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RECEIVED 03 July 2026

REVISED 03 September 2026

ACCEPTED 07 September 2026

PUBLISHED 06 October 2026

CITATION

Agrimonti C, Graziano S, Lamanna G,
Scanferla V, Caldara M, Marra F,
Bevivino A, Donati M, Ma C, Li C and
Marmiroli N (2026) Valorization of
agri-food wastes: impacts of vinasse,
olive pomace, and tomato peel
biochars on plant physiology,
soil health and sustainability.
Front. Soil Sci. 6:1927359.
doi: 10.3389/fsoil.2026.1927359

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Valorization of agri-food wastes: impacts of vinasse, olive pomace, and tomato peel biochars on plant physiology, soil health and sustainability

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Introduction: Utilizing agri-food by-products and residues to create soil improvers reduces waste and promotes environmentally friendly agriculture within a sustainability framework. This study evaluates the agronomic, biological, and economic impacts of biochars produced from three agri-food residues: vinasses, olive pomace, and tomato peels.

Methods: Biochars were applied to tomato (*Solanum lycopersicum* cv Heinz 1301) and wheat (*Triticum durum* cv Svevo) in a greenhouse experiment at concentrations of 0.1–0.25–0.5% w/w, to assess their effects on plant biomass, physiology and gene expression, total countable microorganisms, enzymes involved in nitrogen mineralization (arylamidase), cellulose degradation (β -glucosidase) and phosphorus release (phosphatases). Economic and environmental assessments were also carried out.

Results and discussion: Vinasse biochar contained the highest levels of nitrogen (12000 mg/kg), potassium (11700 mg/kg), and phosphorus (4200 mg/kg), performing comparably to vinasse-based compost. A concentration of 0.5% biochar derived from vinasses increased canopy cover in tomato (+125%), and stem length (+13%), fresh and dry root weight (+97% and 57%, respectively), and stem weight (+41% and 44%, respectively) in wheat. The same biochar modulated genes involved in plant growth and development, cell wall biogenesis, nutrient transport, hormone signaling, and stress response in both plants. All biochars, particularly those derived from vinasses, enhanced arylamidase activity in tomato plants compared with the control. The same biochar enhanced activity of β -glucosidase when added at 0.5% in wheat (+29%) and tomato (+20%) over the control. Biochar derived from tomato peels at 0.5% enhanced the phosphatase

activity (+27%) in tomato plants, while biochar derived from olive at 0.5% enhanced the same activity (+45%) in wheat. Economic and environmental evaluations revealed that these biochars were better than composts derived from the same residues, in terms of sustainability and economic value.

KEYWORDS

biochar, by-products, compost, soil functions, sustainable agriculture, tomato, wheat

1 Introduction

In recent years, agriculture has been moving toward more sustainable approaches to improve soil quality while limiting the use of chemical fertilizers. This is in view of more sustainable practices to mitigate climate change and to guarantee food security for the world population (1). In a sustainability context, the utilization of by-products and residues from the agri-food industry to create soil improvers encompasses the objectives of sustainability reducing waste and promoting environmentally friendly agriculture. Among the derivatives of these residues, biochar, a porous carbonaceous solid, has gained interest in agriculture because of its beneficial effect on soil and its capacity to promote plant growth (2). It is produced by thermochemical conversion of plant and residual animal biomasses in a partial (pyrogasification) or complete (pyrolysis) oxygen-depletion atmosphere, at temperatures ranging from 400–1000 °C (3). Biochar is composed of an organic fraction containing carbon (C) (up to 90%), hydrogen (H), nitrogen (N), oxygen (O), and ashes, and an inorganic fraction with sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), carbonates, phosphates, and oxides. However, chemical composition depends on the starting biomass, temperature, and time of pyrolysis. For example, high temperatures determine an increase in the concentration of zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), Na, K, and Mg (3, 4). These properties make biochar a soil improver and an amendment capable of promoting plant and microbial growth (5, 6). In fact, most of the organic N is converted into stable heterocyclic forms incorporated into the carbon structure, and there is a very low risk of NH₃ emission, ensuring at the same time a slow release of N. Biochar also contains functional groups, such as hydroxyl phenolic, carboxyl, and ketonic groups, that implement absorption of potential contaminants, contributing to soil remediation (6–8). Moreover, biochar improves the growth of rhizospheric microorganisms because it possesses a porous structure suitable for internal and external colonization, sheltering beneficial microbes from predators or other species with which they compete for survival (1, 9, 10). Because the rhizosphere microorganisms promote degradation of organic matter, P solubilization, and N fixation, they can improve the nutrient availability for plant growth (11).

In Italy, the agri-food industry produces huge amounts of by-products and residues that are generally diverted to produce biogas (12, 13). Significant agri-food chains are those involving: *i*) tomato processing (about 5.6 million t processed year⁻¹); *ii*) wine (about 50 million hl year⁻¹); *iii*) olive milling (about 300,000 t year⁻¹) (14). Biochar obtained from tomato residues showed good potential as a soil improver thanks to the content of K and P, as well as the

pollutants and water-trapping capacities (7, 15). Biochar obtained from grape pomace was effective against obligatory endoparasites like root-knot nematodes (RKNs) in tomato cultivation (16). This material revealed a good capacity for removing Pb from water (17) and showed a marked beneficial effect on ryegrass growth (18). The biochar obtained from olive pomace presents a good content of carbon and nutrients (Ca, N, K, P, and Mg) and a good capacity for retention of toxic metals Ni and Cd (19–21). All these beneficial effects on soil can make biochar derived from agri-food industry by-products a promising soil improver to support sustainable agricultural practices in the context of the circular economy. Moreover, the application of biochar can generate carbon credits to be sold on voluntary carbon markets and reduce the liberation of GHG in some crops (22–25). The positive effects of biochar on plant growth and productivity have been observed in different studies in greenhouse and field trials (26–28). However, its interactions with plants and soil are not fully understood; furthermore, the effects of the various types of biochar – whose composition depends on the initial biomass and the pyrolysis process are still largely unknown (29). Therefore, pot experiments in controlled conditions of temperature and humidity can be useful to fill these gaps prior to going into the fields with specific trials.

This work evaluated the impacts of three types of biochars obtained from agri-food by-product (olive pomace, vinasse, and tomato peel) on soil properties and plant response using one horticultural plant (tomato) and one common cereal (wheat) as testers. It consists of an integration of basic research aimed at defining the mechanisms underlying the cross-talk between plants and the soil after the application of these biochars, and at assessing their potential in agriculture and plant nutrient management for more sustainable practices. It was developed through determination of plant physiological parameters, profiling of plant leaf transcriptome, measurements of key soil enzymes involved in nitrogen mineralization (arylamidase), carbon degradation (β -glucosidase), and phosphorus release (phosphatases) combined with economic and carbon credit assessments.

2 Materials and methods

2.1 Biochar production and characterization

2.1.1 Biochar production

Tomato peels were provided by the tomato processing plant “Rodolfi-Mansueto”, in Ozzano Taro, near Parma (Italy); grape

pomace by the coop “Le due Torri”, a grape milling plant located in Montecchio, near Reggio Emilia (Italy); and olive pomace by the olive mill “Frantoio Oleario F.lli Badaracco” located at Recco, Genova (Italy). Biochar was produced using a pilot fixed-bed downdraft pyrogasification plant at the University of Parma, in conjunction with the company Iridenergy (Mantova, Italy). The reaction carried out was a slow pyrolysis process that yields a larger quantity of biochar as the main product. The drying was conducted at 150 °C until constant weight to reduce the initial moisture content below 10 wt %. After drying, the feedstocks were mechanically milled and sieved to obtain a homogeneous particle size of approximately 1–5 mm, ensuring uniform heat distribution during thermal treatment. Pyrolysis classification should not be based solely on the peak temperature but rather on the combination of operating parameters, including heating rate, residence time, reactor configuration, oxygen availability, and the intended product distribution. The reactions were carried out at a heating rate of 20 °C min⁻¹, with a residence time of 45 min, an equivalent ratio (ER) of 0.2, and a gas flow rate of 2 m s⁻¹, using blowers with a diameter of 0.08 m. The materials used were dried before being fed into the pyrogasifier. Grape pomace slow pyrolysis was carried out at a combustion chamber temperature of 874 °C and a pressure of 3.7 bar. Tomato peels were processed at a temperature of 915 °C and a pressure of 4.7 bar, and olive pomace slow pyrolysis was carried out at a combustion chamber temperature of 850 °C and a pressure of 3.6 bar. Differences in parameters were correlated with the levels of humidity of the raw materials employed. A higher humidity level required higher temperatures to reach an adequate biochar yield. The slow pyrolysis process had a duration of between one and two hours, scaling with the chamber temperature. Biochars obtained were named as: BC-O (derived from olive pomace), BC-V (derived from vinasses), BC-T (derived from tomato peels). In addition, a biochar derived from wood (BC-WA4) previously characterized (30) was added to the panel of treatments as a control.

2.1.2 Biochar analysis

Each analysis was conducted with three biological replicates. All samples were prepared according to the International Organization for Standardization (ISO) 13909–4 (31) and sieved before analysis according to UNI EN 15428 (29). Electrical conductivity (EC) and pH were determined on 60 mL of each biochar extract, following European standards protocols (32).

Bulk density was evaluated according to the ISO 23499:2013 protocol (33) with minor modifications. Samples were poured into a graduated cylinder with a capacity of 100 mL, and the mass was determined. Results were expressed in g L⁻¹.

Dry matter content (DMC) was calculated according to the UNI EN 13040:2008 protocol (34).

Total organic matter content (OMC) and ash content were evaluated using UNI EN 13039:2011 (35). Biochar samples were oven-dried at 103 °C to reach a constant weight and then incinerated at 450 °C in a muffle furnace. Results were expressed as a percentage of the initial total dry weight.

Elemental composition (Total Organic Content (TOC), N, K, P) was determined using 0.1 g of dried biochar and the protocol UNI

EN 13654–2 (36). The organic material within biochar (5 g) was mineralized in boiling sulfuric acid and Kjeldahl solution (37). Nitrate and nitrite were determined following the EPA 300.0 protocol. Hydrogen (H), C and oxygen (O) content were determined following the ISO procedures 16948:2015 (38) and 16993:2016 (39). Brunauer-Emmett-Teller surface area (BET), Cation Exchange Capacity (CEC), and volatile compounds were determined following the ISO procedures 9277:2022 (40), and 18123:2015 (41).

