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Microwave pyrolyzed sewage sludge: influence on soil microbiology, nutrient status, and plant biomass

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Abstract

Background: Sewage sludge (SS) has been considered a potent source of soil nutrients. However, its direct application to agricultural soils have been discouraged owing to its toxic nature. Therefore, conversion and modification of SS to decrease its toxicity has resulted in advanced methods. Co-pyrolysis of SS with other amendments is an ideal treatment resulting in an environmentally safe and nutrient rich final products with additional properties to sequester carbon. In the present study, a novel biochar was produced through the microwave pyrolysis of SS mixed with zeolite and sawdust. The pyrolysis product was thus characterized for elemental composition, polycyclic aromatic hydrocarbons, via Fourier Transform Infrared Spectroscopy (FTIR), and for its effects on soil microbial characteristics, soil health and plant biomass after soil application.

Results: Results revealed that, the SS modification resulted in stable product with higher nutrients which further depend on the type and ratio of feedstock used. Its application to soil significantly improved soil chemical and microbiological properties and altered lettuce biomass.

Conclusions: We concluded that sawdust feedstock promoted nutrient availability in the resulting biochar and induced higher activity of nutrient mineralizing enzymes, whereas zeolite slowed down the release of nutrients from soil and putatively immobilized enzymes. This joint effect of sewage sludge biochar, sawdust and zeolite benefited the plant acquisition of nutrients in comparison with the microbial nutrient uptake. We thus conclude that microwave pyrolyzed SS could be used as a soil enhancer.

Keywords: Sustainable agriculture, Soil quality, Bio stimulants, Nutrient cycling, Pyrolysis

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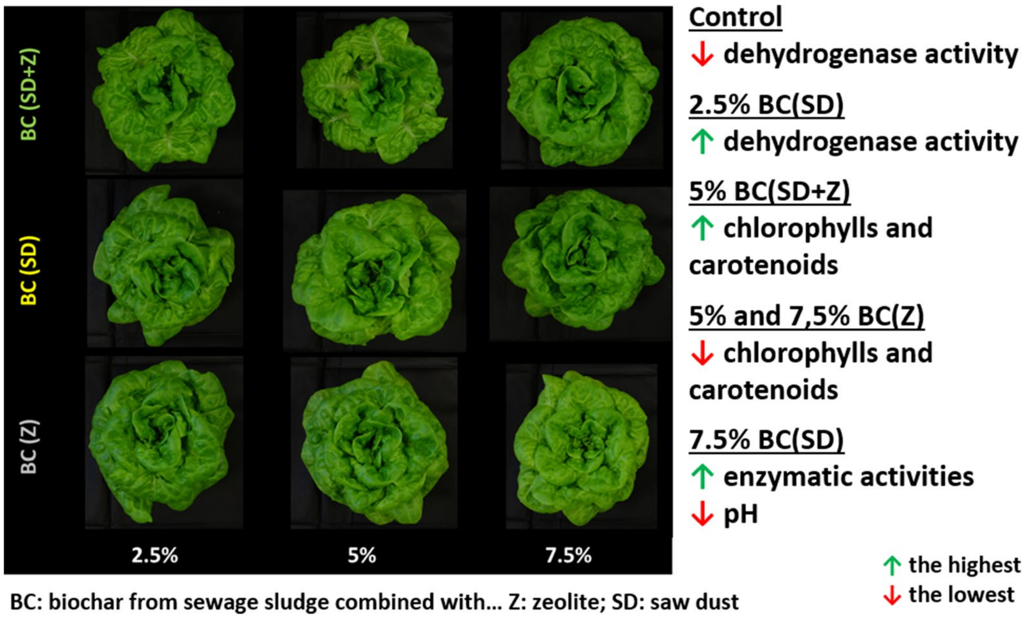
Graphical Abstract



Introduction

Currently, municipal waste management has emerged as a serious societal issue. In this regard, a challenging issue is the increase in the volume of sewage sludge waste (SS), the treatment of which needs energy-intensive and costly processes and safe disposal methods [1]. The Commission by the member states of the Europe reported that more than 10 million tons of dry biosolids including sewage sludge (SS) are being generated in EU annually [2]. This amount is the result of the implementation of European Union Directive, the Urban Wastewater Treatment 91/271/EEC and introduction of advanced technologies in development of wastewater treatment plants (WWTP). In European Union (EU), nearly, 37% of the annually produced dry biosolids (~3.6 million tons) have been recycled in agricultural activities [2] directly through conventional methods used for sewage sludge (SS) disposal or incinerated, compost etc. Currently, recycling to land (directly or via composted SS) has been considered among the most beneficial and economical way for municipal SS management [3]. Nevertheless, despite of many positive impacts of recycling, there are many risks of SS application including the introduction of emerging contaminants (mainly high concentration of heavy metals, organic toxic compounds, pathogens and microplastics) in soil which, may contaminate the food chain or harm the environment by surface runoff into receiving waters [4].

Therefore, there is an increasing interest of developing alternative methods of SS treatment, in particular thermal processing methods such as monoincineration, co-incineration, gasification, hydrothermal carbonation, and pyrolysis have gained momentum in the current era [5–7]. Pyrolysis in this sense is a process of thermal anoxic conversion of organic materials producing gas, oil and solid pyrolyzed residue. This technology of SS processing can be highly advantageous, since it reduces up to 50% of the waste volume [8] and stabilizes the organic matter (OM) in SS. In addition, the liquid and gaseous products can be used as a fuel, whereas the carbon-rich, solid by-product (char) have various agricultural and technological applications [6]. In particular, it is called biochar when applied to soil [9], while when applied technologically, it is called charcoal or coke [10].

Using biochar as the soil amendment or fertilizer is the subject of many recent studies. It is known that biochar can modulate the plant uptake of different nutrients or potentially toxic chemical substances and elements, such as heavy metals. Reduction of heavy metals concentrations and their leachability from fishpond sediment enriched with biochar was established by Mehmood et al. [11]. In the same study, it was observed that biochar addition could also increase the concentration of plants available macronutrients, such as phosphorous, nitrogen or potassium. This beneficial effect of biochar could lead to higher yield of plant biomass production as well as the potential use of contaminated soil for the crop production due to immobilization of toxic elements, such as the cadmium [12]. The efficiency of this effect is dependent on the properties of biochar which are derived from the source of biochar, biochar particle surface, pH and the way of biochar production. Conditions of pyrolysis, such as temperature, residence time, heating rate, feedstock particle size determine both the physical and chemical parameters of resulting biochar [13–15]. The properties of feedstock influence the trace element content (contaminants or aromatic substances) and also its potential to be used in agriculture [16–18]. Depending on conditions of pyrolysis and type of SS [15], biochars obtained from SS pyrolysis are highly macroporous, with the small volumes of the meso- and micro-pores [19] among others, e.g., wood-derived biochar. However, one should bear in mind that the pyrolysis conditions regulate the availability and toxicity of emerging contaminants [20, 21]. For instance, it has been reported that, pyrolysis at lower temperature of 300 °C resulted in the significant reduction in DTPA (diethylenetriaminepentaacetic acid)-extractable metals in the SS-derived biochar [14]. Another study showed that SS conversion to biochar significantly reduces the content of PAHs (polyaromatic hydrocarbons) and their toxicity [22]; however, pyrolysis increased trace metals content in resulting product due to a decrease in resulting mass (Pb, Cd, Zn, Cu, Ni and Cr) [20, 23]. Other studies also reported reduced concentration of volatile organic compounds at higher pyrolysis temperatures (up to 600 °C). In addition, increased temperature led to increased content of stable aromatic carbon, ash, some macro- (Ca, Mg, P, and K) and micronutrients (Cu and Zn) and increased alkali reaction [20]. Nevertheless, a suitable temperature of the SS pyrolysis can be used to transform bioavailable heavy metals into less soluble forms [21, 23].

Addition of zeolite (organo-mineral sorbent) to sewage sludge feedstock before pyrolysis represents efficient approach for improving the quality of SS biochar. Similar enrichment of (composted) SS with zeolite brought the higher water-soluble and total macro-nutrient content, as well as lower phytotoxicity of the obtained blended organo-mineral matter [24]. Co-pyrolysis of pre-biochar feedstock and bentonite or kaolin increased chemical and thermal stability, recalcitrancy, aromatic structures in biochar [25, 26], other co-pyrolyzed organo-mineral clays and biochar improved sorption ability of products: the produced biochars efficiently bound ciprofloxacin [27], removed Cr(VI) from aqueous solution [28], mitigated greenhouse gas emissions (GHG) by sorption of CH_4 and N_2O emitted from soil [29]. Another approach to decrease the toxicity of SS-contaminating heavy metals is co-pyrolysis of SS with other biomass [30–35]. Co-pyrolysis of SS with wooden (bamboo sawdust, willow sawdust) or other lignocellulotic (rice straw) organic materials reduced the mobility and bioavailability of heavy metals in the final biochars [31, 33, 34]. However, concurrent effect of the addition of co-pyrolyzed biomass reduced yield, thermal stability, surface area, and pore volume of biochars, although the contents of organic matter and carbon in biochars significantly increased [31, 33]. Jin et al. showed the increased number of P–H (phosphorus–hydrogen) bonds (in phosphine) under co-pyrolyzed biochars as revealed by Fourier-Transform Infrared Spectroscopy (FTIR) analysis [33]. The feedstock consisting of SS and pinewood sawdust (1:1 w/w) [30] or bagasse [35] reduced vaporization of gaseous carbonaceous products (aromatic compounds, ketones, CO_2), while volatilization of nitrogen-containing products (i.e., NH_3) and sulphur compounds was minimized [35].

