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Above- and below-ground morpho-physiological traits indicate that biochar is a potential peat substitute for grapevine cuttings nursery production

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The growing demand for grapevine planting materials, due to growing global viticulture, is promoting research studies to improve vineyard sustainability. In greenhouse nurseries, peat is the most common growing medium component used although is an expensive and non-renewable material. Indeed, the reduction of peat exploitation is receiving great attention, and currently, several materials are being investigated as peat substitutes for composing the cultivation substrates. Biochar, a carbon-rich, recalcitrant charred organic co-product of the pyrolysis or gasification process, has emerged as a potentially promising replacement for soilless substrates in nursery plant material propagation. Although several studies carried out at greenhouse nurseries have shown that biochar, can improve plant growth, only a few studies have focused on the production of grapevine plant material. To fulfil this knowledge gap and push forward the sustainability of the nursery sector, we evaluated above and below-ground morpho-physiological traits of one-year-old potted grapevine cuttings growing with 30% volume of four different biochar types (i.e., from pyrolysis and gasification) mixed with commercial peat. The present study shows that biochar can be used in growing media mixes without adverse effects on roots, improves soil water retention and leaf water potential, and improves the effects on soil microbiology.

The increase in viticulture worldwide reflects a higher demand for grapevine (*Vitis vinifera* L.) planting materials raising the urgent need to improve the sustainability of nursery activities[1]. Indeed, in greenhouse nurseries, peat is the most common material composing growing media although is expensive and non-renewable[2]. In addition, peatlands exploited for peat extraction are important ecosystems for carbon (C) reserves and for regulating local water quality and regime[3]. Therefore, to reduce this exploitation impact several alternative-to-peat materials are currently receiving great attention from researchers being investigated as growing media components[4]. Biochar, a carbon-rich coproduct of biomass pyrolysis or gasification, can be produced from renewable organic waste material and is a potential candidate material for composing alternative and sustainable growing media[5,6–8]. Indeed, the majority of the studies testing the application of biochar to soils, soil-less growing media, and plant growth showed a series of positive properties[9,10], such as the increase in plant growth[11,12] mainly due to: (i) the higher soil water content occurring during the dry growing period[13,14], (ii) the increase in beneficial microbial microfauna[15,16] and (iii) the increase in cation exchange capacity and micro–macro nutrients availability[17]; Therefore, the valorization through the biochar production of agro-industrial by-products may represent a potential material alternative-to-peat in composing the growing media. Besides environmental concerns[18] the nursery production of forced or dormant grafts grown in containers may represent an effective alternative to open-field propagation, and the selection of alternative-to-peat materials should take into consideration their ability to produce high-quality plant material to be transplanted at any time of the year. To this aim, replacing peat with biochar could represent a powerful tool for improving plant growth and substrate characteristics while reducing

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water consumption. In particular, problems related to biotic and abiotic stresses, such as root dehydration, contamination by soil pathogens, and frost damage can negatively affect vineyard establishment of dormant root vines and ensuing field production[19]. Furthermore, a further critical issue in the nursery sector concerns the quantity and quality of the water used for irrigation since the annual water consumption for irrigation is currently around 1000–1500 mm for each plant in a container[20]. Therefore, the peat replacement with biochar is linked to its inherent characteristics of an increase in water availability of the growing media[21,22] and, thus, a reduction in the quantities of irrigation water needed. Also, container production reduces production cycle timespan, and improves plant quality and seedling performance in the field, especially under harsh site conditions[23]. Specifically, for the soil chemical characteristics of the growing media, an ideal substrate should have balanced air porosity, optimal bulk density, and adequate water-holding capacity. These characteristics guarantee an efficient exchange of oxygen and carbon dioxide, promoting the correct development of fine roots[2,12]. Achieving the right balance between these physical characteristics is important to stimulate root and plant growth throughout the container usage. Finally, most studies on biochar and growing media have focused on increasing crop growth or reducing non-peat environmental concerns, such as carbon sequestration, remediation of contaminants, and reduction of greenhouse gas emissions[24,25]. There is also a fair number of studies focused on the production, characterization, and engineering of biochar[26,27] in soilless substrates[28,12, 29]. All of these studies indicated that plant responses to the addition of biochar to substrates are similar to those found with standard peat-containing substrates, with the added benefits of reducing nutrient and water loss and bulk density and creating a benefit for microbes. Although this plethora of studies done in a greenhouse nursery shows that biochar, used at low doses, can improve plant growth[29], few studies have been done on the production of grapevine planting material[8,30,31]. Therefore, the present research aims to evaluate biochar as a possible substitute for peat in the pot growth of grapevine cuttings. The biochar types used come from pyrolysis and gasification of waste biomass and different temperatures allowing to evaluation of the performance of the different biochar types on the characteristics of the substrate and plant growth development in terms of water retention, microbial biomass, root functional traits, ecophysiological performance, and production. We hypothesized that biochar is a soil component that can be used in growing media mixes in quantities of 30% v/v conferring ideal physicochemical properties to the substrate and, in turn, (i) increasing the substrate water retention and plant water status and reducing the irrigation requirement (ii), improving the fine root development by increasing both length and biomass in all diameter classes, and (iii) increase the community-level physiological profiling (CLPP) of rhizosphere. To test our multiple hypotheses, we used four different biochars in terms of the bioenergy production process (i.e., pyrolysis and gasification) to analyze the morpho-physiological above- and below-ground traits of potted grapevine plants together with the chemical-physical substrate characteristics and the physiological profile of rhizosphere microbial community function.