Semi-volatile organic compounds (SVOCs) were extracted from solid samples by ultrasonic extraction according to EPA Method 3550C (42). The extracts were purified using silica gel cleanup following EPA Method 3630C (43) and subsequently analyzed by gas chromatography–mass spectrometry (GC-MS) according to EPA Method 8270E (44).

The concentrations of metals in biochar and compost samples were determined following aqua regia extraction according to UNI EN ISO 17294-2:2016. Metal concentrations were expressed as mg kg dry matter (DM)⁻¹.

2.2 Compost production and characterization

2.2.1 Compost production

Composts were prepared in rotary drum composters of 100 L, starting from 5 kg kg⁻¹ of olive pomace (C-O), vinasses (C-V), and tomato peels (C-T) mixed with 10 kg kg⁻¹ of wheat straw. The composting system was formed by a high-density polyethylene (HDPE) cylindrical drum with a lid that can be secured tightly to avoid spillage during turning. The drum was rotated manually to provide appropriate mixing and aeration of the feedstock during the composting. The process was conducted in an area protected from direct sun and rain for three months until the composts exhibited a C:N ratio of 20. One week after the inoculum, the biomass reached a temperature of 40 °C, and after one month, the temperature reached 60–65 °C. These phases were followed by two months of maturation and cooling.

2.2.2 Compost analysis

In accordance with what was already reported above for the biochar samples, each analysis was conducted in a biological triplicate. All samples were prepared according to the International Organization for Standardization (ISO) 13909–4 (31) and sieved before analysis according to UNI EN 15428 (32). EC and pH were determined on 10g of each compost diluted in 100ml of demineralized water, following EN European Standards protocols (45).

Dry matter content (DMC) was calculated according to the UNI EN 13040:2008 protocol (34).

Total organic matter content (OMC) and ash content were evaluated using UNI EN 13039:2011 (35). Compost samples were oven-dried at 103 °C to reach a constant weight and then incinerated at 450 °C in a muffle furnace. Results were expressed as a percentage of the initial total dry weight.

Elemental composition (TOC, N, K, P) was determined using 0.1 g of dried compost and the protocol UNI EN 13654–2 (36). The organic material within compost (5 g) was mineralized in boiling sulfuric acid and Kjeldahl solution (37). Nitrate and nitrite were determined following the EPA 300.0 protocol.

2.2.3 Growth chamber treatments with biochars and composts

The potential phytotoxicity of biochars and composts was tested on *Lactuca sativa* and *Hordeum vulgare* L. in a growth chamber according to the UNI EN 16086 – 1 (46) and on *Lepidium sativum* L. following the guidelines for soil improvers as per the UNICHIM Met 1651–2003 protocol (47). Tests were conducted using the following incremental concentrations of biochars and composts: 0.5, 1, 3, 5 g per plate⁻¹.

2.3 Plant growth experimental design

2.3.1 Greenhouse treatments with biochars

The plants chosen for this experiment were: industrial tomato, *Solanum lycopersicum*, Heinz 1301, and durum wheat, *Triticum durum*, cv Svevo. The seeds of both plants were surface sterilized with 10% sodium hypochlorite for 1 h and washed with sterilized dd H₂O. Tomato seeds were germinated in a potting mix for three weeks in a growth chamber before being transplanted into larger pots with soil substrate with various amendments. Seedling growth conditions were: light/dark period 16 h/8 h, and a temperature of 25 °C (light) and 19 °C (dark), under supplemental lighting providing a minimum of 150 mmol m⁻² s⁻¹ of photosynthetic photon flux density (PPFD).

Afterwards, plants were transplanted in pots of 3 L containing universal soil (Gramoflor, profi-substrat) composed of Baltic peat (21%), Irish peat (37%), volcanic pumice (13%), and superfine peat (29%) and with pH (H₂O) = 6.0–7.0 and EC = 30–40 mS m⁻¹ and mixed with 10% of natural sand. Sterilized wheat seeds were vernalized for two weeks at 4 °C in the dark. After germination, they were transferred to pots.

The experiment was set up in randomized blocks with eight independent pots (biological replicates). Five were devoted to the analysis of enzymes and biomass, while, in order to carry out gene expression analysis on plants at an intermediate stage, prior to the plant's destruction, three were devoted to expression analysis. Pot positions were periodically swapped; they were watered daily with water to maintain approximately 80% of the soil's water-holding capacity. Plants were supplemented with the biochars BC-O, BC-T, BC-V, and BC-WA4 at three concentrations: 0.1–0.25–0.5% w/w corresponding to 0.4, 1, and 2 t h⁻¹. For gene expression analysis, leaf tissues were collected at 21 and 60 days after transplantation (DAT) for tomato plants and 30 and 60 days after sowing (DAS) for wheat. Both tomato and wheat plants were harvested at 62 DAT and DAS.

2.3.2 Determination of plant parameters

At 21 and 30 DAT for tomato and 30 and 60 DAS for wheat, the chlorophyll content was determined *in vivo* with the SPAD-502 chlorophyll meter (Konica Minolta Business Solution Italia Spa,

Milan, Italy), measuring on three expanded leaves per plant. Ten measurements were taken along the entire length of the leaf, and the average was recorded. For tomato plants, canopy cover was measured with the Canopeo App (developed by the Soil Physics Research Group and App Center at Oklahoma State University, <http://canopeoapp.com/>, downloaded in April 2025) at 21, 30, and 60 DAT. At 62 DAT/DAS, the plants were removed from the pots, roots were washed with water and dried with absorbent paper. For each plant, length, fresh and dry weight (FW and DW) of roots and stems were recorded.

Shoots and roots were oven-dried at 60 °C for 24 h to reach a constant dry weight. The dry biomass ratio was calculated as the percentage of the dry weight with respect to the fresh weight for both roots and shoots.

2.4 Soil characterization

2.4.1 Chemical-physical analysis of soil

Soil samples from 0 to 20 cm were air-dried and passed through a 2 mm sieve before chemical analysis. EC and pH were determined on 60 mL of each sample, following the EN European Standards protocols (45). Total organic carbon (TOC) was estimated according to UNI EN 15936:2022 (48); the amount of total N, and assimilable P (P₂O₅) were determined according to DM 13/09/1999 SO n 185 GU n 248 21/10/1999 Met XIV.3 (49). While the amount of exchangeable potassium has been determined to DM 13/09/1999 SO n 185 GU n 248 21/10/1999 Met XIII.5 + UNI EN ISO 11885:2009 (49, 50).

2.4.2 Determination of soil microbial activity

Soil sample analyses were carried out at 50 DAS, or DAT, of wheat or tomatoes, respectively. The overall microbial activity in soil samples was determined through the fluorescein diacetate [3',6'-diacetylfluorescein (FDA)] hydrolysis method (51) and plate count. For FDA analysis, 1.0 g of fresh soil was incubated in 6 mL of sterile sodium-phosphate buffer (60 mM, pH 7.6) mixed with 100 µL of FDA stock solution (2000 µg mL⁻¹). The mixture was shaken continuously for 2 hours at 37 °C. The reaction was stopped with a mix of chloroform/methanol (2:1), and fluorescence was measured at 490 nm with the microplate reader iMark (BioRad, Hercules, California, USA).

For plate counts, 1 g of soil was resuspended in 6 mL of sterile sodium-phosphate buffer (60 mM, pH 7.6) and shaken overnight at room temperature. Afterwards, 50 µL of serial dilutions (10⁻⁴ to 10⁻⁵) of the original suspensions were plated in triplicate both on Pikovskayas Agar (Piko) (Sigma Aldrich, St. Louis, Missouri, US), recommended for the detection of phosphate-solubilizing microorganisms, and N-free medium agar (NFM), used to support the growth of N-fixing bacteria (51). The plates were incubated for 48 h at 28 °C. The results were expressed as CFU g⁻¹ of soil.

2.4.3 Soil enzymatic analysis

The activities of enzymes arylamidase (ARN), β-galactosidase (β-GLU), and acid and alkaline phosphatase (PHOS) were

measured following the ISO 20130 procedure (52). An amount of 160 mg of soil was resuspended in 1 ml of distilled water and shaken at room temperature for 10 min. Afterwards 125 µl of each sample was transferred into a plate of 96-wells and mixed with 25 µl of substrate: for β-GLU (4-nitrophenyl β-D-glucopyranoside, 50 mM), PHOS (4-nitro-phenylphosphate disodium salt, 50 mM), and ARN (L-leucine β-naphthylamide hydrochloride, 8 mM). Samples were incubated at 37 °C for 4 h, and reactions were stopped with 25 µl of CaCl₂ (500 mM) and 100 µl of Trizma (100 mM, pH 12) for β-GLU and PHOS, and 150 µl of ethanol 96% for ARN. The plate was centrifuged at 2000g for 5 min, and 100 µl were transferred in a fresh plate and the OD was measured at 405 nm with the microplate reader iMark (BioRad). For ARN, after centrifugation, 100 µl of supernatant was transferred into a fresh plate and mixed with 100 µl of acidified ethanol (2% HCl 37%) and 100 µl of 4-Dimethylamino cinnamaldehyde (0.06%). After 20 min of incubation at room temperature in the dark, the OD was measured at 540nm. The activity was expressed as nmol of product min⁻¹ g⁻¹ of soil. Product quantity was calculated from a standard curve built with serial dilution (0–360 nmol ml⁻¹) of para-nitrophenol (β-GLU and PHOS) and β-naphthylamine (0–200 nmol ml⁻¹) (ARN).

2.5 Gene expression analysis of plants

2.5.1 Primer design

Target genes were selected from the tomato database (<https://solgenomics.net/>, accessed on 23.04.2025), and then the orthologous genes were identified for *T. durum* cv Svevo through a BLAST search on the Ensembl database (plants.ensembl.org, Assembly: GCA_900231445.1). A list of the selected sequences is reported in [Supplementary Table 1](#). Primers were designed using Primer-BLAST tool (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>), and they are listed in [Supplementary Table 2](#). Each primer pair utilized in quantitative reverse transcription PCR (RT-qPCR) was tested to assess its efficiency and specificity for the target genes in *S. lycopersicum* and *T. durum*.

