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Evaluation of Biochar from Agricultural and Forestry Residues: A Comparative Study of Olive and Poplar Prunings

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Abstract

The sustainable valorization of agroforestry residues through thermochemical conversion can support circular bioeconomy and renewable energy production. This study evaluated olive pruning (*Olea europaea* L.) and poplar (*Populus alba* L.) residues from Central Italy as feedstocks for biochar production through a pilot-scale fixed-bed pyrolysis process. The feedstocks and resulting biochar were characterized in terms of their physicochemical properties, while their agronomic potential was assessed through germination assays and lettuce (*Lactuca sativa* L.) pot experiments. The carbon content of the biochar produced from poplar (BFOR) was statistically higher than that produced from olive pruning (BAGR): 92.0% and 79.9%, respectively. BFOR consistently showed a germination index above 100%, indicating phytostimulant potential within the tested conditions, whereas the BAGR produced species-dependent responses. In lettuce pot experiments, biochar alone resulted in limited effects on plant growth. However, when combined with organic fertilization, fresh biomass increased by approximately 10% compared with fertilization alone, reaching 107.88 g with BAGR and 107.08 g with BFOR, compared with 97.53 g for fertilization alone. The combined treatments also showed improved photosynthetic performance. These results highlight the potential of pilot-scale biochar production from locally available agroforestry residues as an integrated strategy for renewable energy production and sustainable soil management.

Keywords: agroforestry residues; soil amendment; pyrolysis; biochar potential; circular bioeconomy; waste thermal conversion; biomass valorization

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1. Introduction

The transition towards more sustainable and climate-neutral production systems has increased interest in strategies that improve resource efficiency, promote renewable energy production and valorize residual biomass [1–4]. In this context, the circular bioeconomy provides a framework for replacing linear biomass management with integrated value chains in which agricultural and forestry residues can be converted into renewable energy and value-added bio-based products rather than being treated as waste [5–8]. Agricultural and forestry activities generate large amounts of lignocellulosic residues, including pruning residues and woody biomass, whose chemical composition varies according to botanical origin, growing conditions and chemical composition [9–11]. This variability strongly influences the suitability of biomass for thermochemical conversion because feedstock characteristics can influence process behaviour and the physicochemical properties and

potential applications of the resulting biochar [12]. Among the biomass resources available in the Mediterranean region, olive pruning is one of the most abundant biomass resources owing to the extensive cultivation of olive orchards throughout Southern Europe, whereas poplar biomass from short-rotation forestry represents a fast-growing renewable woody resource particularly suitable for thermochemical conversion [13–16]. The production of pruning and woody residues can be substantial and represents an important factor when evaluating their potential as raw materials for thermochemical conversion. In Mediterranean olive systems, pruning operations are estimated to generate an average production of approximately 33 kg of dry tree biomass^{-1} [15]. In Italy, estimates indicate that pruning olive range from 1.1 to 2.8 t ha^{-1} y^{-1} depending on the size of the tree and the characteristics of the orchard [17]. For poplar, studies conducted in Central Italy have reported annual dry biomass productions of approximately 4.7–5.8 t ha^{-1} year^{-1} with annual and biennial cutting cycles, respectively, while tests conducted in the province of Rome reported values of approximately 6.7–7.5 t of dry matter ha^{-1} year^{-1} for different poplar clones [18]. Despite their availability, a considerable fraction of these materials remains underutilized or is managed through low-value practices such as open-field burning, which can result in the loss of potentially recoverable carbon and nutrients and contribute to atmospheric emissions [19–24]. Their recovery through integrated biomass management strategies can therefore contribute to simultaneously reducing environmental impacts, using resources more efficiently, and generating renewable energy and value-added co-products [25–27]. Importantly, olive pruning and poplar biomass represent two distinct residue streams. Comparing these two feedstocks under comparable processing conditions provides an opportunity to investigate whether the origin of the biomass is reflected in the characteristics and agronomic behaviour of the resulting biochar. Among the available biomass conversion technologies, thermochemical processes represent a suitable pathway for the valorization of heterogeneous lignocellulosic residues because they can simultaneously generate energy and carbon-rich products. Depending on the process configuration and operating conditions, biomass can be converted through combustion, gasification, pyrolysis or pyro-gasification [28,29]. Among these technologies, pyrolysis and pyro-gasification are particularly relevant for biochar production because they allow part of the biomass carbon to be retained in a stable carbon-rich solid fraction while also producing bio-oil and syngas [30]. Moreover, compared with conventional combustion, pyrolysis and gasification provide higher conversion efficiency and lower atmospheric emissions [31–35]. Biochar is a heterogeneous carbonaceous material whose physicochemical properties are determined by both feedstock composition and thermochemical operating conditions (e.g., reactor configuration, temperature, heating rate and residence time) [36]. These factors affect conditions such as carbonization efficiency, aromatic condensation, pore development, and characteristics such as carbon content, ash and mineral nutrient concentrations, surface properties and carbon stability, which in turn influence the potential applications of the resulting biochar [37–40]. Consequently, the characterization of biochar produced from different feedstocks is essential for establishing its suitability for specific end uses, including soil amendment [41,42]. Woody feedstocks, such as poplar biomass, generally produce biochar with a high fixed carbon content, condensed aromatic structures and long-term environmental stability, whereas agricultural residues tend to generate biochar containing larger ash fractions and greater concentrations of essential plant nutrients [43]. Biochar has attracted considerable interest as a soil amendment due to its potential to modify the physical, chemical and biological soil properties. Previous studies have reported its effect on parameters such as aggregate stability, porosity, water-holding capacity, cation exchange capacity and nutrient retention [44–50]. In addition, biochar can affect plant performance through changes in nutrient availability. Its high sorption capacity enables the

immobilization of heavy metals and other contaminants, reducing their bioavailability and limiting plant uptake, thereby extending its environmental benefits beyond soil fertility improvement [51–55]. Biochar amendments may also influence plant performance indirectly through modifications of rhizosphere processes and microbial activity. Responses have been reported for seed germination, photosynthetic performance and chlorophyll content [56–58]. However, these effects are not consistent across all biochars, since agronomic responses depend on feedstock characteristics, production conditions, application rate, soil properties and crop species. These factors highlight the importance of evaluating each biochar before recommending its practical application [59–61]. More recently, research about the combination of biochar with organic fertilization has also received increasing attention because the two inputs may provide complementary effects on nutrient availability and plant development [50,62,63]. Despite the growing body of research on biochar, knowledge gaps remain. Many studies have focused separately on thermochemical process optimization, physicochemical characterization or agronomic evaluation, while fewer have integrated these aspects within a common experimental framework [64]. Moreover, a substantial part of the available evidence concerns laboratory-produced or commercially available biochar, whereas comparatively fewer investigations have evaluated biochar produced under pilot-scale thermochemical conditions. This distinction is relevant because reactor configuration and operating conditions can influence biochar properties, limiting the direct extrapolation of laboratory-scale results to larger-scale production systems [32]. These issues are particularly relevant for Mediterranean agroforestry residues, whose properties may vary according to local environmental conditions and production practices [65]. Likewise, most studies have investigated only selected response variables on individual plant or soil responses, providing only a partial view of biochar–soil–plant interactions [66,67]. Integrated evaluations combining the characterization of feedstock and biochar with the study of germination, plant physiological and morpho-productive responses, soil respiration and interactions with fertilization remain comparatively limited, particularly for biochar produced from Mediterranean agricultural and forestry residues using pilot-scale pyrolysis systems [29,68]. Accordingly, the aim of this study was to characterize and compare biochar produced from two representative Mediterranean agroforestry residues, olive (*Olea europaea* L.) pruning and poplar (*Populus alba* L.) biomass, using a pilot-scale fixed-bed pyrolysis system under comparable operating conditions. The specific objectives were to: characterize the physicochemical properties of the starting biomasses and the resulting biochar; assess their effects on lettuce (*Lactuca sativa* L.) growth and physiological and morpho-productive responses under pot conditions; and (iv) investigate their interaction with organic fertilization and their effects on CO_2 emission from soil. With regard to this last point, it should be emphasized that the study considered a fertilizer approved for use in organic farming rather than a synthetic one. The aim was to provide experimental evidence in support of sustainable farming techniques. By comparing an agricultural residue with a forestry residue within the same experimental framework, this study aims to determine whether differences in feedstock origin are reflected in biochar characteristics and agronomic responses, thereby contributing to the assessment of locally available agroforestry residues as resources for integrated energy–agriculture value chains. Within this context, biochar should be regarded not merely as a by-product of renewable energy production but as a multifunctional co-product capable of generating environmental, agronomic and economic benefits.

2. Materials and Methods

2.1. Feedstock Availability and Characterization

The feedstocks used for biochar production were selected as representative agricultural and forestry residues widely available in the Mediterranean region of Central Italy. Their selection was carried out based on the provisions of the NEST SO-FAST (Holistic Development of a Smart Agro-Energy Supply Chain for the Energy Transition in Central Italy) project, which evaluated the availability, spatial distribution, and energy potential of agricultural and forestry residues in the Lazio Region. This evaluation identified olive pruning residues and poplar biomass as two of the most representative types of lignocellulosic feedstocks for the development of sustainable thermochemical value chains. Olive pruning residues (*Olea europaea* L.) were chosen as agricultural residues, while poplar (*Populus alba* L.) biomass from short-rotation forestry were selected as the forestry biomass, both from experimental fields at CREA agricultural farms. Therefore, conventionally, it was chosen to indicate in the text and in the reference tables and figures the agricultural biomass with AGR and the forestry biomass with FOR, as well as the relative biochar obtained with the acronyms respectively BAGR and BFOR. Before the physicochemical analysis, part of the material was ground using a Retsch SM 100 kneading mill Retsch GmbH (Haan, Germany) and a Retsch ZM 200 centrifugal mill Retsch GmbH (Haan, Germany). After pyrolysis, the biochar samples were also shredded for characterization. All measurements were performed at the Laboratory for Experimental Activities on Renewable Energy from Biomass (LASER-B) of CREA-IT in Monterotondo (Rome).

2.2. Proximate and Ultimate Analysis

The starting biomass and the corresponding biochar were analyzed to determine moisture content, ash content, ash fusibility, elemental analysis, higher heating value (HHV), lower heating value (LHV) and metal content. The moisture content was determined using a Memmert UFP800 drying oven Memmert GmbH (Schwabach, Germany) according to ISO 18134–2:2024 [69] at 105 ± 2 °C for 24 h. The ash content was determined according to ISO 18122:2022 [70] by using a Lenton EF11/8B muffle furnace Lenton Thermal Designs Ltd. (Hope Valley, United Kingdom), up to 250 °C at 6.5 °C min^{-1} and then up to 550 °C at 10 °C min^{-1}. The fusibility of ashes was determined by using a SYLAB SHV-IF 1550 tubular furnace SYLAB SARL (Metz, France) in accordance with UNI EN ISO 21404:2020 [71]. Cylindrical ash samples were prepared and placed in a chamber with controlled temperature rates until 1550 °C. The elemental composition of carbon (C), hydrogen (H) and nitrogen (N) was evaluated using a Costech ECS 4010 CHNS-O elemental analyzer Costech Analytical Technologies Inc. (Valencia, CA, USA) according to ISO 16948:2015 [72]. Biomass samples were prepared by a pellet press (Pellet Press 2810, Parr Instruments, Moline, IL, USA) and analyzed to determine HHV using an isoperibol calorimeter (6400 Parr Instruments, Moline, IL, USA) according to ISO 18125:2018 [73]. The LHV was calculated starting from the HHV and the hydrogen content as described by the same standard method.