Currently, the interest in producing SS biochar has gained momentum. The research is focused on the wide range of SS biochar applications. One of these approaches is the use as a soil amendment. Thus, biochar produced from SS blended with other type of biomass may be improved in its nutrient content and binding properties. This biochar used as soil amendments, could have the beneficial effect to reduce leaching of soil nutrients, to enhance the fertilizing properties, and nutrient retention capacity in degraded soils [36–40]. Based on many of the studies, it seems that using of SS biochar in agriculture as the soil amendment or fertilizer is the potentially beneficial strategy. The review by Xiao et al. (2022) [41] mentioned positive effects of SS biochar: decrease of desorption capacity in soil for PAHs, increase of the soil N retention, immobilization of heavy metals—Cu [42], Pb and Cd [43, 44] etc. Taking this background into account, this work aimed to use the sawdust-blended SS to produce biochar with decreased availability of toxic contaminants and to utilize the carbon-enriched final biochar for improvement of soil chemical and biological properties, plant nutrition and crop growth. It was intended to compare the effect of co-pyrolyzed SS and zeolite on the resulting biochar properties. Recently, negative or "no effect" of SS biochar on soil was also reported, e.g., no effect on available nutrient concentration [45] or phytotoxic impact of volatile organic compounds in SS (unless they were removed by short washing or weathering) [46]. Hence, the need for further research on biochar SS as a potential soil activator and evaluation of its benefits might be highly desireable. Therefore, the specific objectives of this work were to (i) assess the efficacy of value addition of zeolite and sawdust in co-pyrolyzed SS in terms of increased soil

Fig. 1 Raw dried sewage sludge

Fig. 2 Pelletized raw material

nutrient contents and reduced PAHs and (ii) evaluate the effects of soil applied co-pyrolyzed SS on microbial soil health indicators and plant biomass. To achieve the objectives, following hypotheses were tested.

Materials and methods

Biochar preparation and characterization

Treatments of biochar were prepared by microwave pyrolysis from SS mixed with zeolite or/and sawdust, as shown in Table 1. Biochar was applied to soil in 3 doses corresponding to the indicated weight percentages of biochar in soil: 2.5%, 5% and 7.5% (25, 50, 75 $t \cdot ha^{-1}$). The sludge was obtained from the municipal WWTP.

Sewage water and sludge characterization and processing

The WWTP has a capacity of around 530,000 population equivalents. WW is predominantly municipal WW originating from households. Only 12–15% are industrial influents, but generally this WW mostly has the character of typical municipal WW. Despite the relatively low industrial WW ratio, SS tends to contain relatively high concentrations of heavy metals. The anaerobically digested SS was dried using a contact blade paddle dryer at temperature below 100 °C. Tested raw dried samples of SS (Fig. 1) had dry solids around 91% and output fraction from dryer was a powder-like material with particle fraction 1–8 mm. Random tests revealed that the hygroscopic water content was below 2.0%.

Pelletized feedstock

The mixtures of dried raw SS with very fine sawdust (from softwood) and zeolite were pelletized by industrial pelletizing press (Fig. 2). In this work, a synthetic zeolite (Purmol 13) was chosen—zeolite-type ZSM-5 with admixtures of other zeolites (faujasite, wassalite) with a fineness of about < 100 µm. This synthetic zeolite has demonstrated the efficiency of the process of microwave depolymerization of lignocellulosic biomass [47].

For these experiments, the pelletization process used an extrusion die having diameter of 6.4 mm. The temperature during pelletization was measured on the metal matrix of the pelletizer. The pyrolyzed feedstock were approx. 6.4 mm diameter pellets of mixed SS with additives made by pelletizing press (Fig. 3).

Table 1 Treatments amended with biochar prepared from sewage sludge, sawdust, and zeolite

Treatment	Composition of biomass for pyrolysis	Abbrev.
2.5 wt% biochar (sewage sludge + zeolite)	95 wt% SS + 5 wt% zeolite	2.5% BC (Z)
5 wt% biochar (sewage sludge + zeolite)	95 wt% SS + 5 wt% zeolite	5% BC (Z)
7.5 wt% biochar (sewage sludge + zeolite)	95 wt% SS + 5 wt% zeolite	7.5% BC (Z)
2.5 wt% biochar (sewage sludge + sawdust)	75 wt% SS + 25 wt% sawdust	2.5% BC (SD)
5 wt% biochar (sewage sludge + sawdust)	75 wt% SS + 25 wt% sawdust	5% BC (SD)
7.5 wt% biochar (sewage sludge + sawdust)	75 wt% SS + 25 wt% sawdust	7.5% BC (SD)
2.5 wt% biochar (sewage sludge + sawdust + zeolite)	75 wt% SS + 20 wt% sawdust + 5 wt% zeolite	2.5% BC (SD + Z)
5 wt% biochar (sewage sludge + sawdust + zeolite)	75 wt% SS + 20 wt% sawdust + 5 wt% zeolite	5% BC (SD + Z)
7.5 wt% biochar (sewage sludge + sawdust + zeolite)	75 wt% SS + 20 wt% sawdust + 5 wt% zeolite	7.5% BC (SD + Z)

Fig. 3 Biochar

Microwave pyrolysis unit

Experiments were performed by slow microwave pyrolysis unit which works at low pressure 800 hPa. Microwave was generated by magnetron with 3.0 kW input power, regulated output power, and with 2.45 GHz. This unit works discontinuously, and the maximum capacity is approx. 3 kg·batch^{-1} of feedstock. The glass condenser attached to the pyrolyzer was used for the separation of gaseous products and the oil. For incoming and reflected waves a tuner was installed. The infrared (IR) thermometer was introduced into the center of the input feedstock.

The input weight of feedstock samples was 1.0 kg·batch^{-1}. During the experiments, the output regulated power of magnetron was 1.2 kW, residence time was 60 min, and the temperature during the tests did not exceed 250 °C.

Fourier transform infrared spectroscopy (FTIR) analysis

The Fourier transform infrared spectroscopy (FTIR) spectra of the obtained biochar samples was recorded on a Bruker diffused reflectance infrared Fourier transform (DRIFT) spectrometer. The spectra were collected at transmission mode between 4000 and 400 cm^{-1} with resolution of 8 cm^{-1} and 128 scans using OPUS computer-based software. Prior analysis the samples were prepared by mixing with KBr to form a homogenous mixture.

Polyaromatic hydrocarbons (PAH) determination in resulting biochar

The extraction of homogenized samples (1 g of grounded BC sample, Retsch MM 200) was carried out by pressurized solvent extraction (one PSE, Applied Separations). Toluene was used as a solvent, the extraction was carried out at 130 °C, 120 bar and 3 cycles. Before extraction, internal standard (100 /10 ul, 5 deuterated PAH) was added to samples. Toluene was evaporated to approximately 1 mL of final volume. Gas chromatography with mass spectrometry (Bruker EVOQ GC-TQ) was used for the analysis of 16 EPA PAHs in the extracts. 16 EPA PAHs were separated in column DB-EUPAH (20 m × 0.180 mm; 0.14 um), the temperature program was 80 °C for 1 min, then an increase to 320 °C (5 min) with heating rate 15 °C/min, spitless injection at 270 °C, EI 70 eV, SIM mode. Quantification was carried out by internal standard calibration.

PO_4 determination

For the analysis of PO_4–P in water leachate (5 g of BC and 50 ml of MilliQ water, filtration after 24 h) was used the spectrophotometric method according ČSN EN ISO 6878 (MQuantTM Phosphate Test, Merck) [48].

Determination of leachable heavy metals

Heavy metals such as mercury (Hg), cupper (Cu), chromium (Cr), zinc (Zn), led (Pb), arsenic (As), nickel (Ni) and cadmium (Cd) were determined in the water extract using atomic absorption spectrometer with electrothermal atomization ZEEnit 60 from Analytik Jena (Germany) with Zeeman background correction and selected hollow cathode lamp by Photron (Australia) according to the method described in the work of Racek et al. (2019) [49].

Pot experiments and sampling

The pot experiment with lettuce (*Lactuca sativa* L. var. Brilant; SEMO a.s, Czech Republic) was performed in 1-L capacity pots. The pots were filled up with 300 g of commercial garden substrate TS 3 medium basic 425 standard (Klasmann–Deilmann GmbH, Germany), thoroughly mixed with a dose of biochar according to treatments reported in Table 1. The substrate was a mixture of light peat (0–25 mm) with wetting agent, dry matter 45%, pH 6.0, salts 1 g L^{-1}, nutrient content (according to manufacturer): N 140 mg·L^{-1}, P 44 mg·L^{-1}, K 150 mg·L^{-1}, Mg 100 mg·L^{-1}. Altogether, 10 treatments were tested (substrate amended with 9 types/doses of biochar, and unamended substrate = negative control), each treatment was prepared in four replicates.

Three seeds were sown in each pot, then watered with 200 mL of deionized water. After a week of germination, only one plant per pot was established. The watering was regularly carried out during cultivation to maintain the same soil moisture and plants did not wilt. The experiment was carried out for 8 weeks in greenhouse under controlled conditions (day/night): temperature 22/18 °C, relative air humidity 50/50%, photoperiod 14/10 h. At the end of the cultivation, the chlorophyll *a* fluorescence in dark adapted leaves was measured and the above ground plant biomass was harvested for the analyses of pigments, and for plant fresh and dry biomass estimation. In addition, mixed soil sample was taken from each pot for determination of basic physical, chemical and biological soil quality indicator.

