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Effects of biochar derived from the pyrolysis of either biosolids, manure or spent coffee grounds on the growth, physiology and quality attributes of field-grown lettuce plants

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ABSTRACT

The positive effects of biochar applied as soil conditioner for boosting soil fertility and thus plant growth are sufficiently documented, mostly through *in-situ* experimentation. This field study evaluated the effects of soil amendment (5% v/v) with distinct types of biochar (biosolids-derived biochar, BDB; cattle manure-derived biochar, MDB; spent coffee grounds-derived biochar, SCGDB) on the growth, physiology and quality attributes of lettuce (*Lactuca sativa* L.) plants grown in sandy clay loam-textured soil, under temperate climatic conditions. Peat moss (PM) was also used, to serve as internal control due to its wide use. Plants grown on MDB-amended soil showed a significant increase of biomass production rate, evident through elevated mean fresh and dry weight values compared with all other treatments. Increased growth was also found in BDB treatment, though failed to reach significance. None of the treatments impacted the photosynthetic pigment content, however they did reduce the nitrates content in leaves. Leaves sampled from BDB and MDB treatments showed reduced soluble solids content (SSC) and titratable acidity (TA), and increased sucrose content. MDB-treated plants showed increased fructose content while BDB-treated plants showed increased total soluble sugar and total phenolics content. Moreover, plants grown in MDB- and BDB-amended soil showed increased total antioxidant capacity, despite maintaining ascorbic acid content in values similar to control. SCGDB and PM treatment did not affect any of the parameters tested. Overall, results showed that BDB and MDB applied as soil amendments may serve as means for enhancing the growth, and partially the nutritional value of lettuce plants.

1. Introduction

Experience obtained from agricultural applications, along with data derived from experiments performed either *in situ* or in control environment (greenhouse and lab) over the last years has render biochar a promising material for

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boosting the agricultural productivity, thus tackling the challenges of securing food supply, in climate change and circular economy conditions (Maroušek et al., 2019). Biochar is a carbonaceous material produced from the pyrolysis of any type of biomass in the absence or limited supply of oxygen, which displays an array of physico-chemical properties depending on the feedstock source and production temperatures (Tripathi et al., 2016). Its high aromaticity, surface area and pore-size distribution, along with its neutralizing and cation exchange capacity (among other properties), enable biochar to be used as soil amendment for the improvement of degraded soils (saline, sandy soils), the enhancement of nutrient availability, the increase of water use efficiency, the remediation and/or protection from organic or inorganic pollutants, and the mitigation of greenhouse gasses (nitrous oxide, methane) emissions (Ali et al., 2017; Kamali et al., 2020). To this effect, the use of biochar as soil conditioner induces modulations on the physico-chemical and biological properties of soils which improve soil health and fertility, eventually resulting in increased plant growth, stress responses and yield (Agegnehu et al., 2017; Ali et al., 2017; Yu et al., 2019; Dai et al., 2020).

Biochar derived from four different feedstocks (pine needle, maize stalk, *Lantana camara* L., black gram) has been reported to exert beneficial effects on maize and black gram plants grown on acidic soil in pots during their seedling and vegetative stage, Das et al. (2020), whereas woody chips-derived biochar improved the growth of pepper plants grown on acidic and alkaline soils in pots (Liopa-Tsakalidi and Barouchas, 2017), with both studies being performed under greenhouse conditions. A meta-analysis of literature data regarding the impacts of biochar (derived from various feedstocks) application on root traits revealed a significant positive effect in all examined traits, including biomass and volume (Xiang et al., 2017). Moreover, biochar has been shown to improve the performance of crop plants grown under optimum or unfavorable environmental conditions (i.e., drought, degraded soil, extreme temperature) (Ali et al., 2017; Shetty et al., 2021). Such effects were attributed to the better use of nutrients and water by plants, improved soil pH, structure and water holding capacity and changes in soil microbial dynamics (Agegnehu et al., 2017). Studies have also revealed the positive affects of biochar on the quality attributes (i.e., nutritional value, antioxidant capacity) of agricultural products. Sani et al. (2020) reported that the co-application of *Trichoderma* and timber waste-derived biochar under reduced fertilizer application (50%) can increase the yield and enhance the quality of field-grown tomato fruits. Agbna et al. (2017) reported that wheat straw-derived biochar improved irrigation water use efficiency and the quality of fruits of tomato plants grown in soil in greenhouse, even under limited water supply. Furthermore, biochar derived from the pyrolysis of pistachio shell managed to mitigate the Ni-mediated stress in lettuce and mungbean plants grown in pots by boosting their antioxidant defense system, while also improving their nutritional quality (Turan, 2019, 2021a).