The metal content was determined using an ICP-MS 7700 (Agilent 7700, Agilent, Santa Clara, CA, USA), according to ISO 16967:2015 [74] and ISO 16968:2015 [75]. The solution for the analysis was prepared with about 500 mg of samples subjected to an acid attack (6.0 mL of HNO_3 and 3.0 mL of H_2O_2) and mineralized in a microwave digester (Start D, Milestone) at 180 ∘C, 650 W for 42 min, according to the U.S. EPA Method 3051A [76]. All measurements were performed in triplicate using independent replicates.

2.3. Pyrolysis Conditions and Biochar Production

Pyrolysis tests were carried out using a 1.5 kW prototype system (Figure 1), developed by CREA-IT and DIMA (Department of Mechanical and Aerospace Engineering, Sapienza

University of Rome, Italy), which can operate in both fixed and fluidized beds. The system can operate in either gasification or pyrolysis mode, with the operating conditions and type of gas used being modulated. The reactor consists of an AISI 310 stainless steel cylinder with a diameter of 8.3 cm and a height of 59.0 cm, equipped with an electric heating system and a Watlow® controller for temperature regulation. At the top, a feed screw transports the biomass into the reactor, where (from the bottom) a micro-perforated metal plate acts as a support for the gasification bed, allowing the oxidant to flow through the bed itself. Below there is a "wind box" for preheating the oxidizing or reducing agent, which is injected from below. In fact, using air in sub-stoichiometric quantities or nitrogen, it is possible to operate in gasification or pyrolysis mode. Using LabView®, it was possible to control and record the values of all the sensors present (thermocouples, pressure sensors, mass flow metres and mass flow controllers).

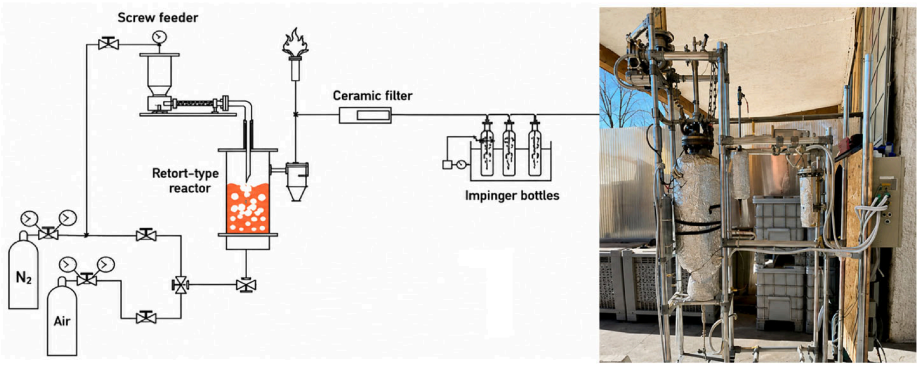


Figure 1. Schematic diagram and photograph of the prototype retort-type reactor used for biochar production under pyrolysis conditions.

The olive and poplar pruning residues were used as received, following natural drying, with a size reduction before being fed into the reactor for biochar production. Tests were performed under the operating conditions reported in Table 1, starting from ambient temperature to 700 °C, at a heating rate of 20 °C min^{-1}, under a nitrogen gas atmosphere (flow rate was set at 5 NL min^{-1}). The pyrolysis tests were carried out in fixed-bed mode. The reactor temperature was increased from ambient temperature to 700 °C at a heating rate of 20 °C min^{-1}, and the final temperature was maintained for 15 min to ensure a consistent treatment of the feedstock. The final pyrolysis temperature was selected to promote a high degree of devolatilization and carbonization of the lignocellulosic feedstocks, with the aim of obtaining a carbon-rich solid fraction with relatively low volatile matter. Previous studies have shown that increasing pyrolysis temperature promotes the removal of volatile compounds and generally increases the proportion of stable and aromatic carbon structures in biochar [14,39]. Pyrolysis of agroforestry residues is commonly performed over a broad temperature range, approximately 400–800 °C [14]. The same final temperature was applied to both feedstocks to ensure identical and reproducible thermochemical conditions and to enable a direct comparison of the resulting biochar. At the end of the pyrolysis process, the reactor was kept closed, and the biochar was allowed to cool naturally and gradually, with N_2 gas flow, inside the reactor until reaching ambient temperature. After pyrolysis, the cooled biochar was collected, weighed, and ground using a Retsch SM 100 cutting mill Retsch GmbH (Haan, Germany) and a Retsch ZM 200 centrifugal mill Retsch GmbH (Haan, Germany). The obtained biochar was then analyzed, with measurements performed in triplicate using independent replicates. The biochar yield was calculated as the ratio between the mass of biochar recovered after pyrolysis and the initial mass of naturally dried feedstock. The weight of condensable products and their ratio in the mass balance were determined through gravimetric analysis of condensate trapped in the bubbler system

(filled with isopropanol solution 90%). The syngas yield was estimated by difference from the mass balance, accounting for the initial feedstock mass, the recovered biochar and condensable products. The lower heating value (LHV) of the syngas was calculated from its composition determined using a gas chromatograph Micro GC INFICON 3000 INFICON Instruments (Shanghai, China). In particular, from iso 6976:2016 [77], the LHV was calculated as the sum of the lower heating values of the individual combustible components, weighted by their respective volumetric fractions [77,78].

Table 1. Operative parameters.

Parameters	Values	Unit
Biomass rate	2.5	kg/h
Reactor T	700	°C
Wind box T	400	°C
Ceramic Filter T	450	°C

In addition to the parameters described in the previous paragraph, the biochar was also analyzed to determine pH, volatile matter, fixed carbon and C/N ratio. The pH was measured using the Eutech Instruments pH700 pH/mV/°C/°F Bench Meter Eutech Instruments Pte Ltd. (Singapore). with a glass electrode in a solution of deionized water (50 mL), in which about 5 g of the sample was added, according to UNI EN 13037:2012 [79]. For the determination of volatile matter, about 1 g of the dried sample was placed into a crucible and into a muffle furnace at 925 ± 10 °C for 7 min according to ASTM D 3175-89. After heating, the difference in weight loss before and after heating gave the volatile content. The fixed carbon was determined by difference, considering moisture content, volatile matter and ash content, according to the following equation:

$$FC(\%) = 100\% - [MC(\%) + VM(\%) + Ash(\%)] \quad (1)$$

where

FC = fixed carbon
MC = moisture content
VM = volatile matter
AC = ash content

2.4. Biochar Agronomic Assessment

The evaluation of biochar as a soil amendment was conducted through a comprehensive experimental approach designed to assess its effects at different stages of crop development. The experimental work consisted of three consecutive phases. The first phase focused on assessing the biological quality of the biochar by determining the germination index (GI). The second phase involved a pot experiment using lettuce (*Lactuca sativa* L.) to evaluate the crop's growth and morphological responses. Finally, the third phase investigated the interaction between biochar application and organic fertilization to assess their potential synergistic effects on nutrient use efficiency and crop performance.

2.4.1. Determination of Germination Index

Tests were carried out to determine the germination index, which is used to evaluate the degree of compost maturation [80,81] as well as for biochar [82] and to check for any negative effects of the biochar. The protocol followed that of Gascò et al. [83] with minor modifications.

Aqueous extracts at a concentration of 1:10 (weight/volume) were prepared by dissolving 10 g of BAGR or BFOR biochar in 100 mL of distilled water. The solutions were

stirred for about 20 min and then left at 26–28 °C for 24 h. The next day, the solutions were stirred for 15 min and then filtered on gauze. The filtered solution was dispensed into Petri dishes where two discs of Whatman® filter paper had been arranged. For each capsule, 3 mL of each solution was distributed. The same procedure was followed using distilled water as a control treatment. The extracts and control treatment were tested on pea, tomato, sorghum and sunflower seeds. For each species, 10 seeds were sown in three Petri dishes (30 seeds), considered as replicates. Petri dishes were placed in a growth chamber in the dark and at a temperature of approximately 24 °C. Germination was checked after four days. The germination index (%) was calculated as follows [83,84]:

$$GI(\%) = \frac{((G1/G2) \times 100) \times ((R1/R2) \times 100)}{100} \quad (2)$$

where G1 and G2 are respectively the average number of germinated seeds for each Petri dish in the extract and control; R1 and R2 are respectively the average length of rooted seeds for each Petri dish in the extract and control.

2.4.2. Pot Experiments

Increasing doses of BAGR and BFOR biochar were compared in pot trials on lettuce (*Lactuca sativa* L.). The aim was to verify the influence on plant growth and evaluate possible positive or negative effects. The tests were carried out in open air (Figure 2) using the soil of the experimental Research Centre for Engineering and Agri-food Transformation of CREA in Monterotondo, Italy (42°05′56.86″ N, 12°37′26.23″ E). The soil had already been characterized for its physical and chemical properties (Table 2).



Figure 2. Pots with biochar dosages (**a**) and plant growth after transplantation (**b**).

Table 2. Chemical and physical properties of the soil used in the tests [85].

Parameters	Values	Unit
Sand	25	%
Silt	38	%
Clay	37	%
pH	7.9	
Organic substance	2.6	%
Total nitrogen	0.16	%
Assimilable phosphorus	14.1	mg kg^{-1}
Exchangeable potassium	365.3	mg kg^{-1}
Cation exchange capacity	31.8	meq 100 g^{-1}

The pots (Ø 16 cm) were filled with 1 kg of previously air-dried soil and sieved with a 2 mm mesh. Four dosages of real application of biochar were compared based on the available bibliography [86]: 5, 10, 20, 30 t ha^{-1}. The corresponding amount of biochar

was added to the soil. The control consisted of soil without biochar. A randomized block experimental design was adopted, with nine treatments (four dosages of BAGR and BFOR biochar plus the control medium for each biochar) and four replicates per treatment. The total number of pots was 40. The codes for the comparison treatments are reported in Table 3.

Table 3. Comparison treatments and identification codes for biochar doses.