Plant biomass quantification and quality parameters determination

Lettuce fresh aboveground biomass (AGB) was determined gravimetrically by weighing the shoots on the laboratory scales. To determine plant dry biomass, fresh plant material was dried at 60 °C to constant weight and obtained dry biomass was again estimated gravimetrically. Dry matter content in fresh plant biomass was calculated (data are not presented). Changes in the photosynthetic apparatus were evaluated using chlorophyll fluorescence parameters measured in dark adapted leaves of intact plants using the portable fluorometer FluorPen FP 100 (Photon System Instrument, Czech Republic). It was measured the fast fluorescent kinetic presented as the OJIP transient curves. Prior to drying of fresh plant biomass, 0.5 g of fresh material was taken from each plant to determine the content of leaf pigments, lyophilized and stored at −18 °C. Lyophilized samples were homogenized with 10 mL acetone. Acetone extracts were analyzed with spectrophotometer (Spetronic 20 Genesys, Thermo Spectronic, USA) at wavelength 662, 645 and 470 nm. The content of individual pigments was calculated according to the methodology Lichtenthaler and Buschmann (2005) [50].

Determination of soil quality properties

The substrate from each pot was homogenized by sieving it through 2 mm mesh and stored at 4 °C (for determination of soil respiration), lyophilized and stored at −18 °C (for determination of soil enzyme activities), and the rest were air-dried for quantification of nutrient content and pH measurement. Soil reaction, pH ($CaCl_2$)—was determined according to ISO 10390:2005 [51], dehydrogenase (DHA) activity was measured according to Voberkova et al. [52] and expressed in µg (triphenyl formazan) TPF·g^{-1}·h^{-1}, other enzymatic activities—β-glucosidase (GLU), arylsulfatase (ARS), phosphatase (Phos), urease (Ure) and N-acetyl-β-D-glucosaminidase (NAG)—were measured spectrophotometrically according to ISO 20130:2018 [53] and the values expressed in µmol (p-nitrophenol) PNP·g^{-1}·h^{-1} and in µmol NH_3·g^{-1}·h^{-1} (urease).

Statistical analyses

Data obtained from the determination of plant biomass, qualitative properties, and soil chemical and biological parameters were statistically analyzed using the methods of principal component analysis (PCA), one-way analysis of variance (ANOVA), Tukey HSD post-hoc test (at significance level $p = 0.05$), and Pearson correlation analysis via Program R, version 3.6.1 [54, 55]. For testing of the normality of distribution, it was used Kolmogorov and Smirnov test and data homoscedasticity was examined by Bartlett's test, both at significance levels of 0.05. Besides, assumptions of all tests were also checked by different diagnostic plots. The minimal level of statistical significance for most of the used methods was 0.05. The results of Pearson's correlation analysis were interpreted (according to the value of correlation coefficient r) as follows: $0.5 < r < 0.7$ (moderate correlation) and $0.7 < r < 0.9$ (high correlation) [56].

Results

Biochar characteristics and results of FTIR analysis

The concentrations of Hg, Cr, Pb, As and Cd in the water extract from biochar types were below detection limits, for Cu it was 1 mg L^{-1}, for Zn 2.2 mg L^{-1} and for Ni 0.7 mg L^{-1}. As the concentrations of all the metals were very low and did not reach minimal inhibition/toxic levels for plants, they are not further discussed in the text. The total nitrogen (N_{tot}) and total hydrogen (H_{tot}) contents were both significantly highest in the BC (SD) biochar and lowest in the BC (SD + *Z*) treatment (Fig. 4).

The mutual ratios between macroelements in the three biochar types showed significant differences too. C:N ratio was highest in the BC (SD + *Z*) biochar (around 12.0) and the lowest in the BC (*Z*) biochar (8.0). H:C ratio on the other hand was highest (> 1.0) in the BC (SD) biochar and the lowest (< 0.8) in the treatment BC (SD + *Z*). Similarly, O:C was highest (> 0.4) under biochar BC (*Z*), followed by BC (SD + *Z*) and BC (SD). These diverse results anticipated no significant mutual correlations between the ratio properties (Fig. 5).

The significantly highest content of leachable phosphate (calculated to dry biochar weight—around 600 mg·kg^{-1}) was detected in the water leachate of BC (SD) biochar, compared to the SD (*Z*) treatment (around 300 mg·kg^{-1}) and the lowest content was in the BC (SD + *Z*) biochar (< 100 mg·kg^{-1}). No significant difference in the sum content of 16 EPA PAHs between all three biochar types was detected. No correlation between PAH content was observed with other biochar properties.

The FTIR analysis of the three types of biochar samples revealed similarities in their composition in terms of aromatic and aliphatic moieties. Importantly, the biochar prepared from 25 wt% sawdust (Fig. 5B), which showed an enhanced intensity signal at 2927 cm^{-1}, depicting characteristic aliphatic C–H stretching as compared to other biochar types (Fig. 5A, C). Moreover, unlike (25% sawdust) and (5% zeolite) derived biochar (Fig. 5B, A), the (20% sawdust + 5% zeolite) derived biochar (Fig. 5C) lack

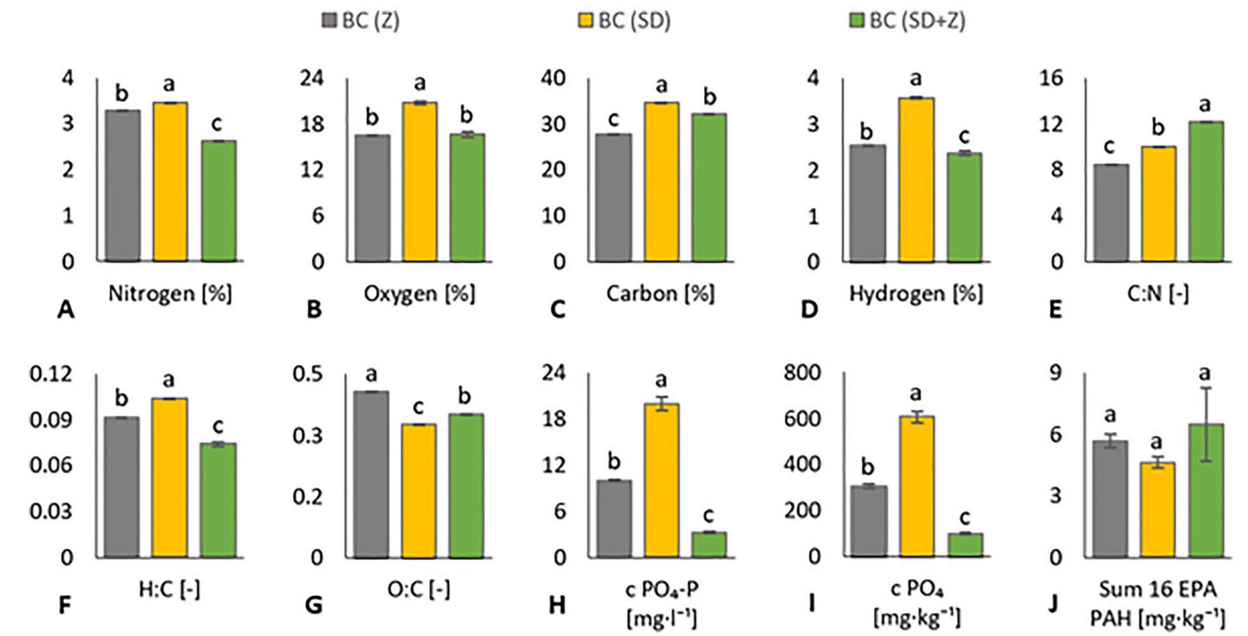


Fig. 4 Chemical properties of biochar treatments made of sewage sludge, sawdust, and zeolite, content of **A** nitrogen, **B** oxygen, **C** carbon, **D** hydrogen, **E** carbon and nitrogen ratio, **F** hydrogen and carbon ratio, **G** oxygen and carbon ratio, **H** concentration of phosphorous in form of phosphate in water leachate of biochar (mg·l^{-1}), **I** concentration of phosphate in biochar (mg·kg^{-1}), **J** concentration of 16 priority PAHs according to U.S. EPA (mg·kg^{-1}). Mean ± standard deviation (error bars) calculated from independent values ($n=4$), different letters express the statistical differences at significance level $p \leq 0.05$

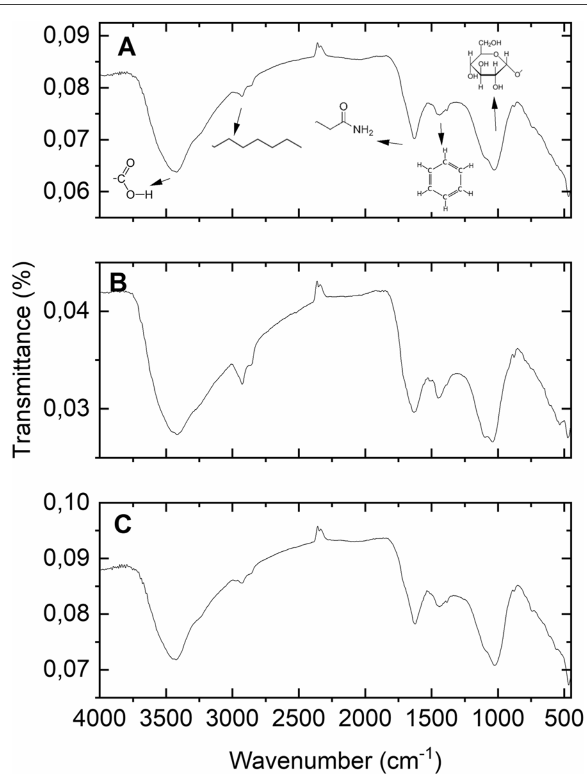


Fig. 5 FTIR spectra of **A** 5% zeolite–biochar, **B** 25% sawdust–biochar and **C** 20% sawdust + 5% zeolite–biochar

a characteristic peak between 3900 and 3500 cm^{-1}, representing OH-stretching of carboxyl functional groups. Furthermore, all the three types of biochar showed strong peak above 1500–400 cm^{-1}, depicting compounds derived from polysaccharides (1057 cm^{-1}), aromatic C–H stretches (1506 cm^{-1}) and aliphatic amides (1557 cm^{-1}) (Fig. 5A, B, C). In addition, the C–H stretching vibrations were observed at 2970 and 2860 cm^{-1}. This shows that the co-pyrolysis of SS biochar with different rates of sawdust and zeolite resulted in altered functional group chemistry of resultant biochar samples.