Results

Growing media characteristics

All types of biochar used, shown in Table 1, have a high total carbon content (C_{tot}) that was higher than 70 compared to peat which contains 15.91 ± 0.01%. The total macronutrients analyzed (Mg_{tot}, P_{tot}, and K_{tot}) have very

Parameters	Units	Peat	BC-pyr1	BC-pyr2	BC-gas1	BC-gas2
C tot	%	15.91 ± 0.01	77.81 ± 0.01	90.62 ± 0.01	88.02 ± 0.01	89.04 ± 0.01
N tot	%	0.52 ± 0.01	0.91 ± 0.01	0.15 ± 0.01	0.32 ± 0.01	0.31 ± 0.01
Mg tot	%	4.32 ± 0.01	0.87 ± 0.01	0.92 ± 0.03	0.24 ± 0.03	0.22 ± 0.01
P tot	%	8.03 ± 0.02	1.33 ± 0.02	0.78 ± 0.01	0.81 ± 0.02	0.71 ± 0.02
K tot	%	8.49 ± 0.03	1.39 ± 0.01	1.13 ± 0.01	0.06 ± 0.01	0.08 ± 0.01
Pb	mg/kg s.s	b.d.l	5.80 ± 0.01	b.d.l	≤ 5	2
Cd	mg/kg s.s	b.d.l	< 1	b.d.l	< 1	< 0.2
Cu	mg/kg s.s	b.d.l	58 ± 0.01	b.d.l	66	23
Zn	mg/kg s.s	b.d.l	59 ± 0.01	b.d.l	66	49
Ni	mg/kg s.s	b.d.l	10 ± 0.01	b.d.l	33	33
Hg	mg/kg s.s	< 5	< 5	< 10	< 1	< 0.2
Cr VI	mg/kg s.s	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25
PAHs (Σ16 US EPA)	mg/kg s.s	–	≤ 6	≤ 2	≤ 1	≤ 1
PCB	mg/kg s.s	–	< 0.25	< 10	< 0.25	< 0.25
pH		5.8 ± 0.2	8.8 ± 0.1	8.2 ± 0.1	10.0 ± 0.3	9.6 ± 0.1
Bulk density	$Mg\ m^{-3}$	0.25 ± 0.02	0.38 ± 0.02	0.19 ± 0.02	0.40 ± 0.02	0.68 ± 0.02
Max water absorption	% (m/m)	92.51 ± 0.02	162.15 ± 0.02	185.42 ± 0.01	80.75 ± 0.02	92.52 ± 0.01
Particle size distribution	< 0.5 mm-% m/m s.s	–	4.45 ± 0.01	16.14 ± 0.01	76.62 ± 0.01	57.60 ± 0.01
Particle size distributions	5 ≤ ≥ 0.5-mm-% m/m s.s	–	79.61 ± 0.01	75.10 ± 0.01	21.29 ± 0.01	33.30 ± 0.01
Particle size distributions	≥ 5 mm-% m/m s.s	–	15.94 ± 0.01	8.84 ± 0.01	2.09 ± 0.01	9.12 ± 0.01

Table 1. Main chemical and physical characteristics of peat and biochar. b.d.l., below detection limit.

variable values. In particular, Mg_{tot} and K_{tot} values are much higher in pyrolysis than in gasification biochar. The pyrolysis biochars (BC-pyr1 and BC-pyr2) have a pH value of 8.8 ± 0.1 and 8.2 ± 0.1 respectively, while gasification biochars have a relatively more basic pH with values of 10.0 ± 0.3 in BC-gas1 and values of 9.6 ± 0.1 in BC-gas2 (Table 1). Peat has an acidic pH of 5.8 ± 0.2. All types of biochar used in the present study fall within the parameters required by Italian Legislative Decree 75/10 which defines biochar as an agronomic amendment in Italy for PAH, PCB, and heavy metals content (Table 1). Furthermore, all types of biochar analyzed could also fall under the new regulation on fertilizer products (EU/2019/1009), recently adopted by the European Union. All types of biochar used were characterized by a very low bulk density (BD) value with lower values in pyrolysis biochar (0.38 ± 0.02 and 0.19 ± 0.02 Mg m^{-3} respectively in BC-pyr1 and BC-pyr2), compared to gasification biochar (0.40 ± 0.02 and 0.68 ± 0.02 Mg m^{-3} respectively in BC-gas1 and BC-gas2; Table 1). Maximum water absorption was on average 174 ± 1% (w w^{-1}) in pyrolysis biochar and 87 ± 2% (w w^{-1}) in gasification biochar (Table 1). The largest percentage of particle size of pyrolysis biochar is concentrated between 5 and 0.5 mm, while in biochar obtained from gasification, the particle size is concentrated in values lower than 5 mm in dry matter (Table 1).

Growing media measurements

Substrates with biochar from pyrolysis and gasification have a more basic pH than the control, with an average value of 7.3 ± 0.2 for both the substrates (pyrolysis and gasification biochar) compared to the control with a pH of 5.9 ± 0.4 (Table 2). Bulk density values are lower in growing media with pyrolysis biochar (BC-pyr) compared to control. The bulk density values of the substrates with biochar from gasification (BC-gas) showed higher apparent density values than the control (Table 2).

Plant measurements

Plant height did not differ between biochar-amended substrates and control independently of the biochar type considered and during the entire growing period with the only exception of the last sampling point (Fig. 1). In particular, on the last sampling date, the height of the plants grown with BC-pyr1, BC-pyr2, and BC-gas1 was significantly higher than both control and BC-gas2-treated plants, which had similar values (Table 3). The total plant biomass did not differ among control and treatments, independently of the type of biochar analyzed (Table 3). The highest and lowest values of the third internode length were measured respectively in BC-pyr1 and BC-pyr2, and in BC-gas2 treated plants, while control and BC-gas1 treated plants had intermediate values (Table 3). Figure 2 shows the leaf water potential values measured at midday. The data showed that the leaf water potential values for biochar-treated plants, both pyrolysis and gasification were significantly lower to control plants on 16th June and 12th July. On 11th August there were still differences in BC-pyr1 and BC-pyr2 but not statistically different in BC-gas1 and BC-gas2 to control. However, there are no differences between the two biochar treatments (BC-pyr and BCgas) (Fig. 2). Although leaf temperatures (Fig. 3) show lower values in plants treated with biochar, there are no statistically significant differences. Statistically significant differences were noted on the date of 11th August between BC-pyr and C.

