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Influence of biochar feedstock blends on soil enzyme activity, nutrient cycling, lettuce biomass accumulation and photosynthesis

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Abstract

The thermal conversion of municipal sewage sludge (MSS) offers significant potential for sustainable waste management, particularly through the production of biochar. This study investigates the properties and soil application effects of three biochar types produced via pyrolysis: (i) pure sewage sludge (100%), (ii) sewage sludge blended with sawdust (50%+50%), and (iii) sewage sludge combined with sawdust and zeolite (50%+45%+5%). These biochars were applied at rates of 2.5% and 7.5% (w/w) to arable soil and assessed in an 8-week greenhouse experiment using lettuce (*Lactuca sativa* L. var. Brilant) as a model crop. The sewage sludge biochar was characterized by high nitrogen, phosphorus, and water-extractable calcium but exhibited low organic matter and organic carbon content. It enhanced soil enzyme activities related to carbon and nitrogen mineralization without affecting microbial respiration. However, at 7.5% application rate, this biochar caused the highest chlorophyll *b* content in lettuce, despite acidifying the soil. Adding sawdust to the pyrolysis feedstock significantly increased organic matter, organic carbon (with reduced recalcitrance), and the C: N ratio of biochar. This biochar formulation promoted microbial activity (as indicated by changes in soil respiration) and nutrient cycling, particularly through increased glucosidase activity. Conversely, addition of zeolite to the pyrolysis feedstock reduced the organic matter and organic carbon content while increasing biochar recalcitrance and nutrient immobilization, particularly of sulfur, ammonium, phosphorus, and calcium. At the 7.5% dose, the sawdust + zeolite-enriched biochar improved soil pH and potentially enhanced nutrient retention. However, it did not stimulate microbial enzyme activity or respiration, leading to lower photosynthetic pigment levels and reduced biomassin lettuce, especially at higher application rate. For short-term soil applications under the conditions of this pot trial, the sewage sludge-sawdust biochar demonstrated the most beneficial effects, rapidly stimulating microbial activity and nutrient transformation. In contrast, the sewage sludge-sawdust-zeolite biochar limited nutrient availability and plant growth, suggesting it may be less suitable for immediate soil and plant nutrition. Long-term studies are needed to fully assess the

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implications of these biochar types for sustainable agriculture. This study highlights the importance of feedstock composition and selection in tailoring biochar properties to meet specific soil and crop requirements.

Keywords Arable soils, Carbon stabilization, Climate change, Microbial activity, Organic matter, Soil amendment

Introduction

More than 10 million tons of municipal solid waste (MSW), which include sewage sludge, are produced in EU annually for last decade [1]. Demands for safe processing of MSW are gradually increasing. The recycling of municipal sewage sludge (SS) to land either through direct application, or after incineration and landfilling, has been considered an acceptable and beneficial waste management strategy. However, the presence of emerging contaminants including heavy metals, organic toxins, and pathogens, poses considerable risk. These substances can potentially enter the food chain, harm the environment and contaminate surface waters [2].

The SS disposal costs high energy, thus, the interest in valorization technologies increases, because SS represents a raw material wit a perspective to be transformed into a secondary product with added value [3]. Currently, thermal processing of various waste materials including MSW culminates [4, 5], specifically in European Union (EU) [6]. Incineration can significantly reduce the quantity of waste and co-generate energy [7], but pyrolysis, i.e. thermal degradation of biomass in the absence of oxygen, also renders advantages: reduction (up to 50%) of waste volume and content of pathogens [8, 9], and concentration of feedstock's organic carbon [10, 11]. The pyrolysis of SS produces both liquid (pyrolysis oil) and solid (tars and biochar) residues [10, 11]. The SS biochar is often macroporous, but the surface properties and specific area varies upon the used sewage sludge [12]. The content of humic compounds in SS [13] increases carbon yield in the resulting biochar [14], therefore, it is more beneficially applicable in agriculture compared to unprocessed sewage sludge [15, 16].

However, the SS biochar also concentrates harmless compounds such as polycyclic aromatic hydrocarbons (PAH) or metal contaminants [17]. The content of contaminants as well as further chemical and physical biochar properties are determined by conditions of pyrolysis process (maximum temperature, heating rate, feedstock particle size) [18, 19]. The pyrolysis process determines availability and toxicity of emerging contaminants in sewage sludge [20, 21]. In particular, lower temperature (approx. 300 °C) pyrolysis results in a decreased content of PAHs and their toxicity [19], but conversely, trace metal contents such as Pb, Cd, Zn, Cu, Ni and Cr rise. In addition, higher-temperature pyrolysis (up to 600–700 °C) of sewage sludge reduces concentration of volatile organic compounds (VOC) and availability of heavy metals [22], but increases alkalinity and content of stable aromatic carbon ash, some macro- (Ca, Mg, P_2O_5, and K_2O) and micronutrients (Cu and Zn) [20].

Nevertheless, pyrolysis represents a possible technology for decreasing bioavailability (solubility) of heavy metals in sewage sludge by conversion into a stabilized biochar [21, 22]. In contrast to unstabilized heavy metals of non-pyrolyzed SS, sewage sludge biochar could be more widely used in agriculture as a fertilizer, but its use still entails non-negligible risk of soil contamination [23]. The effect of individual heavy metal (HM) on plants is specific and depends on its concentration, but in general, most HM depress the vitality of plants [17, 24–26]. The risk of the soil contamination with heavy metals (HM), originating from sewage sludge, could be mitigated by modulating pyrolysis treatment of the feedstock by: (i) either higher temperature, as already mentioned [22], or co-pyrolysis with inorganic or organic additives [17, 27, 28], resulting in further enhanced HM immobilization [29].

The decrease in toxicity of SS contaminants is efficiently achieved by co-pyrolysis with various types of biomass [30–32]. Mobility and bioavailability of HM in the produced biochar was reduced via co-pyrolysis of SS with sawdust [30, 33] or lignocellulose waste material (rice straw, nut shells) [31, 34]. Biochar derived from sewage sludge blended with additional feedstock poses beneficial nutrient-binding properties in soil, thereby reducing nutrient losses [33, 35, 36], enhancing plant growth and nutrition [17, 36], supporting C sequestration in soil organic matter (SOM) [36–38]. Additionally, the stabilization of toxic HM can be improved by incorporating organo-mineral clays such as zeolite into the feedstock [39, 40].

Positive catalytic effects of zeolite addition to SS pyrolytic feedstock, leading to HM stabilization and enhancement of biochar's beneficial properties, have been observed [39, 41]. Some studies has already reported about advantages of co-pyrolyzed feedstock blends of SS, zeolite, and other organic material for biochar properties and its effect on soil quality and fertility [40, 42, 43]. However, a comprehensive evaluation of how such biochar type impact broad range of soil chemical and biologic properties as well as plant viability and primary production (determined by photosynthetic indicators) is still missing. Similar studies made on other types of waste-derived biochar emphasized the importance of this approach (utilizing organo-mineral clays e.g. zeolite, bentonite, as a sorption material) [44] for enhancing nutritional and health traits of amended soil [45–47]. Due to

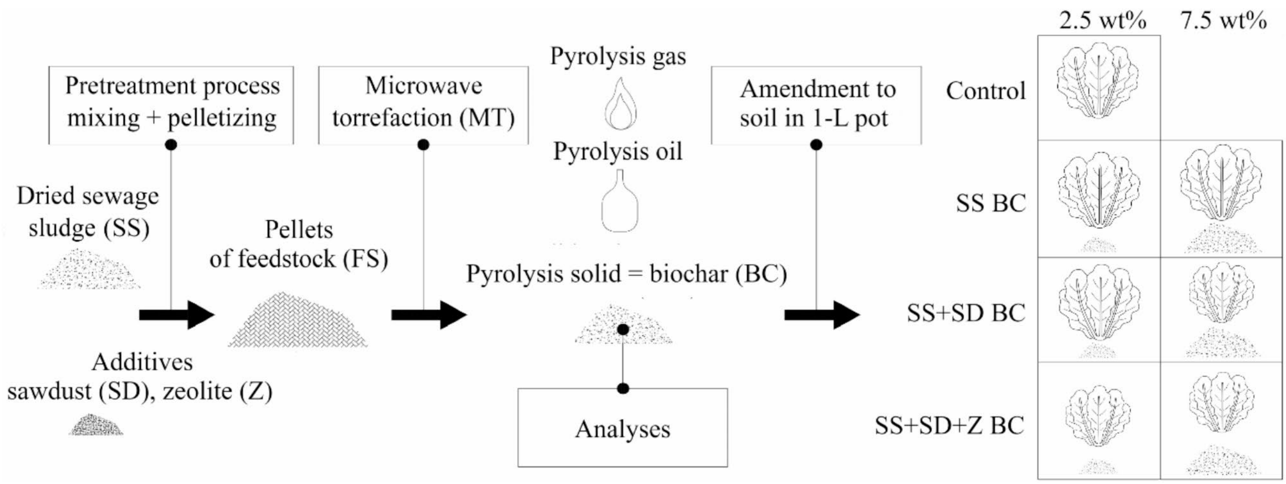

Fig. 1 Experimental design

Table 1 Experimental variants of Biochar prepared by microwave pyrolysis and variants of soil amended with the respective Biochar types

Biomass for pyrolysis	Abbrev. biochar	Soil variant	Abbrev. soil
-	-	control (no biochar)	Control
100 wt% SS	BC	2.5 wt% SS biochar	2.5% BC
		7.5 wt% SS biochar	7.5% BC
50 wt% SS + 50 wt% sawdust	BC(SD)	2.5 wt% SS + sawdust biochar	2.5% BC(SD)
		7.5 wt% SS + sawdust biochar	7.5% BC(SD)
50 wt% SS + 45 wt% SD + 5 wt% zeolite	BC(SD + Z)	2.5 wt% SS + sawdust + zeolite biochar	2.5% BC(SD + Z)
		7.5 wt% SS + sawdust + zeolite biochar	7.5% BC(SD + Z)

above-mentioned large availability of SS as a ubiquitous waste with demand for recovering after disposal, we consider the research coarse outlined in this study as highly important and promising from both scientific and social point of view.

