	A

Comment: Different letters indicate significant differences between individual variants within 3 or 6 days of testing. Symbol * represents significant differences between 3 and 6 days of testing within one variant.

The trends regarding the soil toxicity after 3 and 6 days of testing are contrary for *Lepidium sativum* L. and *Sinapis alba* L. Three days of exposure of burnt soil and burnt soil supplemented with compost had a less inhibitory effect on root growth of *Lepidium sativum* L. than it had after 6 days of exposure (Figure 7) which matches with the research conducted by Radziemska et al. [25] where *Lepidium sativum* L. proved to be more sensitive to soil contamination than *Sinapis alba* L. Although differences between 3- and 6-day testing were detected, they were not significant for any of the indicator plants (*Sinapis alba* L. or *Lepidium sativum* L.). The significance of differences in phytotoxicity after 3- and 6-day test was excluded based on the pair T-test and no significant difference was discovered.

The efficacy of compost application to burnt soil increases with time according to Figure 8. The lowest inhibition effect on root growth was seen with the proportion of 3% *w/w* and 15% *w/w* of compost in 3-day testing but this effect was insignificant. The potential positive effect of compost is based on its composition, i.e., the content of a large amount of stable organic substances [33]. When comparing the effect of compost and biochar on the phytotoxicity (test setup with OECD soil) (Table 6), it was found out that despite of the positive effect of compost no significant difference was confirmed.

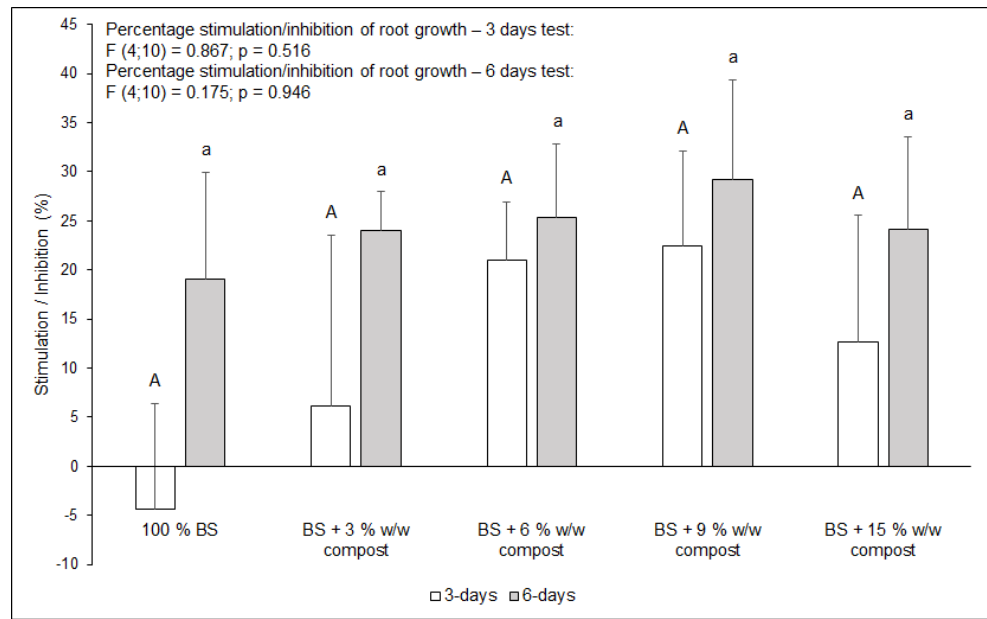


Figure 7. Comparison of inhibition/stimulation of root growth of *Lepidium Sativum* L. after 3 and 6 days of exposure to burnt soil supplemented with compost in increasing proportions (compared to OECD soil). Average values (n = 3) + SE are presented, different uppercase letters indicate significant differences between individual variants after 3 days of testing and small letters after 6 days (one-way ANOVA with post hoc Tukey’s HSD test). The results of the F test are shown at the top of the graph.