Soil reaction and enzyme activities

Soil reaction pH ($CaCl_2$) value was significantly lowest in the treatments 2.5% BC (SD), 7.5% BC (SD), and 7.5% BC (SD + *Z*) as compared to control (Fig. 6). The soil pH did not show any significant and considerable correlation with other measured parameters.

The dehydrogenase activity (DHA) was increased in all treatments except for 2.5% BC (SD + *Z*) and 7.5% BC (SD + *Z*) as compared to the control (Fig. 6). The highest DHA value was detected in the 2.5% BC (SD) treatments and was increased as compared to all treatments of control, with BC (*Z*), with BC (SD + *Z*), and 7.5% BC (SD) treatment. The Pearson's correlation analysis revealed no

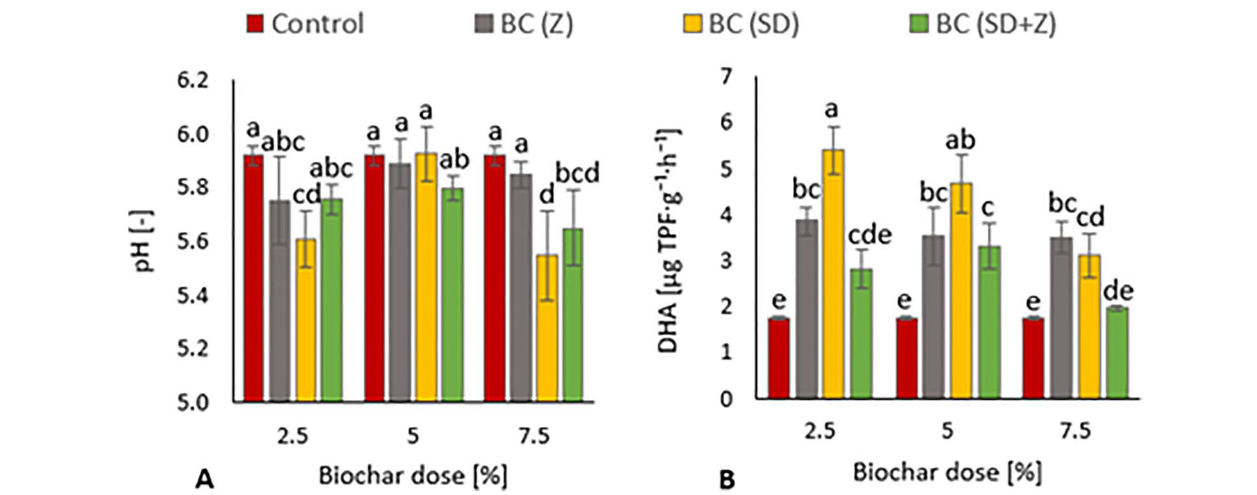


Fig. 6 Soil pH and dehydrogenase activity (DHA) of substrate treatments amended with biochar made of sewage sludge, sawdust, and zeolite. Mean ± standard deviation (error bars) calculated from independent values ($n = 4$), different letters express the statistical differences at significance level $p \leq 0.05$.

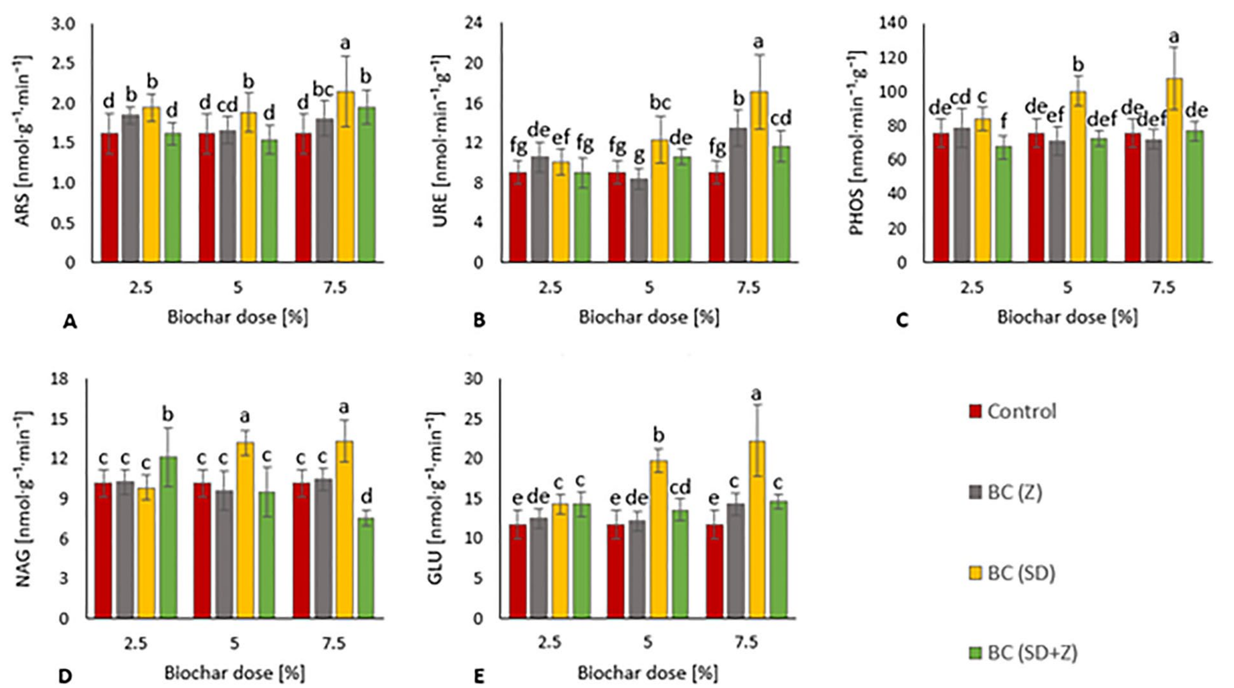


Fig. 7 Soil enzyme activities—**A** arylsulfatase (ARS), **B** urease (URE), **C** phosphatase (PHOS), **D** N-acetyl-β-D-glucosaminidase (NAG), **E** β-glucosidase (GLU)—of substrate treatments amended with biochar made of sewage sludge, sawdust, and zeolite. Mean ± standard deviation (error bars) calculated from independent values ($n = 36$), different letters express the statistical differences at significance level $p \leq 0.05$

significant and considerable correlation of DHA with any other plant or soil property.

The activity of arylsulfatase (ARS) was increased in all treatments with BC (SD), under both 2.5% and 7.5% BC (Z), and 7.5% BC (SD + *Z*) as compared to control (Fig. 7).

The highest ARS value was revealed in the 7.5% BC (SD) as compared to all other treatments, while the lowest ARS was in 2.5% BC (SD + *Z*) and 5% BC (SD + *Z*) relative to all other biochar-amended treatments. The Pearson's correlation analysis showed that ARS was

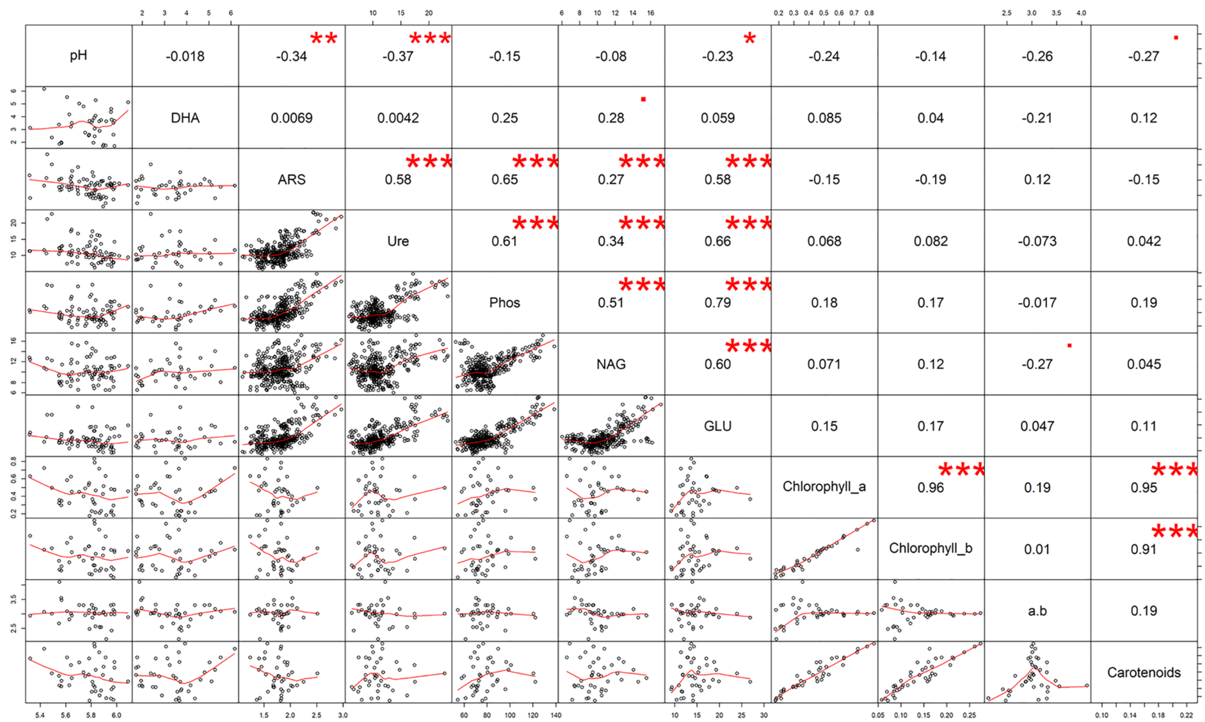


Fig. 8 Results of Pearson's correlation analysis among soil and plant properties. Values in the cells = correlation coefficient *r*, calculated on the level of significance *p*: ≤ 0.1 (·), ≤ 0.05 (*), ≤ 0.01 (**), ≤ 0.001 (***)

significantly ($p \leq 0.001$) and moderately positively correlated with PHOS ($r = 0.65$), URE (r = 0.58), and GLU ($r = 0.58$) (Fig. 8).