Therefore, the biochar beneficial effects in numerous aspects of plant growth, development, and physiology are well documented and reviewed. However, these results were mostly based on studies performed under controlled laboratory or greenhouses conditions (pot experiments), rather than in real field conditions. Furthermore, studies often evaluated the effects of single biomass feedstock for biochar production, with biochar often obtained from laboratory of experimentally designed pyrolizer (Agegnehu et al., 2017). Also, the benefits of biochar applied as soil amendment on plants' performance have been widely illustrated in tropical regions with nutrient and organic matter-rich soils, but only rarely have been studied in temperate regions or in sandy soils with poor fertility and high pH values (Atkinson et al., 2010; Borchard et al., 2014). To this effect, this study aimed to explore the effects of the application of three different types of biochar applied in soil as conditioners on the growth, physiology and quality attributes of field-grown lettuce (*Lactuca sativa* L.) plants. The tested hypothesis was that distinct biochars, produced from totally different biomass feedstocks such as biosolids obtained from wastewater treatment plant, manure and spent coffee grounds, would cause distinct effects on lettuce plants, in a positive way. The fact that this study was performed in field and therefore common practices for the production of lettuce plants were followed, and the fact that the plants were grown in a typical Mediterranean non-fertile soil (sandy clay loam with low fertility) under temperate conditions, enforced the novelty of the present study. Moreover, a variety of biochemical assays were employed for the comprehensive assessment of the tested hypothesis, including major and widely applied markers concerning the growth and quality of lettuce plants

2. Materials and methods

2.1. Plant material and experimental treatments

Green coral lettuce 'Lollo Bionda' (*Lactuca sativa* L. var. crispa) transplants were planted in a sandy clay loam-textured experimental field, at Nicosia, Cyprus. Prior planting, the soil at the root zone of plants (topsoil; 0–25 cm depth) was amended or not with biochar produced from the pyrolysis of distinct biowaste materials (see Section 2.2), or with peat moss (used as internal control as this substrate is commonly used as soil amendment) at a rate of 5% v/v, simulating common agricultural practices. The biochar used was derived either from the pyrolysis of biosolids (BDB; biosolids-derived biochar), cattle manure (MDB; manure-derived biochar) or spent coffee grounds (SCGDB; spent coffee grounds-derived biochar), while peat moss was purchased from the market (100% blend of peat with pH value of 5.5–6.2; Freepeat, Holland). A detailed physicochemical and structural characterization of the three applied biochars can be found in Stylianou et al. (2020). The growing period lasted for 40 days (i.e., from mid-May to late June). Overall, five treatments were applied. More precisely, treatments concerned the control (C) in which plants were grown in non-amended soil, and the DBD, MDB and SCGDB treatments, in which plants were grown in soil amended (5% v/v) with each of the three biochars, respectively. PM treatment refers to plants grown in soil amended with peat moss (5% v/v). The topsoil of the experimental field was constituted of 52% sand, 21% silt and 27% clay, with 0.6% total organic matter, pH value 8.74 and electrical conductivity of 0.84 mS cm^{-1}. All common agricultural practices concerning the cultivation of lettuce plants were followed, while it is worth to note that no pesticides were used. All plants were drip-irrigated with tap water based on their irrigation needs (Christou et al., 2017). Five replicates per treatment were applied in a randomized block experiment, with 6 individual lettuce plants in each, resulting in the use of 150 lettuce plants, in total.

