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Distinct and Combined Applications of Biochar and Wood Distillate Modulate Functional Responses in Young *Vitis vinifera* L.

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Abstract

Biochar (BC) and wood distillate (WD), from agri-waste biomass, are promising sustainable soil amendments. This study evaluates their single and combined effects on morpho-physiological, biochemical, and nutritional responses of young grapevine (*Vitis vinifera* L.) plants. Grapevines were grown for 45 days in pots filled with commercial growing medium treated with BC (20%, w/w), WD (0.5%, v/v), or their combination (BC + WD). Plant morpho-biometric traits, as well as chlorophyll, sugars, amino acids, proteins, phenols, and nutrient contents, were determined. Post-harvest, growing media were analyzed for chemical and thermal properties, elemental composition, and nutrient bioavailability. Wood distillate alone significantly reduced chlorophyll content, likely due to decreased molybdenum bioavailability, and lowered root uptake of molybdenum, copper, iron, calcium, magnesium, and sodium, potentially enhancing salt tolerance. Both WD and BC individually reduced leaf glucose content. Biochar alone also decreased chlorophyll, probably because of high potassium content causing nutrient imbalances and increased root sodium accumulation, while limiting sodium translocation to leaves. The combined BC + WD treatment restored chlorophyll and glucose to control levels, suggesting a synergistic interaction that alleviated single negative impacts. Wood distillate induced stress-related metabolic changes, increasing amino acids but limiting protein synthesis. In contrast, BC partially mitigated these stress effects by enhancing protein accumulation and reducing phenol content. Co-application of BC and WD has the potential to improve nutrient use efficiency in young grapevines. The treatment-specific effects observed in plant and the growing medium underscore the importance of tailoring BC- and WD-based amendments for achieving optimal agronomic benefits.

Keywords Amino acids · Antioxidants · Carbon sequestration · Natural products · Plant growth · Sugars

Introduction

In view of a steadily growing world population, a primary challenge facing humanity is the urgent need to ensure planetary food security (FAO 2001; Kopittke et al. 2019). However, this action will lead to the inevitable exploitation of arable lands, leading to a decrease of soil fertility and crop yields due to soil degradation over time, especially when sustainable management practices are not adopted (FAO and ITPS 2015; Singh Jatav et al. 2020). Moreover, the rapid increase in global waste production presents a significant challenge. However, within the framework of a circular economy, where waste is transformed into a resource, this issue also offers new opportunities and paves the way for innovative scenarios (Singh et al. 2014).

Pyrolysis/gasification of agricultural and forestry waste biomass emerges as a *green* processing technology. Through thermochemical reactions under varying process parameters

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(Gvero et al. 2016; Wang et al. 2020), woody biomass is converted into syngas and a solid carbonaceous material, i.e., biochar (BC) (Grewal et al. 2018; Lehmann and Joseph 2015), while the vapors produced are recovered by recondensation to obtain a liquid product, the wood distillate (WD) (Grewal et al. 2018).

Feedstocks and processing conditions are key factors affecting the composition of both BC and WD. Biochar, mainly known for its role in carbon (C) sequestration, generally exhibits a characteristic high pH that can effectively enhance nutrient solubility in acidic soils, thereby improving nutrient uptake by plants and consequently enhancing crop growth and yield (Chan and Xu 2009; Hussain et al. 2017; Major et al. 2010; Van Zwieten et al. 2010). Similarly, WD, with its complex chemical composition including alcohols, organic acids, polyphenols, and tannins (Wei et al. 2010), has been observed to positively influence various aspects of plant growth and resilience to environmental stress (Ma et al. 2022; Mohd Amnan et al. 2023). In particular, numerous studies have shown that WD, when applied at optimal concentrations, typically ranging from 0.2 to 0.5% (v/v), can enhance plant yield, as well as leaf and fruit quality, while also reinforcing plant defenses against salinity and drought (Fedeli et al. 2024a, 2025; Mungkunkamchao et al. 2013; Zhu et al. 2021).

Given these beneficial properties, both BC and WD have found diverse applications in agriculture. Specifically, BC is included among the soil amendments thanks to its ability to improve physical and chemical soil properties, beside sequestering C in the long term (Ding et al. 2016; Giannetta et al. 2023, 2024; Glaser et al. 2002; Hussain et al. 2017; Nepal et al. 2023). Moreover, BC often serves as an effective carrier for beneficial microorganisms, facilitating their colonization and activity in the rhizosphere (Carril et al. 2024; Lehmann et al. 2003; Quilliam et al. 2013; Singh Jatav et al. 2020). On the other hand, WD is considered a soil amendment when applied *via* fertigation technique, whereas it is classified as a plant corroborant, when used as a foliar spray, modulating plant metabolism and defense mechanisms (Becagli et al. 2023). Due to their inherent C richness and chemical recalcitrance, the application of both BC and WD is regarded as a sustainable practice in agriculture.

Recent studies (Genesio et al. 2015; Giagnoni et al. 2019; Hussain et al. 2017; Marshall et al. 2019) investigated the use of pyrolyzed agricultural waste in vineyard soils, aimed at optimizing grape production while preserving soil quality. This growing emphasis on sustainable practices in viticulture is particularly relevant considering the global expansion of vineyard areas, which reached over 7.3 million hectares in 2022 (OIV, 2023). Climate change is a key driver of this expansion, but it simultaneously presents significant challenges that demand robust adaptation and mitigation strategies within the sector (Fraga 2019).

While there is increasing interest in identifying and implementing sustainable cultivation practices that can support the growth of healthy young grapevines, ensuring their successful transplanting throughout all seasons (Ronga et al. 2019), current literature offers limited insight into the direct effects of BC and WD applications on the growth of young grapevines. Most existing studies have evaluated these bioproducts only in combination with fertilizers (Ronga et al. 2019; Schmidt et al. 2014), leaving a knowledge gap regarding their independent and synergistic impacts. Therefore, this study aims to fill this gap by investigating the morpho-physiological, biochemical, and nutritional responses of young grapevine plants to single and combined applications of BC and WD. We hypothesized that (i) BC and WD, when applied individually, would elicit contrasting effects on morpho-physiological, biochemical, and nutritional traits of young grapevines due to differences in their composition and mode of action; and (ii) their co-application would result in synergistic or compensatory effects, improving overall plant performance and nutrient uptake while minimizing stress-related responses.

Materials and Methods

Biochar and Wood Distillate

Biochar and WD, the solid and the liquid by-product, respectively, obtained from the pyrolysis of woody biomass (i.e., *Castanea sativa* Mill., *Robinia pseudoacacia* L., *Fraxinus* L., *Alnus glutinosa* (L.) Gaertn., and *Quercus robur* L.), were provided by Bio-Esperia S.r.l. (Italy). The woody biomass was pyrolyzed at 1200 °C for approximately 30 min; at the end of this process, BC was dropped into a reservoir at the bottom of the reactor, while WD was obtained by countercurrent steam condensation of the combustion gases that flowed into a separate reservoir. The main chemical characteristics of BC and WD starting materials are listed in Table S1.

Plant Growth and Treatments

Frigo-conserved young grapevine (*Vitis vinifera* L., cv. Sangiovese, VCR23 clone/110R VCR114 rootstock) plants were provided by FareVigneti S.r.l.s. (Italy). The root system of these plants was first shortened by about 2 cm and then soaked in tanks containing tap water (H_2O), which were placed for 2 days at room temperature to allow the vegetative recovery of plants. Afterwards, 20 sprouted plants were transferred to 3.4-L black plastic pots and

subjected to grow under 4 treatments (5 biological replicates/treatment). According to the treatments, pots were filled only with 1.2 kg of a commercial peat-based growing medium (VigorPlant Italia S.r.l.; Table S1) (control, CK) or the medium was mixed with 20% (w/w) biochar (BC treatment). Half of CK and BC plants were fertigated 7 times, once a week, with 0.5% (v/v) wood distillate for the entire experimental growing period (45 days), generating WD and BC + WD treatments, respectively. It is worth noting that the present short-term pot experiment was designed as a preliminary study to evaluate early-stage responses, providing a mechanistic basis for future long-term trials under vineyard conditions. Furthermore, WD was applied at 0.5% (v/v), corresponding to the highest effective concentration identified in our previous studies (Fedeli et al. 2024a, 2025), while a high dose of BC (5 times greater than typical field applications) was deliberately used in this experiment to explore its potential as a substitute for peat-based substrates.

Plants were grown in a climatic chamber under controlled conditions: a 16/8 h light/dark photoperiod, day/night temperatures of 24/19°C, a photosynthetic photon flux density (PPFD) of 250 µmol m^{-2} s^{-1}, 70% relative humidity, and 70% water holding capacity. The latter was kept constant by weighing each pot every three days. The pots were also randomly rotated every three days in order to minimize any possible influence of microclimatic conditions within the growth chamber.

