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Research Article

The Effects of Biochar and Its Combination with Compost on Lettuce (*Lactuca sativa* L.) Growth, Soil Properties, and Soil Microbial Activity and Abundance

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Impacts of biochar application in combination with organic fertilizer, such as compost, are not fully understood. In this study, we tested the effects of biochar amendment, compost addition, and their combination on lettuce plants grown in a soil poor in nutrients; soil microbiological, chemical, and physical characteristics were analyzed, together with plant growth and physiology. An initial screening was also done to evaluate the effect of biochar and compost toxicity, using cress plants and earthworms. Results showed that compost amendment had clear and positive effects on plant growth and yield and on soil chemical characteristics. However, we demonstrated that also the biochar alone stimulated lettuce leaves number and total biomass, improving soil total nitrogen and phosphorus contents, as well as total carbon, and enhancing related microbial communities. Nevertheless, combining biochar and compost, no positive synergic and summative effects were observed. Our results thus demonstrate that in a soil poor in nutrients the biochar alone could be effectively used to enhance soil fertility and plant growth and biomass yield. However, we can speculate that the combination of compost and biochar may enhance and sustain soil biophysical and chemical characteristics and improve crop productivity over time.

1. Introduction

Soil fertility degradation, caused by erosion and depletion or imbalance of organic matter/nutrients, is affecting world agricultural productivity [1]. Inorganic fertilizers have played a significant role in increasing crop production since the "green revolution" [2]; however, they are not a sustainable solution for maintenance of crop yields [3]. Long-term overuse of mineral fertilizers may accelerate soil acidification, affecting both the soil biota and biogeochemical processes, thus posing an environmental risk and decreasing crop production [4]. Organic amendments, such as compost and biochar, could therefore be useful tools to sustainably maintain or increase soil organic matter, preserving and improving soil fertility and crop yield.

Biochar is a carbon-rich material obtained from thermochemical conversion (slow, intermediate, and fast pyrolysis or gasification) of biomass in an oxygen-limited environment. It can be produced from a range of feedstock, including forest and agriculture residues, such as straw, nut shells, rice hulls, wood chips/pellets, tree bark, and switch grass [5]. Biochar has been described as a possible tool for soil fertility improvement, potential toxic element adsorption, and climate change mitigation [6–8].

Indeed, several studies have shown that biochar application to soil can (i) improve soil physical and chemical properties [9, 10], (ii) enhance plant nutrient availability and correlated growth and yield [11, 12], (iii) increase microbial population and activities [13–15], and (iv) reduce greenhouse gas emissions through C sequestration [16].

Table 1: *Biochar and compost characteristics*. The complete biochar and compost characterization and related methods are detailed in Amendola et al. [31] and Alfano et al. [30], respectively. All concentrations refer to dry matter and represent the means of three replicates ± standard error.

	Biochar	Compost
pH	9.7 ± 0.1	7.5 ± 0.1
Alkalinity (% $CaCO_3$)	18.2 ± 0.7	6.5 ± 0.4
EC (dS/m)	7.5 ± 0.4	4.9 ± 0.3
Moisture (g/kg)	62.4 ± 1.3	3.4 ± 0.9
CEC (cmol/kg)	21.3 ± 0.2	21.0 ± 0.2
P_{tot} (g/kg)	12.2 ± 3.0	5.5 ± 0.6
N_{tot} (g/kg)	9.1 ± 0.2	12.0 ± 4.0
C_{tot} (g/kg)	778.1 ± 0.0	337.2 ± 0.3
C/N	125.5	28.1
Cultivable aerobic bacteria (log CFU/g)	Absent	7.6 ± 0.3
Eumycetes (log CFU/g)	Absent	4.9 ± 0.1
Actinomycetes (log CFU/g)	Absent	5.18 ± 0.2
Coliform bacteria (log CFU/g)	Absent	Absent
E. coli (log CFU/g)	Absent	Absent
Salmonellae spp. (log CFU/g)	Absent	Absent

The beneficial effects of biochar on plant productivity and soil microbial population are related to the improvement of specific surface area, cation exchange capacity, bulk density, pH, water, and nutrients within the soil matrix [17]. Beside the generally positive plant growth responses to biochar amendment, especially in acidic coarse texture soil, negligible or negative effects also occur due to types of feedstock and pyrolysis process, biochar application rate, plant species, and soil characteristics [18, 19]. Furthermore, in most cases, biochar does not provide high amounts of nutrients [20, 21].

Some recent studies have indicated that combined applications of biochar with organic or inorganic fertilizers could lead to enhanced soil physical, chemical, and biological properties, as well as plant growth. In particular, several composted materials represent a sustainable source of available nutrients that could enhance plant growth, ameliorating soil physicochemical characteristics and microbiological properties [22–26]. Liu et al. [26] showed that the combined application of compost and biochar had a positive synergistic effect on soil nutrient contents and water-holding capacity under field conditions. In addition, the combination of biochar with compost has proved to be suitable, allowing the reduction of fertilizer inputs, stabilizing the soil structure, and improving its nutrient content and water retention capacity [27, 28]. Again, these studies underline that compost and biochar combination could enhance compost properties, leading to a higher added value and a much better carbon sequestration potential due to the long-term stability of biochar [24, 25].

However, the literature shows that compost effects, as also reported above for biochar, can differ on soil biophysical-chemical properties and plant growth and yield on the basis of feedstock types, methods of producing, and application [29]. The objectives of this study were thus to determine the effect of biochar application alone, obtained from orchard pruning biomass by slow pyrolysis (550°C), or combined with compost, obtained from olive mill residues, on (i) plant growth, physiology, and yield, (ii) soil chemical characteristics, and (iii) soil microbiological abundance and enzyme activities. For this purpose, a short-term potting experiment was performed, using *Lactuca sativa* L. as reference plants and a soil poor in nutrients as growing substrate, to test the following hypotheses: biochar addition together with compost improves (1) soil chemical and (2) microbiological properties and enhances (3) plant growth and physiology more than compost and biochar alone.

