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Effect of Biochar and Inorganic or Organic Fertilizer Co-Application on Soil Properties, Plant Growth and Nutrient Content in Swiss Chard

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Abstract: From the perspective of sustainable agri-food production, farmers need to make the best use of natural resources. Biochar can be a solution to adopt a more sustainable way of farming. Despite its environmental and agronomic advantages, biochar has a low plant nutrient value. This study evaluated the effect of biochar and the co-application of an inorganic or organic fertilizer on the soil properties, growth and nutrient content of Swiss chard (*Beta vulgaris* L. var. *cycla*, *Caryophyllales* order, *Chenopodiaceae* family). The experiment consisted of two factors: biochar type (from vineyard prunings and wood chips) and fertilizing source (ammonium nitrate and vermicompost). Biochars were applied at a 2% rate (*w*/*w*) and fertilizers at a dose providing 280 kg N ha^{-1}. The soil properties (pH, EC, extractable anions, cations, total N, Corg and C/N ratio) were measured before the plants were transplanted and at the end of the growing cycle, along with the growth parameters (leaf number, length and fresh weight) of each leaf cut, the productive parameters (total number of leaves and yield per plant) at the end of the growing cycle and the leaf content of anions (NO_3^-, $P_2O_4^{3-}$, SO_4^{2-}), cations (NH_4^+, Na^+, K^+, Ca^{2+}, Mg^{2+}) and total N. The co-application of biochar and a fertilizing source had a positive effect on soil properties and leaf nutrient content. Vermicompost increased plant growth by 22% and plant yield by 116%, in contrast to biochar, and increased limited leaf NO_3^- accumulation by about 81% in comparison to ammonium nitrate. The co-application of biochar and vermicompost is the better option to increase Swiss chard yield while preserving the nutritional and health qualities of the product.

Keywords: sustainable agri-food systems; vineyard pruning biochar; wood chip biochar; vermicompost; mineral fertilizer; organic amendments; *Beta vulgaris* L. var. *cycla*; soil fertility; plant production; plant nutrients

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1. Introduction

The agricultural systems of the next decades will be more based on the adoption of farming practices that reduce environmental impacts and mitigate and adapt to climate change to improve the sustainability of crop production and guarantee food security and safety. By 2050, a global population of 9.7 billion people will demand 70% more food than is consumed today [1]. To ensure sufficient nutritious food, agri-food systems will have to improve their resource use efficiency and environmental performance significantly. In Europe, the Farm to Fork (F2F) strategy [2], which is at the center of the EU Green Deal [3], aims to make food systems fair, healthy, and environmentally friendly. By means of the F2F strategy, the EU aims to achieve 50% reductions in pesticide and antibiotics use, a 50% reduction in nutrient losses by 2030, at least a 20% subsequent reduction in fertilizer use, and at least 25% of EU's agricultural land using organic farming. In this view, farmers need to reduce and optimize the use of inputs and make the best use of natural resources. In a circular economy approach, to which the F2F strategy is also related, and from a long-term sustainability perspective, the agricultural sector can exploit the significant amount of wastes, co-products and by-products deriving from crops, livestock, non-food crops and forestry, urban green areas and agri-food industries, and organic material in municipal solid waste. The management of these materials represents a burden, both in terms of economic and environmental impact; however, they are still rich in residual substances that can be recycled and used again in agriculture as biofertilizers, soil amendments, biostimulants, bioactive compounds and biopesticides [4].