The objectives of this study were to evaluate the multiple effect co-pyrolyzed blend of SS + sawdust(+ zeolite) on the properties of both biochar, biochar-treated soil, and growth and vitality of plants grown in amended soil. It was hypothesized that:

1. The blending of sewage sludge (SS) feedstock for pyrolysis with sawdust improves biochar's organic carbon content and C: N ratio.
2. The SS + sawdust blended biochar, applied to soil, increases SOM and total carbon content, resulting in enhanced microbial activity and transformation of nutrients.
3. The blending of sewage sludge pyrolysis feedstock with zeolite and sawdust decreases biochar's carbon aromaticity, mineral nitrogen volatilization, and increases nutrient stabilization compared to the sewage sludge + sawdust biochar.
4. Soil amended with SS + sawdust + zeolite blended biochar also exerts increased stabilization of nutrient, decreases soil nitrogen transformation and carbon respiratory utilization, despite zeolite-derived decline in the more stable aromatic compounds [48].

Materials and methods

The scheme to illustrate the experimental design is displayed in Fig. 1.

Biochar preparation by co-pyrolysis

Variants of biochar, prepared by microwave pyrolysis (MP) from sewage sludge mixed with sawdust or with sawdust and zeolite, are listed in Table 1. Biochar was applied to soil in two doses (weight%): 2.5% and 7.5%. The source of dried sewage sludge was the Brno Modřice Waste Water Treatment Plant.

Sewage sludge properties (origin, composition, processing prior to co-pyrolysis)

The Brno Modřice Waste Water Treatment Plant (WWTP) has a treatment capacity of approximately 640,000 equivalent inhabitants and is a classic mechanical-biological treatment plant. Wastewater is predominantly of a municipal nature originating from households. Only 12–15% is industrial wastewater, but in general, even these wastewaters are mostly municipal wastewater. Sewage sludge is dried directly at the treatment plant using a contact paddle sludge dryer at a temperature below 100 °C. The dry matter content in the raw dried sample was 91%, the output fraction from the SS dryer was in powder form with a particle range of 1–8 mm, the TOC content was 30.8%. Heavy metal content in dried SS was as stated in Table 2.

Sawdust (origin, composition, processing prior to co-pyrolysis)

Sawdust was added to dried SS to increase the ratio of organic part in produced biochar. Sawdust from soft woods (spruce) was chosen for research activities due to its availability and ease of handling. Processing prior to co-pyrolysis involved mixing sawdust in a weight ratio 50: 50% with dried sewage sludge. Subsequently, pelletization was done using a briquetting press (type JGE 260) for the production of pellets with a size of the extrusion holes of 6 mm and the optional length of the pellets set to approx. 40 mm.

Zeolite (origin, composition, processing prior to co-pyrolysis)

Based on the previous experience [49, 50], synthetic zeolite Purmol 13 was chosen: zeolite type ZSM-5 with admixtures of other zeolites (faujasite, wassalite), particle size < 100 µm. This synthetic zeolite demonstrated the efficiency of the microwave depolymerization process of lignocellulosic biomass. Processing prior to the co-pyrolysis involved mixing dried sewage sludge, sawdust, and zeolite in a weight ratio 50: 45: 5. Subsequently, pelletization took place, with identical conditions as described in the previous Section.

Microwave pyrolysis (description of the used method)

For biochar production, low temperature slow microwave pyrolysis (known as torrefaction) (MT) unit was used, which is located and operated in the AdMaS Center. It is a one segment of full-scale MT (originally twelve segments for continuous operation), internally called as a small full-scale MT unit which is still representative for real conditions in industrial WWTP. The capacity of the device is around 10 kg·batch^{-1} of dried SS, i.e., it works discontinuously. It consists of one batch reactor equipped with one highly efficient microwave generator (magnetron): 3 kW input power, regulated output power, and a frequency of 2.45 GHz. The glass condenser attached to the pyrolyzer was used for separation of the pyrolysis oil and gaseous products. A tuner was installed for incoming and reflected waves. The pyrolyzed feedstock were pellets made of SS, sawdust, and zeolite by pelletizing press (pellets), as described in Sect. Sawdust (origin, ...) and Zeolite (origin, ...). Batches were pyrolyzed at low pressure (800 hPa). The temperature was continuously monitored during the process by an infrared thermometer, the temperature did not exceed 300 °C. During the process, the output regulated power of magnetron was 1.2 kW and residence time 60 min.

Biochar characterization (analytical methods)

Analysis of organic matter (OM) in resulting biochar was performed using a thermogravimetry analyser Q550 TA Instruments (USA, Delaware) by heating the biochar placed on Al_2O_3 pans in the air stream (90 mL min^{-1}) under temperature program from 30 to 950 °C at heating rate 5 °C min^{-1}. Total amount of organic matter was assessed as a mass loss between 200 and 600 °C. Total organic carbon (TOC) and residual oxidizable carbon (ROC) were measured using the Soli TOC® Cube (Elementar Analysensysteme GmbH, Langenselbold, Germany). N, H, and O were measured using the FLASH 2000 Organic Elemental Analyzer/CHNS-O Analyzer (Thermo Fisher Scientific). The Fourier transform infrared spectroscopy (FTIR) analysis was used to record spectra of biochar samples on a Bruker diffused reflectance infrared Fourier transform (DRIFT) spectrometer at transmission mode 4000–400 cm^{-1} with resolution of 8 cm^{-1} and 128 scans using OPUS computer-based software was recorded. The samples were prepared by mixing with KBr to form a homogenous mixture for prior analysis. The content of polyaromatic hydrocarbons (PAH) in the obtained biochar types was determined by the pressurized solvent extraction (one PSE, Applied Separations), using toluene as a solvent, and by final gas chromatography with mass spectrometry (Bruker EVOQ GC-TQ) [40]. None of 16 detected PAHs exceeded the permitted content in any of biochars according to the EU guidelines [51]. Biochar's content of phosphorus was determined as PO_4–P in water (5 g of BC and 50 ml of MilliQ water, filtration after 24 h) and acidic leachate and measured by spectrophotometric method according ČSN EN ISO 6878 (using MQuantTM Phosphate Test, Merck) [52]. Leachable heavy metals (HMs) such as Hg, Cu, Cr, Zn, Pb, As, Ni, and Cd were determined in the water extract using atomic absorption spectrometer with electrothermal atomization ZEEnit 60 from Analytik Jena (Germany) with Zeeman background correction

Table 2 The heavy metal content in the dry matter of sewage sludge from modřice WWTP

Selected heavy metals	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
	[mg·kg^{-1} dry matter ± standard deviation]							
dried SS	10.72 ± 0.33	< LOQ	222 ± 12	337 ± 3.9	2.45 ± 0.24	98.6 ± 1.5	5.24 ± 0.23	1 681 ± 17

The metal content of *mercury (Hg), cupper (Cu), chromium (Cr), zinc (Zn), led (Pb), arsenic (As), nickel (Ni) and cadmium (Cd)* and selected hollow cathode lamp by Photron (Australia) [40]. None of determined HMs exceeded the permitted content in any of biochars according to the EU guidelines [51].

Pot experiment, sampling and assessment of plant growth and photosynthetic parameters

The pot experiment with lettuce (*Lactuca sativa* L. var. Brilant, SEMO a.s., Czech Republic) was performed in 1-L pots (diameter 11 cm, height 12 cm). The variety was chosen due to its high resistance to mildew (*Bremia lactucae*). The pots were lined inside with non-woven fabric and 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 variants in Table 1, i.e. with 7.7 g of biochar for the dose 2.5 wt% and 24.3 g for the dose 7.5 wt%. The substrate was a mixture of light peat (0–25 mm) with wetting agent, dry matter 45%, pH 6.0, salts 1 $g{\cdot}L^{-1}$, nutrient content (according to manufacturer): N 140 $mg{\cdot}L^{-1}$, P 44 $mg{\cdot}L^{-1}$, K 150 $mg{\cdot}L^{-1}$, Mg 100 $mg{\cdot}L^{-1}$. Altogether, 7 variants were tested (substrate amended with three types of biochar, each in two doses, and unamended substrate = negative control), each variant was prepared in four replicates.