2. Materials and Methods

2.1. Biochar, Compost, and Toxicity Test. The biochar used was a commercial charcoal (provided by Romagna Carbone s.n.c., Italy), obtained from orchard pruning biomass through a slow pyrolysis process at a temperature of 500°C in a transportable ring kiln 2.2 m in diameter that holds around 2 t of feedstock.

An olive waste compost was used, prepared in a specific experimental composting process, following Alfano et al. [30]. Briefly, compost was prepared mixing humid olive husks, from a two-phase extraction plant, with olive leaves (8% w/w); one-year-old, humid, composted husks (25% w/w) were then added to this mixture. Biochar and compost characteristics are summarized in Table 1 and analytical methods are provided in Amendola et al. [31] and Alfano et al. [30].

Biochar and compost phytotoxicity was assessed through the germination index ($GI_{\%}$) of cress plants (*Lepidium sativum* L.) [26]. Three different solutions were used to evaluate the biochar and compost toxicity on seed germination: sterile deionized water as control solution and solutions containing 50% and 75% extract of biochar or compost. Solutions were added to Petri dishes containing 10 sterile seeds of *L. sativum*. Germination percentage and plant root length were recorded after incubation for 42 h at 27°C in the dark (according to Vitullo et al. [32]). Seeds

were scored as germinated if the radicle exceeded the length of the longest seed coat dimension. The seed germination percentage was assessed according to the formula: $GI_{\%} = (G_s L_s)/(G_c L_c) \times 100$, where G_s and L_s are seed germination and root elongation (mm) for the samples and G_c and L_c the corresponding values for controls. The test was repeated in triplicate. The $GI_{\%}$ was obtained by means of $GI_{50\%}$ and $GI_{75\%}$ values. Potential toxicity of biochar was also tested on earthworms (*Lumbricus terrestris* L). For the earthworm avoidance test, equal amounts of unfertilized soil with and without biochar (65 g kg^{-1} of dry soil) were placed in two halves of a pot (50 × 30 cm). Forty earthworms were released between the two substrates. After 48 hours, the pot was examined to determine the soil selected by earthworms [33].

2.2. Experimental Design. One-month-old lettuce (*Lactuca sativa* L. var. *longifolia*) seedlings (Vivaio Mignogna, Ripamolisana, Molise, Italy) were transplanted into plastic pots (3.5 l) prepared with four different substrates: (i) unfertilized soil (PS); (ii) unfertilized soil plus compost (PSC); (iii) unfertilized soil plus biochar (PSB); and (iv) unfertilized soil plus compost and biochar (PSCB). Plants were then grown until maturity (9 weeks) in a screened greenhouse (University of Molise, Pesche, Italy; Lat 41°37′00″N; Long. 14°17′00″E; 510 m a.s.l.) under a controlled water regime, temperatures ranging between 12 and 25°C, and natural day length corresponding to spring-summer season (May–July). Soil was collected from an uncultivated pasture area, located in Pesche, with a floral composition predominantly of graminoid grasses, not under a rotation system and that includes hedges. This area is mainly used for grazing, but the fodder is harvested mechanically. The preplanting physicochemical properties of the soil are given in Supporting Information (Table S1 in Supplementary Material available online at https://doi.org/10.1155/2017/3158207). Briefly, the soil was moderately subalkaline (0–30 cm) with a clay texture according to United States Department of Agriculture classification [34]; it was characterized by low electrical conductivity (EC), cation exchange capacity (CEC), and nitrogen and carbon content; it is unlikely that this soil already contained charcoal as there had been no tradition of crop residue or other burning on the land. For the experiment, soil was air-dried for 72 h, weighed and finely crushed, and then mixed thoroughly before packing lightly in the pots on top of 100 g of pebbles placed on the base to improve drainage. The weight of each filled pot was 3000 g.

Biochar and compost application rates were set up on the basis of previous lettuce pot and field researches [12, 35–39]. In detail, data reported by Carter et al. [35] showed that 50 and 150 g kg^{-1} rice-husk biochar application rate led to a highly positive effect on lettuce growth in compost fertilized and unfertilized soils, respectively. Based on this finding, in the present study, biochar and compost were supplied at an application rate of 65 g and 50 g per kg of dry soil, respectively. After mixing, the pots were filled in order to ensure the same soil bulk density. There were ten pots (one plant per pot) for each treatment arranged in a complete randomized block design and rotated each day to a different position within the block for the duration of the trial. The pots were fully irrigated to prevent water stress (twice a day, as required) and a suspended shade cover net was used to reduce exposure to sunlight.

2.3. Soil Analyses. Soils were sampled at the end of the experiment and air-dried for 72 h. The moisture content was calculated according to the Black method [40] as the difference in sample weight before and after oven drying to constant weight at 105°C. The pH was measured by potentiometry (pH meter Eutech Instruments) in H_2O and 0.01 M $CaCl_2$ using a 1 : 2.5 soil weight : extract-volume ratio. Alkalinity of samples with a pH value greater than 7.0 was determined by titrimetry according to the Higginson and Rayment method [41, 42]. Electrical conductivity (EC) was determined by a conductivity meter (Cond 510, XS Instruments) on a 1 : 5 soil : water suspension [41, 42]. Ash content was determined by igniting an oven-dried sample in a muffle furnace at 440 ± 40°C, according to the American Society for Testing and Materials [43]. Cation exchange capacity (CEC) was assessed according Mehlich [44] using the $BaCl_2$. For total nitrogen (N_{tot}) determination a modified Kjeldahl procedure was used with Devarda's alloy pretreatment, important to recover both NO_3^--N and NO_2^--N [45]. Total phosphorus (P_{tot}) was detected by spectrophotometry (UV-1601 Shimadzu) according to the test method described by Bowman [46]. Available phosphorus (P_{av}) was extracted by a $NaHCO_3$ solution at pH 8.5 and evaluated by spectrophotometry according to the Olsen test method [47]. Total carbon (C_{tot}) content was determined using a CHN autoanalyzer (CHN 1500, Carlo Erba) [48].