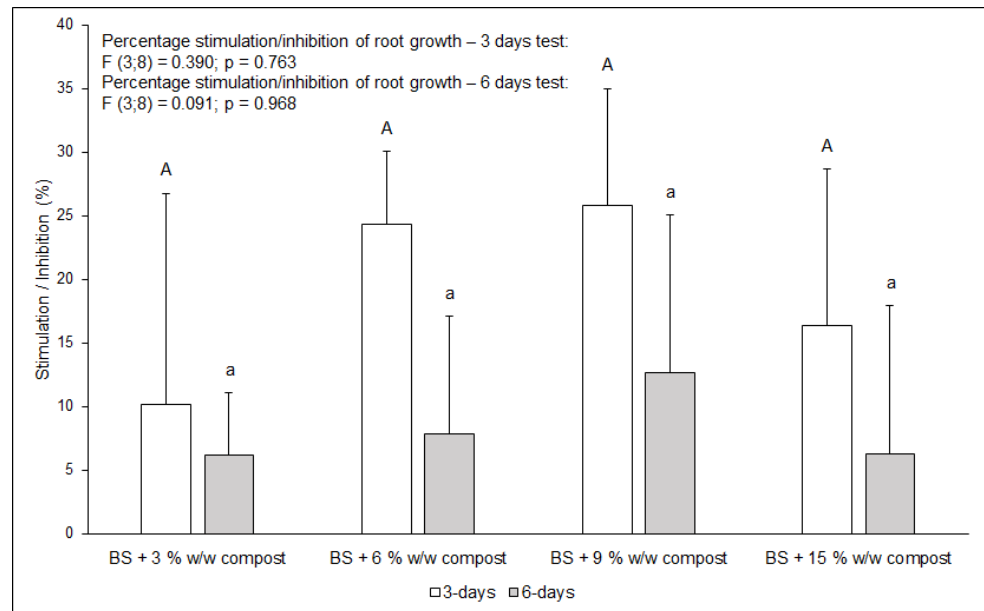


Figure 8. Comparison of inhibition/stimulation of root growth of *Lepidium Sativum* L. after 3 and 6 days of exposure to burnt soil supplemented with compost in increasing proportions (compared to burnt soil). Average values (n = 3) + SE are presented, different uppercase letters indicate significant differences between individual variants after 3 days of testing and small letters after 6 days (one-way ANOVA with post hoc Tukey’s HSD test). The results of the F test are shown at the top of the graph.

Table 6. Comparison of total average of stimulation/inhibition of root growth of *Lepidium sativum* L. after 3 and 6 days of exposure to burnt soil supplemented with compost in increasing proportions (compared to OECD soil).

Treatment	After 3 Days			After 6 Days		
	Stimul. Inhibition (%)	±SE	HSD	Stimul. Inhibition (%)	±SE	HSD
100% BS	−4.42	6.81	A	19.01	6.91	A
BS + 3% w/w compost	6.14	17.38	A	24.03	3.95	A
BS + 6% w/w compost	21.01	5.96	A	25.36	7.51	A *
BS + 9% w/w compost	22.48	9.64	A	29.23	10.07	A
BS + 15% w/w compost	12.65	12.89	A	24.09	9.47	A
BS + 3% w/w biochar	−2.33	10.98	A	18.70	12.06	A
BS + 6% w/w biochar	7.13	7.13	A	24.55	4.15	A *
BS + 9% w/w biochar	8.60	5.90	A	30.78	5.65	A *
BS + 15% w/w biochar	12.29	20.27	A	34.56	8.00	A

Comment: Different letters indicate significant differences between individual variants within 3 or 6 days of testing. Symbol * represents significant differences between 3 and 6 days of testing within one variant.

Guerrero et al. [33] carried out a one-year study investigating the impact of compost application to burnt soils in the Mediterranean. The importance of reclamation of fire-affected soils lies in minimising erosion and recovering the chemical quality of the soil thanks to the addition of the organic amendment which can be supplied by compost [33]. There is a big difference between compost and biochar as biochar contains mainly C-substances without a presence of larger quantities of organic matter [32].

Conditions for *Lepidium Sativum* L. growth in burnt soil enriched by different proportions of biochar, when compared to optimum conditions that OECD soil should provide, are more inhibitory after 6 days of exposure than after 3 days of exposure (Figure 9). Differences between 3- and 6-day test were confirmed by pair T-test which detected the only significant difference within the whole experiment (Table 6). Despite the measured values indicate a positive effect on reducing the soil phytotoxicity compared to the effect of biochar, this effect has not been statistically proven in either test setup with OECD soil (Table 6) or burnt soil (Table 7). The positive effect of compost on reducing the soil phytotoxicity was expected because of its physicochemical properties [34] which are the result of the composting process and the properties of input materials [35–37]. Compost is, thanks to its sorption capacity which is similar to the one of the soil sorption complex, able to bond positively charged particles (heavy metals and other compounds) which can cause soil phytotoxicity [34].