2.5.2 RNA extraction and reverse transcription real-time PCR

Total RNA was isolated from 100 mg of leaf tissue using the RNeasy[®] Plant Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. RNA amount and quality were determined by reading optical density at 260 and 280 nm with a Varian Cary 50 spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). The ratio 260/280 values were in the range of 1.9–2.0 in all samples. The integrity assessment, checked on an agarose gel 0.8% (w/v), revealed a size in the range of 500–5000 nt. An aliquot of 1 µg of total RNA extracted from three biological replicates was reverse transcribed into cDNA using the Quantitect[®] Reverse Transcription kit (Qiagen), according to the manufacturer's instructions. 10 ng of cDNA from each sample was amplified in triplicate, using 2X Power SYBR[®] Green PCR (Life Technologies, CA) in the presence of 250nM of primers listed in [Supplementary Table S2](#). The reaction was conducted in a CFX96 Touch Real-Time PCR Detection System (Bio-Rad) using the

following procedures: 50 °C for 2 min, 95 °C for 10 min, 40 cycles of 95 °C for 15 s and 60 °C for one min. The melting temperature (T_m) for each amplicon was determined through a dissociation step from 60 °C to 95 °C at a rate of 0.2 °C s⁻¹.

2.5.3 Analysis of gene expression data

RT-qPCR data were collected from three biological replicates, each evaluated in technical triplicate. Technical replicates were averaged prior to statistical evaluation. Relative gene expression was calculated using the ΔΔC_t method (53), with *EF1* (elongation factor 1) for tomato and *18S rRNA* for wheat serving as internal reference genes (54) ([Supplementary Table S2](#)). Target gene details are provided in File S1. Reaction quality was confirmed by non-template controls, and reactions returning C_t > 36 were defined as non-detected and excluded from comparative analyses. Statistical significance between control and treated conditions was evaluated on ΔC_t values using an ANOVA test. Differentially expressed genes were defined by a fold change threshold (FC ≥ 2.0 or FC ≤ 0.5). Log₂-transformed fold-change values were visualized via heatmaps generated in Heatmapper (<http://www.heatmapper.ca>, accessed 23 May 2025).

2.6 Statistical analysis

Statistical analyses were performed using the software Past 4.03 (© Copyright Hammer 1999-2020). Data are presented as mean ± SD and were analyzed by ANOVA and Tukey's *post-hoc* test, after verification of normality and homogeneity of variances. *p* values ≤ 0.05 were considered statistically significant.

2.7 Economic and environmental evaluation

The analysis considered two perspectives: (i) the potential economic value of carbon storage associated with biochar application and the feasibility of participation in voluntary carbon markets. (ii) a market perspective, assessing the economic performance of the different amendment treatments based on production costs and crop revenues.

Carbon sequestration potential was calculated with the formula: $M_{\text{biochar}} \cdot C_{\text{org}} \cdot 3.67 \cdot 0.80$, where M_{biochar} is the mass of biochar applied (assuming 3 t h⁻¹), C_{org} is the percentage of TOC, 3.67 is the elemental carbon mass into its equivalent mass of CO₂, and 0.80 is the mass of biochar effectively applied, considering a dispersion factor, commonly accepted of 20%, that takes into account biochar losses during transport, distribution and incorporation into the soil (55).

Production costs were estimated from records collected during the greenhouse experiment, including material inputs and management operations. Revenues were calculated based on tomato yield and average market prices. Common Agricultural Policy (CAP) payments were excluded from the analysis. The cost of biochar was assumed to be €500 Mg⁻¹, based on the estimated price of certified material supplied in bulk, whereas compost costs were estimated from the production costs of the composting process for each agro-food residue.

TABLE 1 Chemical-physical characteristics of biochars (BC) and composts (C).

Parameters		BC-WA4	BC-O	BC-V	BC-T	C-O	C-V	C-T	
pH		8.1± 0.1	7.7 ± 0.1	8 ± 0.1	7.4 ± 0.1	7.5 ± 0.1	7.8 ± 0.1	7.8± 0.1	
EC (mS m ⁻¹)		140 ± 0.10	143.2 ± 0.9	166.2 ± 2	82.7 ± 0.6	43 ± 0.5	45.9 ± 0.6	53.4 ± 0.2	
CEC (cmol (c) kg ⁻¹)		80 ± 0.5	32 ± 1	35 ± 1	31 ± 0.8	74.2 ± 1.4	100 ± 1.5	81.5 ± 0.8	
Bulk density (g cm ³⁻¹)		0.4 ± 0.02	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.01	0.4 ± 0.01	0.2 ± 0.01	0.2 ± 0.01	
ParPParticle (%)	> 20mm	0	0	0	0	N.D	N.D	N.D	N.D
	20mm>x>10mm	0	0	1.1 ± 0.2	0	N.D	N.D	N.D	N.D
	10mm>x>5mm	8.4 ± 1.8	3.7 ± 1.2	9.8 ± 0.5	13 ± 3.8	N.D	N.D	N.D	N.D
	5mm>x>2mm	61.7 ± 1	38.4 ± 2.4	40.8 ± 0.5	52 ± 8.9	N.D	N.D	N.D	N.D
	2mm>x>0.5mm	10.4 ± 1.4	16.9 ± 0.1	11.6 ± 0.3	12.6 ± 2.2	N.D	N.D	N.D	N.D
	<1mm	19.5 ± 1.5	41 ± 3.5	36.7 ± 0.9	22.5 ± 10.4	N.D	N.D	N.D	N.D
Pore size distribution		Micro/mesopores				Mesoporosa			
Phytotoxicity (<i>Hordeum vulgare</i> L)		Non Phytotoxic at tested concentrations*							
Phytotoxicity (<i>Lactuca sativa</i> L)		Non Phytotoxic at tested concentrations*							
TOC (%)		87.1 ± 23	72 ± 22	75 ± 22	86 ± 26	19 ± 5.9	47 ± 14	52 ± 15	
Fixed C (% of dry matter)		68.5 ± 6.3	36.5 ± 5.7	66.2 ± 5.9	34.5 ± 6.6	N.D	N.D	N.D	
NH ₄ + (mg kg ⁻¹)		240 ± 15	220 ± 18	210 ± 17	160 ± 13	580 ± 15	3800 ± 25	4100 ± 28	
O (%)		5.4 ± 1.3	6 ± 1.3	5.8 ± 1	7.2 ± 1.1	33.4 ± 2.1	35 ± 3	38 ± 2.7	
H (%)		1.6 ± 0.2	2. ± 0.8	1.9 ± 0.9	2.2 ± 0.6	5.1 ± 0.4	4.5 ± 1.1	5.6 ± 1.3	
O/C molar ratio		0.06 ± 0.05	0.08 ± 0.05	0.07 ± 0.04	0.08 ± 0.04	1.76 ± 0.36	0.74 ± 0.21	0.73 ± 0.17	
H/C molar ratio		0.02 ± 0.01	0.03 ± 0.03	0.02 ± 0.04	0.02 ± 0.02	0.27 ± 0.07	0.09 ± 0.07	0.11 ± 0.08	
Total N (mg kg ⁻¹)		4900 ± 200	5105 ± 388	12008 ± 674	4606 ± 253	7105 ± 150	35360 ± 475	32305 ± 270	
Nitrite (mg kg ⁻¹)		1.4 ± 0.2	1.4 ± 0.1	0.9 ± 0.1	1.9 ± 0.2	3.9 ± 0.2	360 ± 0.60	1300 ± 1.10	
Nitrate (mg kg ⁻¹)		3.8 ± 1.2	4.5 ± 0.6	7.8 ± 0.5	4.5 ± 0.4	1.4 ± 0.2	1800 ± 1.80	4100 ± 2.30	
P tot (mg kg ⁻¹)		3400 ± 203	2900 ± 118	4200 ± 202	3200 ± 295	330 ± 1.30	1500 ± 1.80	3200 ± 1.90	
K (mg kg ⁻¹)		11000 ± 1900	4000 ± 258	11700 ± 2900	9100 ± 765	2390 ± 2.30	15000 ± 4.10	10400 ± 2.90	
Residual humidity (%)		6.3 ± 0.3	8.9 ± 0.4	5.1 ± 1.5	10.2 ± 1.1	15.5 ± 7.7	10.1 ± 0.6	5.5± 0.3	
Organic matter		95.6 ± 0.1	81.2 ± 1.1	84.7 ± 0.8	92.3 ± 0.7	N.D.	N.D	N.D	
Volatile matter (%)		19 ± 1.1	22 ± 2.2	25.6 ± 1.4	20.6 ± 2	59.9 ± 3	70.9 ± 1	60.6± 2	
BET surface area (m² g ⁻¹)		512 ± 21	532 ± 37	554 ± 20	501 ± 19.6	12.2 ± 2	9.9 ± 1.8	10.6 ± 1.2	
Dry Matter Content (%)		93.3 ± 1	91.1 ± 0.4	94.9± 1.5	89.8 ± 1.1	84.5 ± 7.7	89.9 ± 0.6	94.5 ± 0.3	
Contaminants and Metals									
Polycyclic Aromatic Compounds (IPA) (mg kg ⁻¹) **		<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	
Ni (mg kg ⁻¹)		<1	31.1 ± 1.3	41.5 ± 8.9	27.2 ± 11.5	N.D.	N.D.	1 ± 1.4	
Cu (mg kg ⁻¹)		8.7 ± 2.5	30.2 ± 3.7	103.8 ± 11.5	25.5 ± 7	18 ± 0.6	77.3 ± 6	25.1 ± 0.6	
Pb (mg kg ⁻¹)		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	
Cr (mg kg ⁻¹)		N.D.	38.9 ± 9.6	N.D.	N.D.	4 ± 0.9	N.D.	10.2 ± 1.8	
Fe (mg kg ⁻¹)		2069 ± 80	6119 ± 73	4610 ± 163	4835 ± 190	1371 ± 84	423 ± 25	1199 ± 73	
Zn (mg kg ⁻¹)		46.5 ± 4.1	N.D.	68.2 ± 7.2	52 ± 7.9	25.3 ± 5	28.7 ± 2.9	40.5 ± 6.8	

*Phytotoxicity tests (*Hordeum vulgare* L) - (*Lactuca sativa* L) were conducted at the following concentrations: 0.5, 1, 3, 5 g plate⁻¹. Both tests yielded negative results for all tested samples of biochar and compost.

**List of analyzed IPA: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, epa - benzo(j)fluoranthene, benzo(k)fluoranthene, benzo(e)pyrene, benzo(a)pyrene, perylene, indeno(1,2,3-c,d)pyrene, dibenzo(a,h)anthracene benzo(g,h,i) perylene, dibenzo(a,l)pyrene, dibenzo(a,e)pyrene, dibenzo(a,h)pyrene, dibenzo(a,i)pyrene.

N.D. not determined.