Plant Analyses

Biometric and Morphological Measurements in Leaves and Roots

The height of the main stem and the length of internodes were measured in intact plants at harvest using a ruler, while the number of leaves and stem nodes were counted. Subsequently, the leaf apparatus of each plant was separated from the root apparatus, weighed using a precision balance, and finally kept at −20 °C for the subsequent biochemical analyses, whereas the root apparatus was first washed thoroughly under running tap H_2O and then gently rinsed with distilled H_2O, to remove solid particles, dried with soft paper, and weighed using precision balance. Prior to being dried for phenol and nutrient analyses (see 2.3.6. and 2.3.7. paragraphs, respectively), the root apparatus of each plant was photographed with a digital camera (Canon EOS 400), and the images were used to measure two root morphological parameters (i.e., length and surface area) using the "Segmented Line" and the "Threshold" tools of the Fiji/ImageJ software, with the scale set at 109.4 pixels corresponding to 1 mm (Carril et al. 2023).

Total Chlorophyll

The total chlorophyll content was determined at harvest in the attached leaves of young grapevine plants using a portable, non-destructive chlorophyll content meter (CCM-300, Opti-Science Inc., Hudson, NH, USA). Two readings were recorded on the central part of the youngest, fully expanded leaf of each plant, resulting in a mean value of 10 measurements per treatment (5 biological replicates) (Fedeli et al. 2023a).

Total Soluble Proteins in Leaves

The extraction and the determination of total soluble proteins was performed according to Celletti et al. (2023a). An aliquot (1 g) of frozen young grapevine leaves was homogenized in 6 mL of deionized H_2O with an ULTRA-TURRAX® (T 10 basic, Werke GmbH & Co. KG, Staufen, Germany), and then centrifuged (PK110 centrifuge, Alc International S.r.l., Cologno Monzese, MI, Italy) at 3000 rpm for 5 min. Subsequently, the supernatant was recovered and centrifuged (Z 233 MK-2, Hermle, LaborTechnik GmbH, Wehingen, Germany) at 12,000 rpm for 7 min. An aliquot of the extract (20 µL) was diluted to 1 mL with deionized H_2O and then 0.4 mL of the diluted sample were added to 1.6 mL of Bradford reagent (Thermo Fisher Scientific Inc., Waltham, MA, USA) (Bradford 1976). After 20 min of reaction, the absorbance of the samples was read at 595 nm (UV–Vis spectrophotometer – 8453, Agilent, Santa Clara, CA, USA). The quantification of proteins was calculated using a calibration curve of bovine serum albumin as a standard.

Total Soluble Sugars in Leaves

Total soluble sugars (such as fructose and glucose) were extracted following the method described above for proteins and determined according to Fedeli et al. (2023b). The recovered extracts (about 4 mL) were filtered through cellulose acetate syringe filters (0.45 µm pore size, Lab Logistic Group GmbH, Meckenheim, Germany), completely dried under vacuum at 40 °C, resuspended in 30 µL of deionized H_2O, and analyzed by High-Performance Liquid Chromatography (HPLC, Waters 600E, Milford, MA, USA). The sugars were separated using an ion-exchange column (10 µm, 300 × 6.5 mm, Sugar-Pak I, Waters, Milford, MA, USA), kept at 90 °C *via* an external temperature controller (Column Heater Module, Waters, Milford, MA, USA), and using ultrapure H_2O as mobile phase (flow rate: 0.5 mL min^{-1}). For the detection of the sugars, the HPLC was equipped with a refractive index detector (2410 RI, Waters, Milford, MA, USA). Standards for fructose and glucose were prepared as individual stock solutions, using sugar

reagent-grade analytical standards (D-Fructose and alpha-D-Glucose, Merk KGaA, Darmstadt, Germany).

Free Amino Acids in Leaves

Free amino acids (FAAs) analysis in the frozen leaves was performed by HPLC (Waters LC1, Milford, MA, USA), following the method described by Fedeli et al. (2023b), with slight modifications. This system was equipped with a reversed-phase Zorbax 5 µm C18 column (4.6 × 250 mm, Agilent, Santa Clara, CA, USA), thermostatically regulated at 37 °C. Detection was facilitated by a Waters 470 scanning fluorescence detector with excitation set at 250 nm and detection at 395 nm. The mobile phase, flowing at a rate of 1 mL min^{-1}, consisted of two solvents: (A) 127 mM sodium acetate with 19 mM triethylamine titrated to pH 5 with phosphoric acid; (B) 60% (v/v) acetonitrile/H_2O. According to AccQ-Tag protocol (Waters Corporation, Milford, MA, USA), each reconstituted sample (10 µL) was amino acid derivatized with the AQC fluorescent reagent and 0.02 M borate buffer (pH 8.6) (Cohen and Michaud 1993). Quantification of the content of individual amino acids was estimated by matching the area under the peak of the chromatogram to the amino acid standard (WAT088122, Waters Corporation, Milford, MA, USA.), using the OpenLab ChemStation software (Chromatography Data Systems, Agilent, Santa Clara, CA, USA).

Total Phenols in Leaves

The content of total phenols was determined in the leaf extracts, previously air-dried in the dark according to the procedure described by Borella et al. (2023). For the extraction, the plant material was soaked in 10 mL of 80% (v/v) methanol in water in a ratio of 1:10 (w: v) and placed on an orbital shaker for 30 min. Subsequently, the samples were incubated for 48 h in the dark at 4 °C. After incubation, the samples were filtered through Whatman filter paper no. 1 and the filtrates were used for phenol content determination. The phenol content was quantified using the Folin-Ciocalteu's reagent (Al-Duais et al. 2009). After reaction in the dark to avoid photooxidation, 1.25 mL of 7% (w/v) Na_2CO_3 and 1 mL of deionized H_2O were added to the samples. They were first incubated for 90 min in the dark and then their absorbances measured at 760 nm with an Agilent UV-Vis 8453 spectrophotometer (Santa Clara, CA, USA). The amount of the extract was substituted by the same amount of 80% (v/v) methanol in blank. Gallic acid (98%, Thermo Fisher Scientific Inc., Rodano, Milano, Italy) was the standard of choice and used for the calibration curve preparation in the 5–300 µg mL^{-1} concentration range.

Nutrient Content in Leaves and Roots

Leaves and roots were oven-dried at 65 °C for 5 days and then ground to a fine powder. The resulting plant material was used to determine the total content of calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), zinc (Zn), and sodium (Na) according to the acid digestion method reported by Vannini et al. (2021). In brief, samples of approximately 0.25 g were mineralized with 3 mL of 67% (v/v) HNO_3 and 1 mL of 30% (v/v) H_2O_2 using a microwave lab station. Subsequently, samples were filtered and adjusted to a final volume of 50 mL with ultrapure H_2O. All elements were quantified by inductively coupled plasma – mass spectrometry (ICP-MS; Perkin Elmer NexION 350, Waltham, MA, USA). GBW 07604 (*Poplar leaves*) and GBW 07603 (*Bush branches and leaves*) were used as certified reference materials. Recoveries ranged from 98 to 106% for all elements. The precision of the analysis was estimated by the coefficient of variation of 5 biological replicates and was always > 98%.

Growing Media Analyses

The growing media were collected at harvest, i.e., once plants were completely removed from the potting substrates, and air-dried until a constant weight was reached. Subsequently, the samples were ground for further analyses.

pH, Electrical Conductivity, and Cation Exchange Capacity

The pH and electrical conductivity (EC) were determined in the clear supernatant of the growing medium obtained after shaking the samples in deionized H_2O (1:20 w/v) for 30 min and subsequent centrifugation, as described by Celletti et al. (2021).

Cation exchange capacity (CEC) was determined by saturating the sample with a 0.1 M barium chloride ($BaCl_2$) solution buffered at pH 8.1, followed by titration with 0.05 N disodium ethylenediaminetetraacetic acid (Na_2EDTA). Two drops of 0.1% (w/v) Eriochrome Black T were used as a colorimetric indicator. The endpoint was detected by a color change from light blue to reddish purple.

Thermal Analysis

The stability, and in turn the recalcitrance, of organic matter in CK, BC, WD, and WD + BC was assessed by thermogravimetric analysis coupled with simultaneous differential scanning calorimetry (TGA-DSC 3+, Mettler Toledo, Switzerland). An aliquot (approximately 25–30 mg) of each sample was placed in an alumina crucible and heated from

30 to 700 °C at 10 °C min^{-1} under an oxidizing atmosphere (100 mL min^{-1}). The energy density was calculated by integrating the DSC heat flux over the exothermic region 105 to 550 °C and normalizing it by the mass loss (loss-of-ignition, LOI) (Bona et al. 2023). Ratios between weight losses (WL) occurring within different temperature ranges (400–550/200–300 and 400–550/300–400 °C), and the temperatures at which half of biomass was lost (TG-T_{50}) and half of the energy was released (DSC-T_{50}) were also calculated.

CHN Analysis

The total content of C, hydrogen (H), and nitrogen (N) was determined *via* flash combustion using a CHNS macro-analyzer (vario Macro cube, Elementar, Germany). Soil Standard Peaty OAS (B2176, Elemental Microanalysis Limited, Okehampton, UK) was used as reference material. QA/QC values were 98.9% for C, 97.7% for H, and 100.4% for N.