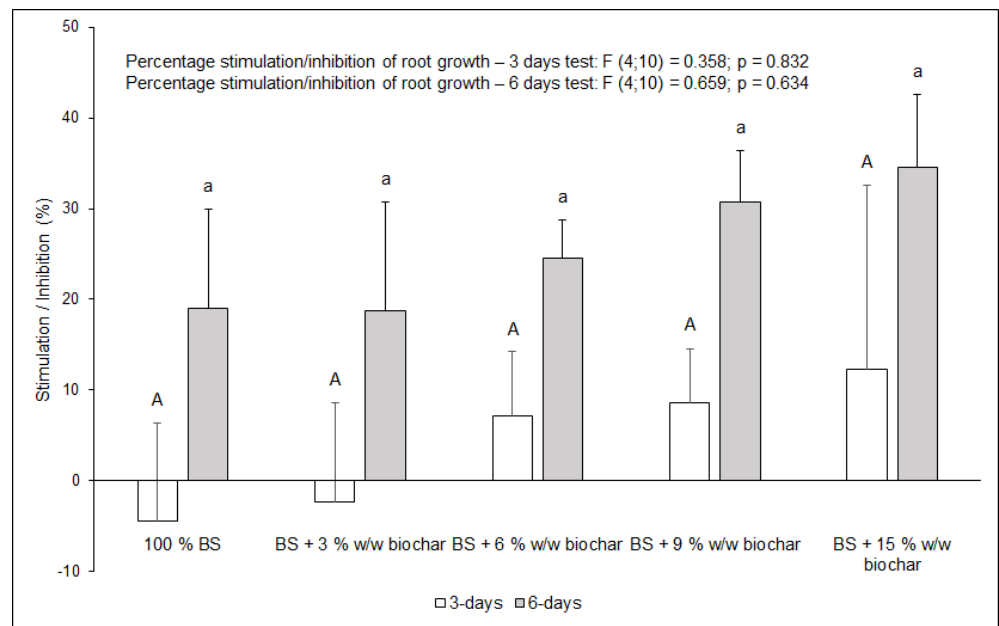


Figure 9. Comparison of inhibition/stimulation of root growth of *Lepidium Sativum* L. after 3 and 6 days of exposure to burnt soil supplemented with biochar in increasing proportions (compared to OECD soil). Average values (n = 3) + SE are presented, different uppercase letters indicate significant differences between individual variants after 3 days of testing and small letters after 6 days (one-way ANOVA with post hoc Tukey’s HSD test). The results of the F test are shown at the top of the graph.

Table 7. Comparison of all means of stimulation/inhibition of root growth of *Lepidium sativum* L. after 3 and 6 days of exposure to burnt soil supplemented with compost or biochar in increasing proportions (compared to burnt soil).

Treatment	After 3 Days			After 6 Days		
	Stimul. Inhibition (%)	±SE	HSD *	Stimul. Inhibition (%)	±SE	HSD *
BS + 3% w/w compost	10.12	16.64	A	6.21	4.88	A
BS + 6% w/w compost	24.35	5.71	A	7.85	9.28	A
BS + 9% w/w compost	25.76	9.24	A	12.62	12.44	A
BS + 15% w/w compost	16.35	12.34	A	6.28	11.69	A
BS + 3% w/w biochar	2.00	10.51	A	-8.42	16.25	A
BS + 6% w/w biochar	11.06	6.83	A	6.85	5.12	A
BS + 9% w/w biochar	12.94	5.90	A	7.99	7.55	A
BS + 15% w/w biochar	16.00	19.41	A	12.91	10.75	A

Comment: Different letters indicate significant differences between individual variants within 3 or 6 days of testing. Symbol * represents significant differences between 3 and 6 days of testing within one variant.

However, when evaluating the efficacy of biochar addition on burnt soil properties, the findings are opposite (Figure 10) and a longer exposure promotes root growth more than the shorter one and the proportion of 3% w/w of biochar stimulates the root growth of *Lepidium Sativum* L. after 6 days of exposure. This finding could not be proven statistically

because of the high variance of measured values. Various studies on biochar application to contaminated soils brought contradictory findings. While some show positive effects on crop yield others report decrease in plant growth. The explanation lies in different reaction of biochar application in fertilized and unfertilized soils. Its ability to increase nutrient retention plays a positive role in fertilized soils while in unfertilized soils it reduces its availability for the uptake by plants [29].