Community-level physiological profile of rhizosphere

The community-level physiological profile (CLPP) was analyzed on the rhizosphere. The microbial response in each microplate that expressed average well-colour development (AWCD) index increased proportionally and the most intensive metabolism of carbon substrates was observed at 120 h. The biochar treatments showed an increase in the AWCD compared to the control (Fig. 4a). The highest value of AWCD was found in the rhizosphere of BC-gas2-treated plants, without significant differences with the other biochar treatments, which were similar to the control (Fig. 4a). Concerning the microbial level of substrate uses, no significant differences between different biochar types and controls were found for carboxylic acids, carbohydrates, amino acids, polymers, and amine except phenolic compounds (Fig. 4b-g) that showed the highest value in samples characterized by the BC-pyr1 application (Fig. 4e).

Root growth length and biomass

Both the length and biomass of the totality of fine roots (d < 2 mm) did not differ among the control and the four biochar treatments (Fig. 5a,e), with the only exception of BC-gas2-treated plants that had the highest biomass than control (Fig. 5e). The fine-roots analysis performed according to three different diameter classes revealed that this pattern remained the same (Fig. 5b,d,f,h). Moreover, the BC-gas2 treated plants showed significantly higher biomass than control plants only in the 0.5–1 mm root class (Fig. 5g). Independently of the treatment,

Parameters	units	Control	BC-pyr1	BC-pyr2	BC-gas1	BC-gas2
pH		5.9 ± 0.4^{a}	7.3 ± 0.2^{b}	7.3 ± 0.2^{b}	7.5 ± 0.2^{b}	7.2 ± 0.1^{b}
BD	Mg m^{-3}	0.24 ± 0.01^{a}	0.28 ± 0.04^{ab}	0.19 ± 0.01^{b}	0.37 ± 0.02^{c}	0.54 ± 0.06^{d}

Table 2. Growing media physical characteristics at the end of the experiment (23rd September 2022). Data (n = 5) are reported as mean ± standard deviation. Statistically significant differences ($p < 0.05$) were identified by the post hoc Dunnett's test for multiple comparisons (SPSS Statistics 25 IBM) and marked with the letters a, b, c.

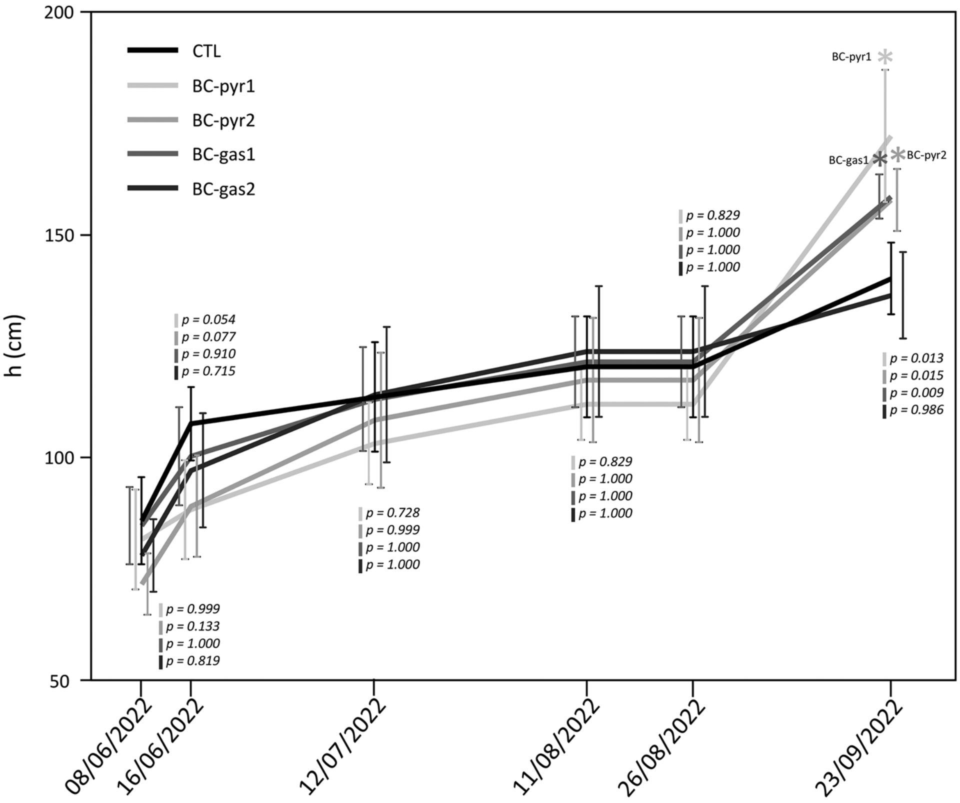

Figure 1. Plant growth rate (cm) during the period of measurement. The curves represent the mean values of 10 plants for treatment (n = 10). Error bars represent the 95% confidence interval while asterisks ($p < 0.05$) and p values indicate statistical differences between the control and the respective treatment.