Total, Bioavailable, and Non-bioavailable Nutrient Fractions

The total content of Ca, Mg, P, K, Cu, Fe, Mn, Mo, Zn, and Na was determined following the alkaline melting method reported by Jones and Dreher (1996). Briefly, 0.1 g of sample were solubilized by adding 0.1 g of lithium tetraborate and 0.4 g of lithium metaborate and mixed in a platinum vessel. The mixtures were melted at 950 °C in a muffle furnace (LM 312, Linn Elektro Therm) for 90 min. The resulting samples were dissolved by adding 40 mL of 5% (v/v) nitric acid, filtered, and adjusted to a final volume of 50 mL with ultrapure H_2O.

The bioavailable fraction of these elements was extracted according to Vannini et al. (2021). Briefly, 1 g of each sample was shaken with 40 mL of 0.11 M acetic acid for 16 h at room temperature. Afterwards, the extracts were centrifuged at 3500 rpm for 15 min and the obtained supernatants were filtered and stored at 4 °C. Total and bioavailable contents of all elements were quantified by inductively coupled plasma – mass spectrometry (Perkin Elmer NexION 350, Waltham, MA, USA). GBW 07411 (*Chinese soil*) and BRC 701 (*Lake sediment*) were used as certified reference materials.

The non-bioavailable fraction for each element was calculated by subtracting the bioavailable fraction from the total content.

Statistical Analyses

The data approached a normal distribution (Shapiro-Wilk test, $p<0.05$) and are presented as the mean of five biological replicates ± standard error. To test differences in treatments, the data were analyzed with one-way ANOVA and LSD as a post-hoc test ($p<0.05$), using CoStat software (version 6.45, CoHort, Barkeley, CA, USA). To describe the variation in the content of FAAs between different treatments, fold changes (treatment/control) were calculated, and the dataset was represented through heatmap visualization performed using R (version 4.3.1; R Core Team 2025). Principal component analyses (PCAs) were also performed using R software to explore the multivariate relationships among treatments (CK, BC, WD, and BC + WD) based on the morpho-physiological, biochemical, and nutritional parameters measured in leaves and roots. Thermal analysis data were obtained from composite samples prepared by pooling equal amounts of material from the five biological replicates.

Results

Plant Responses

Chlorophyll and Growth

Total leaf chlorophyll content significantly decreased under BC (−17%) and WD (−16%), compared to CK plants (Table 1). Stem height was significantly lower in plants exposed to WD (−16%), relative to the CK (Table 1), while the number of leaves was, on average, 37% lower, irrespective of the BC, WD, or BC + WD treatments, compared to CK plants (Table 1). No significant differences were found in the number of nodes or internode length across the different treatments (Table 1). Shoot fresh weight was notably reduced by approximately 24% in plants amended with WD, regardless of the presence of BC, compared to CK plants (Table 1). In contrast, root fresh weight increased significantly under WD and BC + WD treatments (+29 and +34%, respectively), compared to the CK (Table 1). Root length increased significantly across all treatments (+24% for BC, +19% for WD, and +24% for BC + WD), while root surface area expanded significantly only in the presence of BC, with or without WD (+33% for BC and +39% for BC + WD), relative to CK plants (Table 1).

Primary Metabolites

No significant changes were observed in fructose content. Glucose content significantly decreased in plants grown under BC and WD treatments by 51 and 25%, respectively, whereas no significant change was observed under the combined BC + WD treatment, compared to CK plants (Fig. 1).

Leaf total soluble protein content increased significantly by 12% only in the BC-treated plants (Fig. 2).

Table 1 Physiological (total chlorophyll content) and biometric parameters (stem height, leaf number, node number, internode length, shoot fresh weight, root fresh weight, root length, and root surface area) of young grapevine plants grown under four different treatments of the growing medium: CK = control; BC = plants grown with Biochar (BC); WD = plants grown with wood distillate (WD); BC + WD = plants grown with both Biochar and wood distillate

Plant parameters	CK	BC	WD	BC + WD
Total chlorophyll content (mg m^{-2})	564.1 ± 32.2 [a]	470.1 ± 13.4 [b]	473.3 ± 39.6 [b]	585.1 ± 33.5 [a]
Stem height (cm)	96.3 ± 2.9 [a]	82.7 ± 5.0 [ab]	80.8 ± 1.0 [b]	86.0 ± 5.2 [ab]
Leaf number	49.8 ± 2.4 [a]	33.3 ± 1.5 [b]	32.7 ± 4.3 [b]	29.4 ± 2.4 [b]
Node number	23.0 ± 2.3 [a]	19.3 ± 2.9 [a]	18.0 ± 0.6 [a]	20.6 ± 1.4 [a]
Internode length (cm)	4.6 ± 0.4 [a]	4.7 ± 0.5 [a]	4.5 ± 0.4 [a]	4.3 ± 0.4 [a]
Shoot fresh weight (g $plant^{-1}$)	40.8 ± 1.2 [a]	33.2 ± 0.7 [ab]	31.1 ± 3.6 [b]	31.4 ± 2.8 [b]
Root fresh weight (g $plant^{-1}$)	21.9 ± 1.4 [b]	21.4 ± 1.4 [b]	28.2 ± 0.7 [a]	29.3 ± 3.9 [a]
Root length (cm)	16.1 ± 0.8 [b]	20.0 ± 0.6 [a]	19.1 ± 0.7 [a]	20.0 ± 0.8 [a]
Root surface area (cm^2)	151.0 ± 12.9 [b]	200.8 ± 13.9 [a]	196.3 ± 18.8 [ab]	209.6 ± 19.7 [a]

Data are presented as means ± standard error. Different letters indicate significant statistical differences ($p < 0.05$) among treatments

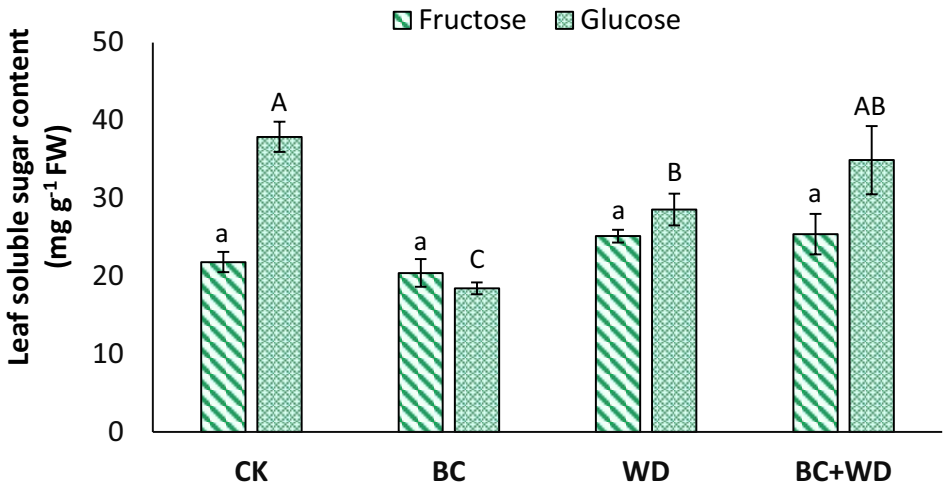

Fig. 1 Content of soluble sugars (fructose and glucose) in the leaves of young grapevine plants grown under four different treatments of the growing medium: CK = control; BC = plants grown with biochar (BC); WD = plants grown with wood distillate (WD); BC + WD = plants grown with both biochar and wood distillate. Data are presented as means ± standard error. Different lowercase letters indicate significant statistical differences ($p < 0.05$) among treatments for fructose, while different uppercase letters indicate significant statistical differences ($p < 0.05$) among treatments for glucose

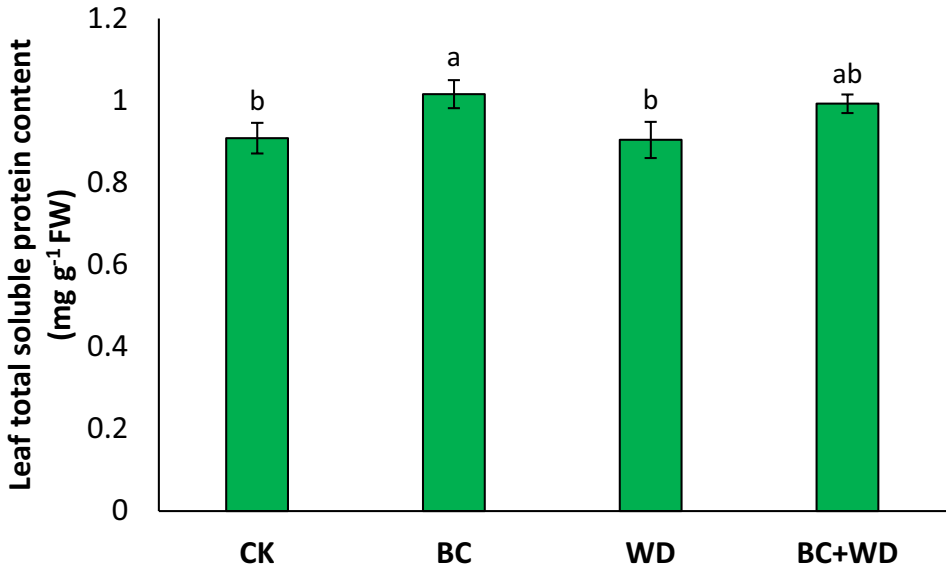

Fig. 2 Content of total soluble proteins in the leaves of young grapevine plants grown under four different treatments of the growing medium: CK = control; BC = plants grown with biochar (BC); WD = plants grown with wood distillate (WD); BC + WD = plants grown with both biochar and wood distillate. Data are presented as means ± standard error. Different letters indicate significant statistical differences ($p < 0.05$) among treatments

Free Amino Acid Profiling

The heatmap in Fig. 3 shows the relative changes in specific amino acid contents comparing BC, WD, and BC + WD to CK. The color intensity emphasizes these variations, with redder shades indicating more substantial increases (Fig. 3).