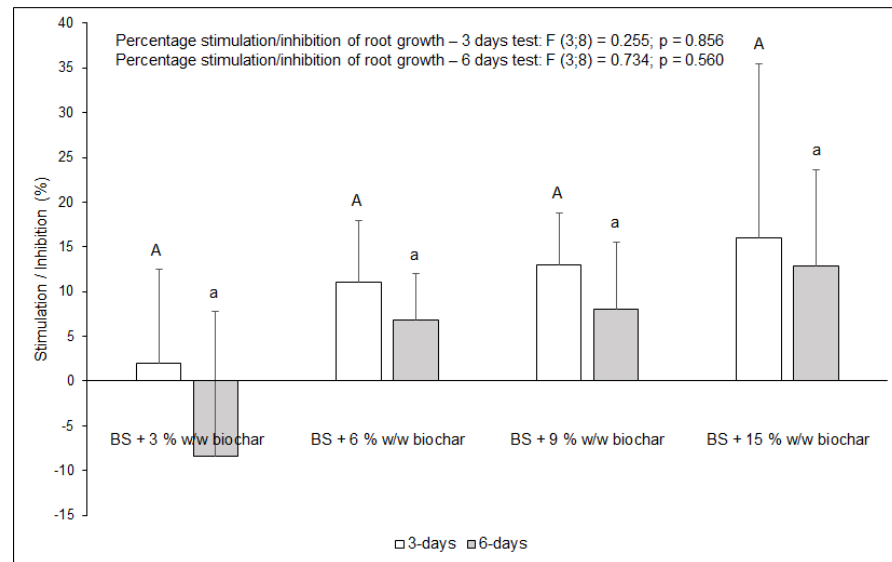


Figure 10. Comparison of inhibition/stimulation of root growth of *Lepidium Sativum* L. after 3 and 6 days of exposure to burnt soil supplemented with biochar in increasing proportions (compared to burnt soil). Average values ($n = 3$) + SE are presented, different uppercase letters indicate significant differences between individual variants after 3 days of testing and small letters after 6 days (one-way ANOVA with post hoc Tukey's HSD test). The results of the F test are shown at the top of the graph.

3.2. Results of Pot Experiment

The aboveground biomass yield from individual treatments are shown in Figure 11. The tested plant species were chosen for their common usage in aided phytomanagement and their ability to immobilize PTEs [25]. Biomass yield among individual plant species differ. *Festuca rubra* L. biomass is much finer than *Lolium perenne* L. biomass which is naturally more robust. *Brassica juncea* L. is an annual herb used as a leaf vegetable or oil crop. Measured values of aboveground biomass yield can be evaluated from several points of view: (a) a factor of the effect of soil amendment application (fertilization); (b) a factor of the effect of the plant species. Stated factors were analyzed with one-way and two-way ANOVA (Figure 11, Appendix A, Figure A1).

The measured values show evident effect of individual soil amendments and type of soil (unburnt/burnt; Appendix B; Table A1). The lowest values of aboveground biomass yield were detected in the treatments with 100% unburnt soil (control) and with burnt soil amended with 3% w/w of biochar (Figure 11). A positive effect of a synergic application of biochar because of elimination of its potential phytotoxicity was confirmed by Yu et al. (2012) [35]. The highest average values of aboveground biomass yield were achieved in the treatment with 3% w/w of bentonite followed by treatment with compost and diatomite. These data are statistically significant (Figure 11) and indicate an important effect of bentonite and diatomite application into the burnt soil with respect to fire-affected soil recultivation.

The effect of individual plant species on the biomass yield was analyzed with one-way ANOVA in combination with post hoc Tukey's HSD test (Figure 12). The results of the statistical analysis confirm that the effect of plant species is partial because from the point of view of average values of aboveground biomass yields differences were found out only

between *Festuca rubra* L. and a *Lolium perenne* L. with *Brassica juncea* L. Therefore, it is obvious that application of individual soil amendments to the burnt soil and sensibility of the plants on these amendments were more important than the plant species.