Parameters	Units	Control	BC-pyr1	BC-pyr2	BC-gas1	BC-gas2
Total biomass	(g)	71 ± 0^{a}	80 ± 1^{a}	80 ± 1^{a}	76 ± 0^{a}	75 ± 1^{a}
3rd internode length	cm	1.7 ± 0.5^{ab}	2.2 ± 0.2^{b}	2.5 ± 0.0^{b}	2.2 ± 0.6^{ab}	1.5 ± 0.3^{a}
Total height	cm	140 ± 6^{a}	172 ± 12^{b}	158 ± 6^{b}	159 ± 4^{b}	136 ± 8^{a}

Table 3. Morphological and physiological parameters of the plants at the end of experiment (23rd September 2022). Data (n = 5) are reported as mean ± standard deviation. Statistically significant differences ($p < 0.05$) were identified by the post hoc Dunnett's test for multiple comparisons (SPSS Statistics 25 IBM) and marked with the letters a, b, c.

the fine-root length decreased with the increase of the root diameter (Fig. 5b,d), and, vice versa, the fine-root biomass proportionally increased with the different root sizes (Fig. 5f,h).

Discussion

Grapevine propagation in the nursery greenhouse is relatively easy. However, high skills and organization are required to produce planting materials with the high-quality standards required every year by growers for new plantings, replanting of uneconomical vineyards, or replacing plants affected by stem disease pathogens[19]. The chemical and physical characteristics of the growing media can influence the cutting system[32]. Growing media should be friable, free of weeds and pathogens, with good water capacity and drainage[33]. Peat is the most commonly used growing media due to its positive hydrological, physicochemical, and agronomic characteristics[34,19]. However, to improve agricultural sustainability, researchers are called to study alternatives to the use of peat as innovative growing substrates. Among the different components suitable for growing media, biochar materials belong to a relatively new and not yet established group in the growing media market.

The main focus of the growing media industry is on growth-supporting properties such as slightly acidic pH, high cation exchange capacity, good aeration, and high-water holding capacity to ensure germination and good plant growth in the youth. In areas where peat is not available, carbonized materials are often used successfully as reported by Steiner et al.[35] using rotten tree trunks and biochar in the Brazilian Amazon. Careful consideration of the use of peat has led to various alternatives being considered, some of which have been evaluated as 1:1

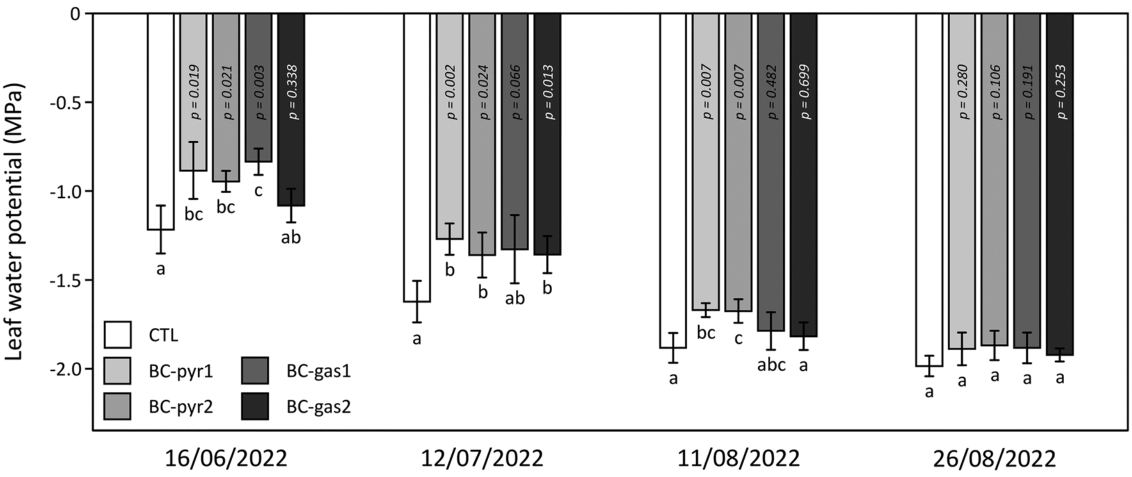

Figure 2. Leaf water potential (MPa) on midday measurement. Each column represents the mean value of 5 measurements (n = 5). Error bars represent the 95% confidence interval while *p* values indicate statistical differences between the control and the respective treatment.

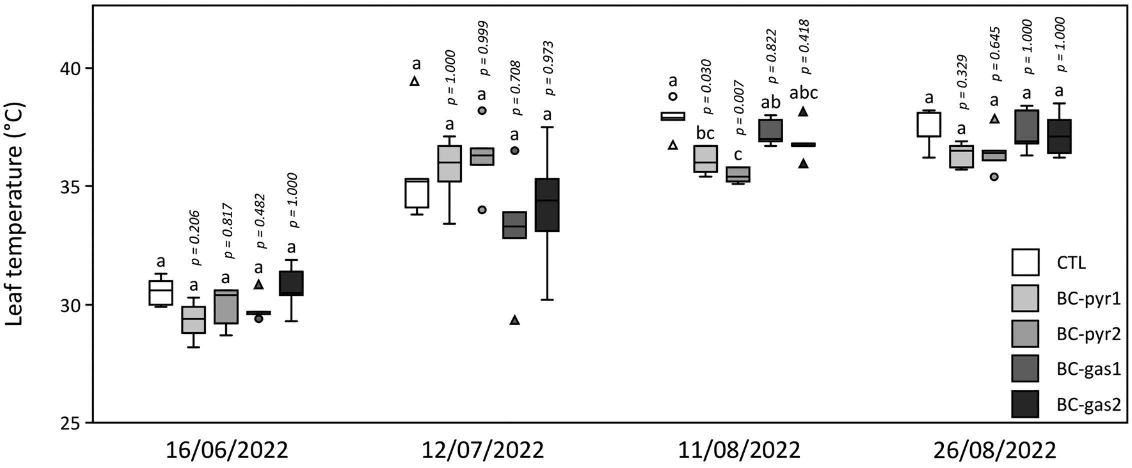

Figure 3. Leaf temperature measurement (°C) made on 5 independent plants (n = 5). Vertical boxes represent approximately 50% of the observations and lines extending from each box are the upper and lower 25% values of the distribution. Within each box, the solid horizontal line is the median value, while circles and triangles represent outliers and extreme outliers. *p* values indicate statistical differences between the control and the respective treatment.