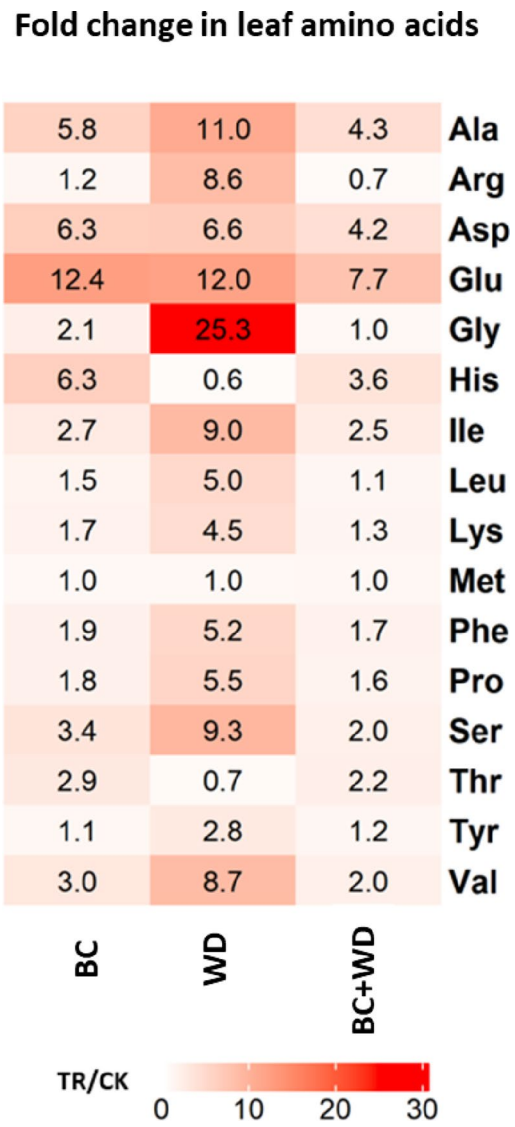

Fig. 3 Heatmap showing the fold changes in individual free amino acid (FAA) contents in the leaves of young grapevine plants grown under four different treatments of the growing medium: CK = control; BC = plants grown with biochar (BC); WD = plants grown with wood distillate (WD); BC + WD = plants grown with both biochar and wood distillate. The fold changes are expressed as the ratio between treated (TR) and CK plants. Values greater than 1 indicate an increase, while values lower than 1 indicate a decrease relative to the CK. Color intensity corresponds to the magnitude of the fold change. Amino acids analyzed include alanine (Ala), arginine (Arg), aspartic acid (Asp), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tyrosine (Tyr), and valine (Val)

The BC treatment generally showed moderate increases in several amino acids, including Glu (12.4-fold), Asp (6.3-fold), His (6.3-fold), and Ala (5.8-fold). The remaining amino acids displayed smaller changes. The WD treatment resulted in substantial increases, especially in Gly (25.3-fold), Glu (12.0-fold), Ala (11.0-fold), and Ser (9.3-fold). Overall, the combined treatment (BC + WD) exhibited less pronounced effects across most amino acids, compared to the individual BC or WD treatments, with the most significant change observed in Glu (7.7-fold) (Fig. 3).

Total Phenols

The leaf total phenol content was significantly reduced by BC, regardless of WD presence, with reductions of 15 and 22% in BC- and BC + WD-treated plants, respectively, compared to CK plants (Fig. 4).

Nutrients

The contents of the main macronutrients (Ca, K, Mg, and P), micronutrients (Cu, Fe, Mn, Mo, and Zn), and Na in leaf and root tissues of the plants for each treatment are reported in Fig. 5. Calcium and Mg contents showed similar trends, being lowest in the leaves of plants treated with BC, whether alone or in combination with WD (Ca: −31% for both BC and BC + WD; Mg: −17% for BC and −13% for BC + WD) (Figs. 5A, C, respectively). In roots, Ca and Mg contents also declined across all treatments, ranked as follows: WD (−7% for Ca and −6% for Mg) < BC + WD (−14% for Ca and −19% for Mg) < BC (−22% for Ca and −41% for Mg), compared to CK plants (Figs. 5A, C, respectively). In contrast, K content showed an opposite pattern, particularly in leaves where BC and BC + WD treatments determined notable increases (+80% for BC and +62% for BC + WD) (Fig. 5B). Root K content increased even more significantly, by +125% for BC and +122% for BC + WD, relative to CK plants (Fig. 5B). Phosphorus content significantly decreased under BC treatment, both in leaves (−25%) and in roots (−22%) (Fig. 5D); a similar trend was observed in the BC + WD treatment, where P showed decreases of 24% in leaves and 21% in roots (Fig. 5D). In contrast, P content increased in WD-treated plants, by 14% in leaves and 9% in roots (Fig. 5D).

In leaves, micronutrient contents did not show treatment-dependent variations (Figs. 5E-H), except for Zn which decreased by 26% only under BC treatment (Fig. 5I). In roots, Cu content was lowest in both BC and WD treatments (−19 and −16%, respectively), relative to CK plants (Fig. 5E). Also, Fe content decreased significantly under individual BC and WD treatments (−16 and −12%, respectively), while Mn content was significantly lower (−10%) only in

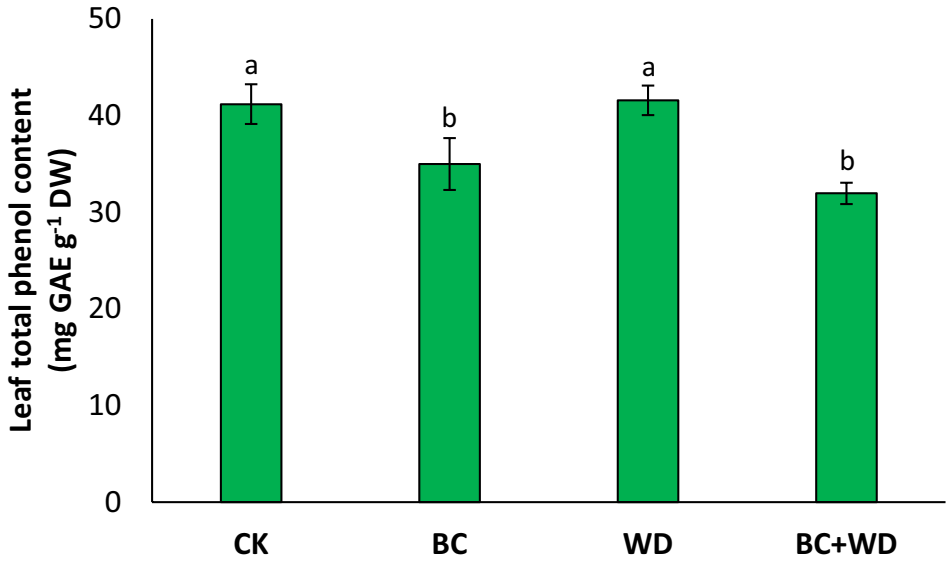

Fig. 4 Content of total phenols in the leaves of young grapevine plants grown under four different treatments of the growing medium: CK = control; BC = plants grown with biochar (BC); WD = plants grown with wood distillate (WD); BC + WD = plants grown with both biochar and wood distillate. Data are presented as means ± standard error. Different letters indicate significant statistical differences ($p < 0.05$) among treatments

BC-treated plants, compared to CKs (Figs. 5F, G, respectively). Conversely, Fe and Mn content in roots increased significantly under the combined BC + WD treatment, by 20 and 6%, respectively, compared to CKs (Figs. 5F, G, respectively). Molybdenum content was notably reduced by 31% only in the roots of plants grown under the WD treatment, whereas Zn content decreased by 16% only under BC treatment, compared to CK (Figs. 5H, I, respectively).

As for the micronutrients, Na content in leaves remained constant across treatments (Fig. 5J). However, in roots, Na content significantly increased (+18%) in BC-treated plants and significantly decreased (−15%) in WD-treated plants, compared to CK plants (Fig. 5J).

PCA Analyses

For leaves, the first two principal components explained 64.0% of the total variance (Dim1 = 46.9%; Dim2 = 17.1%). The PCA plot clearly separated the treatments, with CK and WD located on the negative side of Dim1, while BC and BC + WD clustered on the positive side. Leaf proteins, K, and Na were positively associated with BC and BC + WD treatments, whereas parameters such as leaf number, shoot fresh weight, stem height, glucose, Fe, Zn, Mg, Ca, P, and total phenols were more closely related to CK and WD (Fig. 6A).