Each pot was sown with 3 sprouted lettuce seeds into 2 mm depth, then watered with 200 mL of deionized water. The watering was regularly carried out during cultivation in order to maintain the same soil moisture and prevent the plants to wilt. The experiment was carried out for eight weeks in greenhouse of Faculty of Agrisciences, Mendel University in Brno, Czech Republic (49° 12’ 37” N, 16° 36’ 49.8“ E), under controlled conditions (day/night): temperature 22/18°C, photoperiod 14/10 hours. After 10 days, the seedlings were thinned to one per pot; one representative was left in each pot. All pots were manually watered with approx. 50 mL of deionized water every other day, to maintain ~ 70% of water holding capacity (WHC). At the end of the experiment, the fast chlorophyll fluorescence induction curves (OJIP test) were measured by handheld fluorometer FluorPen FP 100 (Photon Systems Instruments, Czech Republic) at the settings f-pulse 30%, F-pulse 70% and A-pulse 30%. The estimation of chlorophyll fluorescence induction curves (OJIP test) is a useful method to quantify and characterize the intrinsic action of photosystem II (PSII) in plants [53]. For the measurement, fully developed leaves from the middle part of leaf rosette were selected and covered by aluminium foil for 15 min to deactivate photosynthetic electron transport. The obtained data were processed by original software, and then graphically edited in MS Excel. Presented parameters Fv/Fm, Vj and Vi were automatically calculated by original software. The meaning of the individual measured parameters is explained below in the description of Figs. 2 and 3. After the photosynthetic parameters measurement, the above ground plant biomass was harvested for analyses of pigments, and for plant fresh and dry matter estimation. From each fresh plant was collected 0.5 g of leaves for the pigments analyses. These samples were lyophilized and used for acetone extraction. The contents of chlorophyll *a*, chlorophyll *b* and carotenoids were determined by spectroscopic method according to [54] using Spetronic 20 Genesys (Thermo Spectronic, USA). To determine plant fresh biomass, the plant material was weighted on analytical scales immediately after harvest, and then were dried at 60 °C to constant weight to obtained dry biomass. Soil samples were taken from each pot for determination of basic physical, chemical and biological quality indicator.

Determination of soil quality attributes

The substrate from each pot was homogenized by sieving through 2 mm mesh (Retsch sieve 200 × 50, Retsch, Germany) under sterile conditions and stored at 4 °C (for determination of soil respiration), lyophilized and stored at -18 °C (for determination of soil enzyme activities), and dried at 105 °C to constant weight (for pH measurement). Soil reaction – pH ($CaCl_2$) – was determined according to ISO 10390:2005 [55]. Soil basal and substrate-induced respirations (expressed in μg $CO_2{\cdot}g^{-1}{\cdot}h^{-1}$) was determined by the MicroResp® method according to the official supplier protocol (Technical Manual v2.1, The James Hutton Institute) and ref [56]. with terminal spectrophotometric measurement. Following compounds, representing the most common carbon substrates in SOM and plant necromass (e.g. monosaccharides as units of plant structural polymers), were used for substrate-induced respiration: D-glucose (Glc-SIR), D-mannose (Man-SIR), protocatechuic acid (Pro-SIR), D-trehalose (Tre-SIR), N-acetyl-β-D-glucosamine (NAG-SIR), and L-alanine (Ala-SIR). Enzymatic activities, representing indicators of nitrogen transformation in soil - urease (Ure), N-acetyl-β-D-glucosaminidase - and carbon utilization in soil - β-glucosidase (GLU), were determined in enzymatic assays according to [57] and measured spectrophotometrically: the values were expressed in nmol $NH_3{\cdot}g^{-1}{\cdot}h^{-1}$ (urease) and in nmol (p-nitrophenol) $PNP{\cdot}g^{-1}{\cdot}h^{-1}$ (other two enzymes). A microplate reader Tecan Infinite® 200 PRO (Tecan Group Ltd., Switzerland) was used for all spectrophotometric measurements.

Statistical analyses

Data obtained from the determination of plant biomass and qualitative properties, soil chemical and biological parameters, and biochar properties, were statistically analysed using R Program, version 4.3.1 [58]. The methods of principal component analysis (PCA) and Pearson correlation analysis [58, 59] were employed to

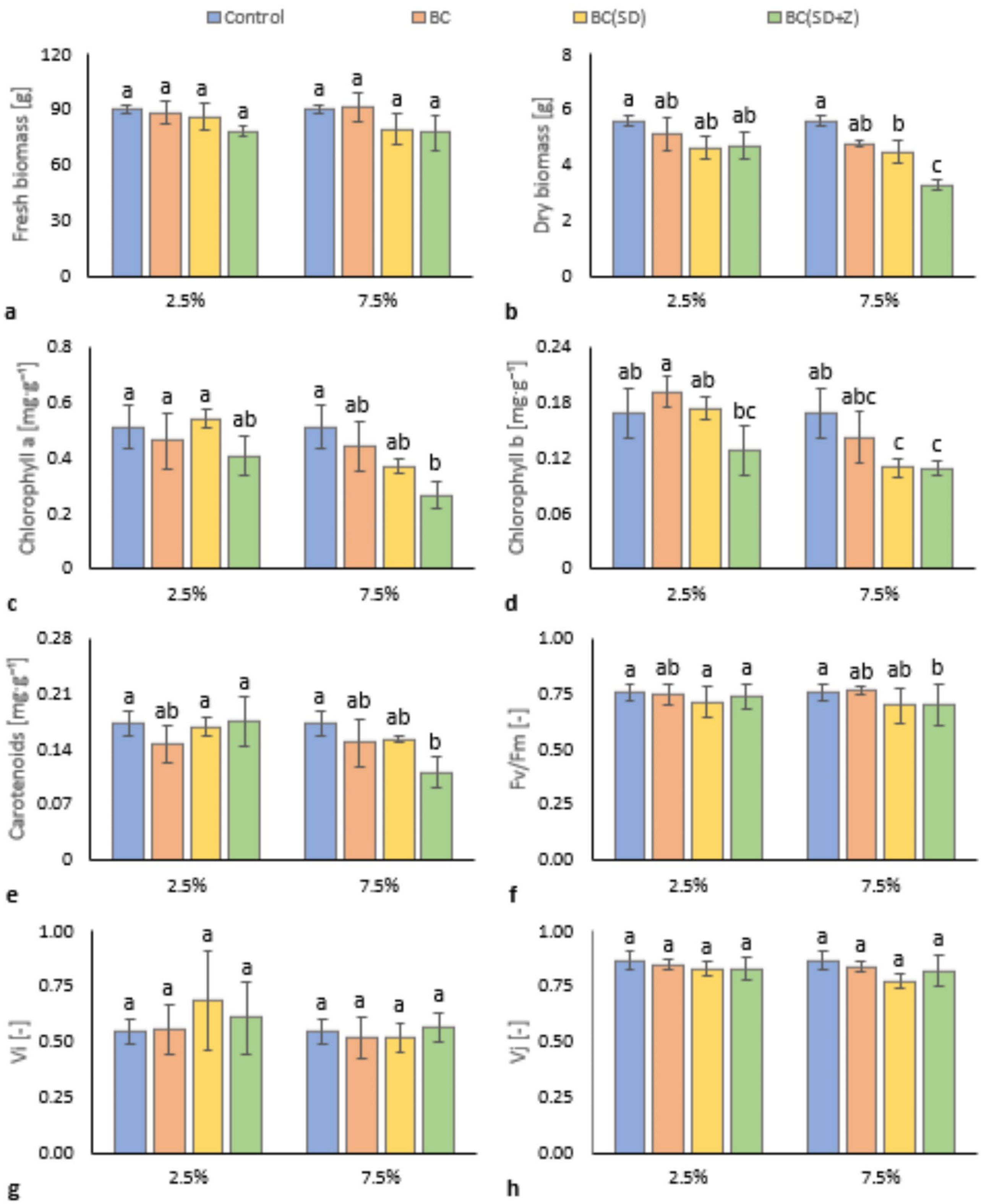

Fig. 2 Growth and photosynthetic parameters of plants cultivated in pot conditions treated with different types and doses of biochar
Displayed properties: fresh biomass [g], dry biomass after drying at 60 °C [g], content of photosynthetic pigments [mg·g^{-1} FW] – chlorophyll *a*, chlorophyll *b* and total carotenoids. Fluorescent parameters automatically calculated from OJIP curves measure (Fig. 5) – maximum quantum yield of photosystem II = Fv/Fm = (Fm-Fo)/Fm, where Fm is maximal fluorescence, Fo is the minimal fluorescence and Fv is variable fluorescence. Vj is the size of wave J in the OJIP curve and Vi is size of wave I in OJIP curve. Fluorescent parameters are given in arbitrary units [a.u.]; mean values ± error bars (= standard deviation), letters indicate statistical differences (calculated by Tukey's HSD posthoc test) among variants at the significance level $p \leq 0.05$