Biochar is one of the bio-based compounds deriving from the transformation of wastes into valuable and useful products for agriculture [5–7]. Several scientific outcomes have showed that biochar has an important role in achieving the environmental sustainability of agriculture [8,9]. Biochar is a carbon-rich material obtained by the thermal treatment (pyrolysis) of organic materials under a limited supply of oxygen [10]. It has a high degree of stability due the high proportion of aromatic C and condensed aromatic structures, a high porosity and a large surface area [11,12]. These characteristics are associated with the environmental and agronomic benefits of biochar soil application. Indeed, biochar is suggested as a beneficial soil amendment to mitigate climate changes through carbon (C) sequestration and the reduction in greenhouse gas (GHG) emissions [13–15]. It is highly durable and can remain in the soil for hundreds to thousands of years [16]. Biochar is also suggested as a good option to enhance soil quality for plant growth, through several mechanisms related to improvements in soil structure and porosity, water and nutrient retention, and cation exchange capacity [17–24]. Moreover, biochar can promote the immobilization of heavy metals, organic pollutants and pesticides in the soil [25–27], resulting in an effective practice for the restoration of the functionality of degraded agricultural soils. Recently, biochar has been recognized to comply with the objectives, criteria and principles of organic production in the EU's agriculture and has been admitted to the list of fertilizers/soil conditioners that can be used in organic farming. It has been included in the Annex I of the Regulation (EC) No. 889/2008 and the implementation of EU Regulation (EU) 2019/2164 (Official Journal of the European Union, L328/61, 18 December 2019), which has been in force since 2020.

Soil and plant responses to biochar application can be positive, negative or neutral, depending on feedstock type, pyrolysis temperature, application rate and method, crop and soil type, and environmental conditions [28]. Moreover, despite its numerous potential functions, biochar is usually characterized by a low nutrient content and nutrient release capacity [29–31]. In particular, biochar nutrient availability can vary in relation to the feedstock composition and the pyrolysis conditions, such as temperature, heating rate and holding time [32,33]. To further increase biochar's effect on plant growth and soil properties, it should be applied in combination with a source of plant nutrients, such as inorganic or organic fertilizers, or a combination of both of these [34–36]. In recent times, some studies observed a significant improvement in soil fertility and crop yield when biochar and inorganic fertilizer were co-applied. A meta-analysis [37] focusing on short-term (1 year) field responses in crop yield across different climates, soils, biochars and management practices worldwide reported that the application of biochar soil along with inorganic fertilizers led to a 48% yield increase. Zhu et al. [38] reported a 75% increase in maize biomass when biochar and NPK fertilizer were combined. An over 30% increase in barley yield was observed by Gathorne-Hardy et al. [39] following the combination of biochar and N fertilizer. Significant interactions between biochar and N fertilizer on rice grain and straw yield were reported by MacCarthy et al. [40] and, similarly, on rainfed rice yield and yield components by Oladele et al. [41]. In two-year field experiments, biochar and N applications significantly increased grain yield and the above ground biomass of maize [42]. Nowadays, the combination of biochar with compost is also regarded as a promising approach to improve soil quality and crop growth. In particular, compost is an excellent source of plant nutrients and improves microorganism activity and the biological properties of the soil [43]. Similarly to biochar, the use of compost is recognized as a tool to recycle organic waste from the perspective of the circular economy and sustainability of agricultural practices. There is a growing interest in the co-application of biochar and compost in agro-ecosystems. In combination, both components could mutually improve each other's properties [44]. Soil addition with biochar and compost was found to improve maize growth and nutrient status in two agricultural Mediterranean soils [45]. A positive interactive effect of biochar and compost on soil's organic carbon, nutrient content and water-storage capacity was observed by Liu et al. [46] Agegnehu et al. [47] found that the application of biochar and compost was more effective in improving soil properties and yields of field and horticulture crops than biochar alone. Biochar–compost application was also reported to improve water and nutrient retention by soil and water, and nutrient uptake by peanut plants [48].