For roots, the first two principal components explained 64.8% of the total variance (Dim1 = 43.3%; Dim2 = 21.5%). The PCA plot clearly distinguished BC from the other treatments along Dim1, while BC + WD, CK, and WD formed distinct but partially overlapping groups. Root Na and K were positively associated with BC, whereas root length, surface area, fresh weight, and Mo were closely related to BC + WD. In contrast, Ca, Mg, Zn, P, Cu, Mn, and Fe were mainly associated with CK and WD (Fig. 6B).

Growing Media Modifications

Chemical and Thermal Properties

The application of BC led to an increase (~10%) in pH, irrespective of the presence of WD (Fig. 7A). An opposite effect of BC was observed on EC, resulting in decreases of 12% in BC and 17% in BC + WD (Fig. 7B). The CEC increased significantly with the addition of WD, with an increase of 17% observed for WD alone and of 9% for the combined treatment (BC + WD), compared to the CK (Fig. 7C).

Thermal analysis showed similar patterns between CK and WD, as well as between BC and BC + WD (Fig. S1). In detail, TGA and corresponding derivative mass loss (DTG) curves were characterized by three main steps of WL between 100 and 550 °C (Fig. S1A, B): the first common WL occurred between 250 and 350 °C, typically ascribed to the decomposition of polysaccharides, including cellulosic material and aliphatic structures; the second common WL occurred between 350 and 400 °C, while the third WL, occurring around 450 °C in BC and BC + WD and around 500 °C in CK and WD, was possibly related to the thermal degradation of more recalcitrant aromatic compounds such as lignin, charred material, and non-hydrolyzable substances (Bona et al. 2023; Dell'Abate et al. 2000; Plante et al. 2009; Provenzano et al. 2018).

The common peak in DTG curves had a maximum around 300 °C (Fig. S1B) and corresponded to a WL between 250 and 350 °C ranging from 35 to 44% (DW) in BC and WD, respectively (Fig. S1A). The second common peak in DTG curves occurred between 380 °C in WD and 390 °C in CK,

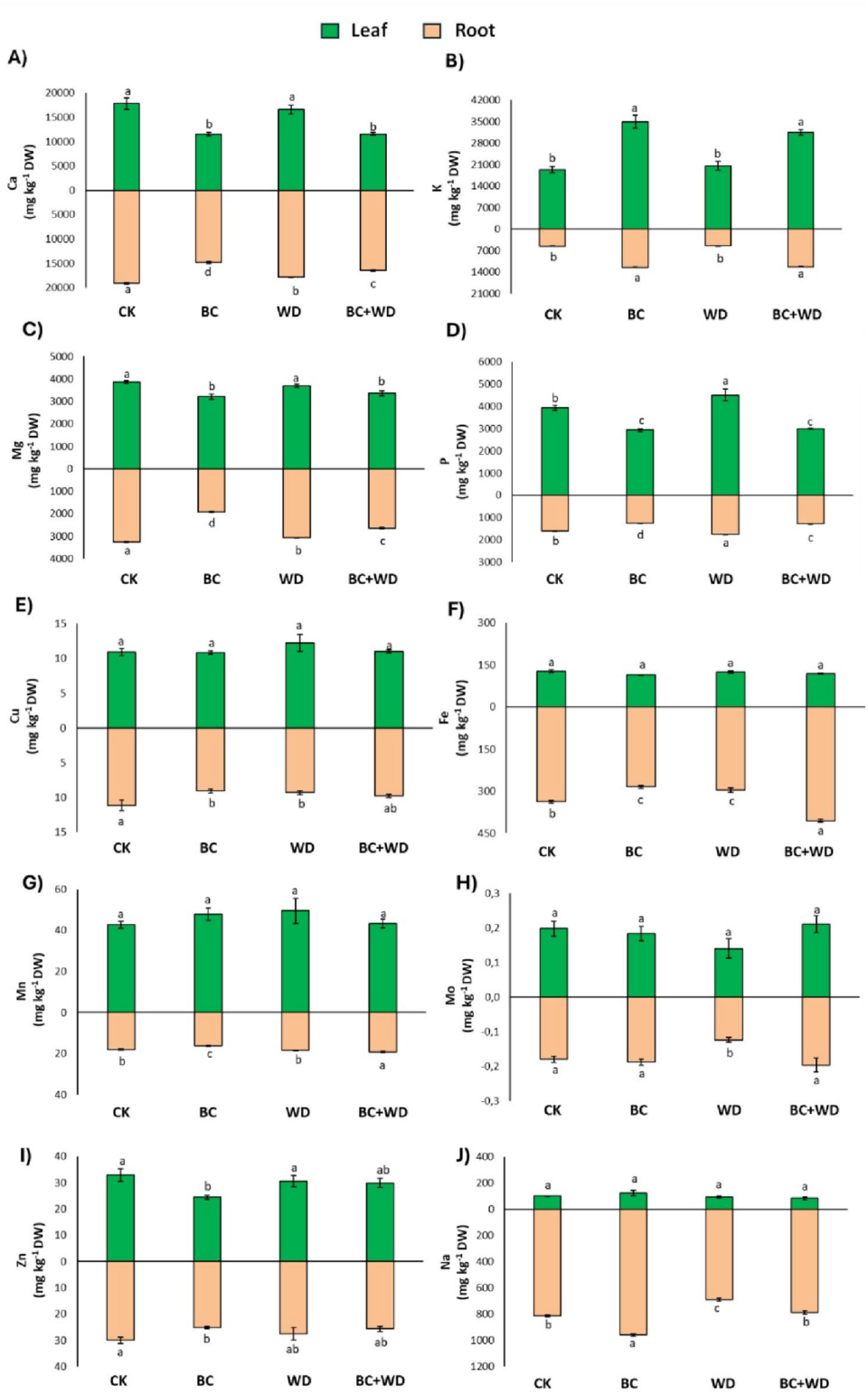

Fig. 5 Total content of different nutrients in the leaves (green bars) and roots (light orange bars) of young grapevine plants grown under four different treatments of the growing medium: CK = control; BC = plants grown with biochar (BC); WD = plants grown with wood distillate (WD); BC + WD = plants grown with both biochar and wood distillate. Data are presented as means ± standard error. Different letters indicate significant statistical differences ($p < 0.05$) among treatments

BC, and BC + WD (Fig. S1B); in all cases, the WL recorded between 350 and 400 °C ranged from 14 to 22% (DW) in BC and WD, respectively (Fig. S1A). The third peak in DTG curves, by contrast, occurred between 425 and 475 °C for BC and BC + WD, and at a slightly higher temperature (~500 °C) for CK and WD (Fig. S1B); in all cases, the WL recorded between 400 and 550 °C ranged from 15 to 29% (DW) in WD and BC, respectively (Fig. S1A).