2.4. Plant Analysis. Plant morphological analyses were performed weekly by measuring the main leaf parameters: number (LN); area (LA); length (LL); width (LW); and perimeter (LP). The Image J 1.41 (https://rsb.info.nih.gov/ij/) software was used for analysis. In addition, at the end of the experiment, leaf and root biomass allocation was determined before (fresh weight, FW) and after (dry weight, DW) two days of drying in an oven at 60°C. The measurements were performed on six plants.

Leaf water potential (ψ) was measured using a pressure chamber (PMS, Instrumentation Co., Corvallis, OR, USA). Leaf gas exchange measurements were performed using a portable gas exchange system (CIRAS-1, PP Systems, Hertz, Hitchin, UK). Leaf gas exchanges and ψ were measured on the fourth fully expanded and sun exposed leaf on a cloud-free day (after 3 months of growth). These measurements were taken on five plants (one leaf per plant) per treatment around midday (between 11.30 a.m. and 1.30 p.m.).

Chlorophyll content was also measured. For this, chlorophylls *a* (Chl*a*) and *b* (Chl*b*) were extracted from three randomly sampled leaf discs (10 mm) with N,N dimethylformamide (DMF). Extraction was performed for 48 h at 4°C in the dark at a ratio of 1 : 20 (plant material : solvent, w : v) [49]. The extinction coefficients proposed by Inskeep and Bloom [50] were used for the quantification by spectrophotometric analysis. The following formulas were used: $\text{Chl } a = 12.70_{A664.5} - 2.79_{A647}$; $\text{Chl } b = 20.70_{A647} - 4.62_{A664.5}$;

Chl = $17.90_{A647} + 8.08_{A664.5}$, where A is absorbance in 1.00 cm cuvettes and Chl is mg per l. The leaf area Chl content was then calculated (mg cm^{-2}) together with the Chl *a*/Chl *b* ratio by dividing the Chl *a* content by the Chl *b* content.

2.5. Microbiological Analyses. Cellular cultivability was assessed by plate-counting. Cultivable aerobic bacteria in soil samples were analyzed on standard plate count agar (Difco Bekton Dikson, Milan, Italy), at 28 and 55°C for 48 h; actinomycetes on actinomyces agar (Difco), at 28°C and 55°C for 48–72 h; eumycetes on malt agar (Difco) + rose bengal 33 mg l^{-1} and tetracycline 100 ml l^{-1}, at 28°C for 72 h, following the method described by Alfano et al. [30]. Cellular counts were done in triplicate by performing quantitative determinations on the basis of colony forming units (CFU) in agarized media, according to Alfano et al. [30]. All results are expressed as log CFU/g DW, after drying aliquots of the samples at 105°C for 48 h.

Soil enzymatic activities were determined by the API-ZYM system (bio-Mérieux Italia, Rome, Italy). With the API-ZYM system, semiquantitative evaluation of the activities of 19 hydrolytic enzymes [alkaline phosphatase, esterase (C4), esterase-lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, phosphoamidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase] was determined [51, 52]. Using a sterile Pasteur pipette, each gallery was inoculated with two drops of 10^{-1} or 10^{-2} suspensions of 20 g of soil in 180 mL of sterile saline solution (0.9% NaCl, w/v). The color that developed in each enzymatic reaction was assigned according to the color chart (range 0–5) supplied with the system, and this, in accordance with reported procedures, provided the conversion evaluation of the hydrolyzed substrates in nanomoles [51]. The samples were analyzed in triplicate and the average data were used. Results were expressed as enzyme relative activity/g of DW of substrate; data were corrected by the dilution factor.

2.6. Statistical Analysis. Analysis of variance (ANOVA) was applied in order to evaluate the effect of each treatment on soil proprieties, chlorophyll, ecophysiology, and microbiological data (one-way ANOVA) and the effect of day, treatments, and their interaction (two-way ANOVA) for plant morphological data. To assess the differences in the measured parameters among treatments, a postmeans comparison was performed using the Fisher least significant difference (LSD) test at the 0.05 significance level. Statistical analysis was conducted with OriginPro version 8.5.1 (OriginLab, Northampton, MA, USA).

3. Results

3.1. Biochar and Compost Characteristics. Biochar and compost used for the experiment were previously analyzed by Amendola et al. [31] and Alfano et al. [30], respectively. Main biochar and compost characteristics are summarized in Table 1. Briefly, as in the majority of biochar, the pH value was within the range of alkalinity. The C_{tot} and N_{tot} contents of biochar were 77.8% and 0.9%, respectively, according to Class 1 of the Guidelines for Certification of the International Biochar Initiative (IBI, http://www.european-biochar.org/en/ebc-ibi). The compost was mature, showing stable chemical and microbiological characteristics, with the potential to be used as an agricultural substrate or soil conditioner.