3 Results

3.1 Biochar and compost characterization

Biochar yields ranged from 0.300 kg kg⁻¹ of raw material for BC-T to 0.380 kg kg⁻¹ for BC-O, with BC-V yielding 0.350 kg kg⁻¹. Compost yields were highest for C-T (0.5950), followed by C-O (0.580 kg kg⁻¹) and C-V 0.550 kg kg⁻¹.

Physico-chemical values of biochars and composts are summarized in Table 1. Biochar derived from vinasses showed the highest values of pH, EC, N concentration (Total N), Nitrates, P, K, and organic matter. The characteristics of BC-WA4 were previously described in Marmiroli et al., 2018, and used in field trials with promising results (30, 56–58).

The biochars showed pH values ranging from 7.4 to 8.1, EC from 82.7 to 166.2 mS m⁻¹, and bulk density from 0.4 to 0.6 g cm⁻³, together with a BET of 501–554 m² g⁻¹. TOC content was high in all biochars (72–87.1%), while fixed carbon ranged from 34.5 to 68.5% of dry matter. With respect to biochars, composts exhibited slightly lower or comparable pH values (7.4–7.8), lower electrical conductivity (43–53.4 mS m⁻¹), higher cation exchange capacity, and a markedly lower BET surface area (9.9–12.2 m² g⁻¹). Compost samples also showed higher concentrations of total N, ammonium, nitrates, and nitrites compared with the biochars, particularly in the materials derived from tomato peels and olive pomace. The biochars exhibited characteristics typical of highly porous and carbon-rich materials, whereas the composts showed greater nutrient availability and cation exchange capacity.

Germination tests did not show adverse effects, and no phytotoxicity was evidenced, neither with *H. vulgare* nor with *L. sativa*, for all three types of biochars and composts (BC-O, BC-V, BC-T, and C-O, C-T, and C-V) (Supplementary Figure 1). As shown in Table 1, C-V presented the highest values of total N and K, C-T the highest TOC and EC, while C-O showed the lowest values for all parameters. The BC-V showed the highest C retention of C and N present in the initial residues (52 and 63%, respectively); these values were slightly lower than those of the respective compost (64 and 68%). The P and K retention ranged from 95–98% in all biochars and 85–90% in all composts.

The levels of the contaminants and metals analyzed were all below the limits defined by EU 1009, 2019 (59).

A general comparison of the most relevant parameters is reported in Figure 1, where a score was assigned to each biochar and compost, following the fuzzy logic approach. The results showed that among biochars, BC-V had the highest score (57), close to those of C-V (58) and C-T (59).

3.2 Effects of biochars on soil and plants

3.2.1 Chemical and physical characterization of soils

Soil samples obtained before and after plant growth were analyzed; these analyses allowed us to estimate chemical and physical characteristics such as pH, EC, and the amount of carbon, N, K, and P. The analysis was performed on controls and

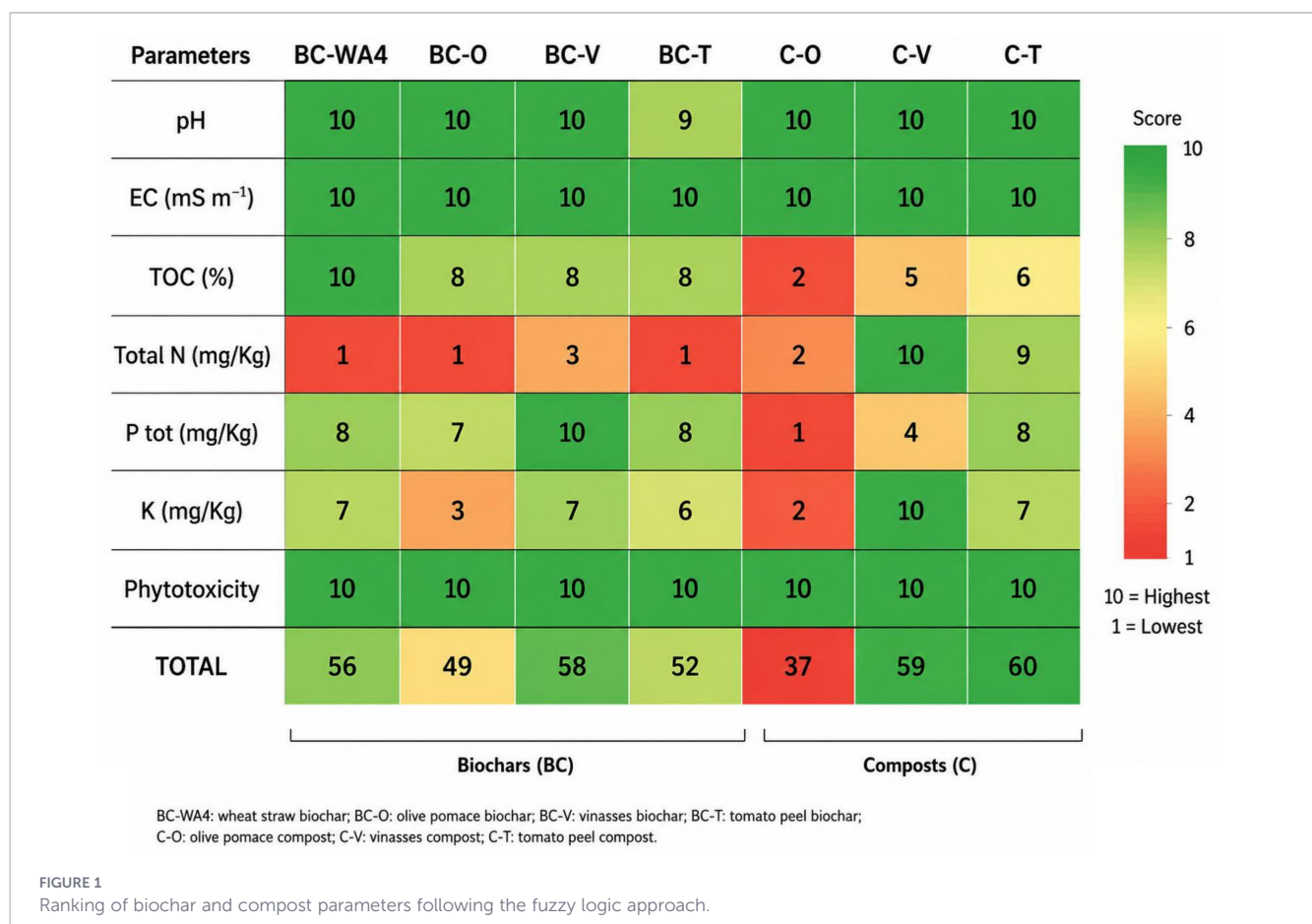


TABLE 2 Measurements of chemical parameters of soils of tomato and wheat with three different biochar at 0.5% (W/W).

Parameters	SOIL BEFORE PLANTING				TOMATO				WHEAT			
	CTR	BC-O	BC-V	BC-T	BC-WA4	CTR	BC-O	BC-V	BC-T	BC-WA4		
pH	5.36 ± 0.01	5.50 ± 0.02	5.39 ± 0.28	5.47 ± 0.19	5.43 ± 0.21	5.56 ± 0.03	5.59 ± 0.02	5.51 ± 0.03	5.55 ± 0.10	5.55 ± 0.04		
EC (mS m ⁻¹)	903 ± 44.65	10338 ± 92	11648 ± 67.5	970 ± 43.5	1017 ± 51.41	22898 ± 32.4	18968 ± 16	1853 ± 14.5	1967 ± 24.9	2000 ± 22.56		
TOC (g kg dm ⁻¹)	445 ± 28.04	446 ± 19.89	446 ± 21.77	446 ± 25.89	446 ± 22.31	445 ± 28.04	446 ± 19.89	446 ± 21.7	446 ± 25.89	446 ± 22.31		
Total N mg kg dm ⁻¹	1.12 ± 0.09	3.67 ± 0.79	7.13 ± 2.55	3.42 ± 1.18	3.83 ± 1.15	1.12 ± 0.09	3.67 ± 0.79	5.71 ± 2.55	3.42 ± 1.18	3.83 ± 1.15		
P ₂ O ₅ (mg kg dm ⁻¹)	1094 ± 52	1109 ± 31.21	1115 ± 22.56	1110 ± 21.42	1107 ± 31.79	1094 ± 52	1109 ± 31.21	1115 ± 22.56	1110 ± 21.42	1107 ± 31.79		
K tot (mg kg dm ⁻¹)	4763 ± 1500	4783 ± 250	4822 ± 230	4809 ± 270	3603 ± 187.5	4763 ± 1500	4783 ± 250	4822 ± 230	4809 ± 270	3603 ± 187.5		

For each trait, the mean value and standard deviation are reported.

soils amended with 0.5% w/w of each biochar, and the results are shown in [Table 2](#), because this concentration exerted the most significant effects on biomass.

The addition of all biochars determined an increase in pH in both tomato and wheat soils. The EC showed different values in tomato and wheat soils. First, it decreased at the end of plant growth more than in the control (-42%) in the soils amended with all biochars; in particular, BC-V showed a less evident decrease (-26%) with respect to the control. In wheat, the trend was opposite: at the end of the plant cycle, the EC increased in all conditions, but more in the control (+ 46) than in the soils amended with biochar. In this case, the BC-V determined the smaller increase (-18%).

In soil obtained at the end of the tomato cycle, the concentration of TOC was lower in all conditions with respect to the initial one. These differences were higher in soils amended with BC-T (-43%), BC-O (-37%) and BC-V (-23%). In soils analyzed at the end of the wheat cycle, the highest difference in TOC concentration was observed in the presence of BC-V. There was also a decrease in N in both plants in the presence of the same treatment ([Table 2](#)). The decrease of P and K was equivalent in all treatments, both in tomato and wheat plants.

3.2.2 Determination of plant biomasses and physiological parameters

The overall results are summarized in [Supplementary Table 3](#) (tomato) and [Supplementary Table 4](#) (wheat) and in [Supplementary Figure 2](#).

In tomato plants, the canopy in the presence of all three concentrations of BC-V has increased by an average of 148%, 141%, and 125% with respect to the control ([Table 3](#); [Figure 2](#)). ANOVA analysis confirmed that these differences were significant ($p < 0.05$) ([Table 3](#); [Figure 2](#)). Other positive/negative differences were not significant.

In wheat, the addition of BC-V at 0.5% w/w increased the biomass with respect to the control, except for root length ([Figure 2](#)). In particular, the fresh weight of the root increased by an average of 97% with respect to the control, but all variations were significant ([Table 3](#)).