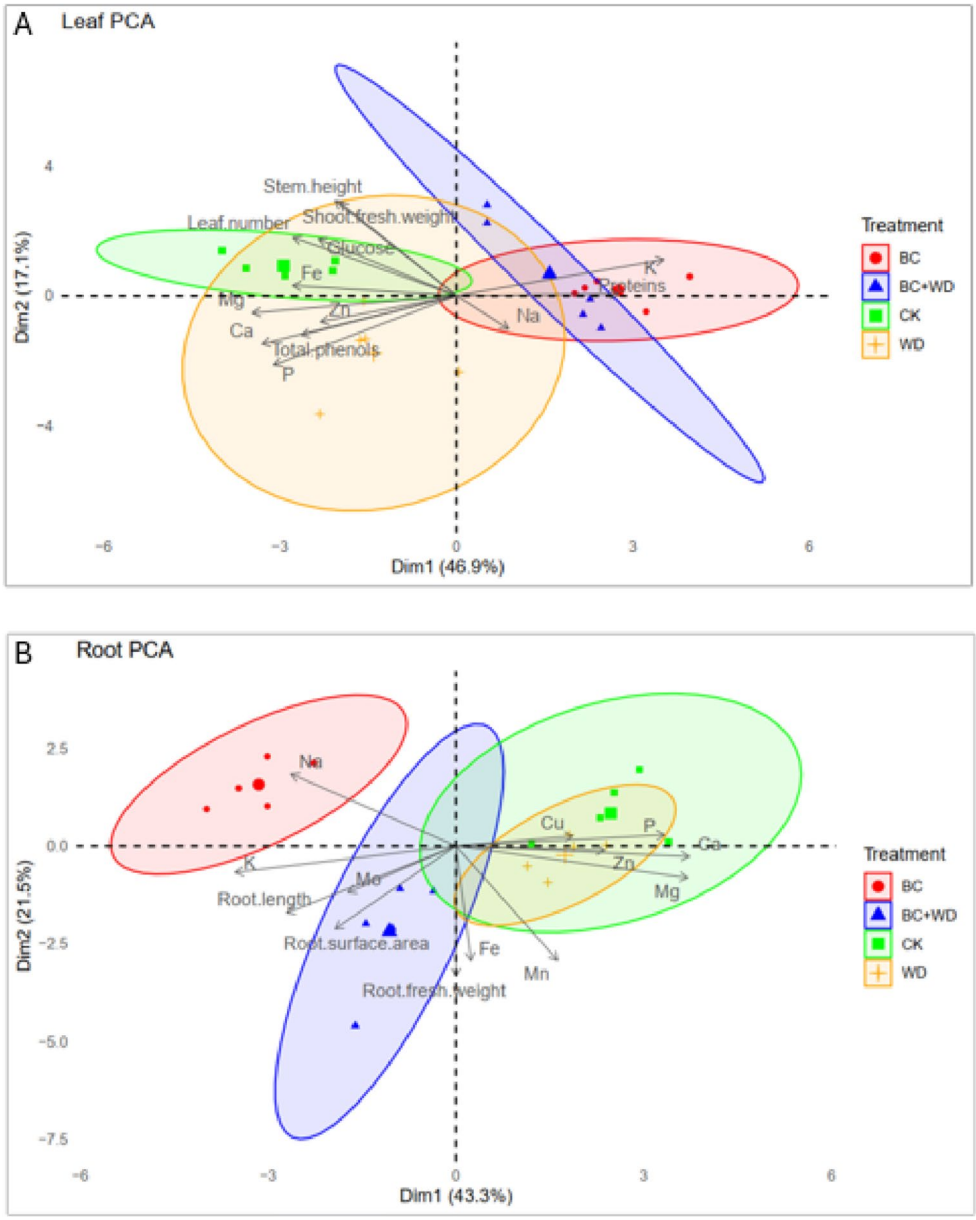

Fig. 6 Principal Component Analysis (PCA) of leaf and root morpho-physiological, biochemical, and nutritional parameters of grapevine plants grown under different treatments: control (CK), biochar (BC), wood distillate (WD), and their combination (BC + WD). Ellipses represent the 95% confidence interval for each treatment group

Normalizing TG data by LOI (Fig. S1C), it is possible to observe that, while the mass lost in all samples was quite similar between 250 and 350 °C (i.e., 42–43% LOI in BC and BC + WD vs. 48–50% LOI in CK and WD) and between 350 and 430 °C (i.e., 24–26% LOI in BC and BC + WD vs. ~32% LOI in CK and WD), much larger differences are recorded between 430 and 550 °C (i.e., 23–25% LOI in BC + WD and BC vs. 9–10% LOI in WD and CK). Therefore, these data suggest a higher proportion of more complex and thermally stable molecular compounds in BC-based substrates, compared to WD and CK. This finding was particularly evident when all thermal indices were compared: in fact, BC and BC + WD showed the highest values of $WL_{400–550/200–300}$, $WL_{400–550/300–400}$, and TG-T_{50} (Table 2).

Thermal indices obtained from DSC analysis (Fig. S1D; Table 2) are in agreement with those obtained from TG, showing lowest values of the energy density (i.e., the energy as heat necessary for organic matter thermal oxidation) and of the temperature at which half of the energy was released in DSC (DSC-T_{50}) for WD compared with BC-based substrates. Therefore, these data confirm that WD was richer in less stable and more decomposable organic matter, as the energy required for the thermal degradation of an organic substrate was assumed comparable to the energy barrier to microbial decomposition (Peltre et al. 2013). Thus, according to the thermal stability and atomic ratios, which are associated with the biological stability, biomasses under study can be ordered as follows: WD ~ CK < BC ~ BC + WD.

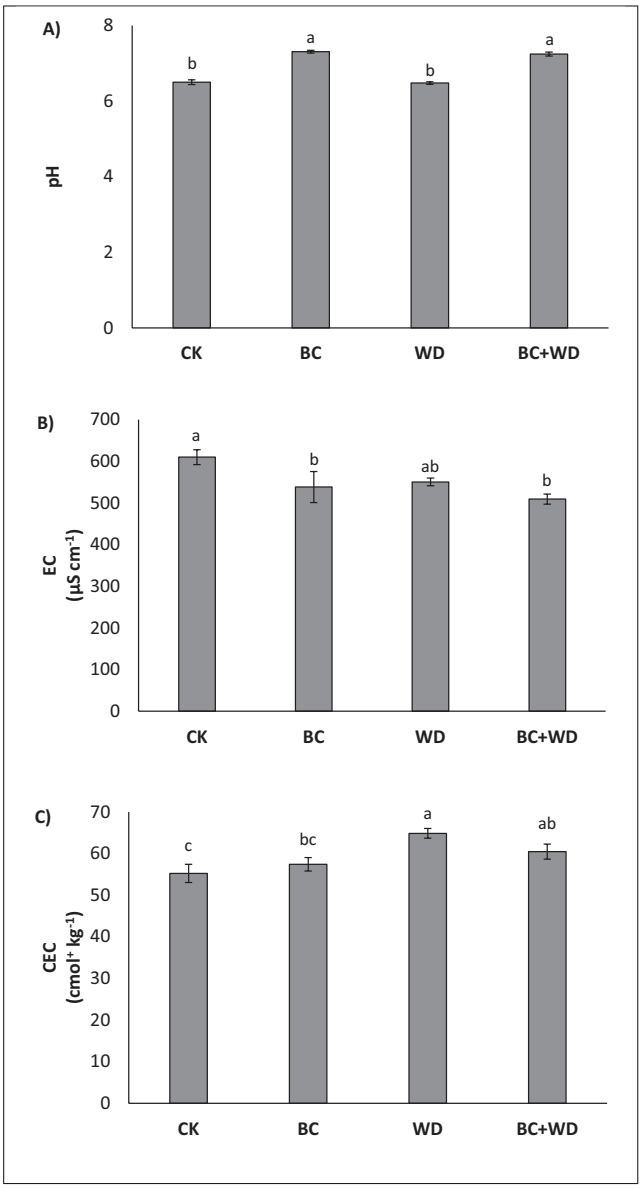

Fig. 7 Chemical parameters [pH, electrical conductivity (EC), and cation exchange capacity (CEC)] of the four different treatments of the growing medium: CK = control; BC = growing medium amended with biochar (BC); WD = growing medium amended with wood distillate (WD); BC + WD = growing medium amended with both biochar and wood distillate. Data are presented as means ± standard error. Different letters indicate significant statistical differences ($p < 0.05$) among treatments

Elemental analysis revealed a significant increase in C content following the application of BC, WD, and their combination, with the highest increases observed in the treatments containing BC (+14% for BC and +13% for BC + WD), and a moderate increase in WD alone (+6%) (Table 2). Conversely, H content was significantly reduced in both BC and BC + WD treatments (−9%), leading to a marked increase in the C/H ratio, which indicates a more aromatic and recalcitrant structure in the presence of BC compared to the CK and also WD (Table 2). Nitrogen content was slightly but significantly lower in BC (−9%) and BC + WD (−10%) than in CK, which showed values comparable to WD (Table 2). These trends were also reflected in the C/N ratios, which exceeded 47 in the BC-containing treatments, highlighting the greater stability of the organic material (Table 2).

Bioavailability of Nutrients

The contents of the bioavailable and non-bioavailable fractions of the main macronutrients (Ca, K, Mg, and P), micronutrients (Cu, Fe, Mn, Mo, and Zn), and Na in the growing media treated with BC, WD, and their combination (BC + WD) are reported in Fig. 8. The bioavailable fraction of Ca remained constant between treatments, while the non-bioavailable fraction showed a significant increase with BC (+13%) and BC + WD (+28%), compared to CK (Fig. 8A). In the case of K, the bioavailable fraction increased 14-fold with BC and 13-fold with BC + WD, compared to CK (Fig. 8B). The non-bioavailable fraction also showed significant increases, i.e., +72% for BC and +166% for BC + WD, compared to CK (Fig. 8B). Similar to Ca, the bioavailable fraction of Mg remained relatively constant across treatments; in contrast, the non-bioavailable fraction increased significantly, with a rise of 2% for the BC treatment and 19% for the BC + WD treatment, compared to CK (Fig. 8C). Also, the bioavailable fraction of P remained consistent across treatments, whereas the non-bioavailable fraction showed a significant increase in the WD and BC + WD treatments, increasing by 6-fold and 10-fold, respectively, compared to CK (Fig. 8D).

No significant variation was observed for Cu across treatments in either the bioavailable or non-bioavailable fractions, in comparison with the CK (Fig. 8E). Also, the bioavailable fraction of Fe remained unvaried across treatments, while the non-bioavailable fraction increased significantly by 28% in BC and 53% in BC + WD, compared to CK (Fig. 8F). The Mn showed a marked increase in both the bioavailable (+64%) and non-bioavailable (+24%) fractions exclusively under the BC treatment, compared to CK (Fig. 8G). The bioavailable fraction of Mo was significantly reduced in presence of WD, both when applied alone (−65%) and in combination with BC (−64%); on the opposite, the non-bioavailable fraction exhibited a pattern similar to that observed for K, with a significant increase under the BC and BC + WD treatments (+85% and +57%, respectively), compared to the CK (Fig. 8H). For Zn, only its bioavailable fraction in the BC-treated soil exhibited a significant increase of 51%, compared to CK (Fig. 8I).