The URE activity was significantly increased in treatments 2.5% and 7.5% BC (*Z*), 5% and 7.5% BC (SD), as well as 5% and 7.5% BC (SD + *Z*) as compared to the control. However, the highest urease activity was detected in the 7.5% BC (SD) treatment and the lowest was in 5% BC (*Z*), as compared to all other treatments. The URE showed moderate positive and significant ($p \leq 0.001$) correlation to PHOS ($r = 0.61$) and GLU ($r = 0.66$), respectively (Fig. 8).

PHOS was significantly increased (compared to the control) in all three treatments amended with BC (SD) at 2.5%, 5%, and 7.5% BC (SD). Moreover, 5% and 7.5% BC (SD) treatments showed higher PHOS compared to any other biochar amended treatment. The highest PHOS activity was recorded in 7.5% BC (SD) and the lowest was in 2.5% BC (SD + *Z*) as compared to control and other treatments (Fig. 6). We also observed a significant ($p \leq 0.001$) correlation between PHOS and NAG (moderate positive, $r = 0.51$) and GLU (high positive, $r = 0.79$).

N-acetyl-β-D-glucosaminidase (NAG) was increased in the treatments 5% BC (SD), 7.5% BC (SD), and 2.5% BC (SD + *Z*) in comparison with the control (Fig. 7) and significantly decreased in 7.5% BC (SD + *Z*) in comparison with all other treatments. NAG correlated significantly ($p \leq 0.001$) and moderately positively with GLU ($r = 0.6$). The activity of GLU was significantly increased in all 3 treatments with BC (SD) and BC (SD + *Z*) as well as in both 5% and 7.5% BC (*Z*), as compared to the control (Fig. 7). The highest GLU values were observed for 5% BC (SD) and 7.5% BC (SD) as compared to controls and other treatments.

Plant biomass and photosynthetic pigments

The fresh and dry aboveground (AGB) biomass showed no significant difference between all amended experimental treatments and the control as well (Fig. 9). The content of chlorophyll *a* and *b* showed highly positive and significant correlation ($p \leq 0.001$, $r = 0.96$) in our study. Their values were considerably decreased in all three treatments with BC (*Z*) and in 7.5% BC (SD + *Z*) treatment, and significantly increased only in 5% BC (SD + *Z*) treatment, as compared to the control (Fig. 9).

A significant decrease in the chlorophyll *a*/*b* ratio was detected in both 2.5% and 7.5% BC (Z) compared to the control.

The total carotenoids content was decreased (as compared to the control) in all treatments amended with BC (*Z*) additive. The highest value was observed for 5% BC

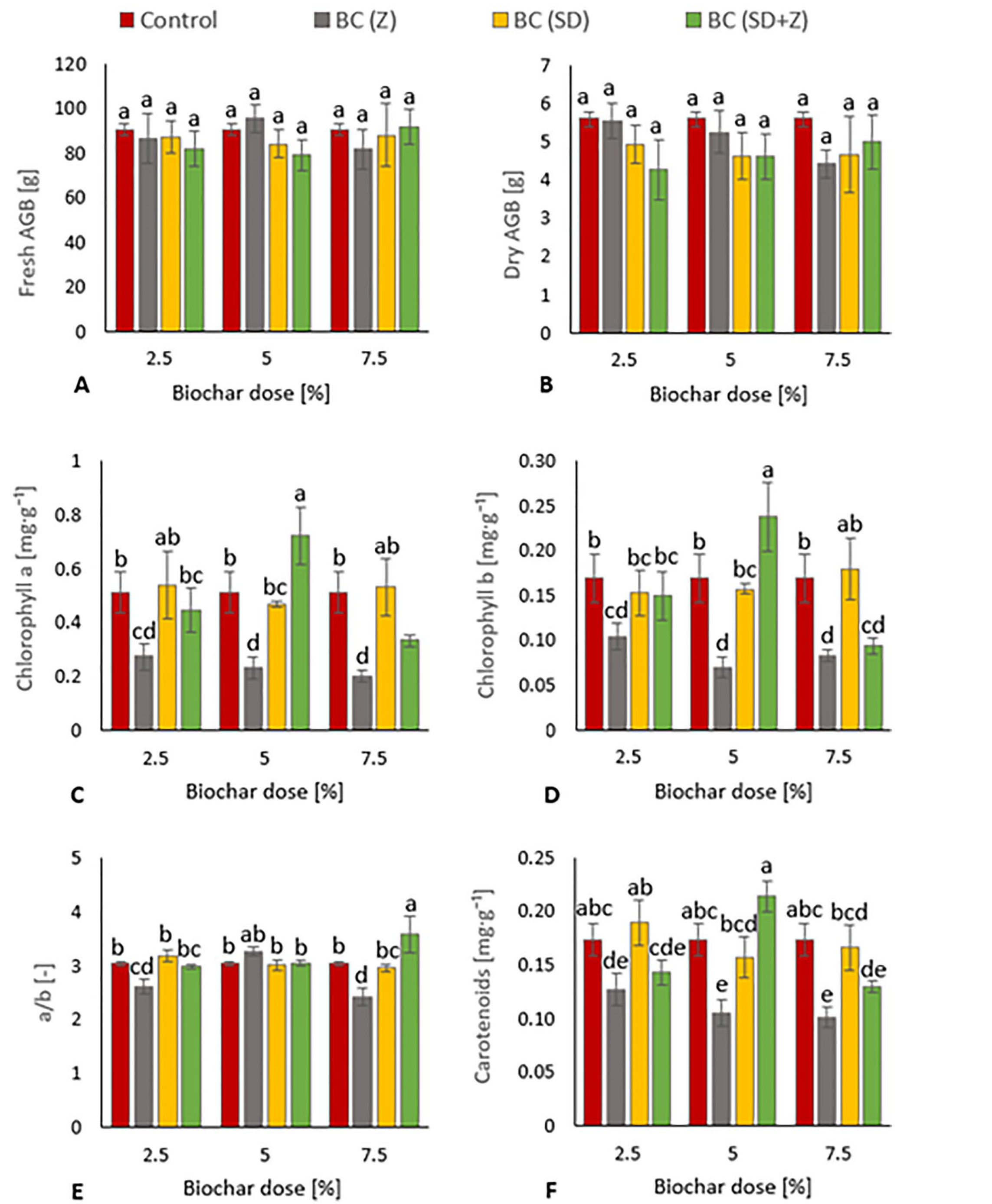


Fig. 9 **A** Fresh and **B** dry aboveground (AGB) lettuce biomass and content of leaf pigments in lettuce cultivated on different substrate treatments—**C** chlorophyll *a*, **D** chlorophyll *b*, **F** carotenoids and **E** the chlorophyll (a/b) ratio. Mean ± standard deviation (error bars) calculated from independent values ($n = 4$), different letters express the statistical differences at significance level $p \leq 0.05$

(SD + *Z*) as compared to control and other experimental treatments (Fig. 9).

The chlorophyll *a* fluorescent induction curves in (Fig. 10) showed the differences between the plants cultivated in substrate with different type and dose of biochar. There were no significant differences in F_0 fluorescence between all treatments. In all experimental treatments except 2.5% BC (*Z*) was measured higher

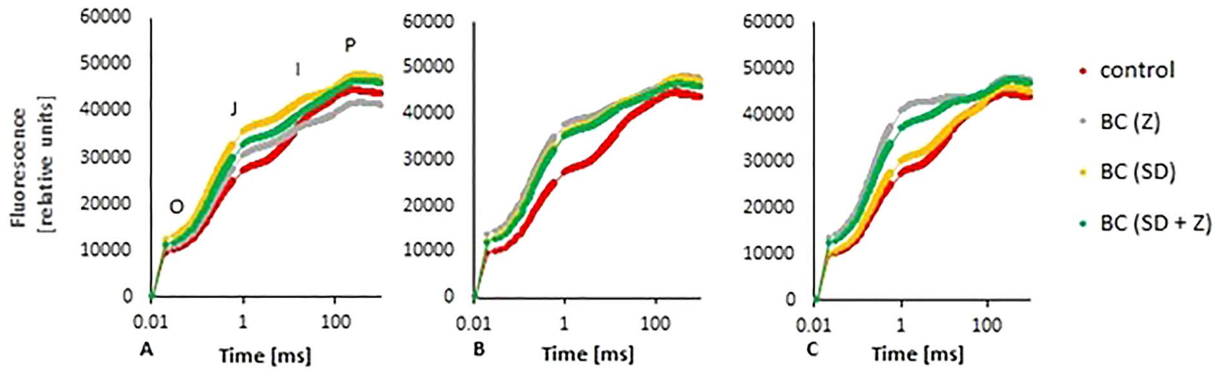


Fig. 10 Chlorophyll *a* fluorescence induction curves (OJIP) of the different cultivation treatments with different doses indicated weight percentages of biochars in soil—**A** 2.5%, **B** 5% and **C** 7.5%

F_m compared to control. The highest fluorescence was observed in 2,5% BC (SD) and 7,5% BC (SD + *Z*) and 7,5% BC (*Z*). It is in the correlation with the results of higher amount of chlorophyll *a* and the chlorophyll *a*/*b* ratio of these treatments Figs. 7, 10). On the other hand, the low J–I and I–P amplitudes are visible from the OJIP curves shapes (Fig. 10) in 7,5% BC (*Z*) and BC (SD + *Z*) reported about possible disruption of photosystem I (PSI) function The O–J and J–I amplitudes were higher in BC (*Z*) and BC (SD + *Z*) in 2,5% and 5% concentration compared with control. The evaluation of QY_{max} and biomass production did not show the significant differences between the experimental treatments (data are not presented).