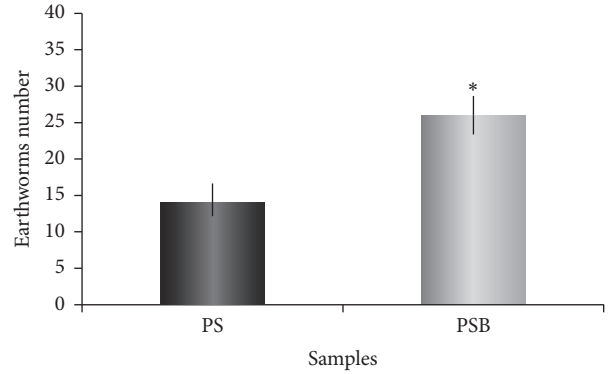


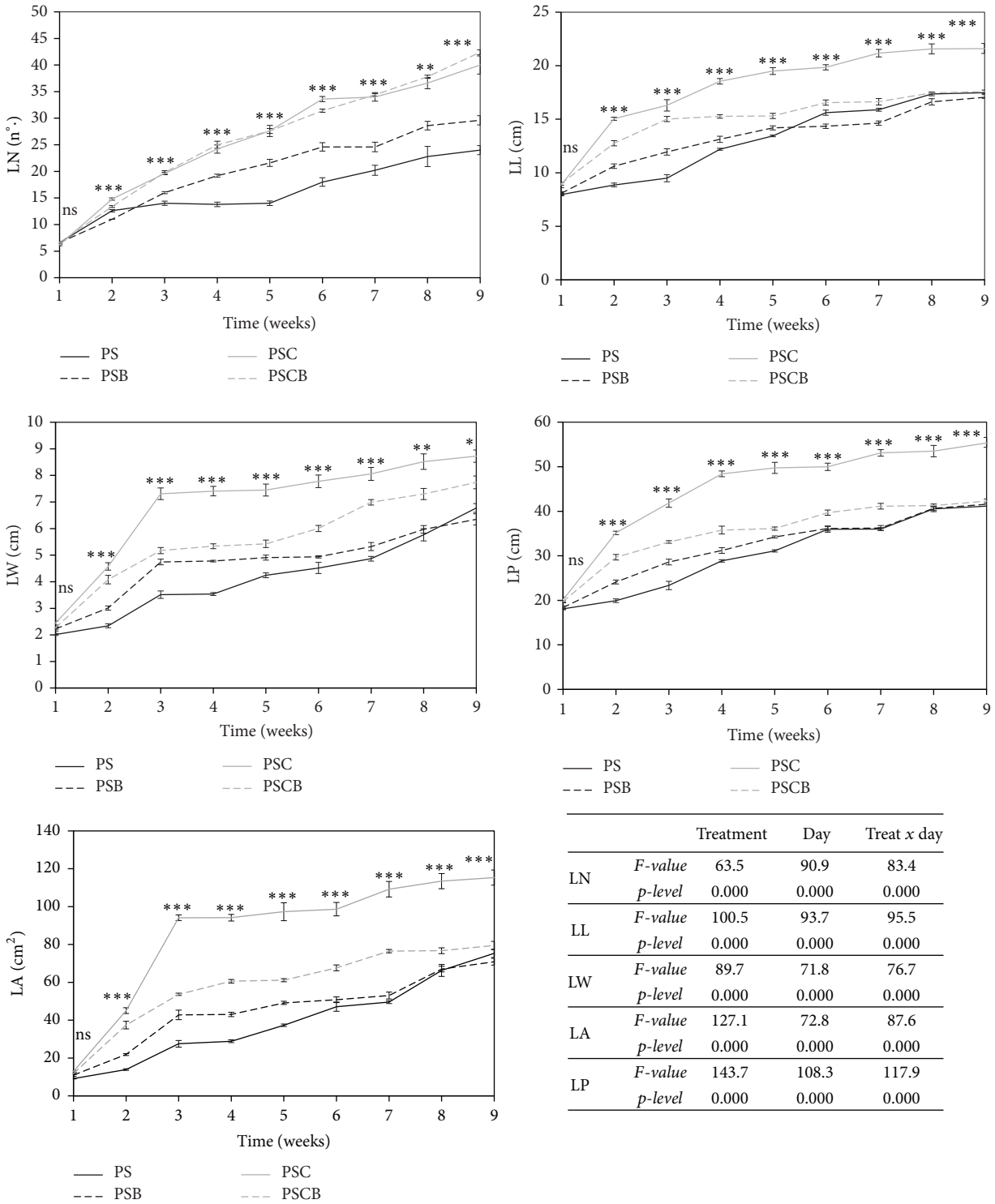
Figure 1: *Earthworms avoidance test*. Number of earthworms (*Lumbricus terrestris* L.) recovered in unfertilized (PS) versus unfertilized plus biochar (PSB) soil compartments after 48 h. Vertical bars represent the standard error of the mean of three replicates of 40 earthworms each. Asterisks indicate significant differences ($p \leq 0.05$).

The results showed that biochar was not toxic for soil biotic communities, *L. sativum* seeds, or earthworms. Indeed, the phytotoxicity test with *Lepidium* showed no effects of biochar and compost on the germination index (82 ± 4 and 95 ± 4%, resp.) compared to the control (water; 100 ± 2%) (data not shown), while the avoidance test showed that *L. terrestris* preferred the biochar-amended soil (Figure 1).

3.2. Soil Characterization. Soil chemical analysis showed that the addition of biochar induced a significant increase of pH values from 6.9 (PS) to 8.0 and from 7.5 (PSC) to 7.7 in PSB and PSCB, respectively. On the contrary, the alkalinity value did not change in PSB and PSCB compared to PS and PSC, respectively (Table 2).

An increase of total N content from 0.8 (PS) to 1.2 was observed in PSB. Conversely, the EC increased about 1.4 and 1.1 fold in PSB and PSCB, respectively, while moisture decreased 0.8-fold in PSCB. Ash content slightly decreased in PSB and PSCB. The P_{tot} was increased about 1.5-fold in PSB, whereas no alteration was observed in PSBC. On the contrary, the P_{av} was 2-fold greater in PSCB than PSC. The C_{tot} content was significantly increased in PSB (5-fold) and PSCB (2-fold) compared to the relative controls (PS and PSC). The CEC value was also slightly increased in PSB and PSCB compared to PS and PSC, respectively. All the above parameters were increased in PSCB compared to PSB, except for pH, moisture, and ash that decreased.