Finally, the bioavailable fraction of Na was not significantly affected by the treatments, while the non-bioavailable

Table 2 Thermal indices and elemental composition (carbon - C, hydrogen - H, and nitrogen - N) of organic matter stability in the four different treatments of the growing medium: CK = control; BC = growing medium amended with Biochar (BC); WD = growing medium amended with wood distillate (WD); BC + WD = growing medium amended with both Biochar and wood distillate

		CK	BC	WD	BC + WD
Thermal indices					
LOI	(%)	89.2	87.3	89.4	87.9
$WL_{400-550/200-300}$		0.963	1.574	0.710	1.436
$WL_{400-550/300-400}$		0.411	0.855	0.309	0.745
$TG\text{-}T_{50}$	(°C)	336	350	331	345
$DSC\text{-}T_{50}$	(°C)	376	398	370	395
En_{dens}	(J/mg_{LOI})	12.6	13.2	12.4	13.3
Elemental composition					
C	(%)	45.3 ± 1.3 [c]	51.5 ± 0.6 [a]	48.2 ± 0.2 [b]	51.2 ± 0.3 [a]
H	(%)	5.3 ± 0.1 [a]	4.8 ± 0.1 [b]	5.3 ± 0.0 [a]	4.9 ± 0.0 [b]
N	(%)	1.2 ± 0.0 [a]	1.1 ± 0.0 [b]	1.2 ± 0.0 [a]	1.1 ± 0.0 [b]
C/H		8.6 ± 0.2 [b]	10.8 ± 0.3 [a]	9.0 ± 0.1 [b]	10.5 ± 0.1 [a]
C/N		37.5 ± 0.5 [c]	47.0 ± 1.4 [a]	40.5 ± 0.4 [b]	47.6 ± 0.3 [a]

The thermal indices include the loss-of-ignition (LOI), ratios between weight losses (WL) occurring within different ranges of temperature ($WL_{400-550/200-300}$, $WL_{400-550/300-400}$), the temperature at which half of mass is lost in thermogravimetry ($TG\text{-}T_{50}$), the temperature at which half of the energy is released in differential scanning calorimetry ($DSC\text{-}T_{50}$), and the energy density (En_{dens}). Thermal analysis was performed on pooled composite samples from biological replicates ($n = 5$). Data for C, H, and N are presented as means ± standard error. All data were determined on on a dry weight basis

fraction showed a significant 10% increase exclusively with BC addition to the soil (Fig. 8J).

Discussion

Several studies (Abdel-Sattar et al. 2024; Fedeli et al. 2022) have reported a stimulating effect on chlorophyll content at low WD concentrations (0.2%, v/v). However, at concentrations exceeding 1% (v/v), WD induces phytotoxicity in certain plant species, particularly weeds, causing chlorophyll degradation and disruption of the photosynthetic machinery, which can ultimately lead to plant death (Liu et al. 2021). These findings suggest that at elevated doses, WD may serve as a natural herbicide by specifically targeting the photosystems (Liu et al. 2021). In our study, we may speculate that the decrease in chlorophyll content in the leaves of plants fertigated with WD was likely related to the significant reduction in the bioavailability of Mo in the growing medium. In fact, among all nutrients investigated, only Mo showed a reduction of its bioavailability when WD was applied. As anionic species ($HMoO_4^-$ and MoO_4^{2-}) are the predominant ones in the soil solution under a wide pH range (Kabata-Pendias and Pendias 2001; Miano et al. 2014), some precipitation phenomena could be hypothesized. This led to a lower root uptake of this micronutrient and slightly affected its allocation in the leaves. In fact, Mo is essential for the activity of some enzymes crucial for plant metabolism and photosynthetic function, and its availability influences the correct assimilation of essential nutrients necessary for chlorophyll production (Zayed et al. 2023). In the roots, besides Mo, WD reduced not only other cofactors of many photosynthetic enzymes, such as Cu and Fe, but also Ca, which is important for maintaining the structural stability of chloroplast membranes, and Mg, which is the central component of chlorophyll. Interestingly, although the reduction in Na bioavailability in the growing medium induced by WD was not statistically significant, it was associated with a significant decrease in Na content in the roots, helping grapevine plants to better tolerate potential Na toxicity. This effect could be attributed to the presence of phenolic compounds and organic acids in WD, that can complex cations and lower Na^+ bioavailability (Allison 1973; Fagernäs et al. 2012).

To the best of our knowledge, no study has specifically explored the interaction between WD and P in grapevine. However, research on other crops, such as the study by Becagli et al. (2023) on faba bean has shown that WD can enhance soil P availability and increase its accumulation in plant tissues. Although our study found no change in P availability in the medium following WD application, a significant increase in P content was observed in both leaves and roots of WD-treated grapevines, possibly due to the activation of specific P transporters or indirect effects mediated by root-microbe interactions.

The BC treatment also reduced chlorophyll content, likely due to nutritional imbalances, especially from the excessive input of K to the growing medium. Potassium likely competed with other cations, primarily Ca and Mg, reducing their uptake and accumulation in plant tissues (Xie et al. 2021). These results show that BC can have a fertilizing potential, in addition to its role as a soil amendment (EU Regulation 2019/1009); at the same time, the observed effects could be the result of applying a high dose of BC to young grapevines grown in pots under these specific environmental conditions. Typically, the maximum

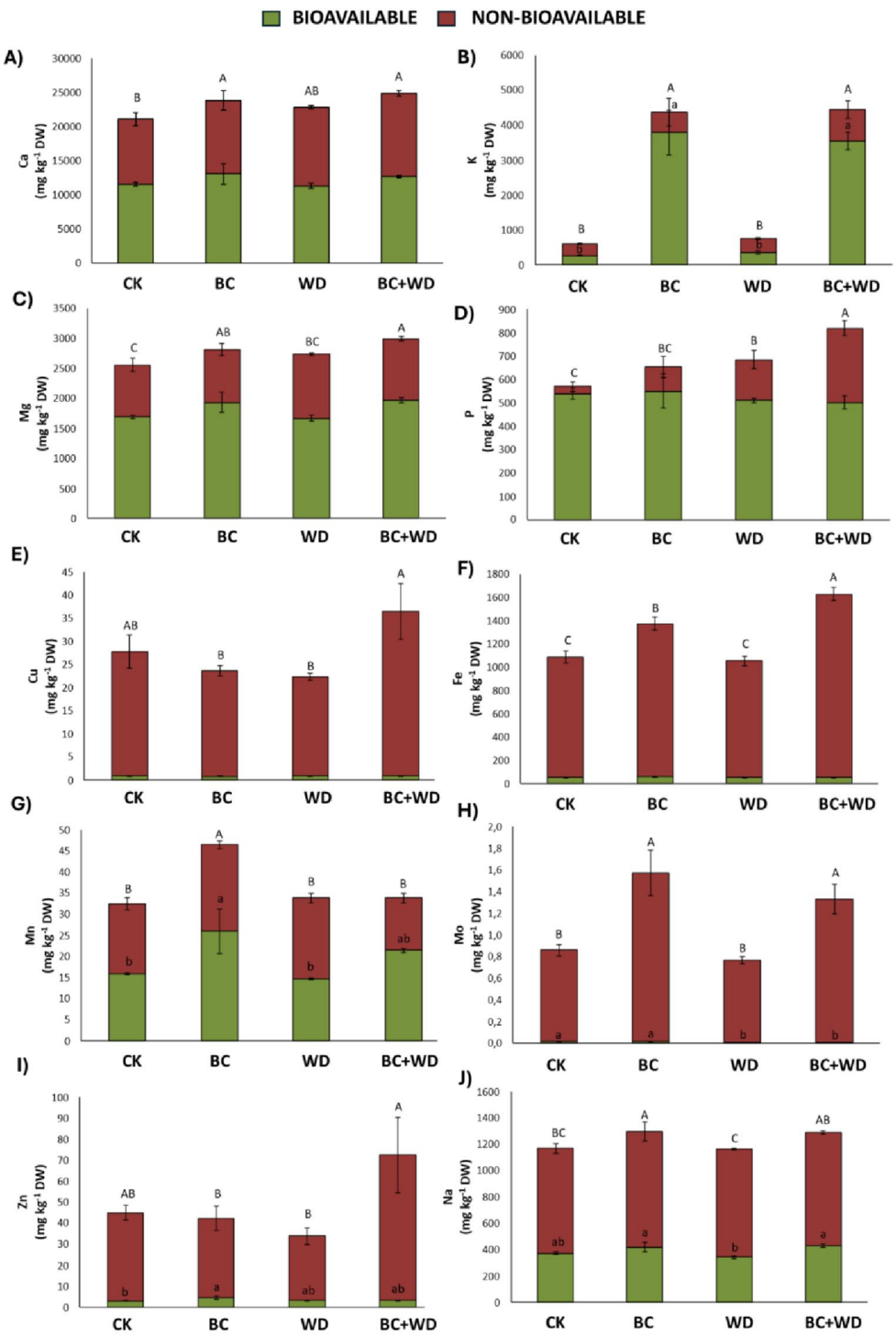

Fig. 8 Bioavailable (green bars) and non-bioavailable (red bars) fractions of the content of different nutrients in the for different treatments of the growing medium: CK = control; BC = growing medium amended with biochar (BC); WD = growing medium amended with wood distillate (WD); BC + WD = growing medium amended with both biochar and wood distillate. Data are presented as means ± standard error. Different letters indicate significant statistical differences ($p < 0.05$) among treatments

recommended BC dose in cultivated fields is 20 t ha^{-1}, corresponding to approximately 4% (w/w) (Gao et al. 2021). In this study, we intentionally applied a dose of BC five times higher than real field conditions with the intention to replace current peat-rich substrates. However, high doses of BC have been reported in the literature to reduce nutrient availability for crops, thereby negatively affecting agricultural productivity (Brtnicky et al. 2021). In this context, it is worth noting that the application of BC also led to a significant increase in Na content in the growing medium. However, despite this increase, Na content in the leaves remained comparable to the CK, while a significant accumulation was observed in the roots. This pattern suggests that grapevine plants restricted Na translocation to the aerial

parts, suggesting activation of ionic exclusion mechanisms known to mitigate salt stress (Tester and Davenport 2003; Zhou-Tsang et al. 2021).