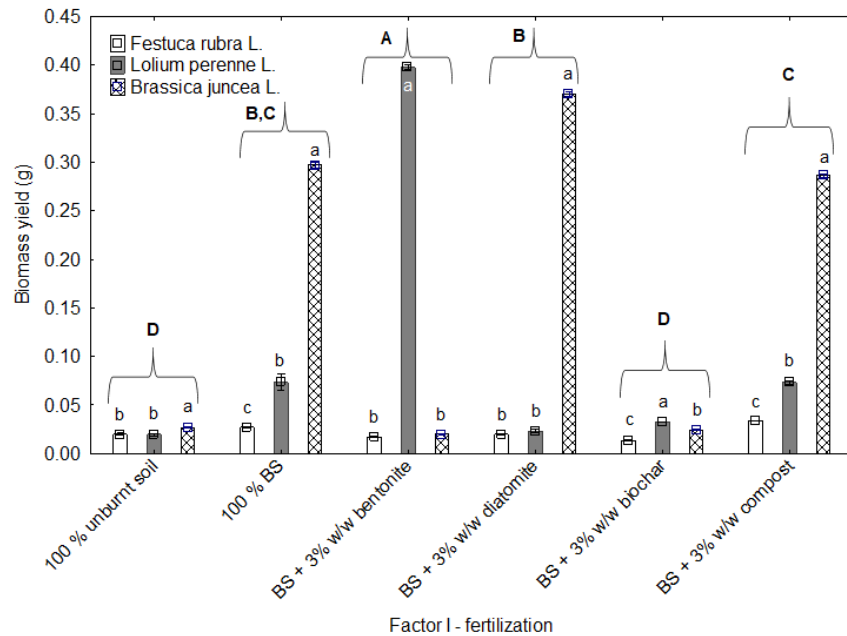


Figure 11. Aboveground biomass yield of *Festuca rubra* L., *Lolium perenne* L. and *Brassica juncea* L. from a pot experiment carried out for unburnt soil, burnt soil, and burnt soil supplemented with different amendments. Average values of plant biomass ($n = 3$) \pm SE are presented; different small letters indicate significant differences between individual plants within one variant. Different uppercase letters in bold indicate significant differences in average ($n = 9$) plant biomass yield between individual variants.

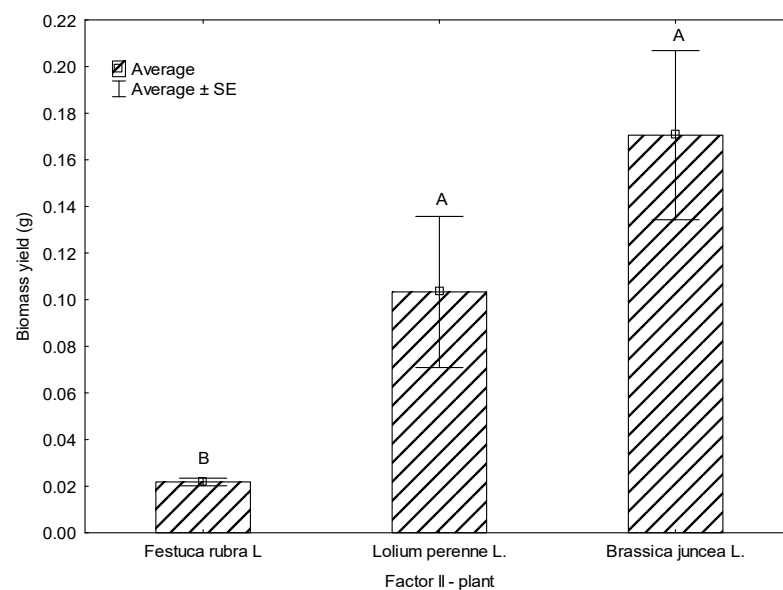


Figure 12. Aboveground biomass yield of *Festuca rubra* L., *Lolium perenne* L. and *Brassica juncea* L. from a pot experiment carried out for unburnt soil, burnt soil and burnt soil supplemented with different amendments. Average values ($n = 18$) \pm SE are presented. Different letters indicate significant differences ($p < 0.05$).

The most favorable treatments for root growth differed for each tested plant species. *Lolium perenne* L. root system benefited from bentonite and biochar application, *Festuca rubra* L. roots grew best in unamended burnt soil and in the treatment with diatomite. *Brassica juncea* L. created the biggest root system in treatment with diatomite as well (Figure 13; Appendix A: Figure A2; Appendix B: Table A2).