Previous studies [49,50] focused on the effects of biochar obtained from vineyard prunings on the growth and quality of Swiss chard (*Beta vulgaris* L. var. *cycla*), a green leafy vegetable belonging to Chenopodiaceae Family. Swiss chard is cultivated all over the world, mostly in Northern India, South America, Mediterranean countries and the USA. Canada, South Africa and Italy are the major producing areas internationally. In the last decades, Swiss chard has gained economic importance among leafy vegetables. It is available at the market year-round thanks to greenhouses, though it is at its peak in early summer and fall. Swiss chard is largely consumed for its nutritional properties and, during the summer, is a useful substitute for other less readily available leafy species, such as spinach. This species, eaten either raw or cooked, plays a considerable role in the Mediterranean diet, because of its nutritional and health benefits as a source of dietary fibers, vitamins, minerals and bioactive molecules [51]. The results of the first experiment [49], comparing the effect of biochar and other organic amendments (vermicompost from cattle manure and three composts, respectively, from olive pomace, cattle anaerobic digestate with wheat straw, and cattle anaerobic digestate with crop residues and wheat straw), each added to the soil at two rates (to provide 140 and 280 kg N ha^{-1}, respectively), showed that biochar did not affect the growth or the qualitative traits of Swiss chard. However, this species responded positively to the vermicompost, followed by the composts from cattle anaerobic digestate and the compost from olive pomace, especially when applied at a higher N rate. In the second experiment [50], biochar was applied in a mixture with the previously tested vermicompost, compost from olive pomace and compost from cattle anaerobic digestate with crop residues and wheat straw, at the N rate that previously provided the best results (280 kg N ha^{-1} with a loading ratio of 50:50). The biochar both alone and in a mixture led to a lower plant height, leaf area and fresh weight, carotenoid and chlorophyll leaf contents; meanwhile, the vermicompost and the compost from cattle anaerobic digestate applied alone had a positive effect.

Following previous findings, it was hypothesized that the co-application of biochar with a source of nutrients could improve soil properties, thus promoting plant growth, yield and quality. Therefore, a further pot experiment on Swiss chard was conducted to test: (i) two biochars from different feedstock (vineyard prunings and wood chips); (ii) two fertilizing sources (ammonium nitrate as an inorganic source and vermicompost as an organic source); and (iii) the co-application of each type of biochar with each type of fertilizing source. The specific objective of the study was to investigate the main and interactive effects of biochar and inorganic or organic fertilizer added together on selected soil properties, plant growth response and nutrient content.

2. Materials and Methods

2.1. Experimental Design

The pot experiment was carried out during the spring–summer 2021 in a greenhouse located at the University of Basilicata (South Italy) in Potenza (PZ, 40°38′ N–15°48′ E, 819 m a.s.l.), under natural conditions of light and temperature. The experimental design consisted of two factors, namely biochar type (B) and fertilizing source (F). In particular, two biochars, respectively deriving from wood chips (Bw) and vineyard prunings (Bv), were used for soil amendment and mixed with inorganic fertilizer (IF), as ammonium nitrate, or organic fertilizer (OF), as vermicompost from cattle manure. The biochars were applied at a rate of 2% of the dry soil weight and the fertilizers at a rate equivalent to 280 kg N ha^{-1}. The rate of biochar can be considered medium based on the literature [52], whereas the N rate corresponds to the findings of previous study [49,50]. To evaluate the effect of each type of biochar, each fertilizing source and their combinations, a full factorial experiment was set-up. The nine treatment combinations (Table 1) were arranged in a randomized complete block design with four replications for a total of 36 experiment units.

Table 1. Treatment combinations used in the experiment.

Biochar (B)		**Fertilizer (F)**		**Abbreviation** [1]
Wood Chips	**Vineyard Prunings**	**Ammonium Nitrate**	**Vermicompost**	
−	−	−	−	B0-F0 (control)
−	−	+	−	B0-IF
−	−	−	+	B0-OF
+	−	−	−	Bw-F0
+	−	+	−	Bw-IF
+	−	−	+	Bw-OF
−	+	−	−	Bv-F0
−	+	+	−	Bv-IF
−	+	−	+	Bv-OF

[1] B0, no biochar; Bw, biochar from wood chips; Bv, biochar from vineyard prunigns; F0, no fertilizer; IF, ammonium nitrate; OF, vermicompost from cattle manure.

The soil used in the experiment was collected from the upper 0–20 cm soil layer in an agricultural field located in the Potenza district (Southern Italy). It was preliminarily analyzed (see Section 2.2) and, accordingly, classified as sandy-loam (USDA classification), with 66.1% sand, 11.5% silt, 22.4% clay, a field capacity (−0.03 MPa) of 22.8% dry weight (dw) and a wilting point (−1.5 MPa) of 11.4% dw. Moreover, soil was characterized by the following chemical properties: pH, 7.6; electrical conductivity (EC) 0.6 dS m^{-1}; organic carbon (Corg), 5.9 g kg^{-1}; organic matter (OM), 1.0%; total nitrogen (total N), 1.5‰; C/N, 3.9; exchangeable Na^+, 63.4 mg kg^{-1}; exchangeable Ca^{2+}, 4489.2 mg kg^{-1}; exchangeable Mg^{2+}, 319.1 mg kg^{-1}; exchangeable K^+, 74.3 mg kg^{-1}. Moreover, the soil resulted in the following contents of extractable anions and cations: NO_3^-, 49.1 mg kg^{-1}; PO_4^{3-}, 8.8 mg kg^{-1}; SO_4^{2-}, 84.3 mg kg^{-1}; Na^+, 74.3 mg kg^{-1}; K^+, 62.9 mg kg^{-1}; Mg^{2+}, 19.9 mg kg^{-1}; Ca^{2+}, 287.1 mg kg^{-1}.