Discussion

Biochar characteristics

We observed the highest average total content of each determined macroelement (N, H, C, O) in the biochar pyrolyzed from the SS amended with 25% of sawdust—BC (SD). We presume that it was due to high organic matter content in the feedstock (namely, due to the presence of sawdust), which decreased the content of ash originating from sewage: BC (SD) biochar contained 30.8% ash (in average). However, in the case of ash coming from feedstock consisting of sewage + sawdust combined with zeolite, the ash content was increased to 40.1% (BC (SD + *Z*)). In case of BC (*Z*), the ash content was the highest, i.e., 42.9% (in average). This illustrates the effect of zeolite, which is an effective catalyzer for cleavage of the chemical bonds in lignocellulose materials in the saw dust [57, 58]. In past, zeolite was effectively used as a pyrolysis catalyzer of feedstock including greenhouse vegetable wastes and lignite coal [58], water hyacinth and algae bloom [59], and *Chlorella vulgaris* and municipal solid waste containing food and fruit waste, wood, paper, and polyvinyl chloride (PVC) [59]. On the contrary, SS contains mainly molecules of microbiological origin, i.e., lipid and proteinaceous moieties, which seem to be less influenced by catalytic activity of zeolite catalyzer. The highest (3.5%) total hydrogen content in biochar pyrolyzed from SS + sawdust contrasted with much less abundant (on H_{tot}) BC (*Z*) biochar (around 2.5%) and BC (SD + *Z*) biochar (< 2.5%). Acidifying effect of sawdust might promote protonation (and joint hydrogenation) of organic compounds in the feedstock. The thermal conversion processes preserved higher content of hydrogen in the final pyrolyzed organic matter probably in the form of highly aromatic carbonaceous structures. Again, the putative (antagonist to the sawdust impact) alkalizing effect of zeolite amendment was presumed to decrease the hydrogen content prior and during the pyrolysis (Fig. 4). The total carbon content was the property to be strictly affected by the amount of organic matter (added to the feedstock) of biochar. Therefore, the BC (SD) with 75% SS and 25% SD showed also the highest total carbon content, followed by BC (SD + *Z*), which was, in contract to the SD (*Z*), supplemented with 20% (of the total 95% of organic content) more OM-abundant sawdust matter (wood commonly contains > 50% carbon, compared to < 40% in sewage sludge). Total oxygen content in the biochar types may be again attributed to the properties of the primary feedstock for pyrolysis. It was reported by studies from different fields of application [60, 61] that zeolite can act as an antioxidant enhancing the pyrolysis reduction process. As a result, we observed a significantly decreased total oxygen content in both treatments amended with zeolite—BC (*Z*) and BC (SD + *Z*) compared to the O_{tot} content of the BC (SD) biochar (Fig. 4).

There were no significant differences (in fresh and dry aboveground lettuce biomass) detected between the treatments, but there were apparent some trends in changes of AGB values. The ratios between macroelements in the 3 biochar types determined the availability of respective nutrient for the soil microbiota and plants as well as indicated the stability rate of the biochars. The highest C:N ratio of BC (SD + *Z*) biochar (around 12.0) indicated the value closest to the plant nutrition optimum 24:1 [62] and subsequently, the highest values of plant biomass quality parameters were observed in the treatments amended with this type of biochar (Sect. 3.3, Fig. 9). The lower C:N ratio of BC (SD) biochar (around 10.0) corresponded to the situation with excess of N, which supports the microbial (bacterial, fungal, actinomycetal) activity [63]. We explained by this the generally most enhanced enzymatic activity in the soil treatments amended with BC (SD), (Fig. 7). Lower H:C ratio indicates higher aromaticity, i.e., abundance of polycondensed structures, which is connected to lower degradability of biochar, according to respected guidelines for biochar quality evaluation [64]. Therefore, the values of H:C ratio from the highest BC (SD) to the lowest BC (SD + *Z*) corresponded to the presumed biodegradability of the biochars, ascribed from the results of dehydrogenase activity (DHA) determination. This (DHA) indicator of decomposition processes in soil was the most enhanced in the soil amended with BC (SD) and the least enhanced in the treatment with BC (SD + *Z*) (Fig. 6). Finally, lower O:C ratio is indicative for low content of O-containing functional groups, i.e., less reactive biochar [65]. The low O:C value is attributed to the lower polarity causing, in general, worse biodegradability and lower microbial activity in the soil treatments amended with BC (*Z*) and BC (SD + *Z*) biochars, which both revealed O:C value < 0.4, compared to the BC (SD) biochar with O:C > 0.4 (Fig. 6).

Similarly, to the close relation between C, N, O content in biochar and soil traits in the treatments of substrate, the demonstrably highest content of leachable phosphate (PO_4–P in water leachate, calculated to biochar dry weight—600 mg·kg^{-1}) in the BC (SD) biochar corresponded to the significantly highest phosphatase (PHOS) activity in the respective soil treatment. The two other biochar treatments showed both much lower (significantly) cPO_4 content and subsequently their application did not enhance any significant PHOS activity (Fig. 7). The content of available P could be affected also with the pyrolysis temperature. It has been observed that the total P content increased with pyrolysis temperature, and highest available P contents were observed from biochar prepared at temperatures not exceeding 300 °C [66]. BC prepared for experiments presented in this study did not exceed 250 °C. This might be another reason for enhanced available P in the present study.

The determination of 16 EPA PAHs revealed no significant difference in their total content between all three biochar types (Fig. 4). However, it was important that the maximum average detected values were below the risk values estimated by EBC guidelines. According to the EBC guidelines [67], the total concentration of the 16 USEPA-priority PAHs must be < 12 µg·kg^{-1} dry mass for basic-grade biochar.

Soil reaction and enzyme activities

Biochar produced from SS and sawdust (at doses 2.5% and 7.5% w/w) significantly decreased (as compared to the control) substrate pH ($CaCl_2$). The highest (7.5%) dose of BC (SD + *Z*) also significantly decreased substrate pH ($CaCl_2$) in comparison with the control (Fig. 6). The sawdust was presumed to change the pH of final pyrolyzed product to acidic, whereas the zeolite was referred to have alkaline reaction in soil. The study by Laghari et al. [68] reported that biochar made from fast pyrolysis of pine sawdust significantly reduced soil pH [68], and we assumed the same effect of BC (SD) on pH in our experiment in all treatments except of 5%. It seems that the zeolite addition mitigated the acidifying effect of co-pyrolyzed sawdust on the amended soil. This is in accordance to previous report, which noted that zeolite combined with biochar increased soil pH during greenhouse pot experiment [69]. Putatively, sewage sludge had higher affinity to zeolite (via its ionic content) than sawdust. At the highest (7.5%) SS dose, the amount of sewage sludge attenuated alkalizing effect of zeolite and sawdust caused drop in final pH.

Dehydrogenase

DHA is the determinant of microbial activity in soil and indicates the rate of decomposition of soil organic matter (SOM) [70]. Amendment of all types of biochar at all doses—except of two, 2.5% BC (SD + *Z*) and 7.5% BC (SD + *Z*)—to the substrate significantly increased DHA values as compared to the control (Fig. 5). The highest DHA value was detected in the treatment with 2.5% biochar pyrolyzed from SS and sawdust, which DHA was significantly increased compared to all treatments with BC (*Z*), with BC (SD + *Z*), and 7.5% BC (SD) treatment. These results indicated that sawdust co-pyrolyzed with SS served as a source of SOM significantly richer (in C, N, O) as compared to the biochar produced from SS + zeolite and SS + sawdust + zeolite (the differences were indicated by C:N and O:C ratio). Wei et al. [71] reported that sewage sludge biochar contained more dissolved organic carbon in comparison with pine wood biochar [71]. Soil DHA was referred to be increased after application of low-temperature SS–biochar to soil [72], due to the nutrient and labile carbon bioavailability. Nevertheless, higher dose of SS + sawdust-based biochar had decreased the beneficial effect on DHA, as well as the interaction between zeolite and sawdust seemed to be adverse for the SOM decomposition, putatively due to the stabilizing effect of zeolite on the available sources of nutrients (e.g., nitrogen).

Arylsulfatase

Arylsulfatase (ARS) catalyzes desulfurization of organic sulfates and is used as an indicator of soil sulphur mineralization [73]. The arylsulfatase was significantly increased (compared to the control) in all biochar-amended treatments except of 5% BC (*Z*), both 2.5% and 7.5% BC (SD + *Z*), (Fig. 7). The highest (7.5%) dose of BC made of sewage sludge and sawdust applied to soil lead to the significantly highest arylsulfatase activity. We assume from these results that SS served as a main source of sulphur in the produced SS-based biochar. Sawdust was reported to protect sulphur losses via volatilization during pyrolysis [74]; therefore, this may explain the observed results. Sulphur stabilization (during co-pyrolysis of SS and SD) seemed to be sawdust-dependent and thus ARS was the most increased in the soil with the highest dose of SD-amended sewage sludge biochar. On the contrary, zeolite that is frequently used as a catalyst in biomass conversion to fuels and chemicals [75]. Thus, yeolite might have counteracted the stabilizing effect of sawdust on SS-derived sulphur, putatively via absorption of compounds in sawdust, involved in the stabilization of volatilizable sulphur of SS.