3.3 Effects of biochars on the soil and plant rhizosphere

3.3.1 Tomato

The effect of the different biochars on rhizospheric soil was determined by total counting of microorganisms, the general microbial enzymatic activity through FDA, and some key enzymes involved in the degradation of organic molecules: ARN, β -GLU, and PHOS.

As shown in [Supplementary Table 5](#), bacterial count showed no difference related to the treatments when compared to the control: the number of bacteria isolated and grown on NFM medium was around 10^8 CFU g⁻¹ of soil in all samples (similar numbers and trend were also displayed when PCA was used, data not shown). Moreover, the number of P-solubilizing bacteria in any condition analyzed was below the limit of detection (LOD). No differences

TABLE 3 Average percentages of significant variation of biochar treated samples with respect to the controls and *p* values obtained through ANOVA.

BIOMASSES				
Tomato				
Parameter	Comparison	% of variation	<i>p</i>	<i>Df</i>
Tomato				
Canopy	CTR/BC-V 0.1%	+148 ± 42	0.028	8
	CTR/BC-V 0.25%	+140 ± 48	0.045	8
	CTR/BC-V 0.5%	+125 ± 38	0.035	8
Wheat				
Length of stem	CTR/BC-V 0.5%	+13 ± 0.44	0.015	8
Fresh weight of root	CTR/BC-V 0.5%	+98 ± 27	0.017	8
Dry weight of root	CTR/BC-V 0.5%	+57 ± 9	0.047	8
Fresh weight of stem	CTR/BC-V 0.5%	+42 ± 8	0.041	8
Dry weight of stem	CTR/BC-V 0.5%	+44 ± 9	0.034	8
ENZYMES				
Tomato				
Parameter	Comparison	% of variation	<i>p</i>	<i>Df</i>
ARN T1	CTR/BC-O 0.25%	+299 ± 196	0.033	10
	CTR/BC-O 0.5%	+817 ± 252	0.001	10
	CTR/BC-V 0.1%	+1013 ± 258	0.001	10
	CTR/BC-V 0.25%	+775 ± 244	0.002	10
	CTR/BC-V 0.5%	+483 ± 60	0.001	10
	CTR/BC-T 0.1%	+150 ± 35	0.004	10
	CTR/BC-T 0.25%	+329 ± 230	0.040	10
	CTR/BC-T 0.5%	+488 ± 58	0.001	10
ARN T2	CTR/BC-V 0.5%	+110 ± 47	0.021	11
	CTR/BC-T 0.25%	+101 ± 19	0.002	10
	CTR/BC-T 0.5%	+75 ± 10	0.023	10
β-GLU T1	CTR/BC-O 0.25%	-31 ± 6	0.001	13
	CTR/BC-O 0.5%	-24 ± 4	0.005	14
	CTR/BC-V 0.25%	+35 ± 3	0.001	13
	CTR/BC-V 0.5%	+20 ± 3	0.018	13
	CTR/BC-WA4 0.1%	-19 ± 6	0.003	14
	CTR/BC-WA4 0.25%	-12 ± 4	0.027	14
	CTR/BC-WA4 0.5%	-34 ± 7	0.001	14
β-GLU T2	CTR/BC-O 0.1%	-25 ± 4	0.006	12
	CTR/BC-O 0.25%	-25 ± 5	0.011	15
	CTR/BC-O 0.5%	-19 ± 2	0.024	12
PHOS T1	CTR/BC-T 0.5%	+2858 ± 5	0.008	13
PHOS T2	CTR/BC-T 0.5%	+24 ± 4	0.014	13
Wheat				
ARN T2	CTR/BC-V 0.5%	+123 ± 31	0.001	19
β-GLU T1	CTR/BC-O 0.1%	-32 ± 6	0.001	19
	CTR/BC-O 0.25%	-21 ± 5	0.008	18

(Continued)

TABLE 3 Continued

ENZYMES				
Tomato				
Parameter	Comparison	% of variation	<i>p</i>	<i>Df</i>
Wheat				
β-GLU T2	CTR/BC-O 0.5%	-11 ± 2	0.006	18
	CTR/BC-V 0.5%	+30± 7	0.011	17
	CTR/BC-O 0.1%	-61 ± 18	0.001	17
	CTR/BC-O 0.25%	-50 ± 10	0.001	17
	CTR/BC-O 0.5%	-48 ± 6	0.001	17
	CTR/BC-V 0.5%	+52 ± 12	0.001	20
PHOST1	CTR/BC-O 0.1%	+26 ± 3	0.005	18
	CTR/BC-O 0.25%	+29 ± 4	0.004	18
	CTR/BC-O 0.5%	+45 ± 7	0.002	17
PHOS T2	CTR/BC-V 0.5%	+31 ± 5	0.005	23

Df means “Degrees of freedom”. The mean enzymatic activities and relative standard deviations of all samples are reported in [Supplementary Table S6](#).

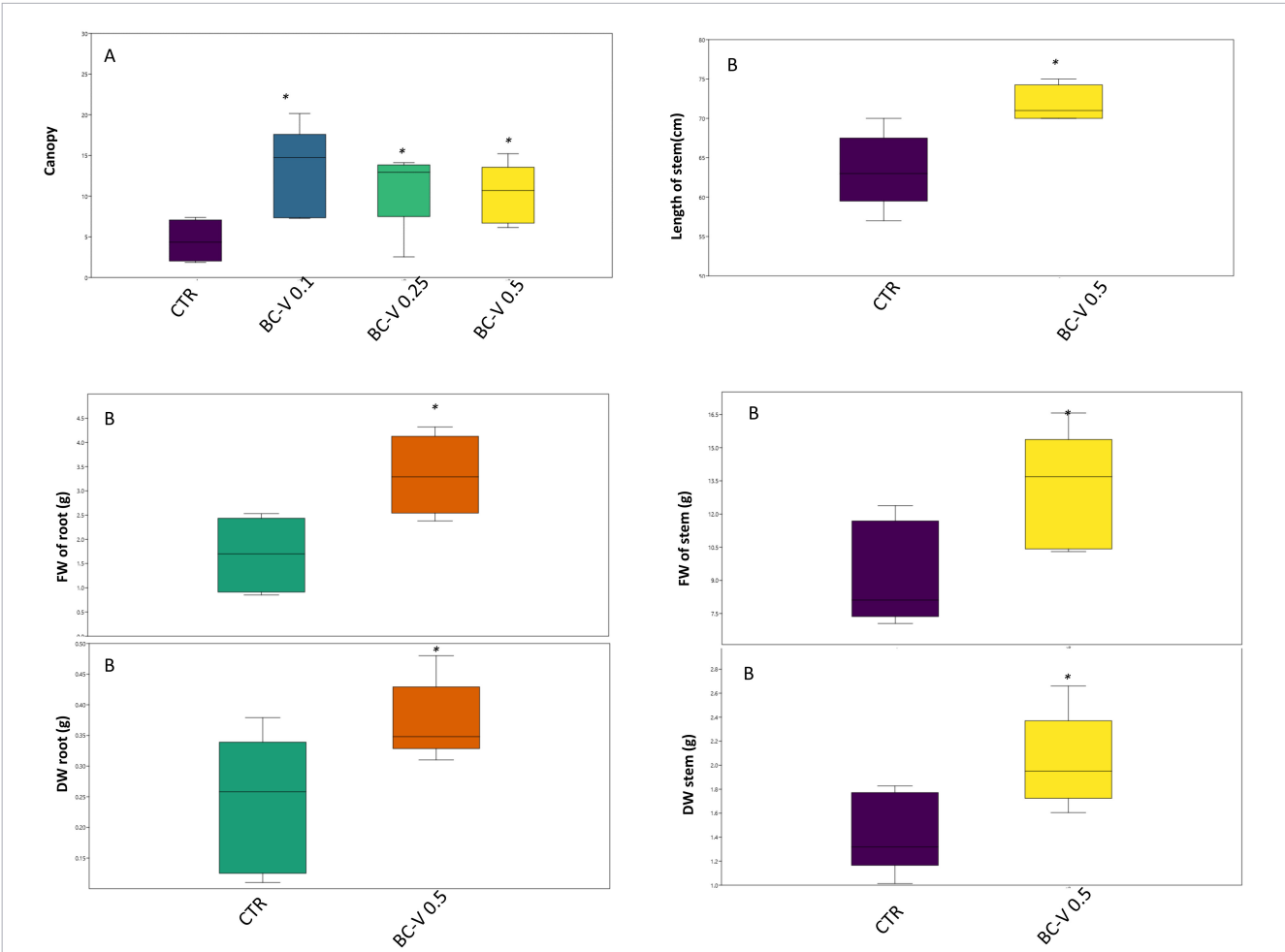


FIGURE 2 Box-plots representing the most significant values in tomato (A) and wheat (B) of biomass with respect to the control (CTR). BC-V: biochar derived from vinasses. The asterisks evidence significant differences: **p*=0.05-0.01; ***p*=0.01-0.001; ****p*=0.001-0.0001; *****p* ≤ 0.00001. The *p*-values are reported in [Table 3](#). Canopy increase is expressed as the difference between the values at 30 days after the transplant and the values at the time of the transplant.