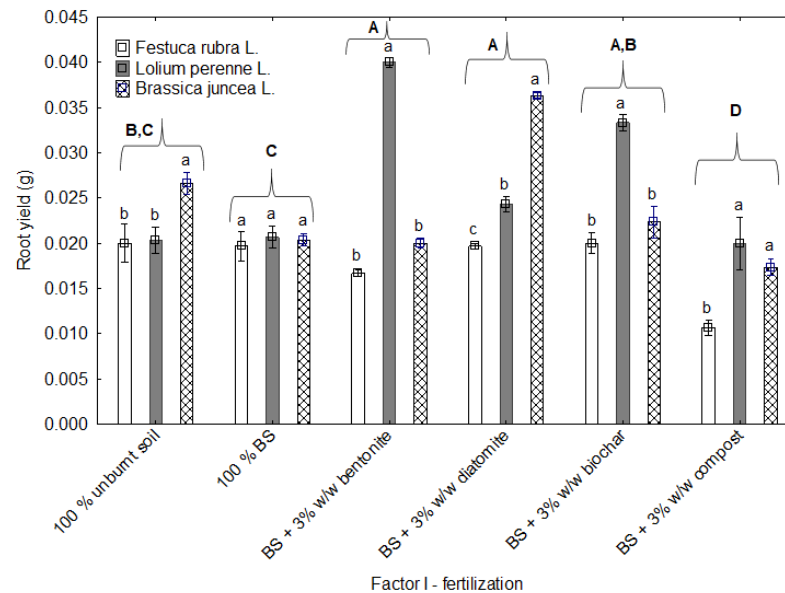


Figure 13. Under-ground biomass (root) yield of *Festuca rubra* L., *Lolium perenne* L. and *Brassica juncea* L. from a pot experiment carried out for unburnt soil, burnt soil and burnt soil supplemented with different amendments. Average values of plant biomass (n = 3) ± SE are presented; different small letters indicate significant differences between individual plants within one variant. Different uppercase letters in bold indicate significant differences in average (n = 9) plant biomass yield between individual variants.

The effect of amendment application to the burnt soil on pH is shown in Table 8. The application of amendments in the proportion of 3% w/w affected the pH only slightly which can be related to the low amendment proportion. Biochar and bentonite decreased the pH of the burnt soil most. The application of biochar with the aim to decrease the pH of alkaline soils was investigated in a study carried out by Liu and Zhang [38] where a decreasing pH trend was detected with increasing biochar application rates. The pH of treatments enriched with diatomite and compost differed from the pH of the unamended burnt very little. Low pH alterations induced by the chosen amendment application proportion suggest that the plants reacted on other soil modifications evoked by the amendments rather than on the altered pH.

Table 8. pH of amended and unamended samples of burnt soil and unburnt soil.

Sample	pH [-]
100% unburnt soil	7.62
100% burnt soil	7.60
Burnt soil amended with 3% w/w bentonite	7.55
Burnt soil amended with 3% w/w diatomite	7.61
Burnt soil amended with 3% w/w biochar	7.53

Yields of under-ground biomass (roots) correspond with the yields of aboveground biomass even though the differences between individual treatments were lower (Figure 14). The highest values were detected in the treatment with bentonite and diatomite. Average values of root yield in the treatment with compost were lower than in the unburnt and burnt

soil where on average exceeded 0.02 g for individual plant species. An unexpected value was seen in the treatment with biochar where the total average value was the third highest.

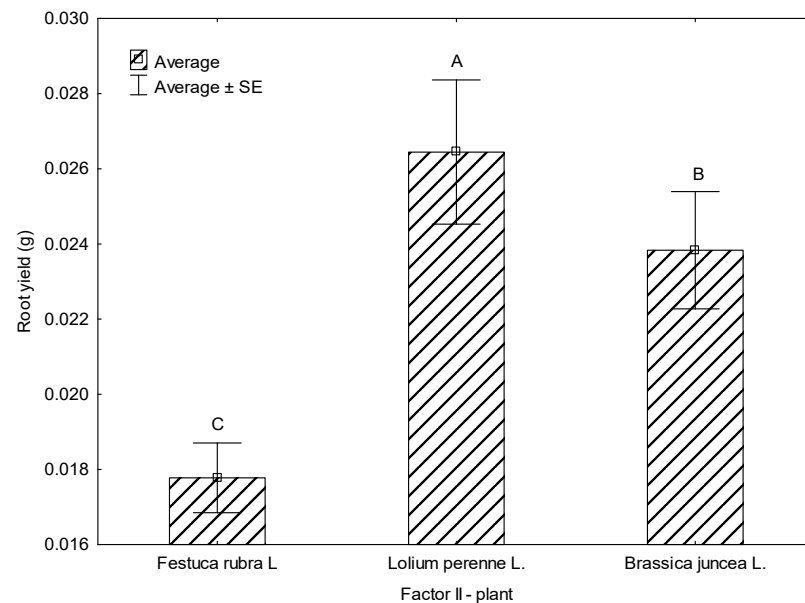


Figure 14. Root yield of *Festuca rubra* L., *Lolium perenne* L. and *Brassica juncea* L. from a pot experiment carried out for unburnt soil, burnt soil and burnt soil supplemented with different amendments. Average values ($n = 18$) \pm SE are presented. Different letters indicate significant differences ($p < 0.05$).