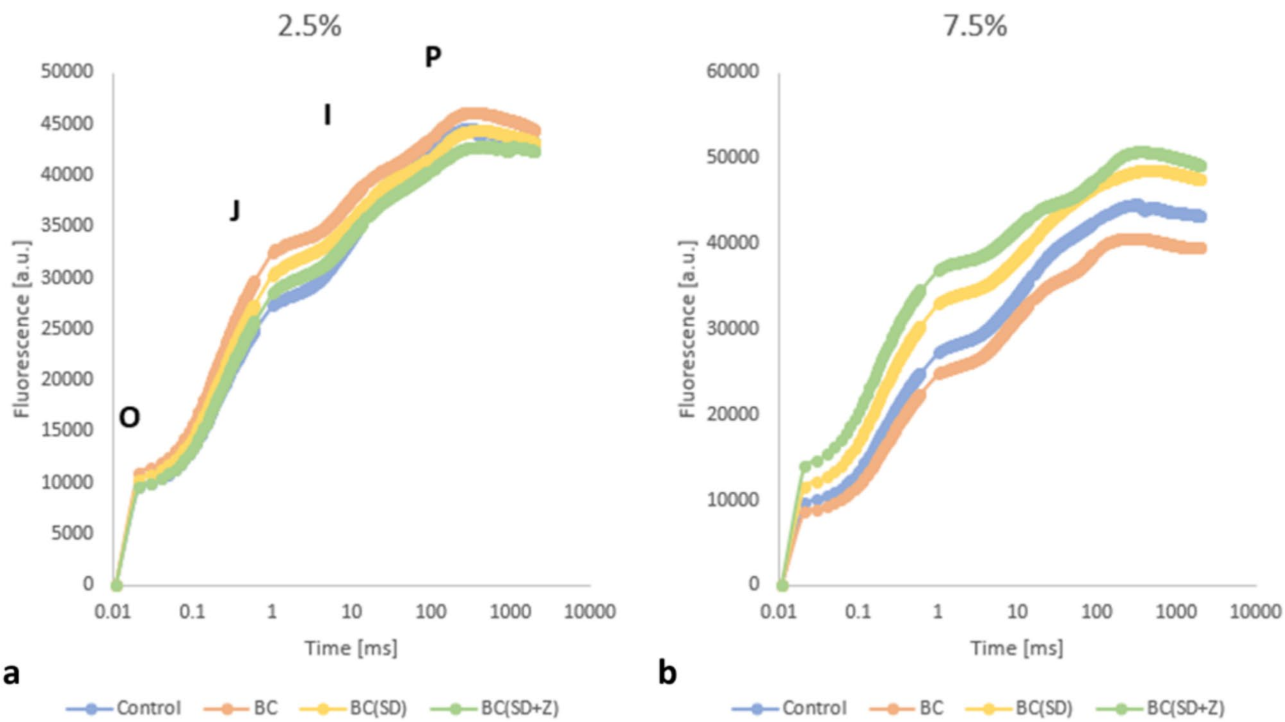

Fig. 3 Fast chlorophyll fluorescence induction curves (OJIP curves) measured on dark adapted leaves of the experimental plants
Curves of fast chlorophyll fluorescence induction (OJIP) measured on dark adapted leaves. The presented curves reported the average values of measured data of all plants of every experimental variant. For better clarity of the curves shape they are presented without the statistics. Statistical evaluation is reported in Fig. 4 f-h (chlorophyll fluorescent parameters). Point O indicate the origin of the OJIP curve and corresponds to minimal fluorescence Fo, part O-J indicate the changes in reduction of primary electron acceptor (Q_A), J-I indicated changes in reduction of secondary electron acceptor (Q_B, plastoquinone and cytochrome), point P corresponded with the value of maximum fluorescence Fm [121, 122]

characterize the relationship between experimental variants and selected soil properties. 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), $0.7 < r < 0.9$ (high correlation), $r > 0.9$ very high correlation [60]. Package "ggplot2" [61] was used for creating advanced statistical graphs.

For statistical comparison, a one-way analysis of variance (ANOVA) Type I (sequential) sum of squares was conducted at a 0.05 significance level [62], to characterize the relationships among the treatments and selected soil properties. For this purpose, it was used package "FactoMineR" [63] and "factoextra" [64]. To identify the difference among factor level means between response and categorial variables after ANOVA, Tukey's honestly significant difference (HSD) test was used, also at a significance level of 0.05. Factor level means with standard error of the mean (SEM) were calculated based on „treatment contrasts" [65].

After all statistical analyses the assumptions of selected models were also checked at significance level of 0.05. For testing the normality, it was used Kolmogorov-Smirnov test and Anderson-Darling normality test from package „nortest" [66] and for testing the homoscedasticity, it was used Bartlett's test and Flinger-Killeen test of homogeneity of variances. Besides, for this purpose it was also used the following diagnostic plots: Plot of residuals versus fitted/predicted values, normal Q-Q plot of standardized residuals, standardized residuals versus fitted/predicted values, Cook's distances, residuals versus leverage and Cook's distance versus leverage [65, 67]. The measuring of data skewness was performed by using D'Agostino's K-squared test and the measuring of kurtosis by using Anscombe-Glynn test from package „moments" [68].

Results

Biochar properties

Organic matter (OM) content, organic carbon forms, and nitrogen forms were the key chemical properties determined in the experimental biochar variants. The organic matter (OM) and total organic carbon (TOC) contents were significantly different within all variants: the values were higher in the sewage sludge + sawdust (BC(SD)) and the sewage sludge + sawdust + zeolite (BC(SD + Z)) variants compared to unamended SS biochar (BC), with expected trend of OM and TOC increasing proportionally to the added organic (sawdust) biomass to the total pyrolyzed matter (Fig. 4a, b). In contrast, residual oxidizable carbon (ROC) was indirectly related to the amount

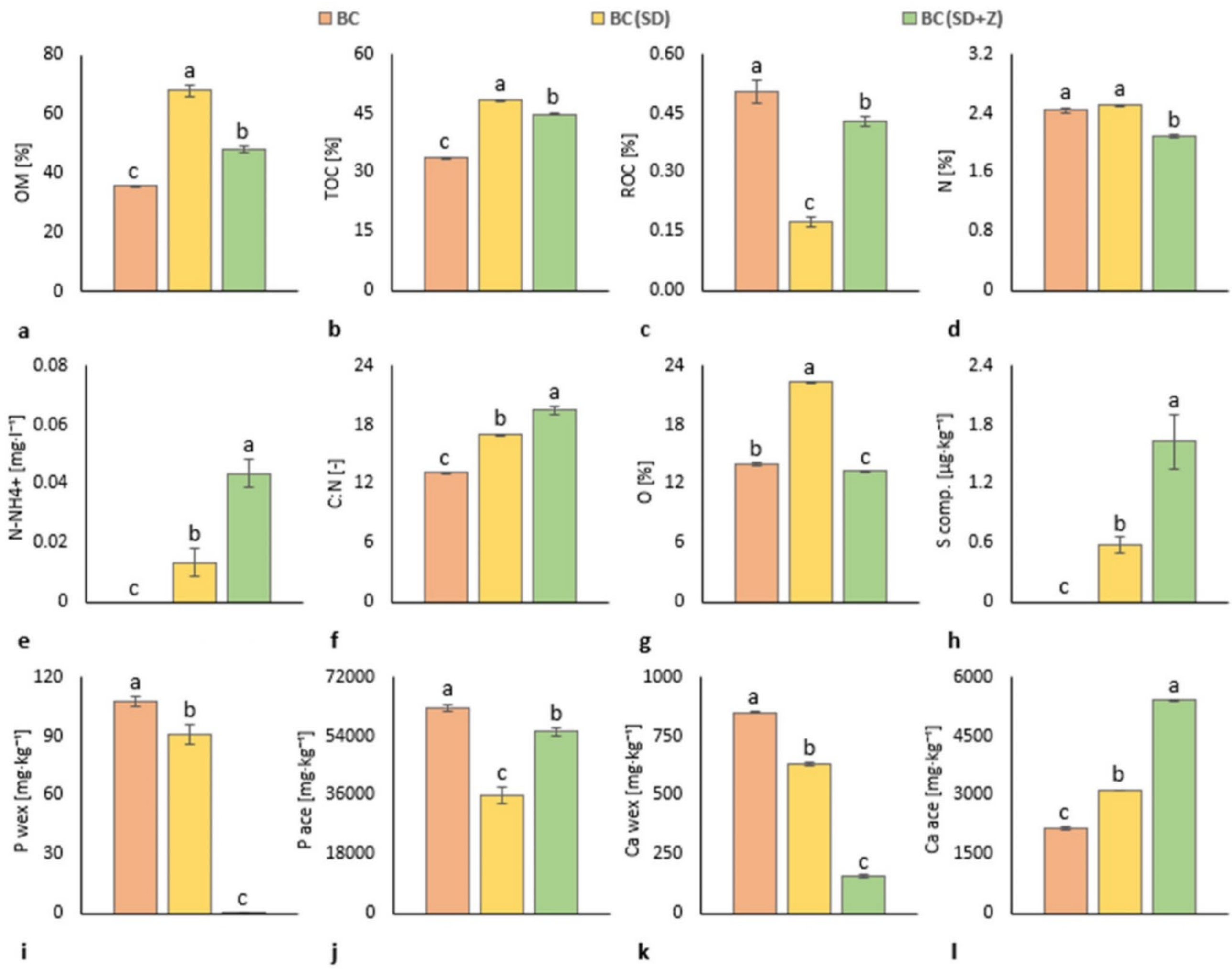

Fig. 4 Properties of biochar pyrolyzed from sewage sludge, *sewage sludge + sawdust, and sewage sludge + sawdust + zeolite*
Displayed properties: OM = organic matter [%], TOC = total organic carbon [%], ROC = residual oxidizable carbon [%], *N* = nitrogen [%], NH_4-*N* = ammonium nitrogen [mg·L^{-1}], C:*N* = carbon: nitrogen content ratio, O = oxygen [%], S comp. = S-compounds [μg·kg^{-1}], P wex = water-extractable phosphorus [mg·kg^{-1}], P ace = acid-extractable phosphorus [mg·kg^{-1}], Ca wex = water-extractable calcium [mg·kg^{-1}], Ca ace = acid-extractable calcium [mg·kg^{-1}]; mean values ± error bars (= standard deviation), letters indicate statistical differences (calculated by Tukey's HSD posthoc test) among variants at the significance level $p \leq 0.05$