Plants reached marketable size 40 days after transplants were planted in field. Half of plants were uprooted and their above ground parts (leaves) were used for biomass measurements, whereas the remaining were used for collecting samples (destructive sampling) that were immediately flash frozen in liquid nitrogen, and subsequently transfer in the laboratory and stored at −80 °C. These samples were used for physiological and biochemical assays.

2.2. Biochar production and handling

Biosolids were collected from an Urban Wastewater Treatment Plant applying Membrane Bioreactor treatment and cattle manure from a farm, both located in the suburbs of Nicosia, Cyprus. A cafeteria located within the campus of the University of Cyprus, Nicosia, provided the spent coffee grounds. All feedstocks were open-air sun dried (under 30–35 °C) for 10 days. Nearly 100 kg of each biowaste material were converted into biochar at 550 °C for 1.5 h, in a prototype pyrolysis kiln (capacity of nearly 15 kg), as previously described in detail (Stylianou et al., 2020). The biochar was then naturally cooled down at ambient temperature and grounded to less than 500 μm, before applied as soil amendment.

2.3. Biomass measurements

The aerial parts (leaves, as a whole) of ten plants were weighted (fresh weight; F.W.) in an electronic balance. Then, samples were oven-dried (60 °C), until constant weight was reached. Dry matter (%) was estimated as the proportion of dry weight to fresh weight.

2.4. Photosynthetic pigment and nitrate content analysis

Photosynthetic pigment was extracted with DMSO from leaf disks obtained from the peripheral edge of the upper middle of fully expanded leaves (at least 10 per replication). The spectrophotometric absorption of the extract at 661, 643, 470, and 534 nm (Thermo Scientific, Helios™ Zeta) was then measured, as proposed by Richardson et al. (2002). The photosynthetic pigment contents of lettuce samples were determined following the equations proposed by Misra and Dey (2013). Nitrate content was estimated by applying the salicylate method proposed by Cataldo et al. (1975). Results were obtained based on a nitrate standard curve.

2.5. Phytochemical measurements

The soluble solids content (SSC) of lettuce samples was quantified with a PAL refractometer (ATAGO, PR-32$^{\alpha}$; Tokyo, Japan). An automatic titrator (862 Compact Titrosampler, Metrohm AG, Switzerland) was used to measure the titratable acidity (TA) of samples. Sugars (TSS, Sucrose, Glucose, Fructose) were extracted from pulp aliquots as analytically described by Hadjipieri et al. (2020). The contents of TSS, sucrose and glucose, as well as that of fructose were spectrophotometrically determined based on the methodology proposed by Jin et al. (2007) and Edewor-Kuponiyi (2013), respectively.

2.6. Ascorbic acid and total phenolics content, and total antioxidant capacity determination

The total ascorbic acid content of lettuce samples was spectrophotometrically quantified by monitoring the reduction of 50 mmol L^{-1} 2,6-dichloroindophenol (900 μL) by the filtrate pulp samples (500 μL) at 520 nm (Thermo Scientific, Helios™ Zeta), and quantified using an ascorbic acid standard curve (Georgiadou et al., 2018a). The methodology proposed by Singleton et al. (1999) was applied for the spectrophotometric quantification of total phenolics content of pulp methanolic extracts. The total antioxidant capacity of lettuce samples was determined by the ferric reducing antioxidant power (FRAP) assay, as analytically described by Georgiadou et al. (2018b).

2.7. Statistical analysis

The *GraphPad Prism* 8.0 software (GraphPad Software, San Diego, CA, United States) was used to perform all statistical analyses. The comparison of treatments' means was made by applying one-way analysis of variance according to Tukey's pairwise comparison test ($P \leq 0.05$).