Biochar Origin	Quantity (t ha^{-1})	Code
BAGR	5	A5
	10	A10
	20	A20
	30	A30
BFOR	5	F5
	10	F10
	20	F20
	30	F30
Soil		S

Poplar wood chips were placed around the experimental area to prevent damage and contain weed development. Two weeks after preparing the pots, the lettuce seedlings were transplanted at the beginning of April 2025 (stage: 5–6 leaves developed). The test lasted for 36 days (early April-mid May). Five days after transplanting, to promote the growth of lettuce, all the plants were fertilized with a synthetic fertilizer (Jolly 20-20-20, Agribios, Mantova, Italy) at 10 g m^{-2}. Water supply was ensured on a regular basis by drip irrigation to restore the available water capacity when it dropped to around 60% of the maximum level. Figure 2 shows the test setup.

In this experiment and in the subsequent ones, the following parameters were considered:

- morphological (plant height, number of leaves per plant);
- physiological (chlorophyll level, photosynthetic efficiency);
- productive (fresh weight, dry weight);
- emission of CO_2 from the substrate.

Following the experimental design (four replicates per treatment), the plant height, the number of leaves per plant, the fresh and dry weight of the plant, photosynthetic efficiency and CO_2 emission were measured for each plant or pot (in the case of CO_2). The sampling method for chlorophyll levels is described below.

Height was measured on all plants immediately after transplanting and before harvesting. A SPAD reader (SPAD-502, Konica Minolta) was used for chlorophyll level monitoring. Before harvesting, three readings were taken on the 4th or 5th leaf starting from the central bud on each plant. The readings of each leaf were considered as technical replicates of the single plant (biological replicate). SPAD values were converted to chlorophyll content (μg cm^{-2}) using the equation reported by [87]:

$$\text{chlorophyll content } (\mu g\ cm^{-2}) = (99 \times \text{SPAD value})/(144 - \text{SPAD value}) \quad (3)$$

At the same time as the SPAD reading, the photosynthetic efficiency was evaluated using the LI-600 porometer/fluorimeter (LI-COR GmbH, Bad Homburg, Germany) operating under full light conditions. For each plant, one reading was taken on the 4th or 5th leaf starting from the central bud. The following parameters were considered: stomatal conductance (g_{sw}), transpiration (E), leaf vapour pressure deficit (VPD), and operational efficiency of photosystem II (Φ_{PSII}).

At harvest, plants were cut at the base, and their fresh weight was determined. Immediately afterwards, they were placed in an oven at 105 °C for 24 h to determine their dry weight and moisture content. Plants from each treatment were collected and analyzed at the LASER-B laboratory for the determination of micro- and macroelement content. CO_2 emissions from the soil were measured before transplanting and after harvesting. The EGM-5 portable analyzer (PP Systems, Amesbury, MA, USA) was used for the determination, which allows continuous and automatic air sampling and CO_2 measurement. The analyzer was set up for an acquisition time of 90–120 s and a CO_2 limit of 200 ppm.

The meteorological data (minimum, average and maximum temperature and total precipitation) for the test period (March–October 2025) were collected from the Arsial Agrometeorological Service database for the locality of Monterotondo–Grotta Marozza (https://siarl.arsial.it/bi/superset/dashboard/27/, accessed on 20 October 2026) [88]. Figure 3 shows the data for the period. The data are presented by decade.

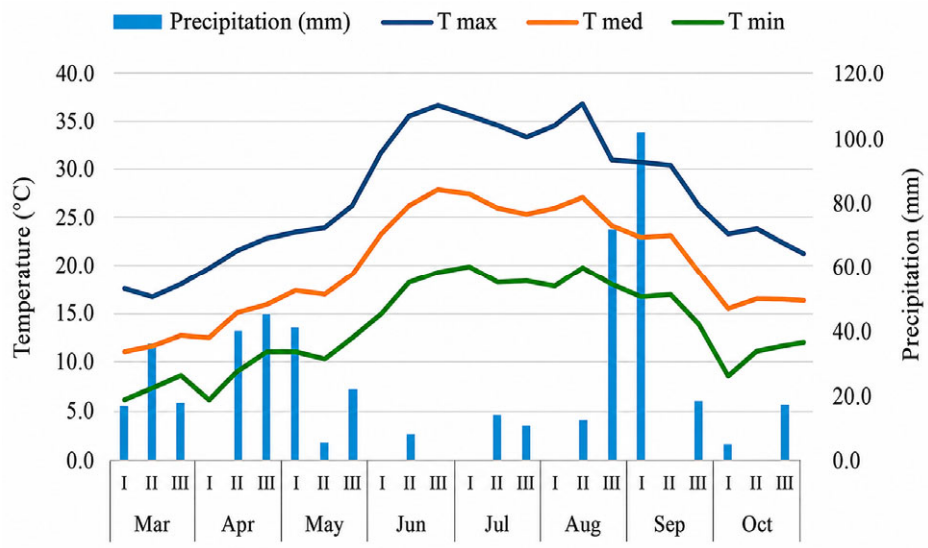


Figure 3. Minimum, average and maximum temperature and total precipitation values (March–October 2025) from the Arsial Agrometeorological Service (Monterotondo-Grotta Marozza) (https://siarl.arsial.it/bi/superset/dashboard/27/, accessed on 20 October 2026) [88].

2.4.3. Combination of Biochar and Organic Fertilizer

The effect of biochar and an organic fertilizer being applied simultaneously was analyzed. The fertilizer used was Organico Plus Bio (Zapi Garden, Conselve, Italy), a pelletized product containing 6% organic nitrogen. The amount of biochar added to the soil corresponded to the maximum amount (30 t ha^{-1}), which was applied in proportion to the surface area of the pot. The choice was based on the results of the previous test for fresh weight and CO_2, as well as the analysis of bibliographic references [86]. At the beginning of August, the pots (Ø 22 cm) were prepared by adding the corresponding doses of the two types of biochar (BAGR and BFOR), where required (Table 4). Transplantation of lettuce seedlings (stage: 5–6 developed leaves) was carried out in early September. Before transplantation, organic fertilizer was incorporated into the soil at a dose of 0.4 kg m^{-2} by applying the amount proportional to the surface area of the vessel. The experiment lasted 33 days (early September–early October). Water supply was ensured on a regular basis by drip irrigation to ensure that the field capacity of the ground was restored. A randomized block experimental design was adopted, with six treatments and four replicates per treatment (24 pots).

Table 4. Comparison of treatments and identification codes in the study of biochar and an organic fertilizser application.

Biochar Origin	Code
Soil with Fertilization	F
Soil with Fertilization and application of AGR biochar	F + BAGR
Soil with Fertilization and application of FOR biochar	F + BFOR
Soil with Application of AGR biochar	BAGR
Soil with Application of FOR biochar	BFOR
Soil	S

The surveys carried out after the transplant were similar to those described for the second-phase test in terms of type and modality. These included morphological, physiological and productive surveys as well as CO_2 emissions from the substrate.

2.4.4. Statistical Analysis

Statistical analyses were conducted using PAST software [89], version 3.22 (2018, Oyvind Hammer, University of Oslo, Norway, https://www.nhm.uio.no/english/research/resources/past/, accessed on 5 August 2026). The differences in biochar composition between BFOR and BAGR were tested using Student's *t*-test. All the data relating to the pot study were checked for normality using the Shapiro–Wilk test and for homogeneity of variances using the Levene test. When both normality and homoscedasticity were met, the data were subjected to one-way analysis of variance, [89]. Significantly different means were separated via Tukey's HSD post hoc test. For data deviating from normality, the Kruskal–Wallis test for non-parametric ANOVA and the Dunn's test for mean separation were used. When Levene's test was significant, meaning the presence of unequal variances, the unequal-variance (Welch test) version of ANOVA was used.

3. Results and Discussion

3.1. Biomass Characterization

The elemental composition of the starting biomasses showed comparable values between olive pruning residues and poplar biomass. Some differences were observed among the analyzed parameters, particularly in terms of ash and hydrogen content, as indicated in Table 5. The higher heating value (HHV) was slightly higher for poplar than for olive, whereas only limited differences were observed in the lower heating value (LHV). Poplar also exhibited a higher ash content, and the corresponding ash melting temperature was approximately 5% lower than that measured for olive biomass. However, this information was not particularly critical for energy conversion activities since the operating temperatures are much lower than those determined for ash fusibility.

Table 5. Physicochemical characterization of starter biomass.

Parameters	Poplar *	Olive *
Moisture %	42.2	46.4
Ash %	4.1	1.8
C %	47.7	40.3
H %	6.1	1.9
N %	0.6	1.4
HHV MJ/kg	19.0	17.4
LHV MJ/kg	17.6	17.2
Ash melting pot (°C)	1335	1416

* Values of starter biomass from Gallucci et al. [90].

The results relating to the metal content of biomass are reported in Table 6 and revealed some differences between the two types. In particular, the olive pruning appeared to be rich in macronutrients compared with poplar biomass, especially for potassium (K), calcium (Ca), magnesium (Mg), and sodium (Na) and had notable concentrations of iron (Fe) and zinc (Zn), whereas a greater presence of cadmium (Cd) and arsenic (As) was observed in the poplar.

Table 6. Metal content (mg kg^{-1}) in starter biomass.

Metals [mg kg^{-1}]	Poplar	Olive
Na	5.50	250.73
K	482.79	2543.00
Mg	106.27	737.79
Ca	1210.04	3253.9
Fe	7.37	546.73
Zn	2.66	11.6
Cu	0.31	0.67
As	0.01	<LOQ
Cd	0.03	0.02
Co	0.003	0.39
Cr	<LOQ	0.87
Ni	0.05	0.56
Pb	0.02	0.06

3.2. Biochar Characterization

The main process outputs obtained during the pyrolysis of olive pruning and poplar residues were evaluated in terms of biochar yield, syngas yield, and the lower heating value (LHV) of the produced syngas. Poplar residues resulted in a biochar yield of 31.7% and a syngas yield of 37.2%, with a syngas LHV of 6.7 MJ Nm^{-3}. In comparison, olive pruning produced a higher biochar yield of 41.9% and a slightly lower syngas yield of 33.8%, while the syngas exhibited a higher LHV of 7.3 MJ Nm^{-3}. These results provide an overview of how the biomass was distributed among the main process products and indicate how differences in the feedstock type influenced the potential energy recovery from the syngas fraction. The two types of biochar differed in their chemical composition (Table 7), reflecting the intrinsic characteristics of the original feedstocks. Both types of biochar showed slightly alkaline pH values; however, BFOR had a higher pH than BAGR. This difference may be due to the different composition of the inorganic fraction (Tables 8 and 9). BFOR showed significantly higher Mg and Ca content than BAGR, whereas BAGR had higher concentrations of Na, Fe, Zn and Cu. The higher abundance of alkaline and alkaline earth elements in BFOR may therefore have contributed to its slightly higher pH. The ash content in the BAGR was almost two and a half times that of the BFOR, while in the latter the percentage of fixed carbon (FC) and carbon was significantly higher. Crespo-Barreiro et al. [82] reported carbon contents ranging from 81.63% to 84.08% and fixed carbon contents from 62.47% to 70.88% for olive-pruning biochar produced at 400–600 °C. In comparison, the BAGR produced in the present study showed a carbon content of 79.9% and a fixed carbon content of 64.69%, within the range reported by these authors. The nitrogen content was found to be quite high. The C/N ratio was 49.8 in BFOR and 46.0 in BAGR. These values were lower than those reported for some poplar-derived biochar [13,14]. Ding et al. [91] reported C/N ratios of 60.89–66.06 for poplar twig biochar produced at 300, 500 and 700 °C, whereas considerably higher values, ranging from 87.71 to 95.58, were reported for poplar bark biochar under the same temperature conditions. The lower C/N ratios observed in the present

study may be related to the relatively high nitrogen content of BFOR and BAGR (2.3% and 1.9%, respectively), as well as to differences in thermochemical conversion conditions.