of organic matter added to the feedstock (Fig. 4c). Furthermore, woody biomass is a nitrogen-poor material, therefore BC(SD) did not show significantly increased total nitrogen as compared to sewage sludge biochar (Fig. 4d). The replacement of 5% sawdust with nitrogen-free zeolite in feedstock decreased the total nitrogen of final pyrolyzed product significantly (Fig. 4d) and determined the highest C: N ratio (close to 20:1) compared to other variants (BC(SD) ~ 17:1, BC ~ 13:1; Fig. 4f). Whereas BC(SD) (compared to the BC) showed significantly increased oxygen content, BC(SD + Z) (compared to BC) exerted decreased (and strongly lower than BC(SD)) values comparable to BC (Fig. 4g). In contrast, BC(SD + Z) exerted the significantly highest ammonium nitrogen, sulfuric compounds and acid-extractable calcium (N-NH_4^+, S comp. and Ca ace) content compared to other variants and the similar trend (Fig. 4h, l). However, zeolite content in BC(SD + Z) significantly decreased values of water-extractable phosphorus and calcium (P wex and Ca wex) as compared to BC(SD) and BC, albeit e.g. the acid-extractable fraction of phosphorus (P ace) was significantly higher in BC(SD + Z) compared to BC(SD) – Fig. 4i-k. The BC showed the significantly highest water- and acid-extractable phosphorus (P wex, P ace) compared to other two variants, as sewage sludge is out of all three materials used for feedstock the one with the highest phosphorus content.

Effects of amendments on soil properties

The above displayed difference in biochar properties among three types were to a large extend projected into the differences in evaluated traits of biochar-amended

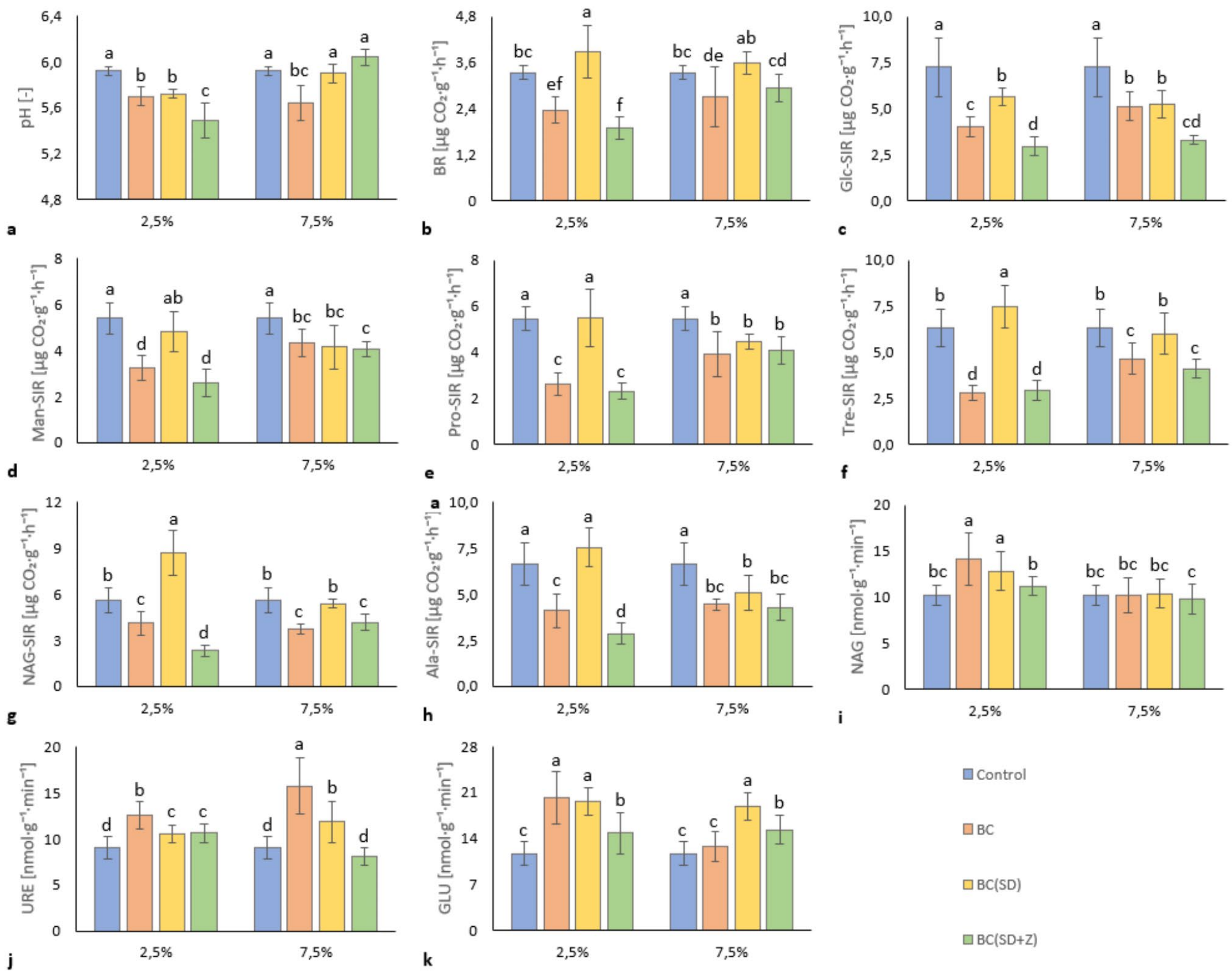

Fig. 5 Soil pH and biological properties of the variants amended with various biochar types

Displayed properties: pH, BR = basal respiration [µg CO_2·g^{-1}·h^{-1}], Glc-SIR = respiration induced by D-glucose [µg CO_2·g^{-1}·h^{-1}], Man-SIR = respiration induced by D-mannose [µg CO_2·g^{-1}·h^{-1}], Pro-SIR = respiration induced by protocatechuic acid [µg CO_2·g^{-1}·h^{-1}], Tre-SIR = respiration induced by D-trehalose [µg CO_2·g^{-1}·h^{-1}], NAG-SIR = respiration induced by N-acetyl-β-D-glucosamine [µg CO_2·g^{-1}·h^{-1}], Ala-SIR = respiration induced by L-alanine [µg CO_2·g^{-1}·h^{-1}], NAG = N-acetyl-β-D-glucosaminidase activity [nmol PNP·min^{-1}·g^{-1}], URE = urease activity [nmol NH_3·min^{-1}·g^{-1}], GLU = β-glucosidase activity [nmol PNP·min^{-1}·g^{-1}]; mean values ± error bars (= standard deviation), letters indicate statistical differences (calculated by Tukey's HSD posthoc test) among variants at the significance level $p \leq 0.05$

soil variants. The impact of soil treatment on the soil pH($CaCl_2$) was determined by different types of co-pyrolysed additives and various doses of biochar. The pH($CaCl_2$) was significantly decreased (compared to the control) in all 2.5% variants, with decreasing trend from BC to BC(SD + Z), while at 7.5% dose, the opposite trend was found (with decreased value in BC variant, Fig. 5a).

The most significant variability between biochar organic matter, organic carbon, and other nutrients (nitrogen, phosphorus) content resulted into differences in the transformation and mineralization of soil carbon, nitrogen and other nutrients. The biochar types with higher ROC content (BC and BC(SD + Z)) determined in the treated soil the significant decrease in the basal respiration (BR) and other respiration types (SIRs) at both doses (2.5%, 7.5%) compared to the respective control variants (Fig. 5b and h). The respiration activity values of soil BC and BC(SD + Z) variants were comparable for the most of respiration types except for Glc- (both doses), NAG- and Ala-SIRs (at 2.5%). Even the BC(SD) biochar, which was the most TOC and the least ROC abundant, contributed at high dose (7.5%) to the soil carbon stabilization, as shown by (compared to the unamended control) similar values of BR, Tre- and NAG-SIR, as well as decreased values of all other SIRs (Fig. 5c-e, h). However, BC(SD) biochar at low dose (2.5%) was able to increase respiration activities BR, Tre- and NAG-SIR (Fig. 5b, f, g) in comparison to the control, while Glc-SIR was decreased. This finding indicated a negative effect of all biochar amendments on the potential respiration capacity of soil and tendence to the soil organic carbon sequestration. High doses (7.5%) of tested biochar types derived mostly negative effects on respiration potential, comparable among various carbon sources, as documented by comparably lowered (in comparison to the control) Glc-, Man-, Pro-, Ala-SIR values in all biochar-treated soil variants.