Equally as in the case of aboveground biomass the effect of plant species was probably secondary which emerges from the measured values (Figures 13 and 14) and from the absence of considerable deviations within individual treatments (Figure 13; Appendix B; Table A2). Average values from all the treatments were significantly highest with *Lolium perenne* L. and *Brassica juncea* L., and lowest with *Festuca rubra* L. Studies employing a pot experiment to investigate the reclamation of fire-affected soils with the application of organic amendment (poultry manure) were carried out by Castro et al. [39] and Villar et al. [40]. These studies focused on determining the lowest effective and the optimum poultry manure dose while Villar et al. [40] evaluated the difference between efficiency of poultry manure and inorganic fertilizer (NPK) application to burnt soil. Based on the findings by Villar et al. [40], restoration of physical and biological soil properties seems to be more relevant for *Lolium perenne* L. growth than improvement of chemical properties induced by inorganic fertilizer. Based on long-term research, it can be stated that ecotoxicological diagnosis is an effective research tool [41] and important for the selection of plant species which have higher chances to tolerate the unfavorable conditions [42].

4. Conclusions

The conducted tests showed that the time was an important factor impacting the overall results of phytotoxicity test. Although the significant difference between 3- and 6-day testing was proven only for *Lepidium sativum* L. and burnt soil amended with biochar, we could see differences in results after 3- and 6-day testing within all the tested soil amendments and both plant species. *Lepidium sativum* L. experienced a contrary trend compared *Sinapis alba* L. as far as reacting to phytotoxicity is concerned.

The efficacy of increased proportions of selected soil amendments were not registered. The only soil amendment that improved the conditions of the burnt soil was 3% w/w of biochar in the case of *Lepidium sativum* L. Due to the high deviation of measured values these results could not be statistically confirmed; therefore, performing the phytotoxicity test in more replicates than it is indicated in SOP should be recommended. The phytotoxicity test does not seem to be a suitable testing tool for longer lasting experiment because a plant is

being affected by more artificial factors like a lack of air, a contact with a degrading filter paper, a restricted space; however, a modified methodology that would allow a comparison of the results with results from the pot experiment could clarify.

The pot experiment showed that the effect of soil amendment application to the burnt soil on the aboveground and under-ground biomass yield was more significant than the plant species which impacted the yield only a partially. The average highest above- and underground biomass yields were acquired when the burnt soil was supplemented with bentonite and diatomite. The pot experiment provides a more realistic evaluation of the effects which a potentially contaminated soil can have on plant growth. It gives the plants more time and space to react. It is essential to choose an appropriate size of a pot that stimulates real natural conditions as faithfully as possible and provide more soil and space for root growth. The main novelty of the manuscript was to show the effects of selected soil amendments and ecotoxicity effect of soil affected by wildfire. The research is still ongoing and further analysis will focus on the determination of soil amendment combinations and chemical analyzes of the samples.

Author Contributions: Conceptualization, P.M.B. and M.D.V.; methodology, P.M.B.; M.D.V. and J.E.; validation, P.M.B.; formal analysis, P.M.B. and J.E.; investigation, P.M.B.; data curation, P.M.B., J.E. and M.D.V.; writing—original draft preparation, P.M.B.; writing—review and editing, M.D.V.; visualization, M.D.V.; supervision, M.D.V. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A. Results of Two-Way Analysis of Variance

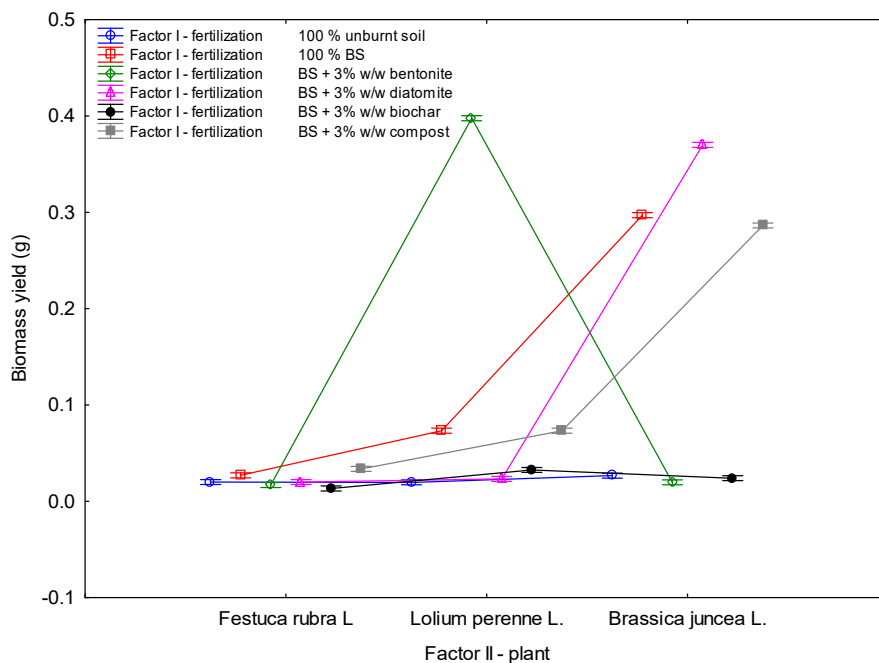


Figure A1. Graphical illustration of the effect of individual factors on the aboveground biomass yield.