3.3. Plant Growth. Significant differences in growth parameters were recorded between treatments (Figure 2). In detail, lettuce plants showed higher leaf number in PSB than PS



		Treatment	Day	Treat *x* day
LN	*F-value*	63.5	90.9	83.4
	p-level	0.000	0.000	0.000
LL	*F-value*	100.5	93.7	95.5
	p-level	0.000	0.000	0.000
LW	*F-value*	89.7	71.8	76.7
	p-level	0.000	0.000	0.000
LA	*F-value*	127.1	72.8	87.6
	p-level	0.000	0.000	0.000
LP	*F-value*	143.7	108.3	117.9
	p-level	0.000	0.000	0.000

Figure 2: *Morphological analysis*. The main leaf parameters were analyzed: LN = leaf number; LL = leaf length; LW = leaf width; LA = leaf area; LP = leaf perimeter. Data represent the mean ($n = 6$) ± standard error. Mean values marked with asterisks are statistically different at $^{***}p \leq 0.0001$, at $^{**}p \leq 0.005$, and at $^{*}p \leq 0.01$. Two-way ANOVA was applied to weigh the effects of day, treatment, and their interactions on plant growth parameters (p and F level values are reported). PS = lettuce plants grown in unfertilized soil; PSB = lettuce plants grown in unfertilized soil plus biochar; PSC = lettuce plants grown in unfertilized soil plus compost; and PSCB = lettuce plants grown in unfertilized soil plus compost and biochar.

Table 2: *Soil chemical properties*. Data represent the mean ($n = 4$) ± standard error. Mean values marked with the same letter are not statistically different. One-way ANOVA was applied to weigh the effects of different treatments ($p < 0.05$).

	PS	PSB	PSC	PSCB
pH	6.9 ± 0.2^{d}	8.0 ± 0.0^{a}	7.5 ± 0.0^{c}	7.7 ± 0.0^{b}
Alkalinity (% $CaCO_3$)	7.7 ± 0.4^{a}	5.9 ± 0.5^{a}	6.4 ± 0.3^{a}	7.1 ± 0.4^{a}
EC (dS/m)	0.71 ± 0.0^{c}	1.0 ± 0.0^{b}	1.3 ± 0.1^{b}	1.5 ± 0.0^{a}
Moisture (g/kg)	63.0 ± 1.6^{a}	58.8 ± 0.3^{b}	61.8 ± 0.9^{a}	47.0 ± 2.1^{c}
Ash (%)	93.8 ± 0.2^{a}	90.0 ± 0.5^{b}	90.8 ± 0.7^{b}	86.0 ± 0.3^{c}
N_{tot} (g/kg)	0.8 ± 0.0^{c}	1.2 ± 0.0^{b}	1.9 ± 0.2^{a}	2.2 ± 0.0^{a}
P_{tot} (mg/kg)	199.9 ± 9.9^{c}	376.6 ± 52.3^{b}	455.6 ± 33.3^{ab}	471.0 ± 1.3^{a}
P_{av} (mg/kg)	$<12.0 \pm 0.0^{c}$	$<12.0 \pm 0.0^{c}$	24.9 ± 3.9^{b}	58.3 ± 4.5^{a}
C_{tot} (g/kg)	12.8 ± 0.5^{c}	59.1 ± 0.0^{a}	29.1 ± 0.8^{b}	65.9 ± 2.2^{a}
CEC (cmol/kg)	39.3 ± 0.0^{d}	40.4 ± 0.0^{b}	39.9 ± 0.0^{c}	43.6 ± 0.0^{a}

PS: unfertilized soil.
PSB: unfertilized soil plus biochar.
PSC: compost fertilized soil.
PSCB: compost fertilized soil plus biochar.

while leaf length, width, area, and perimeter were unchanged. Plants grown in PSCB showed lower leaf length, width, area, and perimeter compared to PSC while leaf number was unchanged. Furthermore, all leaf parameters were increased in PSC compared to PS and also in PSCB compared to PSB, although they were almost unchanged at the end of treatment (after 9 weeks).

Total plant dry weight, considering leaf and root biomass, increased in PSB compared to PS; on the contrary, leaf biomass decreased in PSCB and root biomass was unchanged compared to PSC and PSB (Figure 3). Chlorophyll content did not show significant changes between treatments (data not shown).

The leaf water potential was more negative in plants grown in PS than those grown in other substrates, followed by PSB and PSC and PSCB (Figure 4).

Stomatal conductance showed no significant differences between control and treated plants (Figure 5), while transpiration rate was slightly increased in PSCB and unchanged in PSC and PSB compared to PS (Figure 5). Assimilation rate was not altered by treatment with biochar (PSB) and biochar-compost (PSCB). In particular, plants showed the highest assimilation rate and water use efficiency in PSC (Figure 5).