Table 1
Effects of different biochars and peat moss applied as soil amendments on the biomass production (mean, maximum and minimum weight per plant) and the dry weight of lettuce plants (aboveground parts) (n = 10).

Treatment	Mean weight	Maximum weight	Minimum weight	Dry Weight
	g	g	g	%
Control	103.71 ± 13.77 bc	166.55	49.22	3.76 ± 0.21 b
BDB	140.31 ± 18.17 ab	218.6	81.29	4.23 ± 0.15 b
MDB	161.10 ± 15.11 a	240.35	108.94	5.23 ± 0.56 a
SCGDB	85.07 ± 8.58 c	127.35	48.25	3.94 ± 0.11 b
PM	83.46 ± 16.12 c	136.65	53.41	4.12 ± 0.20 b

Mean values with different letters within each column are statistically different at $P < 0.05$.

3. Results and discussion

3.1. Effects of applied soil amendments on the growth and development of lettuce plants

Lettuce plants did not display any phenotypic alteration or disorder throughout their growing period, thus no additional fertilization beside the basic one (prior planting) was applied, neither any pesticides. Macroscopic observations showed an increase in both growth rate and biomass production in plants grown in soil amended either with BDB or MDB, compared with that in control soil and that in SCGDB and PM treatments. Weight measurements confirmed macroscopic observations, as plants in MDB treatment had 60% higher mean weight per plant compare with control samples, and also their weight was almost doubled compared with samples in SCGDB and PM treatments (Table 1). Plants grown in BDB-amended soil also displayed increased biomass, though such difference did not reach significance compared with the control samples (Table 1). Plants in MDB and BDB showed increased values of maximum and minimum weight per plant compared with other treatments, whereas only plants grown in MDB-amended soil showed significantly increased values of dry weight compared with samples from the control and from the other amended soils (Table 1). Our results supported previous ones (Hagner et al., 2016; Agegnehu et al., 2017) highlighting that the effects of biochar application as soil amendment on the growth and development of crop species is highly variable, depending on the type of biochar (feedstock material and pyrolysis conditions), the application rate and the plant species. Artiola et al. (2012) reported that pine forest waste-derived biochar increased significantly the biomass yield of romaine lettuce grown in pots in greenhouse when applied at a ratio of 2% w/w, but displayed adverse effects when applied at a ratio of 4% w/w. In addition, lettuce plants grown in clay loam soil in pots in greenhouse conditions displayed increased growth rates following application of poultry litter-derived biochar, though in a dosage depended manner (Akça and Namli, 2015). Similar results, showing dosage-depended positive effects of green waste- and rice husk-derived biochar on lettuce grown in greenhouse conditions were reported by Upadhyay et al. (2014) and Carter et al. (2013), respectively. Hagner et al. (2016) reported that birch-derived biochar pyrolyzed at 300, 375 and 475 °C had no impact on biomass production of lettuce plants grown in sandy-silt soil in pots in greenhouse when applied at low ratio (20 g L^{-1}), whereas biochar pyrolyzed at 300 and 375 °C had negative effects when applied at high ratio (80 g L^{-1}). The results of the present study, obtained under real field conditions, corroborate previous results indicating that the effects of biochar are highly depended on the feedstock material used in the pyrolysis process, as only MDB had significant positive effect on the biomass yield of lettuce plants grown in field in sandy clay loam-textured soil.