Before use in the experiment, soil was air-dried, crushed and passed through a 2 mm sieve.

The biochar from wood chips was a commercial product, purchased from a company located in Ivrea (Torino district, North Italy) that uses wood chips and wood processing wastes from the cleaning of green areas and woods within a controlled supply chain, in a dedicated pyrolysis plant of their own design. Biochar from vine pruning was produced at the STAR*Facility Centre of Foggia University (South Italy), using residual vine biomasses (*Vitis vinifera* L.) collected from a local vineyard. The pruning residues (15% humidity) were chipped into particles of approx. 50 mm, mixed and then pyrolized at a temperature of 750 °C for 8 h, in a pilot scale with a fixed-bed tubular reactor (30 L capacity). The heating rate was 10 °C min^{-1}. Once cooled, it was ground and passed through a 2 mm sieve.

The inorganic fertilizer, namely the ammonium nitrate, was a commercial synthetic fertilizer with 34% total N (17% NO_3^- and 17% NH_4^+). The organic fertilizer, namely the vermicompost from cattle manure, was a commercial bio-stabilized amendment with 1.5% total N. The latter was provided by a company located in Montescaglioso (Matera district, South Italy), which produces high-quality soil organic fertilizers and amendments through a bio-stabilization process of composting applied to different organic residues, such as olive mills, crops and livestock.

Plastic pots (13 cm × 13 cm × 24 cm) were first filled with 2 cm layer of expanded clay, placed at the bottom to improve water drainage, and then with 2 kg of air-dried soil. Each biochar and fertilizer was added and thoroughly mixed with the soil. Two months after experimental soil preparation, Swiss chard seedlings of uniform size were transplanted into respective treatment pots (1 plant per pot). The soil surface was covered with a 3 cm layer of polythene beads to prevent water loss by evaporation. Moisture content in each treatment pot was kept constant at the water holding capacity throughout the study period. The latter was checked daily by weighing the pots. Plants were watered weekly 2–3 times with an average irrigation volume equal to 165 mL to compensate for transpiration losses. Leaf harvest started 13 days after transplanting and was performed by cutting from the plant all the mature and fully expanded leaves and leaving the smaller, younger leaves for the next cut. Throughout the growth cycle, five leaf cuts per plant were performed, every ten days approximately. Each leaf cut was performed taking care to not blind the plant, thus allowing the subsequent development of the newly formed basal leaflets.

2.2. Soil Analysis

Before the trial started, three replicated samples of soil used in the experiment were analyzed for a set of physico-chemical properties, according to the following procedures. The particle-size distribution was determined using the pipette-gravimetric method; the field capacity and wilting point, at −0.03 MPa and −1.5 MPa, respectively, were obtained using a pressure plate apparatus (Soilmoisture Equipment Corp.). The pH was determined in the extracts of 1:2.5 (*w*/*v*) soil/water suspension by a digital pH meter (GLP 22 pH-meter, Crison Instruments, Barcelona) and the EC in the saturated soil past extract by a digital conductivity meter (GLP 31 EC-meter, Crison Instruments, Barcelona). The Corg and total N were determined by dry combustion, using a CHN elemental analyzer (CHN LECO 628). In the case of Corg, prior to analysis, samples were treated with HCl to remove carbonates. The organic matter (OM) was appraised by multiplying the percentage of Corg by the factor 1.724. The exchangeable cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) were determined in the extract of soil saturated paste by an atomic absorption spectrometer (AAS 2380, Perkin-Elmer, Seer Green, Beaconsfield, Buckinghamshire, UK). The extractable anions ($NO_3{}^-$, $P_2O_4{}^{3-}$, $SO_4{}^{2-}$) and cations ($NH_4{}^+$, Na^+, K^+, Ca^{2+} and Mg^{2+}) were determined by ion exchange chromatography (Dionex ICS-5000, Dionex Corporation, Sunnyvale, CA, USA) in the extracts of 1:10 (*w*/*v*) soil/deionized water suspensions, after 24 h of shaking [53].