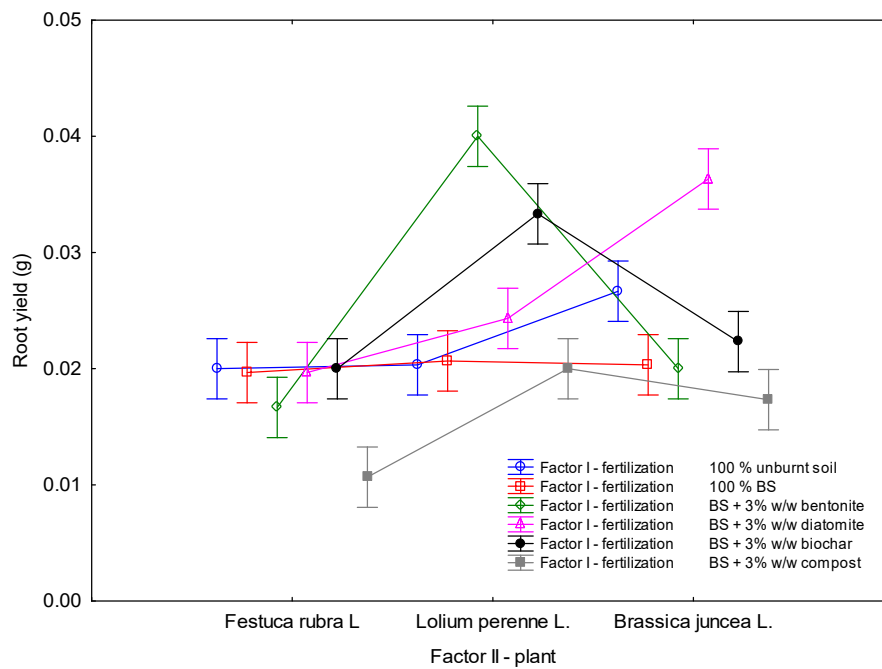


Figure A2. Graphical illustration of the effect of individual factors on the underground (root) biomass yield.

Appendix B. Results of Tukey’s HSD Test

Table A1. Above ground biomass yield—effect of fertilization; significant differences are shown in red.

Tukey’s HSD Test; Variable: Above Ground Biomass Yield (g); Intergroup Error = 0.00002; sv = 36.000

Factor I—Fertilization	100% Unburnt Soil	100% BS	BS + 3% w/w Bentonite	BS + 3% w/w Diatomite	BS + 3% w/w Biochar	BS + 3% w/w Compost
100% unburnt soil		0.000132	0.000132	0.000132	0.992701	0.000132
100% BS	0.000132		0.000142	0.139994	0.000132	0.987639
BS + 3% w/w bentonite	0.000132	0.000142		0.022802	0.000132	0.000133
BS + 3% w/w diatomite	0.000132	0.139994	0.022802		0.000132	0.033794
BS + 3% w/w biochar	0.992701	0.000132	0.000132	0.000132		0.000132
BS + 3% w/w compost	0.000132	0.987639	0.000133	0.033794	0.000132	

Table A2. Underground biomass yield—effect of fertilization; significant differences are shown in red.

Tukey’s HSD Test; Variable: Root Yield (g); Intergroup Error = 0.00001; sv = 36.000

Factor I—Fertilization	100% Unburnt Soil	100% BS	BS + 3% w/w Bentonite	BS + 3% w/w Diatomite	BS + 3% w/w Biochar	BS + 3% w/w Compost
100% unburnt soil		0.350649	0.041734	0.001931	0.086758	0.000137
100% BS	0.350649		0.000262	0.000134	0.000508	0.003454
BS + 3% w/w bentonite	0.041734	0.000262		0.847804	0.999555	0.000132
BS + 3% w/w diatomite	0.001931	0.000134	0.847804		0.672901	0.000132
BS + 3% w/w biochar	0.086758	0.000508	0.999555	0.672901		0.000132
BS + 3% w/w compost	0.000137	0.003454	0.000132	0.000132	0.000132	

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