3.2. Biochar-mediated effects on photosynthetic pigment, soluble solid and nitrate ions content, and titratable acidity of lettuce plants

Biochars have been shown to modulate (mostly in a positive way) photosynthesis in plants either exposed to optimum or adverse environmental conditions via various mechanisms, including increased pigments contents, stomatal conductance, gas exchange and transpiration rates (Ali et al., 2017; He et al., 2020). The quantification of photosynthetic pigment contents showed that the studied biochars, as well as the applied peat moss, did not exert any significant effects on the leaf content of total chlorophyll, chlorophyll a, chlorophyll b and carotenoids (Table 2). Keshavarz Afshar et al. (2016) also reported that the photosynthetic pigment content of milk thistle seedlings, grown in sandy loam soil in pots either in optimum or drought stress conditions, was not affected by the amendment of soil with hardwood (maple)-derived biochar at the rate of 1 or 2%. On the contrary, the application of pistachio shell-derived biochar at a rate of 2% in lettuce plants grown in pots in greenhouse under nickel stress managed to increase the chlorophyll a and chlorophyll b leaf content. Increased total chlorophyll, chlorophyll a, chlorophyll b and carotenoids contents were also reported in the leaves of chickpea plants grown in controlled conditions in pots (peat:pertlite:sand; 1:1:1) amended with biochar derived from the pyrolysis of woody branches of button mangrove plants at a rate of 3% (Hashem et al., 2019). Cotton stalks-derived biochar (applied in pots at a 1% w/w rate in a silty soil) also managed to increase all studied pigments concentration in the leaves of tomato plants (Abid et al., 2017).

Table 3 shows that some of the applied sorbent materials affected significantly the content of soluble solids and nitrate ions, as well as the TA of lettuce leaves. More precisely, plants in BDB and MDB treatments showed increased SSC levels

Table 2
Photosynthetic pigment contents (n = 6) of lettuce leaves as affected by the amendment of soil with the studied soil conditioners.

Treatment	Total Chlorophyll (mg 100 g^{-1} F.W)	Chlorophyll a (Chla) (mg 100 g^{-1} F.W)	Chlorophyll b (Chlb) (mg 100 g^{-1} F.W)	Carotenoids (mg 100 g^{-1} F.W)
Control	7.99 ± 0.77 a	6.44 ± 0.62 a	1.54 ± 0.15 a	3.65 ± 0.37 a
BDB	6.92 ± 0.73 a	5.61 ± 0.60 a	1.32 ± 0.13 a	3.07 ± 0.30 a
MDB	8.13 ± 1.16 a	6.58 ± 0.93 a	1.55 ± 0.23 a	3.75 ± 0.53 a
SCGDB	7.68 ± 0.35 a	6.25 ± 0.29 a	1.43 ± 0.06 a	3.60 ± 0.22 a
PM	8.47 ± 0.80 a	6.85 ± 0.66 a	1.62 ± 0.14 a	3.75 ± 0.34 a

Mean values with different letters within each column are statistically different at $P < 0.05$.

Table 3
Soluble solids content (SSC), titratable acidity (TA) and nitrate ions content in the leaves of lettuce plants grown in soil amended or not with each of the studied biochars or peat moss, for 40 days (n = 6).

Treatment	SSC	TA	NO_3
	% Brix	% citric acid	mg NO_3 100 kg^{-1} F.W.
Control	2.02 ± 0.08 c	0.052 ± 0.002 a	710.67 ± 64.72 a
BDB	2.38 ± 0.05 a	0.035 ± 0.001 c	363.50 ± 34.97 bc
MDB	2.28 ± 0.04 ab	0.042 ± 0.001 b	440.50 ± 104.69 b
SCGDB	2.00 ± 0.03 c	0.052 ± 0.001 a	247.17 ± 27.74 c
PM	2.16 ± 0.07 bc	0.044 ± 0.001 b	251.50 ± 37.14 c

Mean values with different letters within each column are statistically different at $P < 0.05$.

compared with control and SCGDB- and PM-treated plants. Also, plants grown in soil amended with either BDD- and MDB-derived biochar and PM had lower levels of TA in their leaves compared with that of the control samples and of the plants grown in SCGDB-amended soil (Table 3). All applied sorbent materials reduced the concentration of nitrates ions in lettuce leaves compared with control plants (by 38 to 64%), with the reduction in plants grown in soil amended with SCGDB and PM being more pronounced (Table 3). This implies that the used sorbent materials retained nitrogen in soil, thus controlling its soil cycle through mitigating soil nitrogen losses (i.e., NH_3 volarization, *N* leaching) (Gao et al., 2019; Liu et al., 2019), while also enhanced nitrogen use efficiency, as already it has been proved in lettuce plants grown in greenhouse in silt loam soil amended with pine chip- and walnut shell-derived biochar (Pereira et al., 2017) and Chinese cabbage and rice plants grown in field amended with bamboo-derived biochar (Kang et al., 2021).