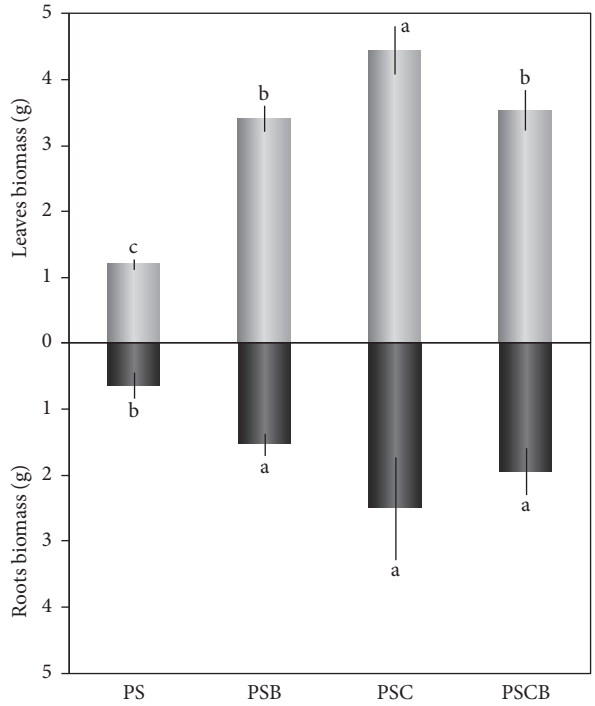


Figure 3: *Effects of biochar and/or compost on leaf and root biomass (g of dry tissue weight)*. Data represent the mean ($n = 6$) ± standard error. Mean values marked with the same letter are not statistically different. One-way ANOVA was applied to weigh the effects of different treatments ($p \leq 0.05$). PS = lettuce plants grown in unfertilized soil; PSB = lettuce plants grown in unfertilized soil plus biochar; PSC = lettuce plants grown in unfertilized soil plus compost; and PSCB = lettuce plants grown in unfertilized soil plus compost and biochar.

3.4. Cultivable Microorganisms. The analysis of cultivable microorganisms showed that in PSB the cultivable aerobic bacteria, actinomycetes, and eumycetes decreased ($p \leq 0.05$) compared to PS while they were unchanged in PSBC compared to PSC (Table 3). Furthermore, the abundance of cultivable aerobic bacteria and eumycetes was higher in PSC than PS while actinomycetes were unchanged; all cultivable microorganisms increased in PSCB compared to PSB (Table 3).

3.5. Soil Enzyme Activities. Enzymatic profile analysis revealed that all enzymatic activities were increased in PSC compared to PS, except lipase-esterase and esterase that were unchanged. Biochar treatment alone or in combination with compost induced specific enzymatic variations. In detail, in PSB, compared to PS, the activities of alkaline phosphatase, acid phosphatase, chymotrypsin, trypsin, phosphohydrolase, lipase-esterase, and esterase were increased, while lipase was

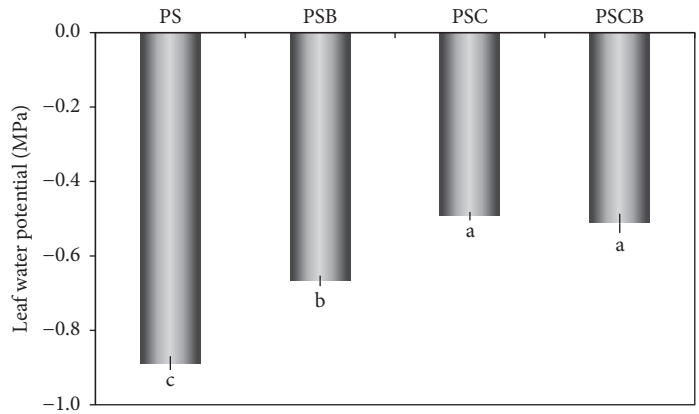


Figure 4: *Leaf water potential at midday of plants grown in different substrates*. Values are means ($n = 5$) ± standard error; significant differences between the means (at least $p \leq 0.05$, according to ANOVA) appear with different letters. PS = lettuce plants grown in unfertilized soil; PSB = lettuce plants grown in unfertilized soil plus biochar; PSC = lettuce plants grown in unfertilized soil plus compost; and PSCB = lettuce plants grown in unfertilized soil plus compost and biochar.