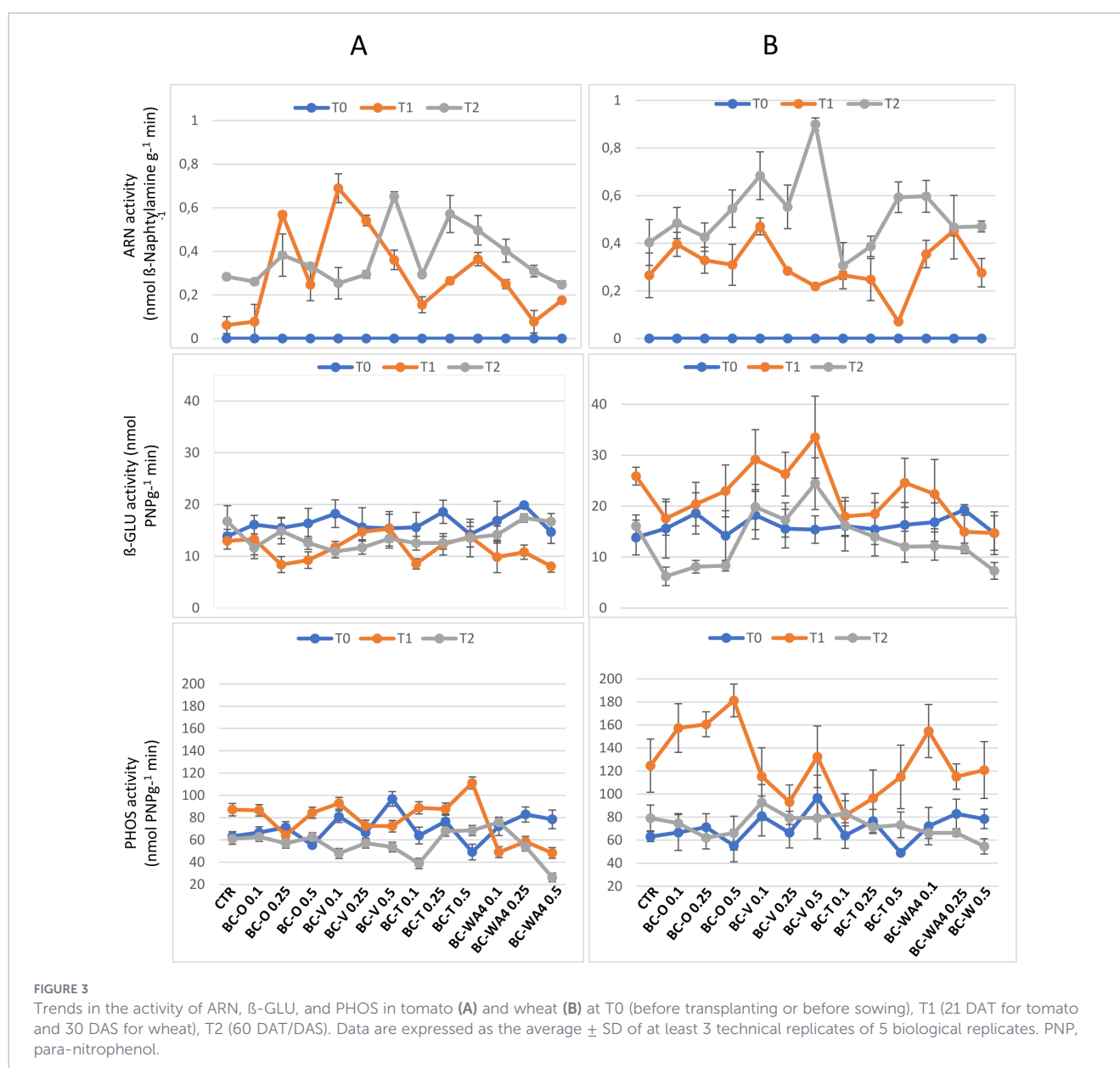
were found in the total microbial activity measured with the FDA assay (Supplementary Table 5).

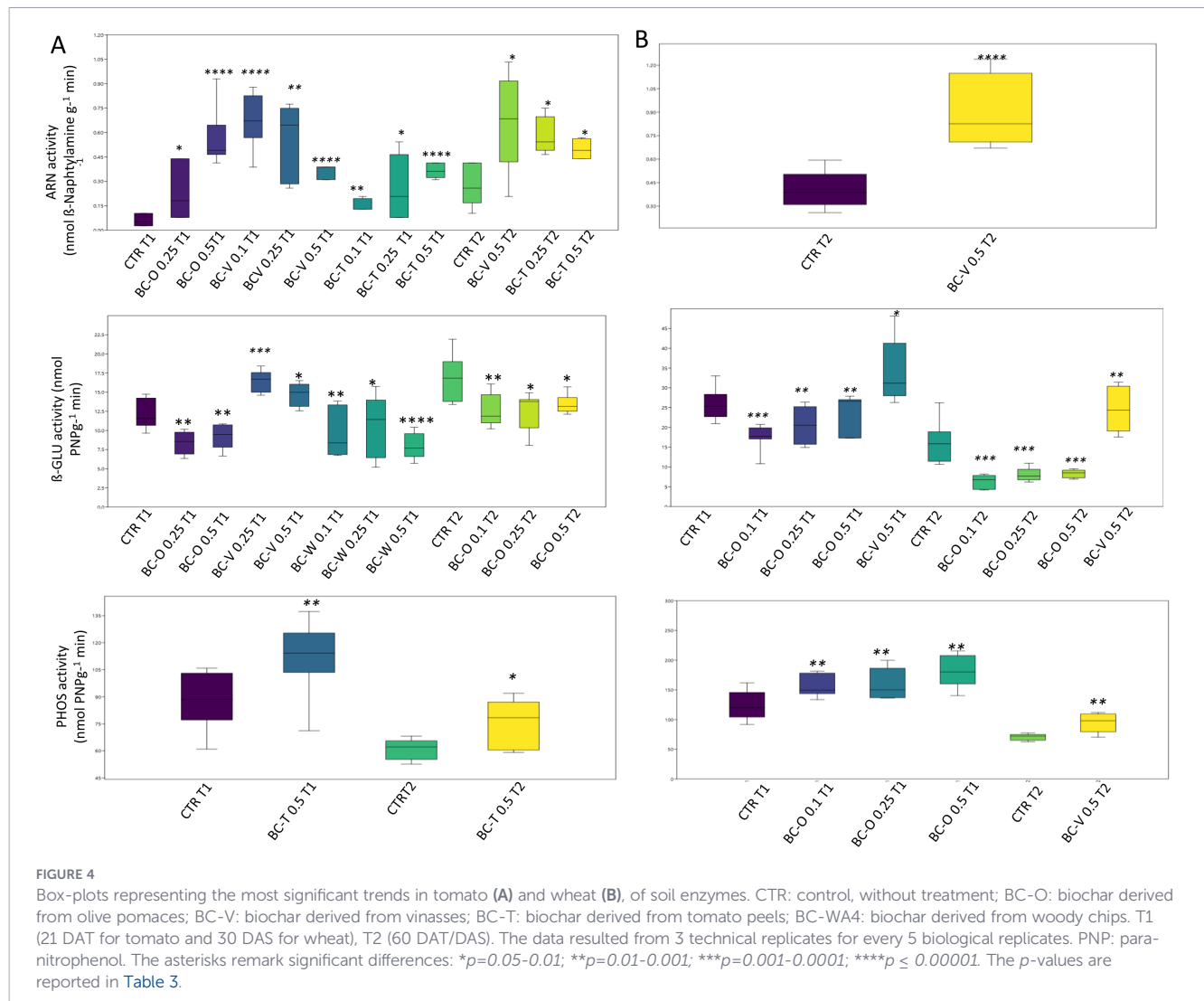
As shown in Figure 3A, the activity of ARN was almost below LOD before plant transplantation (T0) and remained rather low during the vegetative cycle but significantly increased at 21 and 60 DAT. At 21 DAT, in soil samples amended with the biochars, the activity was higher than in the control, except for BC-WA4 at the concentration of 0.25%. These differences resulted in significant differences in the case of BC-O at 0.25 and 0.5%, and BC-V and BC-T at all three concentrations (Figure 4; Table 3). In the samples with BC-O at 0.5% and BC-V at 0.25 and 0.5%, the activity was 9-, 8-, and 11-fold higher than in the control, respectively. Other differences were not significant. At 60 DAT (T2), the activity increased in all the samples except BC-O 0.25% and BC-V 0.1%, and 0.25%. In general, the activity was comparable to that of the control, but in

soil mixed with BC-V at 0.5% and BC-T at 0.25 and 0.5% were significantly higher (Figure 4A; Table 3).

The activity of β -GLU showed a general decrease at 21 and 60 DAT (Figure 3A). At 21 DAT, it was found to be significantly higher in samples amended with BC-V (0.25 and 0.5%) than in the control; at the same time, the activity was significantly lower in soils amended with BC-O at 0.25% and 0.5% and BC-WA4 at all three concentrations (Figure 4A; Table 3). This significantly lower activity in BC-O at all three concentrations was also maintained at 60 DAT (Figure 4A; Table 3).

The activity of acid and alkaline PHOS globally increased at 21 DAT and decreased to 60 DAT, globally below T0 (Figure 3A). No significant differences were found between controls and amended soils, except for BC-T at 0.5%, in which the activity was significantly higher.





3.3.2 Wheat

The bacterial count of soils obtained from wheat growth indicated that there was no significant difference between the different treatments used in terms of CFU g^{-1} of soil. As shown in [Supplementary Table S4](#), all samples contained approximately 10^8 CFU g^{-1} of soil, while P-solubilizing bacteria were below LOD under any of the conditions tested. No significant differences were observed in total microbial activity, as determined by the FDA assay ([Supplementary Table 6](#)).

As observed in tomato samples, the activity of ARN was globally low and almost undetectable before sowing (T0). The activity steadily increased at 30 and 60 DAT, but only in the sample BC-V 0.5% was significantly higher than in the control ([Figures 3B, 4B](#)).

β -GLU activity was globally higher than in tomato samples; it rose at 30 DAS in all samples and was lower at 60 DAS, below the T0 level, except in the BC-V addition. The BC-V activity at 0.5% was significantly higher than in the control at 30 and 60 DAS. As observed in tomato soil samples, the activity of β -GLU was significantly lower in the presence of BC-O at all three concentrations, both at 30 and 60 DAS ([Figure 4B](#); [Table 3](#)). Also, PHOS activity was significantly higher in wheat than in tomato samples,

and like β -glucosidase, it increased at 30 DAS and decreased at 60 DAS. At 30 DAS, samples amended with BC-O at all three concentrations had the highest activity: significantly higher than in the control.