3.3. Effects of applied sorbents on carbohydrate content and the antioxidant machinery of lettuce leaves

Biosolids- and manure-derived biochars applied at soil prior planting have shown to exert, at least to some extent, significant effects on soluble carbohydrate content of lettuce leaves as compared with untreated control samples. It is worth noting that all carbohydrates assessed (fructose, glucose, sucrose) and total soluble sugars were increased in these two treatments when compared with control samples, though not always in a statistically significant manner. On the contrary, SCGDB and PM when amended in soil failed to exert any significant effects on carbohydrate content in lettuce leaves (Table 4). Sucrose content in leaves was significantly increased in plants grown in BDB- and MDB-amended soil, whereas fructose was significantly increased in plants grown is MDB-amended soil, compared with the those in plants grown in control and SCGDB- and PM-amended soil (Table 4). As far as in known, this is the first research showing the biochar-mediated effects on the carbohydrate content of lettuce plants grown under real field conditions. Turan (2019) also reported increased carbohydrates and starch content in lettuce leaves, though plants were exposed to heavy metal stress (Ni) under greenhouse conditions (pot experiment). Moreover, wheat straw-, poplar- and olive residues-derived biochars did not affect significantly the sugar content of tomato fruits from plants grown in pots in greenhouse (Petruccelli et al., 2015), whereas hardwood and coniferous wood chips-derived biochars did not affect the total sugar content and glucose to fructose ratio of grapes, in a 3 years field study (Schmidt et al., 2014).

All treatments applied failed to exert any significant effect on the ascorbic acid content of leaves, compared with control samples (Table 5). Tomato fruits collected from plants grown in soil amended with timber waste-biochar and experienced reduced fertilization (50% reduction) showed similar ascorbic acid content with fruits collected from control non-amended plants (Sani et al., 2020). In contrast, biochar application (pistachio shell) increased ascorbic acid content, as well as the enzymatic activity of ascorbate peroxidase, dehydroascorbate reductase, peroxidase, catalase and superoxide dismutase (resulting in reduced hydrogen peroxide and oxygen radical contents and lipid peroxidation levels) in lettuce plants exposed to Ni stress under controlled conditions (Turan, 2019). It is widely accepted that the application of biochar in soil can enhance the tolerance of plants exposed to adverse environmental conditions, mainly through enhanced antioxidant activity (Ali et al., 2017; Semida et al., 2019). Biosolids- and manure-derived biochar increased significantly the total antioxidant capacity of lettuce leaves compared with control and samples from other treatments (Table 5). In addition, total phenolics' content was also found increased in BDB and MDB treatments compared with all other treatments, though

Table 4
Impacts of soil amendment with each of the three studied biochars or peat moss on the carbohydrate content of lettuce leaves ($n = 6$).

Treatment	Fructose	Glucose	Sucrose	Total soluble sugar
	mg 100 g^{-1} F.W	mg 100 g^{-1} F.W	mg 100 g^{-1} F.W	mg 100 g^{-1} F.W
Control	948.83 ± 36.06 bc	505.32 ± 28.35 ab	82.83 ± 6.12 b	1438.24 ± 82.60 ab
BDB	1021.64 ± 26.65 ab	576.42 ± 31.18 a	102.66 ± 7.07 a	1611.70 ± 44.35 a
MDB	1046.53 ± 35.56 a	563.97 ± 21.21 ab	98.97 ± 7.43 a	1521.80 ± 65.63 ab
SCGDB	893.15 ± 24.45 c	494.54 ± 18.09 b	81.10 ± 3.41 b	1406.73 ± 50.82 b
PM	882.99 ± 32.96 c	498.87 ± 20.28 b	80.65 ± 2.80 b	1418.63 ± 45.06 b

Mean values with different letters within each column are statistically different at $P < 0.05$.