substitute. When considering new growing media ingredients, whether mixed with peat or alone, on a technical level there are important physical, chemical, and biological factors to consider. From a physical point of view, the bulk density of the growing medium components should be low, but the mechanical stability and total pore space should be high[36]. Particle size distribution affects aeration and water-holding capacity[37] The presented results support our general hypothesis demonstrating that biochar can be used as a partial replacement for peat in growing media, in agreement with Dumroese et al.[38] and Steiner and Harttung[3], since it confers ideal physicochemical properties desired in soilless cultivation substrates. According to Yao et al.[39], carbon content is the most significant parameter for biochar quality, and values above the 70% characterize a high-quality biochar. Although the starting material primarily influences the characteristics of the obtained biochar, those used in the present experiment all exceed 70% of carbon content and have little differences in the elemental content and no permanent differences in the chemical characteristics. In agreement with other studies and in support of the first part of the hypothesis, in our study, the biochar application improves the water availability and decreases the leaf water potential, which can be related to the intrinsic characteristics of the biochar used, especially biochar from pyrolysis which showed very high values of maximum water absorption. The biochar used in this experiment has the highest particle size distribution concentration between 0.5 and 5 mm and, as noted by Kern et al.[40], in particular, the < 1 mm fraction plays a key role in water availability and air capacity. Furthermore, the biochar used in our experiment shows lower bulk density values than peat only in the biochar from pyrolysis (BC-pyr2). These properties probably explain how, in our experiment, the addition of biochar improved the leaf water potential during the growing season, confirming and extending the results of other authors[41]. Other studies[42,43,44] highlighted how the biochar production method influences the intrinsic characteristics of the biochar itself

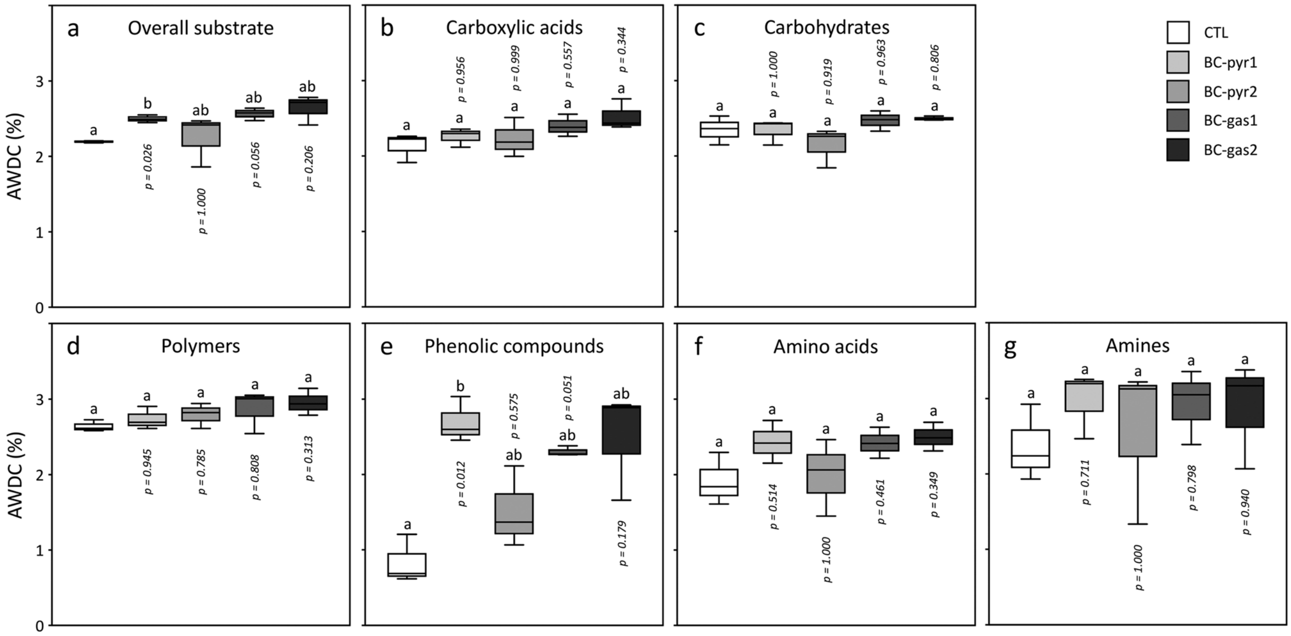

Figure 4. Community-level physiological profiling of the rhizosphere using Biolog EcoPlates. (**a**) Average (n = 3) of well color development (AWCD) at the maximum development drawn from the overall community substrate utilization profile during an incubation period of 12–120 h. (**b–g**) AWCD analyzed considering the microbial activity degrading of each carbon source: (**b**) AWCD at the maximum development of carboxylic acids; (**c**) AWCD at the maximum development of carbohydrates; (**d**) AWCD at the maximum development of polymers; (**e**) AWCD at the maximum development of phenolic compounds; (**f**) AWCD at the maximum development of aminoacidic; (**g**) AWCD at the maximum development of amines. Vertical boxes represent approximately 50% of the observations and lines extending from each box are the upper and lower 25% values of the distribution. Within each box, the solid horizontal line is the median value. *p* values indicate statistical differences between the control and the respective treatment.