Table 7. Physicochemical parameters (mean ± st. dev.) of biochar produced from agricultural (BAGR) and forestry (BFOR) residues (n = 3).

Parameters	BFOR	BAGR	Significance
Moisture %	3.4 ± 0.4	5.0 ± 0.3	**
Ash %	6.2 ± 3.4	14.8 ± 2.7	*
pH	7.5 ± 0.1	7.2 ± 0.1	**
VM %	12.1 ± 3.0	15.6 ± 2.1	ns
FC %	78.8 ± 4.8	64.9 ± 3.5	*
C %	92.0 ± 3.1	79.9 ± 6.8	*
H %	0.6 ± 0.1	0.7 ± 0.2	ns
N %	2.3 ± 1.3	1.9 ± 0.6	ns
C/N	49.8 ± 23.4	46.0 ± 13.8	ns

** Significant for $p \leq 0.01$, * significant for $p \leq 0.05$, ns not significant (after Student's test).

Table 8. Mean (±st. dev.) of macroelement content (g kg^{-1}) in biochar produced from agricultural (BAGR) and forestry (BFOR) residues (n = 3).

Elements (g kg^{-1})	BFOR	BAGR	Significance
Na	0.853 ± 0.099	1.181 ± 0.106	*
K	13.946 ± 2.343	13.517 ± 1.105	ns
P	0.946 ± 0.107	0.987 ± 0.521	ns
Mg	4.818 ± 0.509	3.605 ± 0.309	*
Ca	8.212 ± 1.167	5.798 ± 0.469	*
Fe	6.157 ± 0.837	22.986 ± 1.916	**

** significant at $p \leq 0.01$, * significant at $p \leq 0.05$, ns not significant (following Student's *t*-test).

Table 9. Mean (±st. dev.) of microelement content (mg kg^{-1}) in biochar produced from agricultural (BAGR) and forestry (BFOR) residues (n = 3).

Elements (mg kg^{-1})	BFOR (mg kg^{-1})	BAGR (mg kg^{-1})	Significance	IBI Guideline [a] (mg kg^{-1})	EBC-AgroBio [b] (mg kg^{-1})
As	0.853 ± 0.204	0.667 ± 0.184	ns	13–100	13
Cd	1.222 ± 0.332	<LOQ		1.4–39	0.7–1.5
Cr	8.018 ± 2.618	7.058 ± 0.704	ns	93–1200	—
Co	4.541 ± 1.285	<LOQ		34–100	—
Cu	21.172 ± 2.627	147.383 ± 11.854	***	143–1600	70–100
Ni	6.733 ± 2.942	6.349 ± 0.676	ns	47–600	25–50
Pb	5.172 ± 2.990	2.598 ± 0.319	ns	121–300	45–150
Zn	292.407 ± 41.765	1249.968 ± 100.195	**	416–7400	200–400

*** Significant at $p \leq 0.001$, ** Significant at $p \leq 0.01$, ns not significant (after Student's test). [a] IBI International Biochar Initiative (IBI) Biochar Standards. [b] EBC-AgroBio European Biochar Certificate (EBC) guidelines.

The content of metals (Tables 8 and 9) showed a marked enrichment of inorganic elements compared to the starting biomasses, due to the reduction in the organic fraction during pyrolysis. In both samples, the macro-elements K, Fe and Ca were predominant, with concentrations of the order of g/kg. These concentrations are within the ranges reported for biochar derived from woody residues. Ding et al. [91], for example, reported K concentrations of 9.20–14.68 g kg^{-1} in biochar produced from poplar residues at 300–700 °C. The Na, Fe, Cu and Zn content of BAGR was statistically higher than that of BFOR, consistent with the higher initial levels observed in the biomass. In contrast, BFOR had significantly higher concentrations of Mg and Ca. Overall, the results suggest that

the metallic composition of biochar primarily reflects that of the starting biomass, with differences attributable to the nature of the plant material rather than operating conditions. The results obtained were compared with the reference values and maximum permissible thresholds reported by the Biochar standards of the International Biochar Initiative (IBI) [92] and with the threshold values established by the European Biochar Certificate (EBC) [93] for AgroBio applications. According to IBI guidelines, the concentrations of As, Cd, Cr, Co, Ni and Pb in both biochars were below the corresponding ranges. In particular, As, Cr, Co, Ni and Pb occurred at concentrations significantly below the limits of their respective IBI ranges. Cu and Zn concentrations were below the lower bound of the IBI guideline range in BFOR, whereas BAGR values fell within the respective IBI ranges. Conversely, comparing the results with the EBC-AgroBio threshold values, it emerged that the Cu concentration in BAGR, at 147.0 mg kg^{-1}, exceeded its reference value for EBC-AgroBio. For Zn, BFOR (292.0 mg kg^{-1}) remained within the EBC-AgroBio reference interval (200–400 mg kg^{-1}), whereas BAGR (1250.0 mg kg^{-1}) exceeded the upper reference value. Therefore, while all analyzed elements remained within the IBI guideline ranges, a comparison with the more restrictive EBC-AgroBio reference values identified Cu and Zn as elements requiring particular attention in BAGR. The concentrations of metals were further assessed in relation to the requirements set out in Regulation (EU) 2019/1009 [94], for the assessment of the potential use of biochar as a pyrolysis and gasification material recognized as an organic soil improver. According to the Regulation, contaminants must not exceed 1.5 mg kg^{-1} for Cd, 50 mg kg^{-1} for Ni, 120 mg kg^{-1} for Pb and 40 mg kg^{-1} for inorganic As, while Cu and Zn must not exceed 300 and 800 mg kg^{-1}, respectively. Biochar in the present study had lower values than the statutory limits, except for Zn in BAGR, which showed a concentration of 1250 mg kg^{-1}. This result is consistent with the higher Zn concentration observed in the olive-derived feedstock and further supports the need to consider feedstock characteristics when evaluating the elemental composition of biochar. Working in pruning residues of vineyards, 15 different pesticides used in conventional cultivation were found by Spinelli et al. (2012) [95]. Of these, copper had the highest concentration, though still below the legal limit. These differences may affect the elemental composition of the starting biomass and, consequently, the concentration and distribution of mineral elements in the resulting biochar. Moreover, the different compliance patterns observed according to the IBI and EBC-AgroBio reference values highlight the need to carefully consider both the specific regulatory framework and the intended application when assessing the suitability of each biochar for agricultural use.

3.3. Germination Index (Phase 1)

The aqueous extracts of BAGR or BFOR (w/v) influenced the germination capacity of pea, tomato, sorghum and sunflower seeds. Overall, pea and tomato showed a statistically higher germination percentage than sorghum and sunflower seeds (Figure 4). Regarding the effect of aqueous extracts, a slight positive effect of the BFOR extract was observed in sunflowers, tomatoes and pea compared with the control, while a negative effect was observed for the BAGR extract (Figure 5). This difference assumed statistical significance only for tomato seeds. Pea seeds germinated almost completely regardless of the solution used.

The germination index (GI) showed varying results for the species under study (Figure 6). GI values below 50% indicate high phytotoxicity, values between 50% and 80% indicate moderate phytotoxicity, while values above 80% indicate no phytotoxicity. When the index exceeds 100%, the substrate, whether it be compost, frass or biochar [81,83,96], can be considered a phytonutrient or a phytostimulant [81,83,96]. In this study, a negative effect of the BAGR extract was observed. In the case of sunflowers and tomatoes, this appeared to be associated with phytotoxicity. In all other cases, the GI value was higher

than 80% (absence of phytotoxicity), while the presence of aqueous extracts from BFOR has always recorded values higher than 100%, acting, therefore, as a phytostimulant. The consistently high GI values observed for BFOR indicate a favourable short-term biological response under the experimental conditions tested.

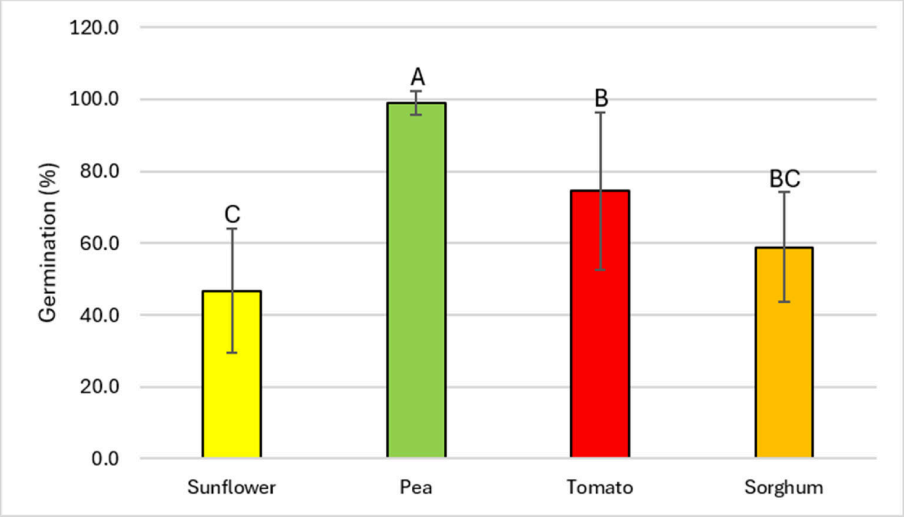


Figure 4. Germination percentages observed for the four species studied. Mean values from nine Petri dishes. The bars indicate the standard deviation. Different letters indicate significant differences after Tukey's test (HSD) for $p < 0.01$.