The recovery of chlorophyll and glucose contents under the BC + WD treatment suggests a synergistic metabolic adjustment, involving the reallocation of energy reserves from leaves to roots to support growth under sub-optimal conditions (Rolland et al. 2006; Schultz et al. 2013). The hypothesized reallocation of metabolic energy, in the form of glucose, to the roots allows plants to strengthen their underground structures, maximizing the acquisition of limited resources such as water and nutrients (Kang et al. 2022). Our results emphasize the importance of these dynamics in the WD treatment, which resulted in a marked increase in root biomass at the expense of leaf biomass and a shortening of the main stem length. These data align with the observations of Nutsukpo et al. (2024), who documented similar metabolic responses in grapevine plants treated with WD, highlighting an increase in root biomass and a reallocation of carbohydrates to support root functions in stressful situations. Furthermore, our results show that both BC and WD, either alone or in combination, on one hand, stimulate an increase in root length and surface area, while on the other cause a reduction in the number of leaves. This supports the hypothesis that glucose is strategically mobilized from the leaves to the roots to promote root proliferation and extension, optimizing plant survival when facing sub-optimal growth environmental conditions.

The increase in leaf protein content observed with the addition of BC to the growing medium suggests an improvement in N availability and assimilation by the plant. Ronga et al. (2019) reported that the application of BC may enhance soil microbial activity in vineyards, increasing N availability and thus supporting plant protein synthesis. Specifically, in our study, BC-treated plants activated some metabolic pathways that favor amino acid accumulation; in particular, Glu, His, and Thr accumulated more in the presence of BC in the growing medium, compared to other treatments. For example, His can be converted to Glu, and Glu gives rise to Pro, a well-known osmoprotectant that accumulates under abiotic stress conditions to maintain cellular homeostasis and mitigate oxidative damage (Forlani et al. 2019). In our study, the addition of BC increased leaf Pro content compared to the CK, though not reaching the much higher levels found with WD. Alternatively, the observed accumulation of Thr, which is the precursor of Ile, could reflect an improvement in N use efficiency. Isoleucine content also increased compared to the CK, but did not reach the levels found with WD addition. In general, WD treatment enhanced the accumulation of all amino acids (except His and Thr), much more than the BC treatment and even more than the combined treatment. However, the conversion of amino acids into proteins in the leaves of WD-treated plants was equal to that of the CK and lower than that recorded with BC alone or BC + WD. It is known that treatments with natural products may lead to a greater activation of biosynthetic pathways, increasing amino acid synthesis; however, in case of limited availability of essential nutrients, there may also be greater degradation of soluble proteins to provide precursors for other metabolic pathways (Wawrzyńska and Sirko 2020). As mentioned previously, Mo was the least bioavailable element in the WD-treated growing medium; consequently, WD plants accumulated less Mo. Importantly, Mo plays a crucial role in N metabolism, as it is a cofactor for key enzymes such as nitrate reductase and nitrogenase (Pienkos et al. 1977). Under WD treatment, the marked accumulation of Gly suggests the activation of antioxidant pathways, as Gly is a precursor for glutathione synthesis, a key tripeptide involved in detoxification of reactive oxygen species and maintenance of cellular redox balance (Hasanuzzaman et al. 2017). This result aligns with the findings of Nutsukpo et al. (2024), who reported that WD application enhanced antioxidant defense mechanisms at the cellular level in grapevines, thereby mitigating oxidative stress induced by the accumulation of bioactive compounds, such as phenols and organic acids, present in the WD formulation. The role of WD in modulating plant stress responses has been widely studied, especially in relation to drought (Ghorbani et al. 2024) and salinity stress (Fedeli et al. 2024a). Wood distillate acts as an elicitor that primes and enhances plant defense mechanisms against both abiotic and biotic stresses. For instance, Fedeli et al. (2024b) reported increased growth in lettuce plants treated with WD, suggesting that WD triggers a controlled stress response known as "eustress." This form of beneficial stress stimulates the synthesis of bioactive secondary metabolites with benefits for plant adaptation, food quality, and human health (Baenas et al. 2014). In our case, however, the analysis of FAA contents suggests that the presence of BC acted synergistically with WD, contributing to the neutralization of oxidative effects that were otherwise observed with WD treatment alone. This hypothesis is further supported by the observed reduction in total phenol content in both the BC and BC + WD treatments, suggesting that BC may have played a key role in modulating the oxidative response triggered by WD.

Phenolic compounds are secondary metabolites often associated with plant defense, acting as antioxidants and protective agents against biotic and abiotic stress (Tuladhar et al. 2021). Therefore, the decrease in phenol content in BC-treated plants could indicate reduced stress perception. In fact, some studies have shown that BC can alleviate oxidative stress by improving soil water retention, due to its porous structure, and nutrient availability, due to its high pH, thereby allowing plants to allocate more resources to

primary growth rather than to defense mechanisms (Akram et al. 2024; Joseph et al. 2021). Moreover, it can be hypothesized that BC may have acted as an adsorbent surface, trapping bioactive compounds present in the WD formulation, thus reducing their oxidative impact on plant tissues.

These plant responses can be more effectively interpreted by considering the chemical and thermal traits of the growing media. Thermal and elemental analyses confirmed that BC created a more stable, recalcitrant organic matrix, while WD contributed more labile fractions, influencing nutrient release and plant response dynamics.

The leaf PCA analysis supported the hypothesis that BC and BC + WD treatments stimulated protein accumulation, likely as a consequence of enhanced N metabolism. In contrast, CK and WD treatments were more closely associated with traits related to vegetative growth and photosynthetic performance, suggesting that these conditions favored C assimilation rather than N investment. At the root level, the PCA indicated that BC application promoted the accumulation of Na and K, while BC + WD enhanced root morphological traits such as length and surface area. These combined effects suggest that BC and WD acted synergistically to stimulate root development, thereby improving the plant's capacity for nutrient acquisition and resource use efficiency. Conversely, CK and WD treatments were more associated with elements essential for structural integrity and metabolic activity (Ca, Mg, P), reflecting distinct nutrient uptake strategies driven by the different amendments.

Conclusions

This study highlights a remarkable impact of BC and WD amendments, applied individually or in combination, on the morpho-physiological, biochemical, and nutritional responses of young grapevines. Overall, while both BC and WD individually induced adaptive metabolic adjustments in grapevine plants, their combination resulted in a more balanced physiological state, suggesting a synergistic interaction. Biochar appears to buffer or modulate the potentially phytotoxic effects of WD, likely by adsorbing reactive bioactive compounds, thereby reducing oxidative stress and promoting a more efficient nutrient use. This synergism was evident in the recovery of chlorophyll and glucose contents, as well as in the improved protein synthesis and reduced phenolic accumulation, pointing to a complex but coordinated plant response that favors resilience and growth under suboptimal conditions.

From an agronomic point of view, these findings emphasize the need for targeted and context-specific use of BC and WD. Doses, properties of the growing media, and crop requirements should be taken into account. However, the combination BC + WD emerges as a promising strategy to enhance crop sustainability while improving plant resilience. However, long-term field studies are required to validate their practical applicability in sustainable viticulture.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00344-026-12092-4.

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Authors' Contributions S.C. conceived and designed the study, conducted laboratory analyses on the plants and growing medium properties, performed data analysis, wrote the original draft, revised the manuscript, and supervised the overall research activity. R.F. assisted with laboratory analyses, data collection, and reviewed parts of the manuscript. M.G. carried out the nutrient analyses. L.C. revised the manuscript. C.Z. contributed to the experimental design, performed elemental and thermal analyses, and revised the manuscript. S.L. supervised the research activity and revised the manuscript. All authors have read and approved the final version of the manuscript.

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Data Availability Data can be made available on reasonable request by the corresponding author.

Declarations

Competing Interests The authors have no relevant financial or non-financial interests to disclose.

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