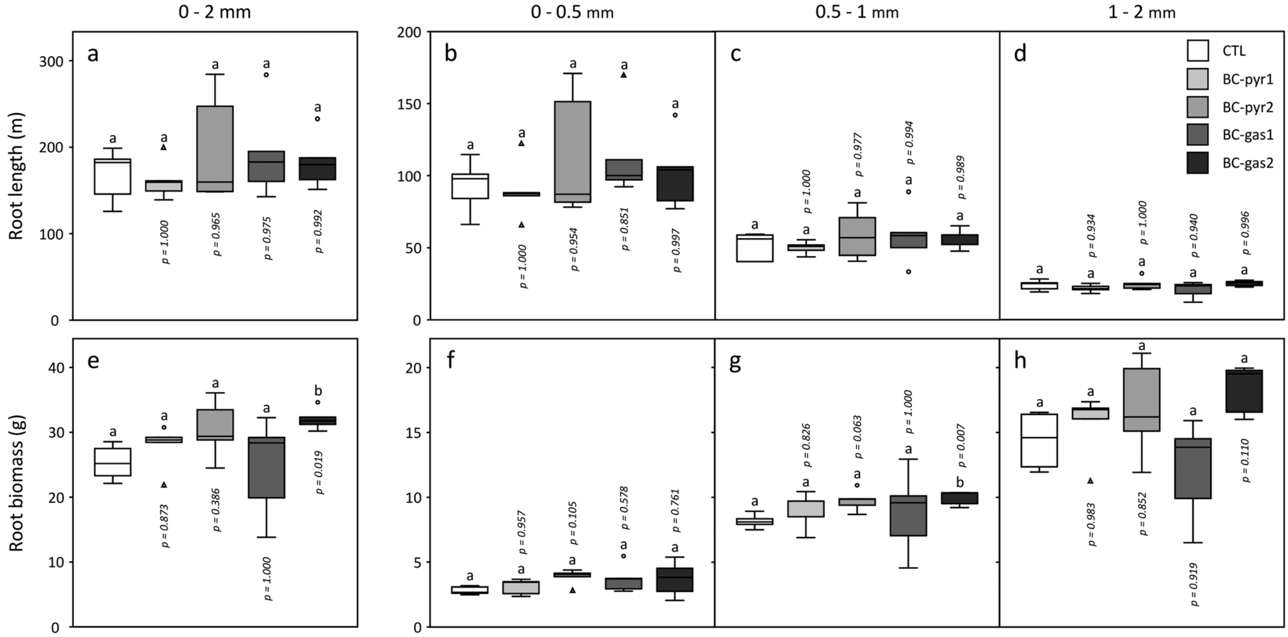

Figure 5. Mean fine-root standing length (m) (**a–d**) and biomass (g) (**e–h**) according to different diameter classes: (**a, e**) 0–2 mm, (**b, f**) 0–0.5 mm, (**c–g**) 0.5–1 mm, and (**d–h**) 1–2 mm. Vertical boxes represent approximately 50% of the observations (n = 5) and lines extending from each box are the upper and lower 25% values of the distribution. Within each box, the solid horizontal line is the median value, while circles and triangles represent outliers and extreme outliers. *p* values indicate statistical differences between the control and the respective treatment.

and, consequently, the interactions with the soil. Furthermore, understanding water-biochar behavior could be further studied by considering molecular interactions at the micro-nano level. For example, Conte et al.[45] suggest that water molecules are bonded to solid carbonaceous material through unconventional hydrogen bonds. The modification of plant water availability induced by the application of biochar could increase the resilience of grapevine cuttings nursery production to water shortage (drought), as demonstrated by the lower leaf potential measured in treated plots compared to control plots. The effect of biochar and its influence on the water-soil relationship depended directly on the type of biochar and the data demonstrate that pyrolysis biochar responds better to the need due to their intrinsic characteristics (Table 1) in terms of maximum water absorbent. The gasification biochars, however, show higher bulk density values compared to the control. In our study we find that replacing peat with biochar leads to an increase in pH, being an alkaline material, as found by Vaughn et al.[4], and a slight increase in bulk density but without negatively affecting plant growth throughout the analyzed period. Indeed, the increase in pH is in line with numerous studies on biochar-based material[46,47]. The increase in pH following the addition of biochar is beneficial for acidic soil or growing media (as is the case for peat in the present work), with the biochar acting as a liming agent and likely replacing the calcium oxide used to increase the pH[48,49]. The biochars ratio used in the present study, maintained pH values around 7.0, as the ideal substrate pH for peat substrate is between 5.0 and 5.5[50]. Furthermore, C_{tot} values are higher in biochar-based growing media.

Moreover, results of plants treated with the two different biochar types showed a similar fine root development independently of the diameter class considered partially supporting the second part of our hypothesis. Also, the ameliorating of the community-level physiological profile observed in biochar-treated rhizosphere supported the third part of our hypothesis.

The results of this experiment were substantially in agreement with those of previous works, which reported that an increase in plant biomass is one of the macroscopic effects induced by the partial replacement of peat in the cultivation substrates with agro-industrial by-products, such as compost, digestate, and biochar[8]. The ability of biochar to increase plant biomass is probably due to better availability and absorption of nutrients by the plant and/or the presence of some microorganisms and compounds capable of increasing plant growth as secondary metabolites[51]. When these substances are added to growing media, they can improve the biochemical activity of plants, similar to plant hormone-like promoters[52]. Interactions among microorganisms and plant roots are essential for the nutritional requirements of the plant. Our results showed how the microbial community was positively influenced by the biochar application for the use of phenolic compounds, which are of great significance in diverse processes of plant development, including rhizogenesis[53].