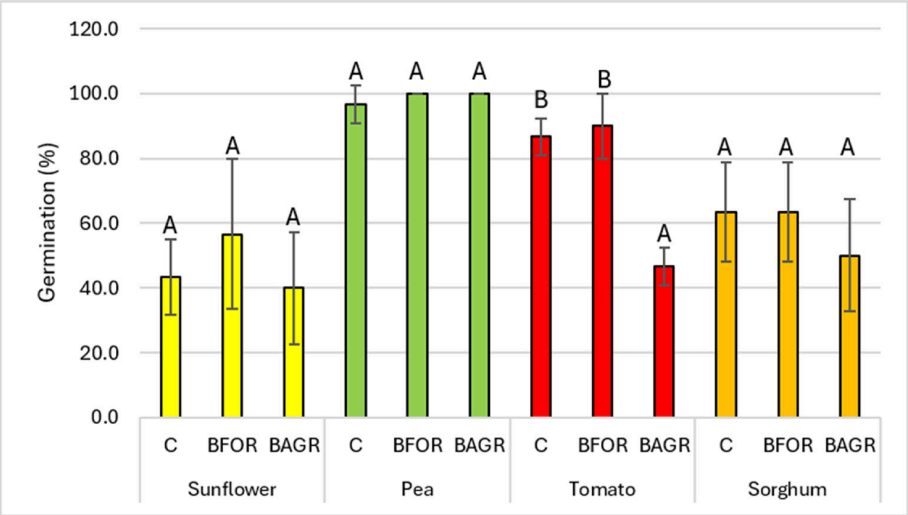


Figure 5. Germination percentages detected on sunflower, pea, tomato and sorghum seeds for aqueous extracts produced by BAGR and BFOR compared with the control (water) after four days. Mean values from three Petri dishes. The bars indicate the standard deviation. For each species, different letters indicate significant differences after Tukey's test (HSD) for $p < 0.01$.

Germination assays have demonstrated that the agronomic suitability of biochar must be carefully evaluated prior to its application in agricultural systems. Previous studies have reported both biostimulant [97] and phytotoxic effects, with the observed responses being strongly influenced by production conditions (including pyrolysis temperature, residence time, and reactor configuration), and the physicochemical characteristics of the original feedstock. These factors determine the composition of water-soluble organic and inorganic compounds released from biochar, which can significantly affect seed germination and early seedling development [98,99]. Rogovska et al. [99] evaluated the aqueous extracts of

six biochars produced from different feedstocks under different thermochemical conditions. The authors observed that diluting the biochar extracts reduced both the pH level and the concentration of inorganic constituents in the extracts, thereby mitigating their inhibitory effects on seed germination. In the same study, Rogovska et al. [99] found that, although the extracts did not significantly impact the final percentage of maize germination, those from three biochars produced at high conversion temperatures significantly reduced shoot growth by an average of 16% compared to deionized water. Furthermore, a corn-residue extract was found to reduce radicle growth by 94%. Conversely, incubation of the extract with leached biochar increased radicle length six to twelvefold. This suggests that biochar may also reduce the phytotoxicity of soluble compounds.

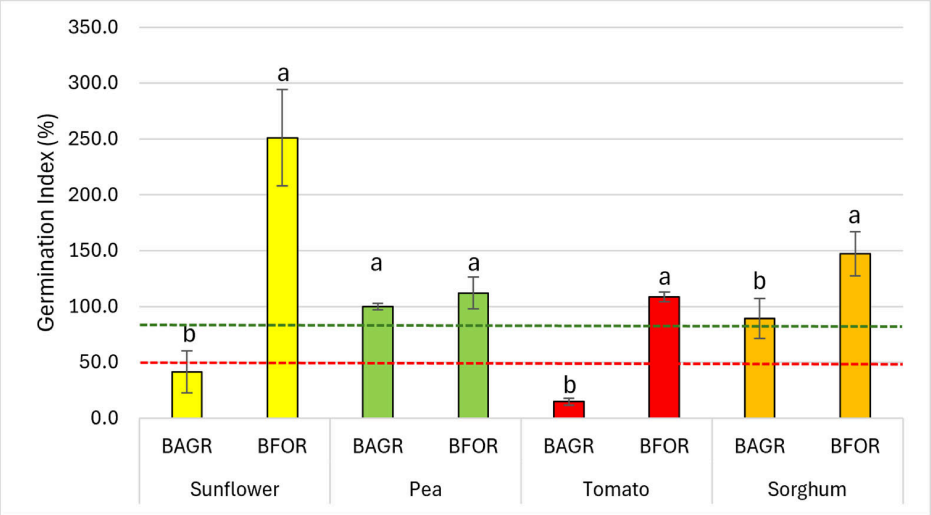


Figure 6. Germination index detected on sunflower, pea, tomato and sorghum seeds for aqueous extracts produced by BAGR and BFOR after four days. The red dotted line indicates the 50% level, and the green dotted line the 80% level. Mean values from three Petri dishes. The bars indicate the standard deviation. For each species, different letters for BAGR and BFOR indicate significant differences.

From a methodological perspective, aqueous extracts provide a simplified but effective simulation of the interaction between seeds and the soil solution during the earliest stages of plant establishment. Consequently, germination assays based on aqueous extracts represent a rapid, reproducible, and cost-effective screening tool for distinguishing the biological responses induced by different biochar types across plant species. The results obtained in the present study further support the usefulness of this approach, highlighting its ability to discriminate feedstock-dependent effects while identifying potential phytostimulatory or phytotoxic responses prior to more time-consuming greenhouse or field evaluations. With regard to the substrates used and the experimental conditions employed in this study, the consistently high GI observed for BFOR, together with the absence of phytotoxic effects across all tested species, made it plausible that biochar produced from poplar pruning using the adopted pyrolysis process had favourable biological properties. In contrast, the more variable response observed for BAGR confirmed the importance of feedstock characteristics in determining the biological quality of biochar.

3.4. Pot Experiment on Lettuce with BFOR and BAGR (Phase 2)

3.4.1. Photosynthetic Parameters (Phase 2)

As regards photosynthetic parameters, no significant differences were observed between the substrates, regardless of individual dosages (Table 10). The chlorophyll content was found to be almost similar, as were the transpiration rate and the leaf vapour pressure

deficit. However, it should be noted that there is a slight reduction in stomatal conductance in the presence of biochar compared to soil.

Table 10. Photosynthetic parameters (mean ± st. dev.) for the analyzed substrates (n = 8 for soil and n = 16 for each biochar).

Parameters	Substrate		
	S	**S + BAGR**	**S + BFOR**
Chlorophyll (μg cm^{-2})	15.2 ± 2.8	15.3 ± 2.7	15.3 ± 3.2
g_{sw} (mol m^{-2} s^{-1})	0.304 ± 0.275	0.217 ± 0.135	0.250 ± 0.0147
E (mmol m^{-2} s^{-1})	2.963 ± 1.816	2.377 ± 1.246	2.734 ± 1.366
VPD (kPa)	1.206 ± 0.133	1.248 ± 0.137	1.224 ± 0.081
Φ_{PSII}	0.671 ± 0.057	0.671 ± 0.050	0.690 ± 0.061

Significant differences were only observed for BAGR regarding individual biochar dosages (Table 11). Overall, a certain correspondence was found between stomatal conductance and transpiration for both types of biochar. This trend is expected under conditions of water availability. From a physiological point of view, the two parameters were found to be correlated [100,101]. Statistical significance was reached for both parameters; for the BAGR, the values were very low at dose A5, while they increased significantly at A10. Quantum efficiency (Φ_{PSII}) is a measure of the efficiency with which light is used in photosynthesis. Under the experimental conditions studied, this parameter was found to be higher in the presence of the highest dose of biochar (from both agricultural and forestry sources), though this did not reach statistical significance. Regarding the BAGR, an increasing trend in quantum efficiency was observed, from A5 to A30.

Table 11. Photosynthetic parameters (mean ± st. dev.) for analyzed substrates by type and dosage of biochar (n = 4). For each substrate type, where reported, different letters indicate significant differences after Tukey's test (HSD) for $p < 0.05$.

Substrate	Chlorophyll (μg cm^{-2})	g_{sw} (mol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})	VPD (kPa)	Φ_{PSII}
A5	15.4 ± 3.9	0.085 ± 0.024 b	1.194 ± 0.366 b	1.425 ± 0.112 a	0.642 ± 0.064
A10	14.6 ± 2.5	0.353 ± 0.125 a	3.584 ± 1.173 a	1.145 ± 0.078 b	0.652 ± 0.031
A20	16.3 ± 1.6	0.187 ± 0.124 ab	2.002± 0.991 ab	1.201 ± 0.106 b	0.678 ± 0.054
A30	15.1 ± 3.3	0.242 ± 0.101 ab	2.728 ± 1.057 ab	1.222 ± 0.061 b	0.712 ± 0.027
F5	17.0 ± 3.9	0.273 ± 0.244	2.881 ± 2.116	1.252 ± 0.114	0.705 ± 0.014
F10	15.3 ± 2.4	0.207 ± 0.093	2.440 ± 1.128	1.244 ± 0.084	0.678 ± 0.044
F20	12.3 ± 2.1	0.184 ± 0.097	2.109 ± 1.008	1.241 ± 0.070	0.648 ± 0.105
F30	16.4 ± 3.0	0.336 ± 0.109	3.507 ± 1.049	1.161 ± 0.031	0.729 ± 0.024

3.4.2. Morpho-Productive Data (Phase 2)

From a statistical point of view, no significant differences emerged for the parameters reported in Table 12. However, some underlying trends can be observed. At harvest, the moisture content of the plants was around 90%. Overall, across all doses, the presence of biochar resulted in a slight increase in fresh weight and dry weight compared to soil without biochar.

The fresh weight was higher in the presence of dosages corresponding to 10 and 30 t ha^{-1} of BAGR, while the highest BFOR values were obtained at 5 and 30 t ha^{-1} (Table 13). No statistically significant differences were found.

Table 12. Mean (±st. dev.) of plant height, fresh weight, dry weight and moisture of lettuce plants grown on the analyzed substrates (n = 8 for soil and n = 16 for each biochar).

Parameters	Substrate		
	S	**S + BAGR**	**S + BFOR**
Plant height (cm)	16.4 ± 1.2	17.1 ± 1.0	17.1 ± 0.8
Fresh weight (g)	47.3 ± 5.1	47.8 ± 10.3	48.1 ± 10.4
Moisture content (%)	90.4 ± 0.8	89.6 ± 1.1	90.2 ± 0.8
Dry weight (g)	4.5 ± 0.6	4.9 ± 1.1	4.7 ± 1.1

Table 13. Mean (±st. dev.) of plant height, fresh weight, dry weight and moisture of lettuce plants grown on the analyzed substrates, by type and dosage of biochar (n = 4).