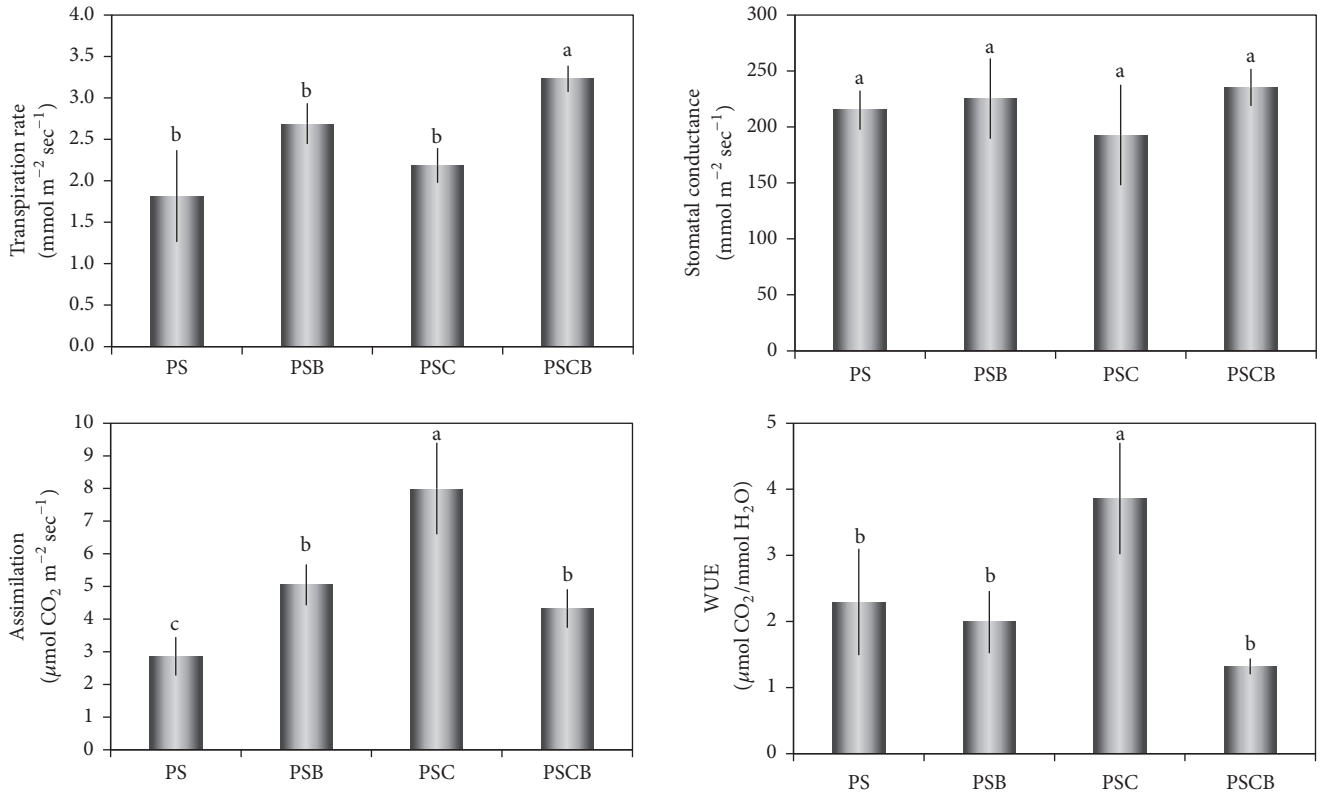


Figure 5: *Transpiration rate, stomatal conductance, assimilation, and water use efficiency (WUE) of plants grown in different soils*. Values are means ($n = 5$) ± standard error; significant differences between the means (at least $p \leq 0.05$, according to ANOVA) appear with different letters. PS = lettuce plants grown in unfertilized soil; PSB = lettuce plants grown in unfertilized soil plus biochar; PSC = lettuce plants grown in unfertilized soil plus compost; and PSCB = lettuce plants grown in unfertilized soil plus compost and biochar.

unchanged (Table 4). In PSBC, compared to PSC, alkaline phosphatase, acid phosphatase, lipase-esterase, and esterase strongly increased ($p \leq 0.01$), whereas chymotrypsin and trypsin activities decreased ($p \leq 0.05$) and phosphohydrolase and lipase activities were lost (Table 4). In PSCB, compared to PSB, we recorded that alkaline phosphatase and acid phosphatase increased and trypsin, phosphohydrolase, and esterase decreased, while chymotrypsin, lipase-esterase, and lipase were unchanged ($p \leq 0.05$).

4. Discussion

The study showed that both biochar amendment and compost addition to a soil poor in nutrients induced a positive effect on

Table 3: *Soil microbiological characteristics*. Values are expressed as log CFU/g dry weight (DW). Data represent the mean ($n = 3$) ± standard error. Mean values marked with the same letter are not statistically different. One-way ANOVA was applied to weigh the effects of different treatments ($p < 0.05$). PS = unfertilized soil; PSB = unfertilized soil plus biochar; PSC = unfertilized soil plus compost; and PSCB = unfertilized soil plus compost and biochar.

	PS	PSB	PSC	PSCB
Cultivable aerobic bacteria	10.97 ± 0.17^{a}	7.88 ± 0.18^{c}	8.84 ± 0.20^{b}	8.35 ± 0.21^{b}
Actinomycetes	8.67 ± 0.15^{a}	5.2 ± 0.12^{c}	6.4 ± 0.3^{b}	6.1 ± 0.2^{b}
Eumycetes	8.85 ± 0.12^{a}	7.89 ± 0.08^{b}	8.74 ± 10.40^{a}	8.52 ± 0.14^{a}

Table 4: *Soil enzymatic activities*. Soil enzymatic activities were determined by the API-ZYM system (bio-Mérieux Italia). Results are expressed as nanomoles/g of dry weight of substrate; the data have been corrected by the dilution factor. PS = unfertilized soil; PSB = unfertilized soil plus biochar; PSC = unfertilized soil plus compost; and PSCB = unfertilized soil plus compost and biochar.

Enzyme	PS	PSB	PSC	PSCB
Alkaline phosphatase	250 ± 25^{c}	500 ± 25^{b}	500 ± 50^{b}	1000 ± 50^{a}
Acid phosphatase	250 ± 25^{c}	500 ± 50^{b}	500 ± 25^{b}	1000 ± 100^{a}
Chymotrypsin	10 ± 5^{c}	250 ± 25^{b}	500 ± 25^{a}	250 ± 25^{b}
Trypsin	10 ± 5^{d}	250 ± 50^{b}	500 ± 50^{a}	100 ± 25^{c}
Phosphohydrolase	10 ± 5^{c}	250 ± 25^{b}	500 ± 50^{a}	10 ± 5^{c}
Lipase-esterase	10 ± 5^{b}	500 ± 50^{a}	10 ± 5^{b}	500 ± 25^{a}
Esterase	10 ± 5^{b}	500 ± 25^{a}	10 ± 5^{b}	10 ± 5^{b}
Lipase	10 ± 5^{b}	10 ± 5^{b}	250 ± 25^{a}	10 ± 5^{b}

lettuce plant growth and physiology and on soil chemical and microbiological characteristics; however, no positive synergic or summative effects exerted by compost and biochar in combination were observed.

In detail, the biochar alone induced a positive lettuce yield response, although transpiration, stomatal conductance, and assimilation rate did not show relevant variations. Positive yield responses to biochar addition have been reported for a wide variety of crops. For example, it is reported that maize yield increased by 98–150% as a result of manure biochar addition [53], lettuce and *Arabidopsis* plant biomass increased by 111% after poplar wood chips biochar addition [54], and wheat grain yield increased by 18% with the use of oil mallee biochar [55].