To widen the practical application of the present study, a few considerations about the economic analysis of the biochar are included. Considering the wholesale prices of the main constituents of the media in cultivation, such as peat, coconut fiber, or green compost, these are much lower than those of biochar and range from €25,00 m^{-3} for peat to €20,00 m^{-3} for coconut fiber, or €12,50 m^{-3} for green compost. If we use peat as a reference and consider an average biochar bulk density of 0.20 t m^{-3}, the reference price level per ton of biochar could stand around €125,00, making it difficult to imagine a future for this material in nursery activities and horticulture (oral communication COST ACTION TD1107 "Biochar as option for sustainable resource management"). However, biochar was recently recognized as a negative emissions technology by the Intergovernmental Panel on Climate Change (IPCC)[54], representing a powerful tool for moving these nursery activities toward higher levels of environmental sustainability. Finally, since environmental considerations have become as important as performance and economic costs, we suggest the need to integrate the environmental and economic assessment to better illustrate the additional costs that could be incurred to reach CO_2 reduction target[55,36]. For example, very recently, Hashemi et al.[56] demonstrated that bio-based peat alternatives (wood fiber, compost, and hydrochar based on willow and degassed fiber from agricultural waste) and their mixtures as growing media (GM) for plant production in Denmark may significantly reduce greenhouse gas emissions and global warming potential compared to peat.

Conclusion

The results obtained in the present study further contribute to expanding the biochar potential applicability and suggesting that it could be a suitable ingredient for alternative-to-peat growing media and an effective strategy to reduce high-quality water requirements in the vine nursery sector. In particular, the different biochars used showed a well above- and below-ground plant and microbial morpho-physiological performance. However, the complexity of interaction between biochar, plant, rhizosphere microbial community, and nutrient-water deserves further investigation, especially considering the wide variability due to different starting materials and thermochemical processes. Finally, although the sale price of biochar is still high when compared with other growing media ingredients, the environmental advantages should be included in a complete analysis of the performance and economic costs.

Material and methods

Experimental design

The experiment was made in 2022 at Vivai New Plants di Barbara Gini, Cenaia (Pisa, Italy). Trials involved 100 grafted cuttings of *V. vinifera* cv. Sangiovese (clone I-SS-F9-A5-48) grafted in 2021 on rootstock 1103 Paulsen (*Vitis berlandieri* × *Vitis rupestris*), grown in pots (15x15x20 cm, approx 4.5 L volume). A randomized block design experiment, with 20 replicates (each replicate composed of one pot), was set up considering five treatments (4 different growing media with biochar and peat) and control without biochar. The experiment started at the end of April (22nd April 2022) transplanting one-year-cuttings of *V. vinifera* for each pot and the plants were harvested at the end of September 2022 (23th September 2022). During the experiment, drip watering was performed once a day at 10 a.m. at a rate of approximately 0.7 l per pot. The evaluated growing media included replacing half of the peat for a 30% in volume of the whole media composition (i.e., Control: peat/pumice 2:1; Treatment: peat/biochar/pumice 1:1:1) with 4 different types of biochar, called BC-pyr1, BC-pyr2, BC-gas3, and BC-gas4.

Growing media (peat and biochar) characteristics

First of all, a series of parameters are recognized that will contribute to a large extent to the different properties of the biochar, among which the production temperature and the type of conversion process influence the characteristics of the biochar[57]. For this reason, we decided to use 4 different types of biochar, 2 of which come from gasification plants and two from pyrolysis plants.

The total content of different elements (Mg, P, K, Pb, Cd, Cu, Zn, Ni, Hg, Cr) was determined after acid digestion with a microwave oven, according to EPA 3052[58], with an ICP-OES (iCAP 6000 Series, Thermo Scientific, Waltham, MA, USA) based on the EPA 6010D 2014 standard. For total carbon (C_{tot}) and total nitrogen (N_{tot}) of biochars and peat, samples were oven-dried at 105 °C for 24 h, acid digested with a microwave oven (CEM, MARSXpress) according to the EPA method 3052[58] and determined using a CHN Elemental Analyzer (Carlo Erba Instruments, mod 1500 series 2). The polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) analysis was carried out via the US-EPA 3550C[59] method; respectively the maximum water retention and the particle size fraction were determined using the method indicated in the DM 1/08/97 SO n. 173 GU 204 2/09/1997 Met.4 and UNI EN 15428[60].

Biochar and peat bulk density were determined using the UNI EN 13041 method[61]. The peat was a commercial type. Following is a brief description of each biochar type and in Table 1 their chemical-physical characteristics along with those of the peat used for composing the growing media.

- Control (C): peats
- Biochar 1 (BC-pyr1) was obtained from the orchard (i.e., olive tree, vines, apricot, and apple tree) pruning biomass through a slow pyrolysis process at a temperature of 500 °C in a transportable ring kiln of 2.2 m in diameter and holding around 2 t of feedstock.
- Biochar 2 (BC-pyr2) was obtained from olive tree pruning biomass through a slow pyrolysis process at a temperature of 550 °C in a rotary kiln of 100 kg per hour process capacity.
- Biochar 3 (BC-gas1) was obtained from the gasification of coppicing residues of beech (*Fagus sylvatica* L.) and oak (*Quercus* spp.) forests at a temperature of 900 °C.
- Biochar 4 (BC-gas2) was obtained from the gasification of coppicing residues of beech (*F. sylvatica* L.), hazel (*Corylus avellana* L.), oak (*Quercus* spp.), and birch (*Betula alba* L.) at a temperature of 900 °C.