Substrate	Plant Height	Fresh Weight	Moisture	Dry Weight	
	(cm)	**(g)**	**(%)**	**(g)**	**(%)**
A5	17.6 ± 0.5	44.5 ± 15.7	89.1 ± 1.6	4.7 ± 1.2	10.9 ± 1.6
A10	16.4 ± 1.1	50.2 ± 7.8	90.5 ± 0.7	4.8 ± 0.9	9.5 ± 0.7
A20	17.3 ± 1.3	47.6 ± 5.5	89.3 ± 0.8	5.1 ± 0.7	10. 7 ± 0.8
A30	17.1 ± 0.9	49.0 ± 12.9	89.4 ± 0.7	5.2 ± 1.6	10.6 ± 0.7
F5	17.6 ± 0.5	49.1 ± 17.3	90.4 ± 0.5	4.8 ± 1.8	9.6 ± 0.5
F10	17.0 ± 0.9	45.4 ± 8.6	90.2 ± 0.6	4.5 ± 1.0	9.8 ± 0.6
F20	16.8 ± 0.9	45.7 ± 7.3	90.0 ± 1.1	4.6 ± 0.7	10.0 ± 1.1
F30	17.0 ± 0.8	52.1 ± 8.9	90.1 ± 0.5	5.1 ± 0.9	9.9 ± 1.3

The limited differences observed in the morpho-productive parameters should also be interpreted considering the intrinsic stability of biochar in soil. Owing to its highly condensed aromatic structure, biochar is characterized by a slow degradation rate and can persist in soils for decades, although the carbon it contains is not completely inert and may be gradually mineralized through both biotic and abiotic processes [6]. Consequently, short-term pot experiments involving fast-growing crops primarily capture the immediate effects of biochar incorporation, providing valuable information on crop compatibility and the initial agronomic response, but they cannot fully represent the long-term interactions occurring between biochar, soil and plants under field conditions. As summarized by Nogués et al. [86], biochar degradation in soil is generally described by a double-exponential model, characterized by an initial rapid decomposition of the labile carbon fraction followed by a much slower oxidation of the recalcitrant aromatic structures. Accordingly, the effects of biochar on plant growth and soil functioning are expected to evolve over time as the different carbon pools progressively interact with the soil environment.

Furthermore, biochar incorporation may alter the mineralization dynamics of native soil organic matter through mechanisms that can either stimulate or suppress microbial activity. The magnitude and direction of these responses depend on several factors, including feedstock characteristics, thermochemical production conditions, soil properties, climatic conditions and the composition of native soil organic matter. Processes such as the adsorption of organic compounds within the porous biochar matrix or the preferential utilization of biochar-derived dissolved organic carbon by soil microorganisms may reduce the mineralization of native organic matter, whereas other mechanisms may promote microbial activity and nutrient turnover. These complex interactions may partly explain the relatively modest short-term responses observed in the present study and further emphasize the importance of long-term field investigations to fully assess the agronomic benefits of biochar under realistic management conditions. Therefore, the present results should be regarded as an assessment of the short-term agronomic compatibility of a fast-growing species when cultivated in the presence of biochar produced under pilot-scale pyrolysis

conditions. Its long-term effects on soil fertility, carbon sequestration, and crop productivity require further validation through multi-season field experiments.

3.4.3. CO_2 from Substrates (Phase 2)

Soil respiration is commonly estimated as the flux of CO_2 emitted from the soil surface. It represents the sum of the CO_2 produced by root respiration and the heterotrophic decomposition of root exudates, soil organic matter, and plant residues. For these reasons, the emitted CO_2 can have different physiological, microbiological and environmental meanings [102,103]. Decomposition processes are the result of complex interactions between soil fauna, fungi, actinomycetes and bacteria. Primarily, gas evolution occurs because of soil microbiome activity that metabolizes complex molecules to low molecular weight substances, which are then oxidized to CO_2 to produce energy or used to provide carbon for cell growth [103].

Pre-transplanting analysis

The incorporation of biochar was carried out fifteen days before lettuce transplanting to stabilize the substrates. The pots were irrigated until the lettuce transplantation to promote microbial activity. Climate trends, with progressively increasing temperatures (Figure 3), have further favoured microbiome metabolism. Indeed, the pre-transplanting survey showed different CO_2 emissions for the three substrates analyzed without distinguishing the biochar dosages (Figure 7). The substrate containing the BFOR was found to have the highest emission, with a statistically significant difference compared to the corresponding substrate containing the BAGR. The average CO_2 emitted by soil without biochar incorporation was intermediate compared to the other two substrates added with biochar. Regarding the different doses of biochar (Figure 8), the A10 and A30 dosages of the BAGR recorded the highest emission values. For BFOR, CO_2 emissions were quite similar except for the F20 treatment, which had the lowest emission rate. In no case was the difference statistically significant.

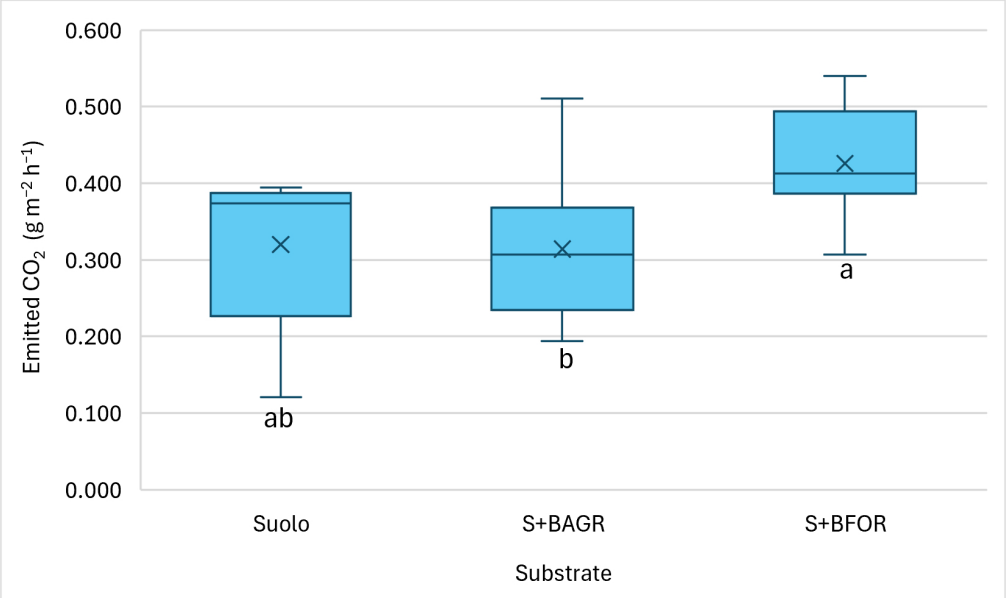


Figure 7. CO_2 emitted (g m^{-2} h^{-1}) for the three analyzed substrates in pre-transplant (n = 8 for soil and n = 16 for each biochar). Different letters below the boxes indicate significant differences after Tukey's test (HSD) for $p < 0.05$. 'x' represents the mean value and the horizontal line represents the median value.

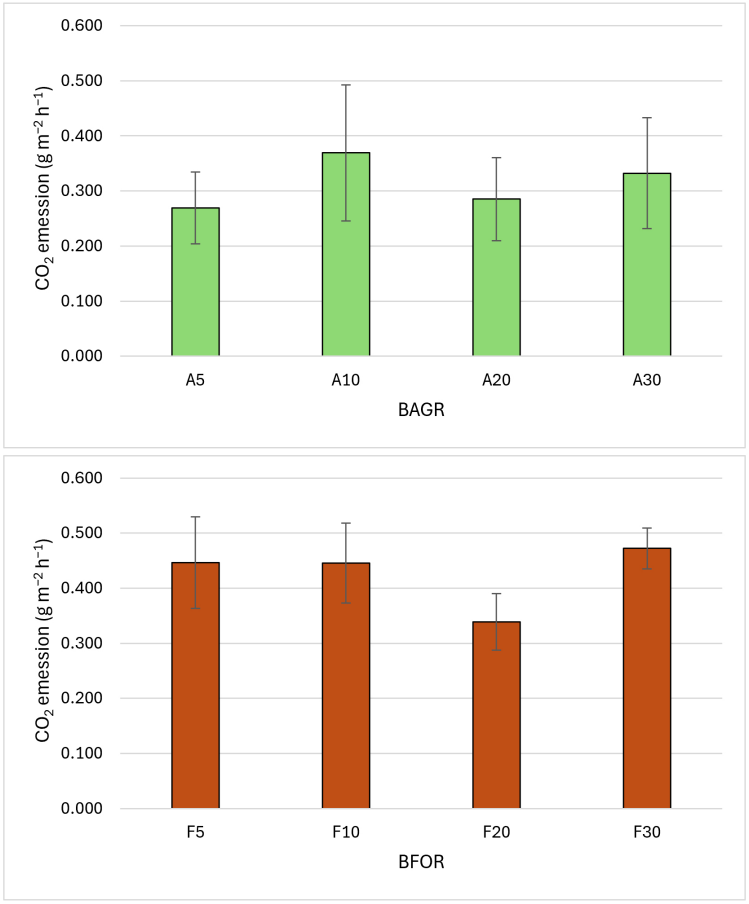


Figure 8. CO_2 emitted (g m^{-2} h^{-1}) from the four analyzed substrates in pre-transplant, distinguishing the application doses for BAGR (**top**) and BFOR (**bottom**). The bars indicate the standard deviation (n = 4).

Post-harvest analysis

The second survey on CO_2 emissions was carried out at the end of June, around one month after the harvest. As with the first pre-transplant survey, irrigation was not suspended. Contrary to the pre-transplanting observations, after one cultivation cycle, the CO_2 fluxes among the substrates became more homogeneous, resulting in statistically non-significant differences in mean values (Figure 9).

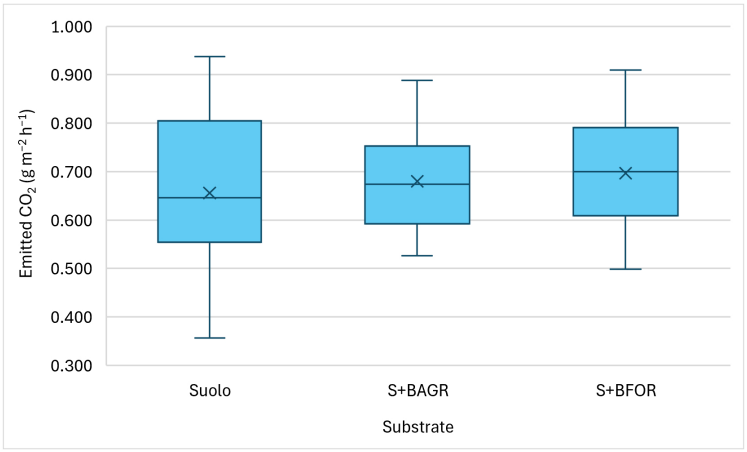


Figure 9. CO_2 emitted (g m^{-2} h^{-1}) for the three analyzed substrates in post-harvest without distinction of application doses (n = 8 for soil and n = 16 for each biochar). 'x' represents the mean value and the horizontal line represents the median value.

However, it should be noted that from the pre-transplanting period to the post-harvest survey, when the soils were free from plants, an increase in emission was recorded (Table 14 and Figure 10). Notably, the largest increase in emissions (higher than 100%) was observed in soil without biochar incorporation and in soil incorporating BAGR. For the BFOR substrate, the increase was found to be just over 60%.

Table 14. ANOVA results on the survey timing and the analyzed substrate related to CO_2 emission.