3.4 Effects of biochars on gene expression

A gene expression analysis was done to assess the relationships between the presence of biochar in soil and the plant response, targeting genes involved in plant development. Genes were chosen following literature reporting the effects of biochar on growth and gene expression in tomato ([57, 58, 60](#)), wheat ([61, 62](#)), and *A. thaliana* ([63](#)). Target genes were chosen following these criteria: (i) gene expression found specifically in leaves; (ii) differential expression observed when biochar was present; and (iii) functions had to be related to plant essential pathways, such as brassinosteroid biosynthesis, cell wall biogenesis, ion transport, jasmonic acid biosynthesis, and ethylene signaling. The list of these genes is reported in [Supplementary Material S1](#) and includes 25 genes for tomato plants and 18 for wheat. According to their biological functions, the genes were divided into two categories: genes involved in plant defense and genes involved in growth stimulation

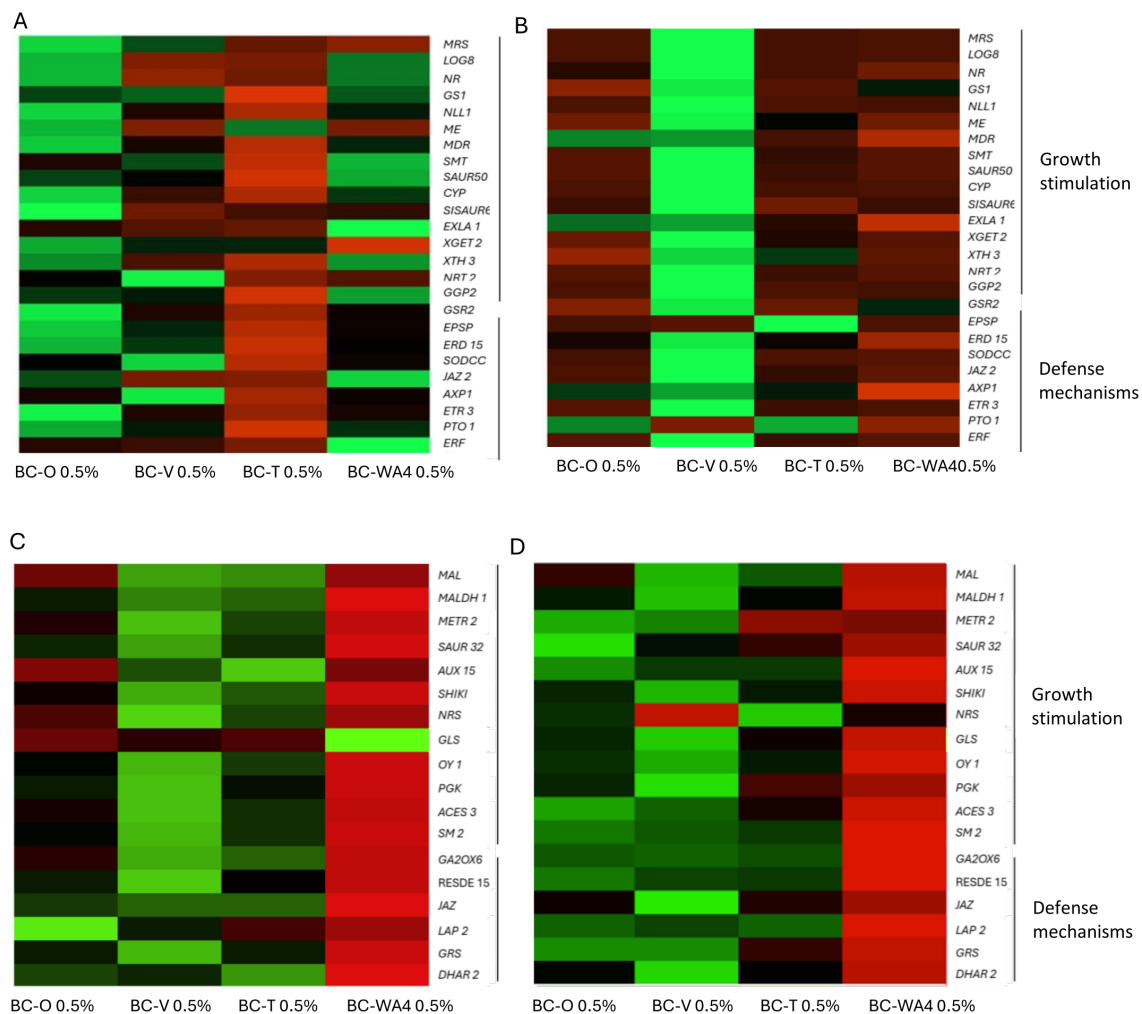


FIGURE 5

Heat map showing the extent of fold-change in the target genes as determined in leaves at 21 and 60 DAT (**A, B**) in tomato and at 30 DAS and 60 DAS (**C, D**) in wheat in response to the five different treatments. The analyzed genes are classified according to their biological function into "Growth stimulation" and "Defense mechanisms". Each row in the picture represents a gene, and each column represents a treatment. Red and green correspond, respectively, to lower and higher expression levels. All values were normalized with respect to the control; therefore, the control is not shown.

pathways (Supplementary Table S2). Wheat and tomato, when cultivated in a greenhouse, are generally more responsive to amendments during the vegetative stages (54). Therefore, the analysis of leaves during the stages of growth at 21–60 DAT for tomato plants and 30–60 DAS for wheat was considered a good approach to study the molecular crosstalk between soil and plant.

Moreover, because the biomass of plants was significantly higher at a concentration of 0.5% w/w of biochar, the gene expression analysis was conducted on plants grown at this concentration and compared with the control.

3.4.1 Gene regulation with biochar in tomato

As reported in Figures 5A, B, the different treatments determined a generalized modulation of gene expression at both 21 and 60 DAT.

At 21 DAT, the genes that are more responsive to treatments are present within different pathways: cell wall biogenesis; nitrate transporter; malate metabolic process; auxin response; ethylene-

activated signaling; regulation of jasmonic acid, and response to oxidative stress.

Each type of biochar determined a specific pattern of expression; with BC-WA4, some pathways were upregulated: cell wall biogenesis (*XTH3*), nitrate transporter (*NTR*), ethylene signaling (*ETR3-ERF*). In this same condition, the genes *EXLA 1* and *XGET 2* that are involved in cell wall biogenesis were downregulated. BC-O regulates genes like *ME* (malate metabolic process), *SISAUR6* (response to auxin), cell wall biogenesis (*XGET 2* and *XTH 3*), nitrate transporter (*NTR*), and ethylene signaling (*ETR 3*). BC-T determined a downregulation of almost all the genes considered, except for the *ME* and *XGET 2* genes that are involved in malate synthase and cell wall biogenesis, respectively. BC-V upregulated the nitrate transporter (*NTR*), response to oxidative stress (*APX*), and ethylene-activated signaling (*ETR 3*); while it downregulated the jasmonic acid pathway (*JAZ 2*).

At 60 DAT, as shown in Figure 5A, except for the treatment with BC-V, the expression patterns show a downregulation of most of the genes considered. Instead, BC-V upregulated genes present in

the following pathways: transport of N (*NII 1* and *NTR*) and magnesium (*MRS*), brassinosteroid (*CYP*) and glucose metabolism (*GGP 2*); response to oxidative stress (*ERD* and *SODC*) and ethylene-activated signaling (*ETR 3* and *EFR*).

3.4.2 Gene regulation with biochar in wheat

As shown in [Figures 5C, D](#), all 18 genes were thought to display modulation in response to the different treatments and as a function of the developmental stages at 30 and 60 DAS.

At 30 DAS, the treatments with BC-O, BC-T, and BC-V led to the upregulation of most of the genes involved in growth stimulation. In this case, specific expression differences were observed for some conditions: for example, with BC-O, the *MAL* (malate metabolic process) and *AUX* (auxin response) genes were down-regulated, while the *ACES 3* (amino acids metabolism) and *RESDE* (defense response) genes showed a modulation similar to the controls, while *RESDE* and *LAP 2* (antioxidant/protection) genes were slightly modulated following BC-T treatment. Treatment with BC-WA4 determined a downregulation of the analyzed genes except for the *GLS* (amino acids metabolism) gene, which was upregulated.

At 60 DAS, the expression patterns were very similar to those observed at 30 DAS, with few exceptions. Overall, as shown in [Figures 5C, D](#), most of the analyzed genes were upregulated. Some pathways were modulated by the three treatments: magnesium transporter (*MALDH*), malate metabolic process (*MAL*), amino acids metabolism (*SHIKI*, *GLS*, *ACES 3*), N-transporter (*NRS*), photosynthesis (*OIL*), glycolysis (*PGK*), and antioxidant/protection (*SM 2*, *RESDE*, *JAZ*, *GRS*, and *DHAR 2*). Treatment with BC-WA4 determined a downregulation of the analyzed genes, as previously reported at 30 DAS.

3.5 Effects of biochar and compost on carbon sequestration and economic value of carbon credits

In our analysis, we compare the carbon sequestration potential of biochar and compost produced from different agri-food residues and applied to agricultural soil. [Figure 6A](#) illustrates the sequestration potential of the various types of biochar and compost, assuming an equal application rate per hectare. Biochars show the highest carbon sequestration capacity, with the tomato peels derived

biochar (BC-T) performing only 1.3% below the wood-based reference biochar (BC-WA4). In contrast, composts derived from olive pomaces (C-O), vinasses (C-V), and tomato peels (C-T) residues exhibit substantially lower sequestration potential, with reductions of 78%, 46%, and 40%, respectively, compared to the reference biochar. The results depend on the amount of biochar and compost applied to the soil, which in this study is assumed to be 3 t ha⁻¹ for both amendments. However, agronomic literature indicates that compost is typically applied at higher rates, generally ranging from 5 to 15 t DM ha⁻¹ (64), a range associated with a substantially greater potential for carbon sequestration. The market price of carbon credits based on international programs is around 5 € tCO₂eq⁻¹, whereas for European programs the average price is about 30 € tCO₂eq⁻¹ (65). However, prices can vary greatly depending on the type of project, the region where the project is developed, and the negotiation process. For this study, we adopted a price of 30 € tCO₂eq⁻¹. [Figure 6B](#) pointed out that farmers can benefit from an additional economic benefit through the carbon credits, ranging from 50 to 230 € ha⁻¹.

5 Discussion

Biochar and compost represent the most common derivatives of agri-food residues. In general, composts are identified as organic fertilizers because they contain nutrients that are directly available to plants, as they are associated with a large amount of organic matter and microorganisms (66). In our case, we compared biochar and compost from the same agri-food supply chains and observed that composts differ mainly in their higher N content, while P, C, and K were comparable or lower than the corresponding biochars. A general comparison conducted between biochars and composts with a symbolic fuzzy logic approach showed that BC-V was analogous to C-T and C-V, but in terms of carbon sequestration potential, biochars were better.

On this basis, we conducted greenhouse experiments using only biochars. This material, derived from agri-food residues, typically contains higher levels of nutrients such as N, P, K, Ca, and Mg, along with increased ash content compared with wood-based biochars, but their properties are more variable because they depend on the raw material used (67–69).