Growing media measurements

At the end of the experiment, growing media samples were collected from 10 plots per treatment including the control. Soil pH was measured in water solution (1:2.5 ratio) using the pH Meter Mettler Toledo S220. The soil bulk density (BD, Mg m^{-3}) was measured using a cylindrical core of 100 cm^3 volume (V)[62]. Samples were weighed at field conditions (FW), dried in an oven at 105 °C for 48 h, and reweighed (DW) for calculating the gravimetric soil moisture content (g g^{-1}) as:

$$[(FW - DW) \div DW] \tag{1}$$

and BD (Mg m^{-3}) as:

$$[DW \div V] \tag{2}$$

Plant measurements

During the experiment, periodic measurements (8th June; 16th June; 12th July; 11th August; 26th August) of plants' height were done. Dry biomass of stems and leaves (at 80 °C in a ventilated oven for 48 h), 3rd internode length, and total height were measured at the final sampling point (23rd September). During summer, the leaf water potential (MPa) was measured on four cloudless and representative days (16th June; 12th July; 11th August; 26th August). Leaf water potential was measured according to the procedures of Padgett-Johnson et al.[63], using a pressure chamber (PMS, Instrumentation Co. Corvallis, OR, USA), on 5 randomly selected plants for each treatment, 25 in total. The measurements were made on the plants after the water had been removed for 48 h, only on mid-day leaf water potential (ψmd) because this method is considered the most suitable for the control of vine water status[64–66]. Specifically, mid-day measurements were taken between 12.30 and 1.30 pm. The time between leaf excision and chamber pressurization was generally less than 15 s. For each sampled plant, the fourth fully expanded and sun-exposed leaf (1 leaf per plant, 5 leaves in total for mid-day measurement for each treatment). Leaf temperature measurements were achieved with a handheld thermal camera Flir i7 (FLIR Systems Inc., Wilsonville, OR, USA). Leaf thermal images have been acquired before sampling on the same leaves used for leaf water potential measurements; then the average surface temperature for each leaf has been calculated with the FLIR Tools software.

Community-level physiological profile of rhizosphere

The community-level physiological profile (CLPP) was analyzed on the rhizosphere at the sampling point of 23rd September. Rhizosphere samples were collected from each pot and stored at 5 °C and a composite sample for each matrix was then made in the laboratory before the analysis. Samples were incubated for one week at 30 °C to stabilize the microbial biomass before the extraction. The CLPP analysis was attained according to a method first developed by Garland and Mills[67] and it's based on the community-level substrate utilization. The soil was inoculated in 96-well plates (Biolog Ecoplate) containing 31 different carbon sources (carbohydrates, carboxylic acids, polymers, phenolic compounds, amino acids, and amine) plus a control well, in three replications. Tetrazolium violet redox dye was used for each well as a color indicator if added microorganisms utilize the substrates[68]. The soil was shaken for 30 min at 250 rotation per minute in NaCl (0.9%) solution in a ratio 1:10 containing glass beads and then centrifugated for 3' at 3000 rotation per minute. Next 150 µL of each sample were inoculated into each well of Biolog EcoPlates and incubated at 25 °C. The rate of utilization was indicated by the reduction of tetrazolium, a redox indicator dye that changes from colorless to purple[69]. Data were recorded with a plate reader at 590 nm every 24 h until 216 h. Microbial response in each microplate that expressed average well-color development (AWCD) was determined as follows[70]:

$$\mathrm{AWCD} = \sum \mathrm{ODi}/31$$

where ODi is the optical density value from each well, corrected by subtracting the blank well (inoculated, but without a carbon source) values from each plate well[68]. AWCD was also analyzed considering the microbial activity degrading each carbon source.

Root analysis

Five plants for each treatment were used for root morphological traits analysis. The stem was cut and the soil clod was carefully pulled out of the pot taking care to leave it intact. Roots were freed from the soil by washing it away over a 2 mm sieve. Grapevine roots were sorted from other species roots, immersed in water/ethanol (5:1 vol.) solution in Petri dishes and stored at 5 °C until further processed. Roots were scanned with a calibrated flatbed scanner coupled to a lighting system for image acquisition (Expression 10000 XL, Epson America Inc., Long Beach, CA, USA) and images were analyzed by WinRHIZO software to measure root length (m) according to different diameter classes (class 1 0–0.5 mm; class 2 0.5–1 mm; class 3 1–2 mm). Afterward, the plant tissues were separately oven-dried at 70 °C for 72 h (until constant weight) and weighed to obtain the dry mass (g) of different diameter roots.

Statistical analysis

SPSS Statistics 25 (IBM) was used to run the post hoc Dunnett's test for multiple comparisons. Statistically significant differences ($p < 0.05$) between the means were marked with the letters a, b, c. In box plots, vertical boxes represent approximately 50% of the observations, and lines extending from each box are the upper and lower 25% values of the distribution. Circles and triangles represent outliers and extreme outliers. In bar charts error bars represent the 95% confidence interval.

Ethical policies

The use of plant materials in this study was carried out in compliance with the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

Data availability

The data presented in this study are available on request from the corresponding author.

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Author contributions

S.B. and S.F.D.G. conceived the research project. F.P.V. supervised the project. S.B. and A.Ma. collected and interpreted aboveground data. A.D. processed the roots and collected data. A.Mo. supervised the root analysis and interpreted root data. P.B. performed the data analysis and provided chart visualization. D.C. produced the biochar. S.B. wrote the manuscript draft. A.Mo., P.B., F.P.V., A.Ma., D.C. revised the manuscript draft. All authors contributed to the article and approved the submitted version.

Competing interests

The authors declare no competing interests.

Additional information

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