Source of Variation	GdL	F	*p*
Timing (T)	1	136.60	<0.0001
Substrate (S)	2	2.44	0.096
T × S	2	1.26	0.292

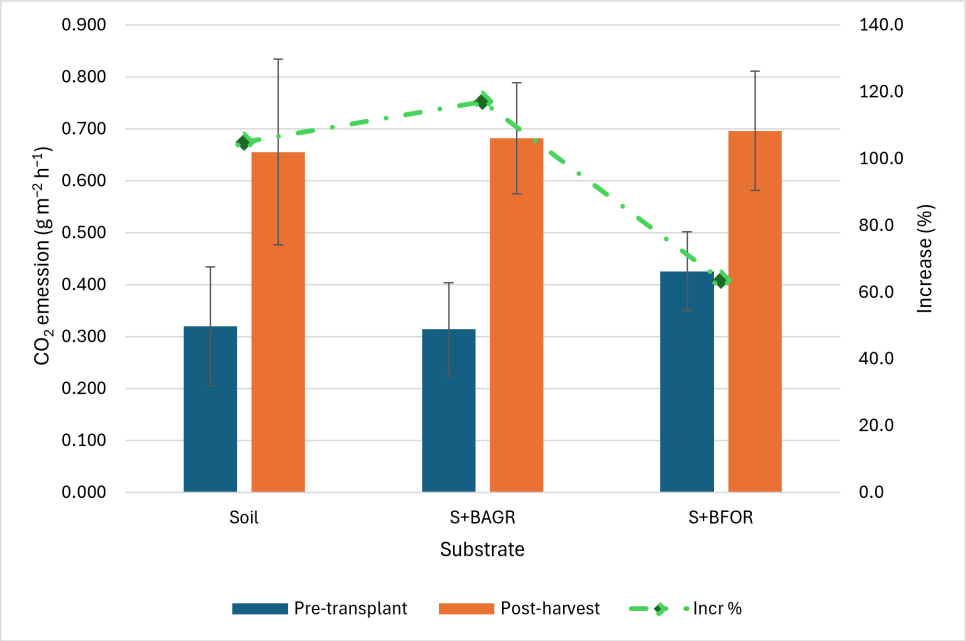


Figure 10. Timing × Substrate interaction values of CO_2 emitted (g m^{-2} h^{-1}) from the three substrates (p = 0.292, not significant). For CO_2 emission, n = 8 for soil and n = 16 for each biochar. The percentage increase (Incr %) between the two significant timings is reported.

The trend observed for single doses was similar to that observed for pre-transplantation with respect to BAGR (higher emissions for A5, A10 and A30). For BFOR, the highest emissions were observed for the F5 and F30 applications (Figure 11). The different magnitude of the increase in CO_2 emissions observed among the substrates between the pre-transplanting and post-harvest measurements is consistent with previous findings showing that the response can vary depending on feedstock characteristics, pyrolysis conditions and soil properties. Ding et al. [91] reported that, after 180 days of incubation, cumulative CO_2 emissions from soils amended with poplar-residue biochar were 33.1–73.8% lower than those measured following the addition of the corresponding untreated biomass residues.

The observed changes in soil CO_2 emissions highlighted the complexity of the processes involved following biochar incorporation into soil. Biochar can simultaneously influence carbon availability, nutrient retention and availability, soil organic matter dynamics and microbial activity, generating interactions that cannot be interpreted through a single mechanism. Consequently, soil respiration should be regarded as the integrated outcome of microbial metabolism, root respiration and organic matter turnover rather than as a simple indicator of carbon mineralization.

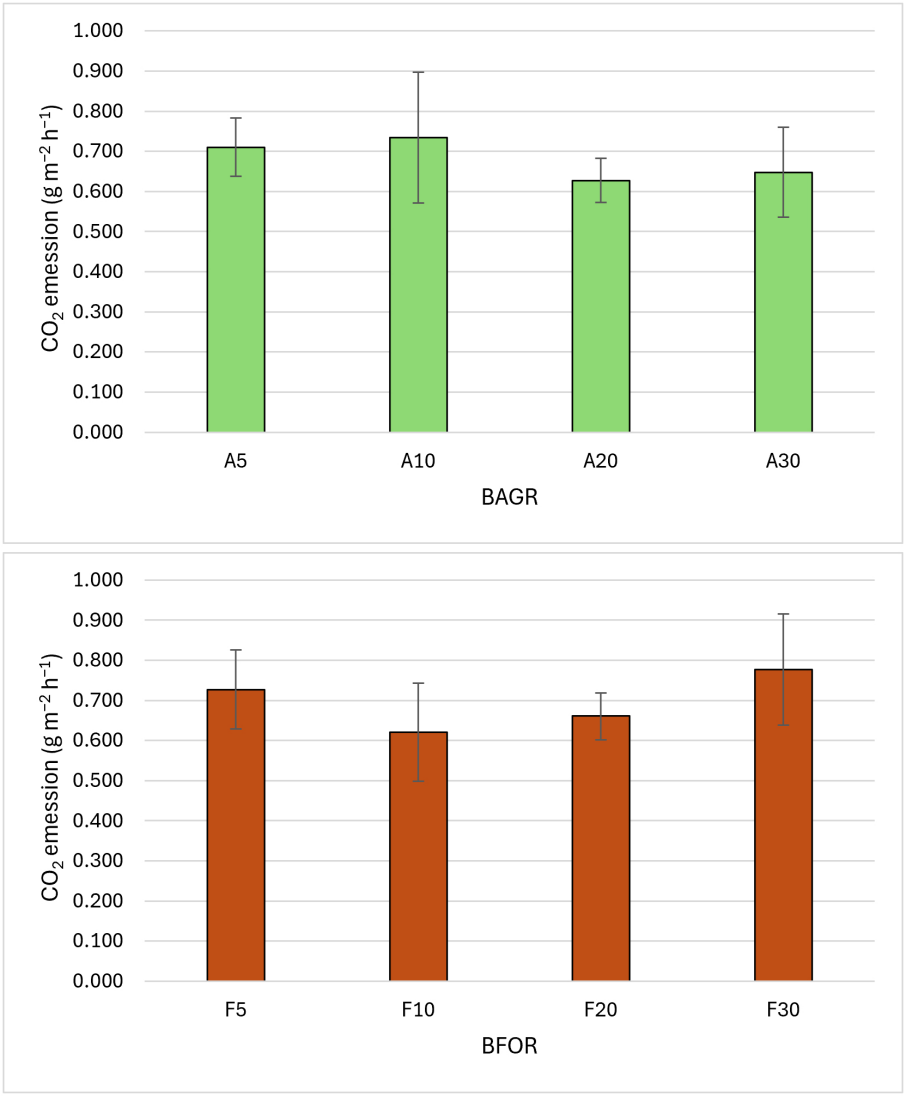


Figure 11. CO_2 emitted (g m^{-2} h^{-1}) for the three analyzed substrates in post-harvest, distinguishing application doses for BAGR (**top**) and BFOR (**bottom**). The bars indicate the standard deviation (n = 4).

The results obtained in the present study indicate that monitoring soil CO_2 fluxes provides useful information on the short-term biological response of biochar-amended soils and can give indications about the effects of biochar incorporation on both soil microbial activity and root respiration. Nevertheless, the interpretation of these measurements requires careful consideration of several environmental and methodological factors known to affect soil respiration. These factors include soil and air temperature, soil moisture, spatial heterogeneity of the sampling area and the proper operation of the measurement system. Consequently, a robust assessment of biochar effects on soil carbon dynamics should be based on repeated measurements performed under different environmental conditions and supported by sufficiently large datasets capable of capturing the temporal and spatial variability of soil biological processes. Overall, these findings indicate that soil respiration measurements, when interpreted together with plant physiological and agronomic responses, provide a useful framework for evaluating the short-term effects of biochar on soil biological functioning and may support future long-term field investigations.

3.5. Combination of Biochar and Organic Fertilizer (Phase 3)

3.5.1. Photosynthetic Parameters (Phase 3)

The most obvious trend, although not statistically significant, was shown by the chlorophyll content (Figure 12). The value decreased from the substrate with fertilizer only (20.77 μg cm^{-2}) to 18–19 μg cm^{-2} in substrates with fertilizer and biochar, to approximately 17 μg cm^{-2} in substrates with only biochar, and to 15.55 μg cm^{-2} for soil without any addition. However, although no significant differences were observed, the photosynthetic parameter data gave some interesting indications (Table 15). The integration of fertilization and biochar (F + BAGR and F + BFOR) resulted in tendentially higher stomatal conductance and transpiration values than fertilization alone or biochar application without fertilization (Table 15). Similarly, quantum efficiency was found to be higher for the F + BAGR and F + BFOR substrates.

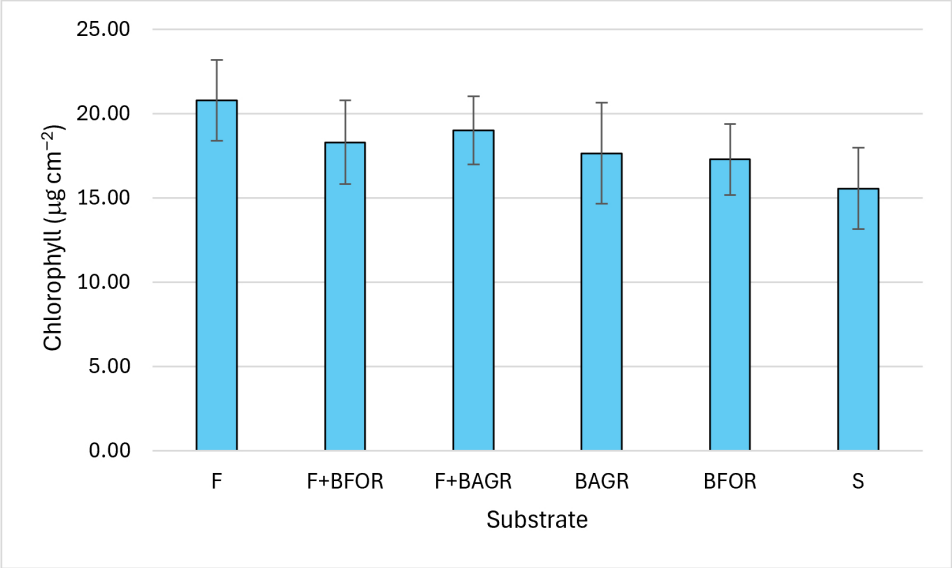


Figure 12. Chlorophyll content (μg m^{-2}) detected for the analyzed substrates. The bars indicate the standard deviation (n = 4).

Table 15. Photosynthetic parameters (mean ± st. dev.) detected for the analyzed substrates (n = 4).