Urease

Urease (URE) is an ubiquitous enzyme that hydrolyzes urea and is involved in the early phase of nitrification processes leading eventually to the nitrogen mineralization. The urease activity was (in comparison with the control) significantly increased in all treatments amended with middle (5%) and high (7.5%) dose of biochar BC (SD) and BC (SD + *Z*), except of soil amended with biochar BC (*Z*). In this case, 2.5% BC (Z) and 7.5% BC (*Z*) showed increased URE (as compared to the control). The highest (7.5%) dose of BC made of sewage sludge and sawdust again mediated the significantly highest enzyme (urease) activity in the treated soil. These results suggest the main contribution of sewage sludge material to the nitrogen content in the produced SS-based biochar(s). Other authors, i.e., [76] also referred to significant increases in urease URE activity, total soil nitrogen, and available phosphorus under SS-biochar amendment (as compared to the control soil) [76], which can be attributed to high nitrogen and phosphorus content of sewage sludge. The supplement of nitrogen in SS to the feedstock putatively increased utilizable (by activity of URE) nitrogen concentration in co-pyrolyzed product, which increased urease activity in soil both with higher dose of sole SS-based biochar and with the contribution of either zeolite or sawdust amendment (Fig. 7). We also presumed the sawdust-derived enrichment of produced SS-biochar with organic nitrogen, or zeolite-mediated nitrogen stabilization and protection against mineralization. There was reported such combined use of biochar and zeolite in the compost, which significantly increased the enzymatic activities and reduced the ammonia loss [24]. Both assumptions may explain the observed highest URE value in the 7.5% BC (SD) treatment. Application of SS–biochar with both additives zeolite and sawdust (co-pyrolyzed) caused again in the respective substrate treatments an adverse effect on URE (when compared to relevant treatments with either sole sawdust or sole zeolite in SS).

Phosphatase

Soil phosphatase (PHOS) is a determinant of phosphorus solubilization and mineralization, it catalyzes dephosphorylation of organophosphates. Only soil amended with biochar produced from SS + SD (5% and 7.5% doses) revealed a significantly increased phosphatase activity, compared to the control and all other treatments. Contrarily, application of 2.5% BC (SD + *Z*) decreased PHOS in comparison with the control (Fig. 6). These findings agreed with knowledge that phosphorus biogeochemical processes are largely influenced by biochar amendments in soils [77]. We presumed that sawdust addition to sawdust facilitated enrichment of the resulting biochar with phosphorus from SS feedstock, while sole zeolite did not improve phosphorus content in BC (*Z*) during pyrolysis. In contrast, zeolite putatively decreased yield of SD-derived phosphorus in final BC (SD + *Z*) during the co-pyrolysis process (see Fig. 4H,)I). Moreover, zeolite could also protect phosphorus from mineralization in soil, which effect was again the strongest under interaction of Z with sawdust. The presumed zeolite hindrance to the phosphorus availability in soil corresponds with the reported negative effect of zeolite on phosphate dissolution [78].

N-acetyl-β-D-glucosaminidase

N-acetyl-β-D-glucosaminidase (NAG) and β-glucosidase (GLU) are enzymes responsible for the decomposition of two the most abundant soil polysaccharides, such as cellulose [79] and chitin [80]. The enzymes are indicators of carbon (NAG, GLU) and nitrogen (NAG) mineralization. Low (2.5%) dose of SS-biochar pyrolyzed with zeolite and higher (5% and 7.5%) doses of BC (SD) significantly increased NAG activity compared to the control, whereas 7.5% BC (SD + *Z*) applied in soil lead to significant decrease of NAG in comparison with all other treatments. Again, we ascribed that the sole sawdust positively affected putative fungal growth and chitin formation, which was subsequently degraded by NAG, whereas sawdust in combination with zeolite and increasing dose of SS-based biochar led to the biochar with seemingly adverse effect on the fungal and chitin abundance. De la Rosa et al. [81] observed that SS-derived pyrogenic organic matter and carbon may be colonized in its pore system by fungus (*Fusarium oxysporum*), which was involved in the degradation of its aromatic network [81]. A significant negative effect of high dose (7.5%) biochar co-pyrolyzed from SS + SD + *Z* might be attributed to putative zeolite-mediated (nitrogen) stabilization in SOM of respective treated soil, which could presumably limit the fungal nutrition and abundance. Positive correlation of NAG with GLU NAG indicated their joint involvement in the saccharide-structured OM degradation.

β-Glucosidase

The β-glucosidase activity was significantly increased in all biochar-amended treatments except of both 2.5% and 5% BC (*Z*), compared to the control (Fig. 7). Amendment with 5% and 7.5% (w/w) biochar produced from SS + SD lead again to the highest GLU values, in comparison with all other treatments. This is evidence that sawdust addition to co-pyrolyzed SS resulted in the SS–biochar which stimulated SOM formation and subsequent decomposition of its carbohydrate fraction more efficiently than the application of biochar with co-pyrolyzed SS + zeolite. We assumed that from the described beneficial effect of sawdust biochar on higher OM (as well as available phosphorus and available potassium) in soil [82]. This additive of zeolite to primary biochar feedstock was only (significantly) beneficial to GLU at highest dose of biochar. Whereas its effect during co-pyrolysis with sawdust was adverse and decreased GLU in soil to levels equal to biochar made of sole zeolite + SS, independently on the increasing SS–biochar dose.

In general, we observed that zeolite amendment to the pyrolyzed biomass resulted in the biochars with mostly adverse effects on soil enzyme activities. An explanation is that zeolite protected the nutrients from mineralization and decreased availability. Mondal et al., (2021) referred to this zeolite features: selectivity for major essential nutrients, including ammonium (NH_4^+), phosphate (PO_4^{2-}), nitrate (NO_3^-), potassium (K^+) and sulfate (SO_4^{2-}) and reduction of nutrient leaching from soil [83]. Nutrients are released slowly from zeolite, which can cause retardation in the release of nitrogen, phosphorus, potassium [84]. Zeolite can also serve as a matrix/scaffold for enzyme immobilization [85], as either catalyst or inhibitor, which may further explain the observed decrease in enzyme activities. A second explanation is that zeolite stabilized the whole structure of biochar thereby preventing the leaching of nutrients in soil.

Plant biomass and its quality parameters—chlorophylls and carotenoids content

The fresh and dry aboveground (AGB) biomass showed no significant statistical difference between all experimental treatments, due to the high variability in the biomass yield between the replicates within each treatment. It was apparent that the random and more significant variability occurred between the dose-differing treatments in the fresh AGB values, whereas dry AGB showed a descending trend in the average values for the BC (*Z*) treatments (with increasing biochar dose) and an ascending trend for the BC (SD + *Z*) treatments (with increasing biochar dose) (Fig. 9). Chlorophylls *a* and *b* (Chl-*a*, Chl-*b*) are the photosynthetic active pigments, that act as the acceptors of light energy and after excitation allow its conversion to the chemical bonds. Their contents reflect leaf photosynthetic ability and plant health condition [86]. Leaf content of chlorophylls *a* and *b* was evidenced to be jointly controlled by climate, soils, and plant nutrient availability [87]. Both properties chlorophyll *a* and *b* correlated highly positively and significantly. They both were significantly lowered by application of biochar from SS + zeolite and by amendment of high dose (7.5%) of BC (SD + *Z*). Nevertheless, application of 5% dose of biochar (co-pyrolyzed from SS + sawdust + zeolite) to the substrate increased both Chl-*a* and Chl-*b* content as compared to the control (Fig. 9). In the study by Hashmi et al., [88], there was also significant increase in Chl-*a* and *b* in response to a *Pongamia pinnata* L. waste leaf biochar + full NPK fertilizer application, which caused a reduction in nutritional stress in pea (*Pisum sativum* L.) [88]. We assume that the combination of SS, sawdust and zeolite resulted in the biochar with less easily utilizable nutrients for soil microbial growth and activity (in comparison with SS + sawdust chars), but which provided more plant-available nutrients (with slow release) and suitable conditions for plant nutrition and thriving. The potential of zeolite to act similar to slow-release fertilizers gradually providing the required essential elements for plant growth was described by Hamidpour et al., [89]. A combined adverse effect of other dose-dependent negative traits of this type of biochar might be responsible for this result as well. Moreover, our presumption of variable soil nutrient availability to microflora and plant was proved by the results of BC (Z) treatments, which Chl-*a* and Chl-*b* values seemed to be significantly decreased due to limited access of nutrients, putatively immobilized by the strong adsorption on zeolite or by stabilization of biochar´s structure. The described features might affect the synthesis and abundance of carotenoids in lettuce too, which is indicated by high positive significant correlation of these pigment levels (Fig. 8). Similar results were observed by Abid et al., [90], who reported impacts of biochar on growth and photosynthetic pigments in tomato (*Solanum lycopersicum* L.) plants by reducing cadmium concentration under various irrigation regimes [90]. The photosynthetic pigments were comparatively low in cadmium contaminated irrigation treatment without biochar application, but increased photosynthetic pigments was observed with 1% biochar and sewage water application. This mentioned positive effect of biochar addition to plant growth on cadmium or lead contaminated soil related to the immobilization of these heavy metals for plant uptake and the improving of physiological and biological soil properties [11, 12].