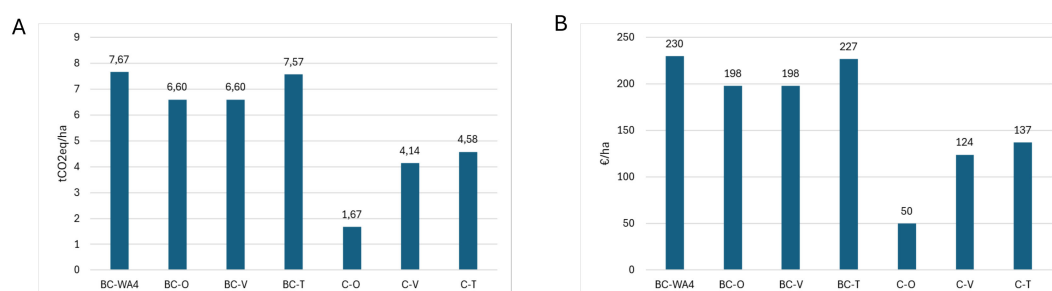


FIGURE 6
Estimated carbon sequestration potential (A) and relative economic value (B).

In accordance with data reported in the literature, all biochars determine an increase in soil pH, because they contain alkaline compounds and functional groups that buffer against acidity (70). This property makes biochar effective for reducing soil acidity and enhancing overall soil health (71).

The BC-V with a high N concentration has more positive effects on plant biomass and soil characteristics. However, these effects were different in the two crops considered. In tomato, the increase in plant biomass was not significant except for the canopy, which increased in the presence of BC-V. The effects of this type of biochar were more evident in wheat, where, at the concentration of 0.5%, there was a significant increase in the biomass, FW, and DW of stems and roots. These results agree with other papers reporting how the presence of biochar in the soil determines an increase in plant biomass (54, 72–74).

Soil enzyme activities exhibited crop-specific dynamics. In tomato, enzyme activities remained relatively stable throughout the growing cycle, whereas in wheat, β -GLU and PHOS activities peaked at 30 DAS before declining, while ARN activity progressively increased. The higher PHOS activity observed in wheat at 30 DAS may account for the greater residual soil phosphorus through enhanced mineralization of organic P.

Biochar effects also vary according to crops and feedstock. In tomato plants, biochar significantly stimulated ARN activity at the early growth stage, although these differences became less pronounced by the end of the crop cycle. Vinasse-derived biochar (BC-V) consistently enhanced β -GLU activity in both tomato and wheat, whereas olive pomace biochar (BC-O) reduced its activity, likely because of substrate adsorption and reduced substrate availability, as previously reported (75, 76). Although BC-O increased PHOS activity in wheat, this response was not associated with improved plant biomass, indicating that enhanced enzyme activity alone did not translate into greater crop growth.

In this complex picture, the role of the N cycle, in which the ARN is a key enzyme, seems to be crucial. Indeed, ARN works by breaking amides and releasing NH_3 , which is further converted into plant-assimilable compounds. Previous studies demonstrated that ARN activity is positively correlated with total N. The higher N content in BC-V may explain the increase in activity exerted by this potential soil improver, and the increase in canopy in tomato and in biomass in wheat (77). This hypothesis is supported by the fact that in the presence of BC-V, the absorption of N is enhanced in both plants.

BC-V also had the highest content in P, K, and organic matter. These factors can further enhance global enzymatic activity (78). From this point of view, BC-V can be a potential fertilizer. A crosstalk between soil activity and plants was demonstrated by the effect of BC-V on the key genes involved in N-transport (*NRT2*) in tomato and assimilation (nitrate reductase-*NR*) in wheat. In tomato leaves, in the presence of BC-V, the gene *NRT2* was upregulated during the entire plant life cycle. In wheat, the gene *NR* is upregulated by BC-V at 30 DAS and downregulated at the end of

the plant life cycle. These results agree with what was obtained previously (79), suggesting that biochar-induced alterations of soil properties can also influence the gene abundance of soil nitrifying bacteria.

With BC-V treatment, a progressive switch on genes involved in growth and plant defense was also observed in both tomato and wheat, confirming the role of this biochar in activating different defense pathways. Genes involved in hormone response showed the highest modulation (> 2-fold change). In tomato plants, at the end-of-life cycle, an evident up-regulation of *LOG8* (encoding the cytokinin-activated phosphoribohydrolase enzyme), *SISAUR6*, *SAUR50*, *XGET*, and *XTH3* was observed, involved in auxin-mediated cell wall biogenesis (80, 81). All these genes promote cell division and leaf vascular tissue differentiation (*LOG8*) (82) and/or cell wall biogenesis; therefore, their induction is compatible with an increase in biomass and canopy. But these genes were also up-regulated in the presence of BC-O at 21 DAT, even though, differently from BC-V, they were repressed at the end-of-life cycle. No significant effect on plant biomass was observed with these treatments, suggesting that BC-O action is limited during the first phases of plant growth, with no visible effects. BC-V also induced genes involved in other hormone-mediated responses, such as *ETR3* (ethylene response factor) and *JAZ2* (repressor of jasmonate response) (83). This suggests that this biochar can induce hormonal-dependent pathways, nowadays only partially known (84, 85).

In wheat, many genes involved in cell growth mechanisms were up-regulated by almost all treatments, except for BC-WA4. Positive effects on genes involved in growth and development were evident: all of them were upregulated by this treatment during the entire life cycle, with a consequent increase in biomass. Specifically, the *NRS* gene was up-regulated in response to BC-V at 30 DAS and down-regulated at 60 DAT, suggesting an alternative pathway for N at the end-of-life cycle. It is interesting to note how the regulation of *JAZ* gene was similar in tomato and wheat: in both cases, in the presence of BC-V, they are up-regulated at the end-of-life cycle. *JAZ* is a family of regulators that repress genes induced by jasmonate, a hormone responsible for stimulating plant defense to different stresses (86). Therefore, in the absence of stress, the upregulation of *JAZ* inhibits the transcription factors activated by jasmonic acid. This is consistent with a growth–defense trade-off, in which the plants slow down their growth to activate defense mechanisms to save energy and vice versa (86, 87). In this reported case, *JAZ* can inhibit the stress response to favor plant growth. This same mechanism was already described previously, where a general biochar depends on upregulation of growth genes and downregulation of defense genes (60, 63).

Compared with previous studies, the main contribution of this work is the integrated evaluation of biochars derived from three important agri-food residues, vinasses, tomato peels, and olive pomace, within the same experimental framework, using both tomato and wheat as representative crops.

This work demonstrated that biochar from agri-food residues can help to reduce the use of chemical fertilizers. In particular, it is described how biochar produced from vinasses (BC-V), with high N, P, and K content, can improve the biomass of tomato and wheat; at the same time stimulates ARN activity in soil and modulates the expression of genes involved in N transport and assimilation, as well as genes involved in plant growth and differentiation. This evidence provides a mechanistic link between biochar properties, soil functions, and plant responses that is still largely unknown.

Furthermore, by comparing biochars and composts produced from the same agri-food residues, our study extends the circular-economy perspective beyond waste valorization and demonstrates the complementary advantages of these two amendment strategies.

The comparison between biochars and composts according to other seven more “economical” parameters linked to production, transport and use, showed that biochars can be competitive with commercial composts if not for the gap in production costs which are still much higher for biochars (260 or more € ton⁻¹ vs 25–86 € ton⁻¹), that are compensated by lower transportation and application costs per hectare (20–10 tons ha⁻¹ vs 10–40 tons ha⁻¹). However, these higher costs may be partly offset by the economic benefits arising from greater carbon sequestration potential.

Biochar’s higher capacity to sequester carbon is due to the high proportion of *sp*²-hybridized C forms, polycyclic aromatic hydrocarbons, which account for around 70–85% of the total C. This form of C resists to microbial degradation for 500–1000 years, improving long-term fertility and C sequestration capacity. By contrast, compost consists mainly of aliphatic, *sp*³-hybridized C forms that are more accessible to microorganisms and consequently utilized. This fraction contributes more rapidly to the release of nutrients, but at the same time is utilized much more quickly, from a few months to a few years, compared with the *sp*² fraction (88, 89). In this way, compost may contribute rapidly to fertility while biochar improves long-term soil physical properties. The latter is significantly better for long-term carbon sequestration, with consequent environmental benefits, and has greater climate mitigation potential in terms of CO₂ sequestration. Moreover, biochar is less odorous, a characteristic that limits the application of compost in proximity to urban centers. All these aspects are summarized in [Supplementary Table S7](#).

Biochar yields, and C and N retention are generally lower than those of composts; our results align with this trend, with the exception of BC-V, which retained C and N levels comparable to its respective compost. Conversely, biochar retains nearly 100% of non-volatile nutrients (P and K) along with highly recalcitrant C (90, 91). However future life-cycle assessments should address these benefits and characteristics on an initial biomass basis to enable a precise evaluation of economic and environmental evaluation.

In conclusion, the combined agronomic, biological, molecular, environmental, and economic evaluation represents an important improvement over studies considering these aspects separately and provides a more comprehensive basis for selecting residue-derived biochars according to their intended agricultural and environmental function. Pot studies offer a high degree of reproducibility and enable understanding of how a single mechanism works in a stable environment. Of course, field-scale trials, nutrient-release studies,

determination of yields and C, N, P, K retention will be necessary to confirm the persistence of these effects under realistic agricultural contexts, in which it is more difficult to control environmental conditions.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary Material](#), further inquiries can be directed to the corresponding author/s.

Author contributions

CA: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. SG: Validation, Writing – original draft, Writing – review & editing, Visualization. GL: Writing – original draft, Formal analysis, Methodology. VS: Methodology, Writing – original draft. MC: Supervision, Writing – review & editing. FM: Methodology, Writing – original draft. AB: Validation, Writing – review & editing. MD: Data curation, Writing – review & editing. CM: Writing – review & editing. CL: Supervision, Writing – review & editing. NM: Funding acquisition, Project administration, Supervision, Writing – review & editing.

Funding

The author(s) declared that financial support was received for this work and/or its publication. This work has received funding from Projects DeliSoil (GA No. 101112855) and MultiSoil (GA No. 101218951) funded by the European Union under the Horizon Europe Program. This output reflects only the authors’ views, and the Research Executive Agency (REA) of the EU cannot be held responsible for any use that may be made of the information contained therein. This project was supported by MAECI-NSFC Italy-China Joint Research Program 2023-2025, Project FUNCHARS (PGR02026).

Acknowledgments

The authors would also like to thank Urbana Bonas for carrying out some of the soil analyses.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The authors AB, NM declared that they were an editorial board member of Frontiers at the time of submission. This had no impact on the peer review process and the final decision.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsoil.2026.1927359/full#supplementary-material>

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