The tendency to lower carbon and nitrogen mineralization rate at high (7.5%) dose of biochar types was observed for soil enzymatic activity of N-acetyl-β-D-glucosaminidase (NAG), degradation indicator of polysaccharide chitin: the values of all biochar-treated soil variants were comparable to the control (Fig. 5i). However, low dose (2.5%) of biochar types still promoted plant and fungi necromass-related carbon mineralization, indicated by the both significantly increased β-glucosidase (GLU) and NAG in all biochar-treated soil variants (or only in BC and BC(SD) in case of NAG; Fig. 5i, k), as well as increased GLU in 7.5% BC(SD) and 7.5% BC(SD + Z). The significantly most enhanced urease (URE) activity, was detected in the 2.5% BC and 7.5% BC variants, amended with biochar possessing the lowest C: N ratio (close to 13:1; Fig. 5j). The amendment of both co-pyrolysed biochar types enhanced URE comparably at 2.5% dose. At higher amended dose of biochar, 7.5% BC(SD) still increased URE compared to the control, but 7.5% BC(SD + Z) did not.

Effects of amendments on plant properties

The three tested biochar types differently affected growth and quality of lettuce cultivated in the pot experiment. The basic growth parameters, chlorophyll fluorescence parameters, and content of photosynthetic pigments were studied (Figs. 2 and 3). There were observed no significant differences in fresh (at both biochar doses) or dry (at 2.5% dose) aboveground biomass among the variants treated with different types of biochar and compared to the control (Fig. 2a, b). Only at 7.5% dose, soil BC(SD) and BC(SD + Z) variants exerted reduced dry biomass compared to the control, and 7.5% also BC(SD + Z) also compared to 7.5% BC variant (Fig. 2b).

The noticeable differences were observed in the levels of photosynthetic pigments. While chlorophyll *a* (Fig. 2c) and carotenoid (Fig. 2e) contents were significantly reduced (compared to the control) only in plants cultivated in 7.5% BC(SD + Z), content of chlorophyll *b* (Fig. 2d) was decreased both in BC(SD), BC(SD + Z) variants compared to the control (at 7.5% dose) and in BC(SD + Z) also compared to the BC variant (at 2.5% dose). There were no significant differences in Fv/Fm ratios (at 2.5% dose), Vi and Vj among the variants, except of significantly decreased value of Fv/Fm ratio in the 7.5% BC(SD + Z) variant (compared to the control; Fig. 2f).

The OJIP curves in the Fig. 3 reflected the Fast chlorophyll fluorescence induction kinetics. From these curves is clearly visible, that the application of 2.5% dose of any tested biochar types had no significant effect on electron transport chain in photosystem II, which is confirmed statistically in Fig. 2f-h. Although the Fig. 3b infers that after the application of higher dose of tested biochar could have some effect to primary photosynthetic processes, the statistical analysis of the basic fluorescent parameters (Fig. 2f-h) showed no noticeable differences between influence of applicated biochar or their different doses, because of the relatively high variability of the measured data.

Discussion

Biochar properties

The properties of produced biochar were strongly influenced by the feedstock used for pyrolysis, particularly the nutrient composition of charred plant biomass (sawdust) and the high adsorption capacity of zeolite. Consequently, the significant reductions in water-extractable calcium and, especially, phosphorus observed in the zeolite-enriched biochar were likely due to decreased leachability of these elements, as well as differences in phosphorus (P) content among zeolite, sawdust and sewage sludge. Sewage sludge, even after incineration, remains a P-rich source [69]. Previous studies by Mosa et al. (2020) and Wang et al. (2021) have reported that zeolite-biochar composites exhibit significantly higher nutrient efficiency compared to single-component biochar [70, 71]. Acid-extractable phosphorus content (P ace) decreased the most in BC(SD) due to the addition of P-deficient sawdust compared to more P-abundant sewage sludge. The BC(SD + Z) exhibited less decreased P ace content than BC(SD), as zeolite causes high post-pyrolytical stabilization of phosphorus [72], due to its natural affinity to phosphate [73]. Conversely, the desorption of bound phosphorus (P wex value) strongly decreased. This has already been reported by Mosa et al. (2020) who referred to the both enhanced P-adsorption and P-desorption in the zeolite-enriched biochar [70].

Zeolite has been reported to have high content as well as high affinity to calcium (and other ions) [74]. Therefore, the highest acid-extractable (Ca ace) calcium was probably caused by natural enrichment of used zeolite with this element. The lowest water-extractable calcium content (Ca wex) in BC(SD + Z) was ascribed to the Ca adsorption. This finding was in the line with reports of Ayalew and Aragaw (2019) that equilibrium between dissolved and precipitated calcium is deflected towards adsorption to zeolite (at room temperature) [75]. Compared to the (sole) sewage sludge biochar, the presumably increased absorbance of calcium to the pyrolysis-derived, sawdust-coupled ash in the BC(SD) was likely the reason for the significantly lower value of water-extractable calcium (Ca wex) but higher (in BC(SD), compared to BC´s value of Ca ace. These results agreed with Johan et al. (2021) who referred to ash-promoted increase in calcium affinity to phosphate [76]. Both P and Ca showed much higher persistence towards water-extraction compared to acid-extraction, as shown by Ca ace values higher two levels of magnitude compared to Ca wex values and by P ace values higher by almost three levels of magnitude than P wex values. Very high significant ($p \leq 0.001$) positive correlation between P wex and Ca wex corroborated their relation, whereas very high negative correlation of Ca ace with P wex and Ca wex documented various biochar amendment-derived differences in the calcium extractability (Figure A1).

Total sulfur content in the biochar variants was presumably related to the variable amendment-related adsorption and chemical properties as well. Albeit the sewage sludge is an important source of sulfur, it can be lost by volatilization during the pyrolysis (or other type of combustion) [77]. However, significantly increased content of sulfur (S_comp.) in the BC(SD + Z) as compared to BC(SD) and BC was likely due to the zeolites, which are capable of efficient adsorption of sulfur dioxide, hydrogen sulfide, and other S-compounds [78]. Similarly, the presumed volatilized sulfur binding to pyrolyzed sawdust and protection from losses by emissions likely caused higher S_comp. in BC(SD) than in BC, which assumption agreed with the previous findings [79].

The thermal processing (e.g. incineration) of sewage sludge causes significant emissions as well as loss of ammonium (Wielgosinski et al. 2016). As the increased ammonium nitrogen content in the BC(SD + Z) was detected, compared to the BC(SD) and BC, an enhanced zeolite-coupled adsorption and mitigation of ammonium emission was assumed. This feature agreed with several authors' references [71, 80] to high affinity of zeolite to ammonium ions (NH_4^+). The affinity of pyrolysed sawdust to ammonium [81] was presumably responsible for the differences in N- NH_4^+ between BC and BC(SD). Several related cation contents were documented by the significant ($p \leq 0.001$) and very high positive correlation of N-NH_4^+ and Ca ace, S_comp, as well as negative correlation between N-NH_4^+ and Ca wex, P wex (Figure A1).

Finally, the presence of zeolite and pyrolyzed sawdust in the sewage sludge biochar markedly affected the content of the organic matter and the key macroelements – carbon, nitrogen and oxygen. The organic matter (OM) and total organic carbon (TOC) contents were significantly highest in BC(SD) biochar, supplied with 50% sawdust (more organic- and carbon-abundant feedstock biomass compared to SS) prior to the pyrolysis, than in BC(SD + Z) with 5% less sawdust supply, and BC biochar (no sawdust supply). OM and TOC significantly ($p \leq 0.001$) and highly positively correlated with each other (Figure A1). BC had approximately half of the organic content of BC(SD), which was in the line with findings of Tomczyk et al. (2020), reporting that biochars produced from solid waste feedstocks exert lower carbon content than biochars produced from wood biomass [82]. However, the organic carbon composition was in BC(SD + Z) biochar disproportionally shifted towards the more abundant recalcitrant fraction ROC in comparison to BC(SD). Whereas BC type showed the highest ROC values, presumably due to the losses of more labile forms of organic carbon (OC) from SS during pyrolysis, complex compounds in SD underwent depolymerization [83] and did not enrich the ROC fraction of OC. Nevertheless, zeolite, which acts as a catalyser accelerates cleavage of bonds in lignocellulose substrates, may enhance the production of gaseous products [84], leading in BC(SD + Z) to lower TOC content, but due to higher rate of labile carbon losses to higher ROC value. This indirect dependence of TOC and ROC contents were documented also by significant ($p \leq 0.01$) and high negative correlated each other, furthermore ROC was significantly ($p \leq 0.001$) highly negatively related to OM as well (Figure A1).