The chlorophyll *a*/*b* ratio is known as an indicator of stress in plants, e.g., under osmotic stress, chlorophyll *a*/*b* ratio tend to increase due to greater reduction in Chl-*b* compared to Chl-*a* [91]. Only the treatment 7.5% BC (SD + *Z*) exerted significantly increased *a*/*b* ratio in comparison with the control. We ascribed that to the presumed adverse effect of high dose of this type of sewage sludge–biochar, in combination with low pH. There was also detected a significant decrease in *a*/*b* in both 2.5% and 7.5% BC (Z) treatments compared to the control, but we attribute this feature to the overall inhibited synthesis of chlorophyll in these treatments. Similarly, to our findings, the study of Sattar et al., [92] revealed that biochar application increased contents of photosynthetic pigments—chlorophyll *a*, *b*, *a* + *b* and *a/b* ratio in maize (*Zea mays* L.) seedlings and it was concluded that biochar application is an efficient way to mitigate adverse effect of drought stress [92].

Carotenoids (Car) are a common group of auxiliary plant pigments in photosynthesis, they transfer the absorbed energy to chlorophyll and protect it from excessive light intensity [93]. The changes of leaf carotenoids content and their proportion to Chl-*a* and Chl-*b* are used as indicators of the physiological state of plants during development, senescence, acclimation, and adaptation to stresses and different environments [94, 95]. Application of biochar pyrolyzed from SS + zeolite into soil at all doses (2.5%, 5%, 7.5%) lead to the significantly decreased Car content compared to the control, as well as the amendment of 5% biochar BC (SD + *Z*) (Fig. 7). These results indicate that lettuce plans responded by lowered synthesis of carotenoids to restricted availability of nutrients in the substrates amended with SS + *Z*-biochars, similar to chlorophylls. Ghassemi-Golezani et al. [96] also described the decrease of photosynthetic pigments such as chlorophylls and carotenoids in safflower (*Carthamus tinctorius* L.) leaves in the case of salt toxicity of soil [96], but the application of biochar and particularly biochar-based nanocomposites of magnesium and manganese oxides reduced sodium accumulation and improved biosynthesis of photosynthetic pigments.

Photosynthesis is one of the basic metabolic processes of the plants. It can be divided into two groups of the reaction—primary processes involving the absorption of light energy and its conversion into the chemical bonds of ATP and NADPH and secondary processes which used primary products for assimilation of CO_2. Function of primary processes which are light dependent can be characterized by the fluorescent parameters [97]. Measuring of these parameters is quick non-invasive method to study the stress effect of the various cultivation condition to the experimental plants [87]. The higher values of F_m in all experimental treatments compared to control are in the correlation with the results of higher amount of chlorophyll *a* and the chlorophyll *a*/*b* ratio of these treatments (Fig. 8). On the other hand, flattering of J–I and I–P amplitudes visible from the OJIP curves shapes in 7,5% BC (*Z*) and BC (SD + *Z*) (Fig. 10) indicates possible disruption of photosystem I (PSI) function, respectively, its capacity [98, 99]. The O–J and J–I amplitudes suggested the reduction of primary, respectively, secondary acceptors (Q_A, Q_B, plastoquinone and cytochromes) [87] and were higher in BC (*Z*) and BC (SD + *Z*) in 2,5% and 5% dose compared with control. It can indicate the better function of photosystem II (PSII); however, the dry or fresh biomass production did not show any significant differences between the vitality of experimental plants from different cultivation treatments (data are not presented).

The photosynthetic apparatus can affect and can be affected by the overall vitality of plants, the content of photosynthetic pigments (chlorophylls) depends on the total amount and availability of nutrients. We assumed the effect of mineralization intensity (and degree of immobilization by sorption on zeolite) on available nutrient levels and competition in their intake between plants and soil microorganisms. The study by Gholamhoseini et al., [100] described how higher doses of zeolite amendment (14–21%) co-applied with fertilizers (urea, manure) improved quantitative and qualitative properties of sunflower more significantly than amendment of less or no zeolite in combination with fertilizers [100]. We assume from this that the effect of zeolite on chlorophyll content in the biomass of lettuce leaves was remarkable. From our results it could be seen that the potential negative effect of the residual toxic chemical structures from sewage sludge can be reduced by microwave pyrolysis. In addition, the biochar with this origin could be used as the soil supplement without the negative effect on cultivated plants. There is the prediction that the long-term cultivation of plants could lead to observation of positive effect of the biochar addition due to the support of soil microflora and improvement of nutrient availability especially in nutrient poor soil [101].

Microwave pyrolyzed biochar—an amendment or fertilizer?

Application of biochar to soil represents a strategy for sequestration of carbon, because biochar's chemical structure is considered to be resistant to microbiological attack [102]. Simultaneously, depending on the feedstock and conditions of preparation, biochar may represent a source of macronutrients and an amendment improving water-holding capacity. The European Biochar Certificate states that, "Biochar is a charcoal-like substance that is pyrolysed from sustainable obtained biomass under controlled conditions and which is used for any purpose which does not involve its rapid mineralization to CO_2" [67]. Based on this definition, Conte et al. [103] concluded that biochar may be produced only from fast growing plants, plant residues from certified forestry management, agricultural residues, and organic wastes from urban areas [103]. However, pyrolysis of sewage sludge pyrolyzed under conditions (i.e., low temperature microwave pyrolysis) used in this work appeared to produce biochar that is apparently biodegradable and intensively stimulates the activity of soil microorganisms. We attribute this observation to the interplay of two important factors: temperature of the pyrolysis and used feedstock. According to Tag et al., [104] the increasing temperature decreases both the H:C and O:C ratios in pyrolyzed biomass, such as vine pruning (VP), poultry litter (PL), orange pomace (OP) and seaweed [104]. Comparing results with those reported by Tag et al., (2016) roughly correspond to the dependence of O:C at respective pyrolysis temperature, but H:C ratio is in our case significantly lower [104]. In other words, biochar from sewage sludge is less aromatic (see Fig. 5) comparing to other sources, which confirms the comparison with other authors [104–106].

In fact, feedstocks for pyrolysis are usually based on lignocellulose materials, such as wood residues, grass and others. On the contrary, sewage sludge is of microbiological origin, i.e., it contains mainly N-rich compounds from protoplasma and cell membranes, such as proteins and fatty acids. Therefore, resulting structure differs compared to lignocellulose-based biochar; the O:C is similar, but lower H:C in SS-based biochar shows that this biochar is significantly less aromatic, i.e., more aliphatic. As it is well-known that aliphatic structures are better biodegradable comparing to aromatic structures [107], biochar prepared from sewage sludge acts as more as a fertilizer instead of an amendment. This is supported by higher content of nitrogen in its structure, which seems to be also bioavailable as suggested by activity of urease.

Microwave pyrolysis is fast, selective and efficient method for production of pyrolyzed materials [49, 108]. However, the conditions such a low temperature may lead to products whose properties are far from definition of biochar. As follows from the results, no every intentionally pyrolyzed organic material is suitable for carbon sequestration; nevertheless, its effect on soil may still be positive. In particular, it may represent a source of labile carbon that supports soil microbial processes together with macronutrients. In addition, these effects may be tuned by addition of zeolite, which either stabilizes the biochar structure or moderate release of nutrients. It remains a question, if the pyrolyzed product of sewage sludge should still be named as a biochar, as the biochar per definition, is microbiologically stable material [67]. Moreover, its application is one of the keys in the long-term strategy of increasing of soil organic carbon in soils and sequestration/storage of carbon in soil.

Despite the enhanced microbial activity of soil microorganisms, the use of this particular biochar neither increased nor decreased biomass yield which can be explained as follows: i) the amount of N released from SS biochar was high enough to support the soil microorganism and no competition between soil microbiome and plant roots of *Lactuca sativa* occurred and ii) the length of the experiment was too short and the effect on plant could not manifest, i.e., biochar affected soil microbiological processes, but the effect on plants appears with a delay, iii) heavy metals, the higher content of which is usually a problem of the municipal SS [49], affected neither soil microbiome nor plant which confirms its fixation and immobilization in biochar structure [49].

Conclusions

This study concluded that microwave pyrolysis produced biochar from sewage sludge exerted a decreased microbiological stability of carbonaceous content and was putatively less efficient in soil carbon sequestration. The produced biochar significantly affected soil chemical and microbiological properties. In particular, soil pH was significantly decreased due to application of biochar produced from sewage sludge and sawdust, whereas dehydrogenase, β-glucosidase, arylsulfatase, phosphatase, urease, N-acetyl-β-D-glucosaminidase was increased. Biochar application level was the crucial factor in governing enzyme activities. Sawdust biomass promoted nutrient availability in the resulting biochars and induced higher activity of nutrient mineralizing enzymes, whereas zeolite slowed down the release of nutrients from soil and putatively immobilized enzymes. This joint effect of sewage sludge biochar, sawdust and zeolite benefited the plant acquisition of nutrients in comparison with the microbial nutrient uptake. However, this effect was not accompanied with a changed lettuce biomass yield as the fresh and dry aboveground (AGB) biomass showed no significant difference between all experimental treatments. Albeit the biochar SS + SD + Z (at dose 5% of sewage sludge) determined no improvement in quantity of lettuce biomass, it showed the highest content of photosynthesis pigment (chlorophyl *a*, *b*, carotenoids) and represent an eventual approach in the production of sewage sludge-based biochar with desired traits for soil/agricultural application.

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Author contributions
AM, JH and MB: conceptualization. KL, JR: methodology. TH, KL, and LM: software. MN, JK, and AK: validation. MB, MM, LM and TH: formal analysis. JR, MM and MG: resources. KL, MG, MM and AK: data curation. KL, JH: writing—original draft preparation. JK, AM, TH, MN, JR and MB: writing—review and editing. MB and JK: supervision. KL, LM and JR: project administration. KL, AK, JK and MB: funding acquisition. All authors read and approved the final manuscript.

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Availability of data and materials
The data sets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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