A possible explanation is that the biochar, increasing the pH, CEC, N_{tot}, C_{tot}, P_{tot}, and water content, could enhance available nutrients for plants and, consequently, biomass accumulation [22, 56]. In fact, the increase in CEC value could be driven by the presence of cation exchange sites on the biochar surface [10, 57], and, as also reported in Vaccari et al. [58], this could contribute to retaining NH_4^+, leading to improved N nutrition in biochar-amended soils [59–61]. This would confirm a direct biochar role in the nutrient supply to plants [20, 62]. The increased pH in biochar treated soil could also be indirectly related to growth stimulation in lettuce. Indeed, Beesley and Dickinson [63] hypothesized that soil alkalization caused by biochar addition might positively influence earthworms, as also observed in our study, with a subsequent positive effect on dissolved organic carbon content. The pH value has also been found to influence the soil microbial population and enzymatic activities; indeed, a high pH might enhance bacteria abundance, whereas it did not change fungi total biomass or dramatically reduce their growth [64, 65]. The activity of alkaline phosphatase, aminopeptidase, and N-acetylglucosaminidase enzymes has also been reported to increase after biochar applications [66, 67]. In accordance with this evidence, our results showed that the biochar alone decreased cultivable microorganisms abundance, while it enhanced the activity of enzymes involved in phosphorus, nitrogen, and carbon cycling (alkaline phosphatase, acid phosphatase, phosphohydrolase, lipase-esterase, esterase, chymotrypsin, and trypsin). These results could indicate that although the bacteria abundance could be reduced by biochar soil alkalization, the microbial communities related to nitrogen, phosphorus, and carbon cycling could be stimulated by biochar-induced increasing of soil P_{tot}, N_{tot}, and C_{tot} availability [68, 69].

Nevertheless, the compost alone amendment showed the best clear and positive effects on plant growth and yield and on soil chemical characteristics. Indeed, according to data reported in the literature [70, 71], in compost amended soil lettuce plants showed the maximum total biomass accumulation, assimilation rate, and water use efficiency, probably due to the high soil nutrients availability (soil C_{tot}, N_{tot}, P_{tot}, and P_{av} content was increased). This high soil nutrient status could also have enhanced the activity of enzymes involved in phosphorus and nitrogen cycling (phosphohydrolase, chymotrypsin, and trypsin), which increased compared to those in the unamended soils; on the other hand, the slight pH increase could be responsible for the decrease of cultivable bacteria.

No synergic or positive effects exerted by compost and biochar combination were observed here compared to the compost alone treatment. Indeed, we showed that lettuce

growth changes were negligible combining compost and biochar amendment, although, as shown above, the amount of compost applied and the nutrients supplied were adequate to produce the highest plant benefits in the compost alone treatment. Furthermore, compared to the addition of compost alone, the compost and biochar combination did not improve soil chemical characteristics, except for an increase in C_{tot} and P_{av} content. These increases could be related to biochar capacity to enhance C accumulation and sequestration and to retain and exchange phosphate ions by its positively charged surface sites [59, 60]. Additionally, in compost and biochar-amended soil, microorganism abundance was unchanged while the activity of enzymes involved in N and P mineralization (chymotrypsin, trypsin, and phosphohydrolase) was reduced or completely lost compared to those in the compost alone treatment.

It is reported that biochar can have significant effects on microbially mediated transformation of nutrients by its surface interaction with substrate and soil microbial enzymes [72, 73] or by inducing soil alkalization [74]. Indeed, we hypothesized that soil microbial abundance and activities were shaped by nutrient availability and pH, which in turn could be balanced by biochar-compost combination. However, the biochar benefits could be amplified over time through surface oxidation and bioactivation with soil microbes and fungi [75, 76]. In addition, given that the beneficial effects of biochar were found to increase more in sandy than in loamy substrates [25], we hypothesized that, in PSCB, the high nutrient status, due to the compost, could have masked biochar effects [77].

In summary, our short-term potting experiment clearly demonstrated that compost addition provided the best solution regarding soil quality and fertility, which were also reflected in best plant growth and biomass yield.

Furthermore, taking into account that the soil used in this study had low nutrient status, suboptimal for plant growth without additions of organic and/or inorganic amendments, these results demonstrate that the application of biochar alone could also be effectively used to enhance soil fertility and plant growth and biomass yield. This may have important implications for sustainable low-input agriculture, with economic and environmental benefits for both marginal and productive cropland.

Nevertheless, unexpectedly, combined application of biochar and compost did not outperform compost amendment in terms of biomass yield and soil fertility. However, it may enhance and sustain soil biophysical and chemical characteristics over time, given that most of compost will disappear through mineralization within 5 years after application whereas most of the biochar will stay in the soil for decades [20, 78].

Further long-term and large-scale field experiments are required to analyze differences over time and in particular to quantify the amount of recalcitrant carbon supplied and sequestered in the soil by both biochar alone and the combination of biochar and compost. Their benefits and effects in terms of improving and sustaining soil fertility, crop productivity, and economic returns to users should be also evaluated over time.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Gabriella Stefania Scippa coordinated the project. Gabriella Stefania Scippa, Dalila Trupiano, Claudia Cocozza, and Roberto Tognetti conceived and designed the experiments. Dalila Trupiano, Carla Amendola, Claudia Cocozza, Francesca Fantasma, Silvia Baronti, Sara Di Lonardo, and Giuseppe Lustrato performed the experiments. Dalila Trupiano, Carla Amendola, Claudia Cocozza, Silvia Baronti, Sara Di Lonardo, and Giuseppe Lustrato analyzed data. Gabriella Stefania Scippa contributed reagents/materials/analysis tools. Dalila Trupiano wrote the manuscript. Claudia Cocozza, Roberto Tognetti, Silvia Baronti, and Francesco Primo Vaccari revised the manuscript. All authors approved the manuscript.

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