Total nitrogen was comparable between BC and BC(SD) types despite the fact that wood material (lignocellulose) typically contains twenty- to 100-fold less nitrogen than sewage sludge in dry biomass. A decreased volatilization of nitrogen compounds during the pyrolysis process was assumed, due to the wood matter addition. Kawamoto (2017) reported that phenolic compounds may be released into the pyrolyzed product during the thermal degradation of lignin from sawdust [85]. Ozdal et al. (2013) reported that protein-polyphenol complexes change secondary and tertiary structures of proteins, presumably causing increased thermal stability [86]. Similar complexing of sawdust-derived polyphenols with polypeptides from sewage sludge was considered in this study resulting in lowered emissions of gaseous and volatile nitrogen compounds in BC(SD). In the biochar variant BC(SD + Z), which exhibited significantly decreased total nitrogen content (compared to the other two variants). This protective effect may be mitigated due to zeolite adsorbed the phenolic compounds and prevent them from complexing with polypeptides. This observation is in line to work of Peréz et al. (2017) who referred to zeolite adsorption and removal of ionizable phenolic compounds from water [87]. Therefore, this impoverishment of BC(SD + Z) with nitrogen determined the significantly highest C: N ratio in this variant as compared to both other variants. In contrast, the lowest TOC value of sewage sludge biochar BC led to the significantly lowest C: N. Pearson's correlation showed significant ($p \leq 0.01$) positive correlation of C: N with TOC and negative correlation ($p \leq 0.05$) with total nitrogen (Figure A1).

As the last evaluated biochar property, the oxygen content in all variants was determined and compared. The BC(SD) showed significantly highest percentage of oxygen compared to the other two variants. The sawdust amendment to sewage sludge may be the reason as Kawamoto (2017) reported that lignin is prone to oxidation at relatively low temperatures of pyrolysis [85]. Han et al. also referred to apparently higher oxygen content in pinewood biochar (pyrolyzed at 250 and 450 °C) compared to the sewage sludge biochar [88]. The increased oxygen content of BC(SD) compared to BC(SD + Z) could be caused by physico-chemical potential of zeolite to reduce electron transfer during pyrolysis. As zeolite is known as a versatile antioxidant [89], the sawdust-mediated tendency to increase pyrolytical oxidation of BC(SD + Z) was presumably mitigated by zeolite amendment. Concluding the results of biochar properties determination, the hypotheses 1. and 3. were verified.

Effects of amendments on soil properties

The specific biochar properties of the respective types (Sect. Biochar properties) influenced the soil pH, albeit in a contrasting way according to the applied dose. Lower doses (2.5%) of all biochar types decreased pH of the treated soil, whereas two biochar types, BC(SD) and BC (SD + Z), did not decrease the soil pH at higher dose (7.5%). This could be ascribed to the effect of biochar on the soil cation exchange capacity (CEC) [90]. We speculate that at the low (2.5%) application rate, the CEC increased to such an extent that nearly all alkaligenic ions (Ca^{2+}, Mg^{2+}) in the treated soil were absorbed by the biochar. This likely altered their availability, preventing them from counteracting the acidifying effect of H^+, NH_4^+, K^+ ions, ultimately leading to a decline in soil pH. However, at higher (7.5%) biochar application rate, the effect of soil CEC became saturated. Instead of further ion adsorption, intrinsic (and previously immobilized) calcium and magnesium ions were replaced by an additional input of these elements, introduced through the amendment, which was applied at a three-fold higher quantity than at the lower biochar dose. Solely pyrolyzed SS has been reported to produce a biochar that lowers soil pH [91], as also evidenced in this study by the lowest total content of alkalizing minerals e.g. Ca^{2+} considering both water- and acid-extractable fractions (Sect. Biochar properties).

Concurrently, the pH decrease in 2.5% BC(SD + Z) variant may have resulted from increased ammonium concentration and its subsequent nitrification, a process known to promote acidification. This process may have been coupled with increased urease activity at the low biochar dose (2.5%), which facilitated NH_4^+ release. However, at the high 7.5% biochar dose, the lower urease activity inhibited nitrification-driven pH decrease. At this higher application rate, the alkalizing effects of both pyrolysis additives (sawdust and zeolite) may have further contributed to soil pH stabilization or increase [92, 93].

The BC type did not stimulate microbial respiratory activity (in the 7.5% BC variant), presumably due to the low OM content, higher recalcitrance of carbon sources (high ROC) and low other nutrients (e.g. N-NH_4^+, S_comp.). Similar positive effect of sewage sludge biochar on higher soil carbon recalcitrance [94] and negative effect on the soil respiration have already been reported [95–97]. None of variants at 7.5% application rate exerted significantly higher basal respiration (BR) compared to the control, the highest BR value was found in the 2.5% BC(SD). All types of biochar exhibited stabilizing effect on the external amended organic carbon and SOM sequestration, which was likely coupled with humus formation [98, 99]. However, significant difference between the BR values of BC(SD) and BC(SD + Z) variants at both doses presumed that zeolite amendment to biochar could affect the aerobic degradation processes in the treated soil. Zeolites may act antagonistically to the soil aerobic decomposition: due to their stable structure and desorption of cations they accelerate the formation of condensed humic molecules from soil organic matter (SOM) [92]. Thus, BC(SD + Z) variant showed the lowest values of many SIR respiration types. Despite relatively high TOC and ROC values, BC(SD + Z) biochar was inefficient to enhance respiration activity in soil to higher CO_2 emission. This finding supports the hypothesis 4. Moreover, BR showed a significant ($p \leq 0.01$) moderate positive correlation with OM and O content in biochar and negative correlation with ROC (Figure A2).

The D-glucose-induced respiration (Glc-SIR), as an indicator of soil respiration potential, was lower compared to the control in all amended soil variants, caused presumably by the negative effect of sewage sludge biochar and zeolite on the soil microbiome, both aerobic microbes [95] and total bacteria [91]. Other studies also showed that compared to positive effect of very low doses of SS biochar (0.5-1%) amendment to soil on microbial biomass carbon (MBC), higher amendment (> 2%) led to MBC values (i.e. soil microbial biomass) comparable to unamended soil [100]. The mutual significant ($p \leq 0.001$) moderate positive correlation of Glc-SIR with all other SIRs, which are related to the metabolism of other nutrients (e.g. nitrogen) too, showed that nutrient (metabolite) sorption and resorption in the soil was biochar-mediated and dependent, as also previously referred by [101]. D-trehalose (Tre)-SIR and N-acetyl-β-D-glucosamine (NAG)-SIR properties are related to decomposers of fungal necromass as the substrates D-trehalose [102] and N-acetyl-β-D-glucosamine (a part of chitin) are common components of fungal cell wall. The significantly highest Tre-SIR (NAG-SIR) in 2.5% BC(SD) assumed increased abundance of soil fungi and their turnover. This indicates a stimulatory effect of sawdust biochar on soil fungal biomass (and the mycorrhiza abundance), which has already been reported [103]. The mutual relation between both indicators (Tre-SIR, NAG-SIR) was evidenced by the significant moderate positive correlation ($p \leq 0.001$, Figure A2). L-alanine (Ala)-SIR, common soil amino acid and nitrogen source, was the indicator of nitrogen mineralization: the control and 2.5% BC(SD) variants showed significantly higher Ala-SIR in comparison to other variants. These results prerequisite higher soil L-alanine availability to decomposition in the respective soil variants, it could be related to the presumably higher turnover of fungal biomass, corroborated by the significant ($p \leq 0.001$) high positive correlation between Ala-, Tre-, and NAG-SIR (Figure A2). These findings corroborated the hypothesis 2. In contrast to positive effect of sawdust biochar on soil induced respiration (Tre- and NA-SIR), zeolite exerted opposite, markedly negative effect on CO_2 emission from soil, which could be ascribed to the affinity of carbon dioxide to the clay surface [104].

The organic matter composition of the biochar variants further determined the activities of soil enzymes involved in the carbon and nitrogen mineralization. N-acetyl-β-D-glucosaminidase (NAG) is the enzyme that catalyzes the cleavage of N-acetyl-β-D-glucosamine, thus it is involved in C and N cycling in soils [105]. β-glucosidase (GLU) catalyzes the hydrolysis of the glycosidic bonds to terminal non-reducing residues in β-D-glucosides (e.g. cellulose, oligosaccharides), it serves as a soil C-cycling and quality indicator [106]. The highest NAG and GLU activities were induced by the amendment of low dose (2.5%) of BC and BC(SD) biochar types, which exhibited high TOC, N, O content (BC(SD) or ROC, N content (BC; see Sect. Biochar properties). The assumption that revealed enzyme activity values are related to the increased accessibility of the organic carbon and the limiting microbial nutrients (e.g. N, P), was corroborated by the significant ($p \leq 0.05$) positive moderate correlation of GLU with N and O, as well as by GLU and NAG mutual correlation (Figure A2). Positive effect of biochar amendment on the soil β-glucosidase activity was already reported [107, 108], however the sewage sludge application to soil showed mostly an adverse impact [96, 109], but this effect was dose-dependent [100]. The hypothesis 2. was not verified as BC(SD) biochar stimulated GLU-mediated carbon mineralization even at higher application dose (7.5%) in comparison to the control unamended soil and in agreement with previous findings [40].