Table 5
Ascorbic acid and total phenolics content, and total antioxidant capacity of lettuce leaves as affected by the growth of plants in soil amended with each of the three studied biochars or peat moss ($n = 6$).

Treatment	Ascorbic acid content	Total phenolics content	Total antioxidant capacity
	mg ascorbic acid 100 g^{-1} F.W.	mg GAE 100 g^{-1} F.W.	mmol ascorbic acid 100 g^{-1} F.W.
Control	306.66 ± 7.73 a	300.36 ± 23.36 bc	3.82 ± 0.32 b
BDB	311.03 ± 12.50 a	451.26 ± 28.91 a	5.51 ± 0.52 a
MDB	317.48 ± 11.49 a	414.12 ± 42.11 ab	5.27 ± 0.43 a
SCGDB	295.10 ± 5.23 a	299.82 ± 22.25 b	2.97 ± 0.29 b
PM	290.82 ± 6.75 a	283.22 ± 24.33 bc	3.60 ± 0.17 b

Mean values with different letters within each column are statistically different at $P < 0.05$.

the increase only reached significance in BDB treatment (Table 5). A linear correlation between the total antioxidant capacity (measured as FRAP) and phenolic compounds following wounding was also revealed in lettuce leaves (Kang and Saltveit, 2002), further supporting the obtained results.

The effects of biochar on the carbohydrate content of tomato and grape fruits (Schmidt et al., 2014; Petruccelli et al., 2015) and on the ascorbic acid and antioxidant capacity of tomato fruit, olive pulp and lettuce leaves (Turan, 2019; Sani et al., 2020; Turan, 2021b), as well as the obtained results of the present study, indicate that the effects of biochar application are largely depended on the feedstock used and the pyrolysis conditions (i.e., type of pyrolysis and temperature), as well as on the application rate and the plant species. Results also suggest that the use of BDB and MDB as soil amendments may contribute, at least to some extent, to an antioxidant-rich diet as far as lettuce is concerned.

4. Conclusions

A vast number of distinct types of biochars are currently produced, depending on the type of feedstock used and the pyrolysis conditions. Agricultural application as soil improver is just one of the numerous environmental applications of biochar. Results of the present study, obtained from a field study under real agricultural practices, showed that the amendment of soil with BDB and MDB has positive effects on the growth and development (increased biomass production) and partially on the nutritional value of lettuce plants, as compared with control untreated plants or plants grown in soil amended with SCGDB or PM. Moreover, all applied biochars may serve for the enhancement of nitrogen use efficiency of lettuce plants. The use of PM (a commonly used soil conditioner) as internal positive control further supported the positive effects of applied biochars (BDB and MDB).

CRediT authorship contribution statement

Anastasis Christou: Conceived and directed this study, Designed the experiment, Writing – original draft, Writing – review & editing. **Marinos Stylianou:** Conceived and directed this study, Designed the experiment. **Egli C. Georgiadou:** Supervised all the analytical assays and analyzed the data. **Stella Gedeon:** Performed part of the biochemical assays. **Andreas Ioannou:** Performed part of the biochemical assays. **Costas Michael:** Conceived and directed this study, Designed the experiment. **Panos Papanastasiou:** Conceived and directed this study, Designed the experiment. **Vasileios Fotopoulos:** Conceived and directed this study, Designed the experiment. **Despo Fatta-Kassinos:** Conceived and directed this study, Designed the experiment.

Declaration of competing interest

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

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