Treatment	gsw (mol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})	VPD (kPa)	ΦPSII
F	0.117 ± 0.077	1.258 ± 0.720	1.178 ± 0.129	0.687 ± 0.043
F + BFOR	0.184 ± 0.035	1.904 ± 0.220	1.119 ± 0.113	0.700 ± 0.019
F + BAGR	0.162 ± 0.093	1.682 ± 0.875	1.160 ± 0.111	0.695 ± 0.006
BAGR	0.131 ± 0.083	1.422 ± 0.704	1.225 ± 0.171	0.620 ± 0.074
BFOR	0.142 ± 0.052	1.587 ± 0.425	1.198 ± 0.119	0.579 ± 0.235
S	0.160 ± 0.053	1.837 ± 0.638	1.206 ± 0.052	0.656 ± 0.056

3.5.2. Morpho-Productive Data (Phase 3)

As shown, photosynthetic parameters were higher for plants grown in substrates where fertilization was combined with biochar application, even though the difference was not statistically significant. However, this significance was detected for the morpho-productive characteristics of the same substrates (Table 16), leading to the hypothesis that improvements in physiological parameters positively affected productivity.

Table 16. Morpho-productive characteristics (mean ± st. dev.) detected on lettuce plants on analyzed substrates (n = 4).

Treatment	Plant Height	Num.	Fresh Weight	Moisture	Dry Weigh	
	(cm)	Leaves [a]	(g) [b]	(%)	(g) [b]	(%)
F	23.3 ± 3.0	22.5 ± 2.9 AB	97.53 ± 32.66 AB	91.95 ± 0.95	7.80 ± 2.38 AB	8.05 ± 0.39
F + BFOR	22.1 ± 2.8	23.8 ± 2.1 A	107.08 ± 11.53 A	92.36 ± 0.56	8.15 ± 0.70 A	7.64 ± 0.56
F + BAGR	23.8 ± 3.5	22.3 ± 1.7 AB	107.88 ± 42.4 A	92.07 ± 0.57	8.45 ± 3.18 A	7.93 ± 0.57
BAGR	20.0 ± 1.7	18.8 ± 2.8 BC	41.15 ± 8.78 C	91.71 ± 0.26	3.40 ± 0.66 C	8.29 ± 0.26
BFOR	20.8 ± 1.7	19.0 ± 2.4 BC	49.93 ± 12.15 BC	91.83 ± 0.09	4.08 ± 0.98 AC	8.17 ± 0.09
S	20.6 ± 0.6	18.3 ± 1.3 C	39.40 ± 2.23 C	92.08 ± 0.40	3.13 ± 0.30 C	7.92 ± 0.40

[a] Different letters indicate significant differences after analysis with Kruskal–Wallis test for non-parametric data and Dunn test (HSD) for $p < 0.01$ for separation of means. [b] Different letters indicate significant differences after analysis with Welch's F-test for unequal variances and Dunn's test (HSD) for $p < 0.01$ for separation of means.

The integration of fertilizer with soil supplemented with biochar (F + BAGR and F + BFOR) resulted in the highest production in terms of both fresh and dry weight compared to fertilization alone (although in a limited and non-significant manner) and compared to the presence of biochar alone or to the soil. The difference was particularly high (and statistically significant) compared to the latter or to soil added with biochar (BAGR or BFOR), with a reduction greater than 50–60% compared to the F + BAGR or F + BFOR substrates. Previous studies have reported that plant response to biochar and its combination with other organic amendments can vary depending on soil properties, biochar characteristics, application rates, and experimental conditions. Trupiano et al. [104] observed positive effects of biochar on lettuce growth and biomass when applied to nutrient-poor soils. Massaccesi et al. [105] reported that, after 45 days of cultivation in a Mediterranean environment, the biomass of well-developed lettuce plants increased approximately 2–2.5-fold in the modified soils compared to the untreated control.

The interaction between biochar and other soil amendments, particularly organic fertilizers, represents one of the most promising research directions for improving sustainable soil management. In the present study, the combined application of biochar and organic fertilizer consistently enhanced crop performance compared with the application of biochar alone and, to a lesser extent, with organic fertilization alone. Although improvements in physiological parameters did not always reach statistical significance, the corresponding increases in fresh and dry biomass suggest that biochar may enhance nutrient use efficiency by improving nutrient retention within the root zone and creating more favourable conditions for plant growth.

The practical relevance of these results is further supported by the current European regulatory framework. Under Regulation (EU) 2019/1009, biochar produced by pyrolysis and gasification has been officially included among the Component Material Categories (CMC 14: Pyrolysis and Gasification Materials) for EU fertilizing products, recognizing its potential role as a constituent material within sustainable fertilization strategies. Therefore, the positive interaction observed in the present study further supports the integration of biochar with organic fertilization as a promising approach for increasing resource-use efficiency while contributing to circular bioeconomy and climate-smart agricultural systems.

3.5.3. CO_2 from Substrates (Phase 3)

As observed in the first test, biochar integration into the soil resulted in different CO_2 emissions in the absence of plants (Figure 13). It was possible to compare substrates in which only biochar was present, as it was carried out before fertilization with the organic fertilizer. As previously reported, in this test the pots were left empty for approximately

one month before lettuce transplantation. Contrary to what was observed for the phase 2 test, the emitted CO_2 was statistically higher for the substrate containing BAGR, while the soil recorded the lowest emission.

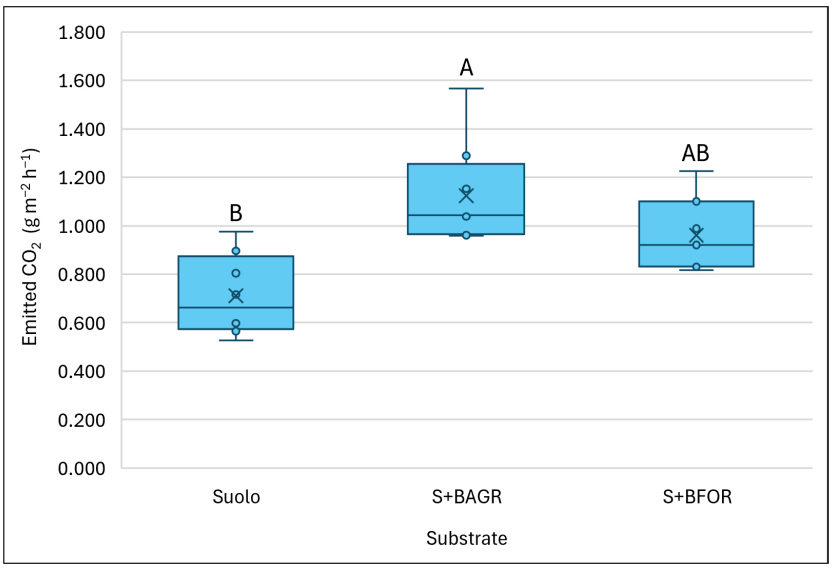


Figure 13. CO_2 emitted (g m^{-2} h^{-1}) for the three analyzed substrates in pre-transplant. Different letters above the boxes indicate significant differences after Tukey's test (HSD) for $p < 0.01$ (n = 8). 'x' represents the mean value and the horizontal line represents the median value.

At the end of the test, the entity of post-harvest CO_2 emission was found to be very different (Figure 14). The emerging trend showed a progressive reduction from the fertilizer treatment to substrates containing both fertilizer and biochar, and finally to substrates containing only biochar or soil alone. However, it should be noted that although quantitatively the CO_2 emission of the F and F + BAGR substrates was quite different, this difference was not statistically significant. The same behaviour was observed for the comparison between F + BAGR and BAGR. Therefore, the observed temporal variability cannot be attributed to the effect of a single amendment and may reflect changes in substrate availability, microbial activity and plant-derived carbon inputs.

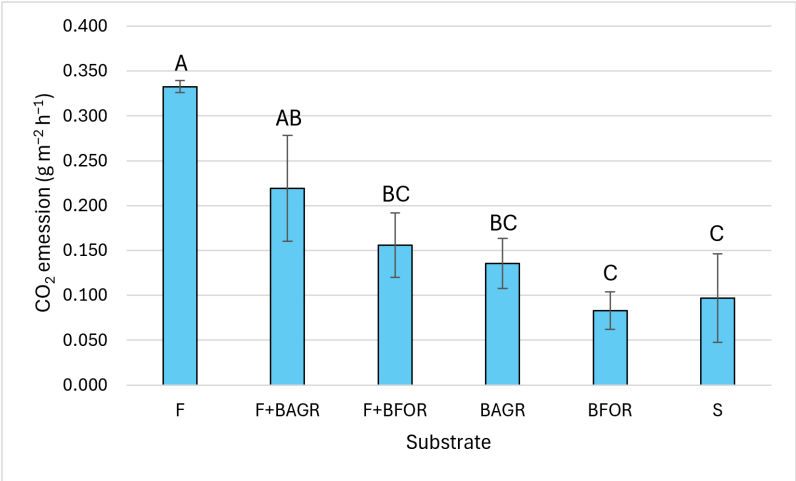


Figure 14. CO_2 emitted (g m^{-2} h^{-1}) for the three analyzed substrates post-transplant. The bars indicate the standard deviation. Different letters indicate significant differences after Tukey's test (HSD) for $p < 0.01$ (n = 4).

4. Conclusions

The results of this study showed that biochar produced from olive pruning and poplar biomass under comparable pilot-scale pyrolysis-gasification conditions differed in their physicochemical characteristics. In particular, significant differences were observed for ash, fixed carbon, carbon and pH, while H, N and C/N did not differ significantly. BAGR exhibited significantly higher Na, Fe, Zn and Cu concentrations, whereas BFOR showed higher Mg and Ca contents. These differences were consistent with the distinct characteristics of the original feedstocks. The germination assays revealed species-dependent responses to biochar type. BFOR exhibited germination indices above 100% for all tested species, whereas BAGR showed more variable responses, with lower germination performance particularly observed in sunflower and tomato. These results support the use of germination assays as a preliminary screening approach for assessing the biological response to biochar before its application in soil–plant systems. Under the pot conditions tested, the application of biochar alone did not result in statistically significant differences in lettuce fresh biomass compared with the control. When biochar was combined with the organic fertilizer, statistically significant responses were observed for some physiological and morpho-productive parameters, whereas other measured traits remained statistically unchanged. The integration of biochar (BAGR and BFOR) in soil supplemented with fertilizer increased fresh biomass by approximately 10%, although without statistical significance. The higher biomass observed in the combined treatments should therefore be interpreted within the specific experimental conditions and does not, by itself, demonstrate a synergistic interaction or increased nutrient use efficiency. Soil CO_2 fluxes varied among treatments and sampling periods, indicating temporal and treatment-related variability in soil respiration under the experimental conditions. Overall, the comparison of olive pruning and poplar-derived biochar within the same experimental framework indicates that feedstock origin was associated with differences in biochar physicochemical properties, while biological and agronomic responses varied according to biochar type, plant species, fertilization regime and experimental conditions. The results must be interpreted in the context of the experimental conditions adopted. The lack of additional parameters, such as electrical conductivity and additional contaminants, represents a limitation of the study. Therefore, these parameters will be included in future investigations and long-term and field studies to further evaluate the performance and agronomic safety of biochar under a wider range of environmental and soil–crop conditions. Overall, the results provide a useful basis for further research into the suitability of the biochar for agricultural applications.

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