The highest enhancement of soil nitrogen mineralization, indicated by urease (URE) activity, due to the amendment of sewage sludge biochar was assumed. Urease converts urea into ammonia and carbon dioxide and it indicates the intensity of nitrogen relevant reactions in the soil [110]. The highest URE in the 2.5% BC and 7.5% BC variants, amended with sewage sludge biochar, was ascribed from high N content and low C: N, N-NH_4^+ in the respective biochar type, which may prerequisite high deamination rate due to promoted high demand for mineral nitrogen in soil. This observation aligns with previous reports of improved urease activity in SS biochar-amended soil [100, 111]. However, the positive effect of a higher SS biochar application contrasts with another study that reported an adverse impact of increased biochar adition [109]. The significant ($p \leq 0.05$ and less) moderate correlations of URE with N (positive relation) and with the C: N ratio (negative relation) further supports these findings (Figure A2). The high dose (7.5%) of BC determined the highest soil urease activity due to the abundant precursors of the urea decomposition pathway. On the other hand, the 7.5% BC(SD + Z) variant exhibited significantly decreased URE values as compared to variants amended with all other (7.5%) biochar types. Presumably, the BC(SD + Z)-mediated SOM stabilization and protection from decomposition (due to recalcitrancy) was coupled with rigid coating of pyrolyzed matter with more complex organic compounds [112] associated with zeolite. This verifies the hypothesis 4, which proposed increased SOM recalcitrance and stabilization of nutrients by BC(SD + Z) biochar, was done.

Effects of amendments on plant properties

Biochar amendment to soil affects plant growth and physiology via alteration of exogenous conditions such as physicochemical properties, nutrition availability, soil microbiome composition and activity [97]. One of the basic physiologic and metabolic processes of green plants is photosynthesis, which was monitored via the determination of photosynthetic pigments chlorophyll *a*, *b*, and carotenoids and measurement of photochemical parameters reported about photosystem II efficiency. Experimental results showed no significant differences between the control and the biochar-amended variants in none of three measured photosynthetic pigments at low application dose (2.5%) of biochar, the only significant decrease in the chlorophyll *b* content was in BC(SD + Z) variant compared to BC variant. At high application dose, the negative effect of BC(SD + Z) type in the respective amended soil on all three leaf pigments was even more markable (compared to unamended control soil). Carotenoids, which had the important role in plant antioxidant system could also secondarily lead to an increase of both chlorophylls content and disrupt the photosynthetic electron transfer chain [113]. This worsened plant quality and reduced biomass production could be related to the observed lowered nutrient transformation activities, microbial biomass and metabolism, due to the findings that zeolite and sawdust-enriched biochar contained lower levels of water-soluble and plant-accessible nutrients compared with soil variants treated with BC or BC(SD). Previously, it has been reported that immobilization of nutrients in soil, which was presumably caused by zeolite presence in soil, was coupled with decreased content of chlorophyll [114]. This assumption was supported by the significant ($p \leq 0.05$) moderate positive correlation of Dry_biomass with P wex and C wex (Figure A2). For example, calcium, whose water-soluble form was significantly reduced in BC(SD + Z) biochar, plays an important role as the signal molecule in plant and it is involved in photosynthetic processes [36, 115]. However, it cannot be stated with certainty that the experimental plants were negatively affected by calcium deficiency, as no typical symptoms of Ca-deficiency, such as the tipburn [116] were observed. The same type of biochar BC(SD + Z) reduced the content of water-soluble phosphorus and nitrogen. It is known that these two nutrients are limiting factors of the plant growth and metabolism. For example, P is essential for the plant energetic metabolism, among other (structural) function, and N is necessary as the element of proteins. Their deficiency also plays a direct and/or indirect role in photosynthetic processes [117]. Although, similar to Ca-deficiency, no typical symptoms of N or P deficiency, such as dwarfism or purple leaf diccoloration, were visible in the plants, the reduction of dry matter and decreased chlorophyll and carotenoids correspond with a potential lack of these nutrients [118–120].

However, information on pigment content must always be complemented by biomass production data and efficiency indicators of the photosynthetic process, such as the quantum yield of photosystem II (Fv/Fm) or the properties of OJIP curves (which represent chlorophyll fluorescence induction), specifically the Vi and Vj values. Moreover, not all plant stress conditions necessarily manifest at the photosynthetic level [121]. Despite the insignificant differences in Fv/Fm, Vi, Vj, and the fresh biomass production between the lettuce plants cultivated in soil amended with various biochar types and doses, the higher dose BC(SD + Z) biochar caused a slight flattering of the Vj part of the OJIP curves. This effect could indicate impaired photosystem II function, especially on the level of the second electron acceptors [122].

Nonetheless, the only detectable reduction in dry biomass production among the experimental lettuce plants was observed at the 7.5% dose in the BC(SD + Z) variant. Therefore, it can be assumed that none of the tested biochar types exerted a strong negative or phytotoxic effect on plant growth. At the same time, the potential growth promoting effects of these biochar types cannot be definitely excluded. Given that long-term application of SS-derived biochar have been reported to positively influence the availability of certain nutrients [123], and considering that this study is based on the relatively short-term pot experiment, further investigation is warranted.

Moreover, zeolite is known to function as a long-term mineral fertilizer, as it naturally provides N, K, Ca, Mg, Fe and other essential minerals. Thus, over a longer cultivation period, the positive and differential effects of the various biochar types may become more pronounced. Furthermore, the slight short-term slightly negative effect of most biochar types on microbial biomass (indicated by lower values of Glc-SIR) could potentially slow the rate of nutrient utilization by the soil microbiome. This tradeoff might, in turn, contribute to prolonged soil fertility by sustaining nutrient availability for plant uptake [124].

Conclusions

The short-term pot experiment, based on soil amendment with biochar produced from three types of pyrolysis feedstock i.e. 100% sewage sludge (BC), 50% sewage sludge + 50% sawdust (BC(SD)), and 50% sewage sludge + 45% sawdust + 5% zeolite (BC(SD + Z)), showed variable effects on soil and lettuce (*Lactuca sativa* L. var. Brilant) properties, depending on the application dose (2.5% or 7.5%). BC biochar, low in organic matter and carbon but rich in nitrogen, phosphorus, and water-extractable calcium, enhanced soil enzymes despite decreased microbial respiration, and acidified soil at 7.5% dose. Partial replacement of sewage sludge with sawdust in feedstock for BC(SD) biochar maximized its organic matter, total organic carbon, oxygen content, and C: N ratio as hypothesized, with low content of stable carbon, and acid-extractable phosphorus. BC(SD) type supplied amended soil with the most accessible form of organic matter and promoted enhanced basal respiration as well as several types of induced respiration, it also increased β-glucosidase activity at 7.5% dose. For short-term soil applications in conditions determined by the described pot trial, the sewage sludge-sawdust biochar demonstrated the most favourable effects, rapidly enhancing microbial activity and nutrient transformation. Zeolite addition to sewage sludge + sawdust feedstock changed BC(SD + Z) biochar in comparison to BC(SD) towards reduced organic matter and total organic carbon but increased sulfur, ammonium, and acid-extractable phosphorus and calcium. These traits were ascribed to zeolite's immobilizing effect on nutrients due to their sorption, which feature promoted the most mitigated respiration and enzyme activity of urease and N-acetyl-β-D-glucosaminidase. The assumed limited availability of nutrients in BC(SD + Z) variant was coupled with the most negatively affected plant traits – dry biomass and content of leaf pigments (chlorophyll *a*, *b*, carotenoids). BC(SD) biochar demonstrated promising short-term benefits for soil microbiome (activity and biomass) and fertility, plant nutrition, while BC(SD + Z) exerted completely opposite effect, with long-termed implication for higher stabilization of organic matter and prolonged and gradual release of bound nutrients, which could benefit to sustainable agriculture practice. However, these assumptions of long-term promising implications of the tested biochar types warrant further investigation, which will necessarily include testing in the field conditions on the small-scaled-plot level.

Supplementary Information

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Supplementary Material 1

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Author contributions

Conceptualization, T.H., J.H., J.K., A.Z. and M.B.; methodology, T.H., K.L., J.H., O.M., L.M., T.CH. and M.B.; software, T.B., M.H.S. and A.K.; validation, M.B., T.H., L.M., M.N. and A.M.; formal analysis, A.M., A.K., S.A. and T.B.; investigation, J.H., A.M. and M.B.; resources, A.K. K.L., L.M., T.CH., and O.M.; data curation, T.H., T.B., K.L. and O.L.; writing—original draft preparation, A.Z., K.L. and J.H.; writing—review and editing, A.Z., T.H., J.H., J.K., A.M., M.N., M.H.S, S.A. and M.B.; visualization, T.H., T.B. and A.K.; supervision, A.M., J.K., T.CH. and M.B. and project administration, J.H., A.K. L.M., K.L., M.H.S., S.A. and T.CH. All authors read and approved the final manuscript.

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Data availability

Data is provided within the manuscript or supplementary information files.

Declarations

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Consent for publication

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Competing interests

The authors declare no competing interests.

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