Subsequently, i.e., just before transplanting (T1) and at the end of the growing cycle (T2), four replicated samples of experimental soil taken from each treatment (1 sample per pot) were oven dried, crushed, passed through a 2 mm sieve and analyzed for pH, EC, anion and cation contents, total N and Corg, according to the above reported analytical procedures.

2.3. Biochar and Organic Fertilizer Analysis

Before soil application, three samples of biochars and organic fertilizer were crushed, passed through a 2 mm sieve and analyzed for the set of chemical properties as shown in Table 2.

The pH and electrical conductivity were determined after 1 h of shaking with deionized water (1:20 *w*/*v*) and after waiting for an equilibrium time of 5 min before measurement using a GLP 22+ pH-meter and a GLP 31+ EC-meter (Crison Instruments, Barcelona, Spain), respectively. Proximate properties, i.e., coisture, volatile solid, ash and fixed carbon, were obtained using a thermogravimetric analyzer unit (LECO-TGA701), according to the ASTM D7582 method. Ultimate analysis was performed by dry combustion, using a CHNS elemental analyzer (CHNS LECO 680) that operates according to the LECO-ASTM D5373 method, to determine the C, N, H and S contents. In the case of Corg, combustion was preceded by treatment of biochar and organic fertilizer samples with HCl in order to destroy the carbonates. For the two biochars only, oxygen (O) was calculated by difference:

O (%) = 100-C-H-N-S-ash. Carbon stability of biochars was evaluated indirectly by the molar ratios of hydrogen to organic carbon (H/Corg) and oxygen to organic carbon (O/Corg).

Table 2. Chemical properties of the two biochars and the organic fertilizer used in the experiment.

Parameter	Unit	Biochar		Organic Fertilizer
		Wood Chips	**Vineyard Prunings**	**Vermicompost**
pH	-	8.9 ± 0.13	10.6 ± 0.06	7.6 ± 0.07
EC	$mS\ m^{-1}$	52.0 ± 0.04	249.0 ± 0.04	265.0 ± 0.03
Moisture	% dw	5.6 ± 0.11	15.3 ± 0.31	4.0 ± 0.17
Volatile solids	% dw	42.3 ± 0.44	15.3 ± 0.31	27.5 ± 0.58
Ash	% dw	4.4 ± 0.21	9.9 ± 0.04	72.2 ± 0.57
Fixed carbon	% dw	53.3 ± 0.24	74.8 ± 0.33	0.2 ± 0.02
C	% dw	68.3 ± 0.11	67.7 ± 0.87	11.3 ± 0.05
H	% dw	4.0 ± 0.04	2.1 ± 0.04	1.5 ± 0.06
N	% dw	1.0 ± 0.03	1.0 ± 0.01	1.5 ± 0.05
C_{org}	% dw	66.3 ± 0.06	67.0 ± 0.86	7.8 ± 0.08
C/N	-	67.2 ± 1.96	66.2 ± 0.15	5.2± 0.24
S	% dw	0.03 ± 0.01	0.2 ± 0.01	0.3 ± 0.01
O	% dw	22.3 ± 0.29	17.9 ± 1.46	5.2 ± 0.24
H/C_{org} ratio	-	0.7 ± 0.01	0.4 ± 0.01	-
O/C_{org} ratio	-	0.4 ± 0.01	0.2 ± 0.01	-

Values are means (*n* = 3) ± s.e.

Expectedly, both biochar from wood chips and biochar from vineyard prunings showed an alkaline pH and were C-rich. In particular, the C content was well within the threshold fixed by the European Biochar Certificate (EBC) [54] and Corg content according to the Class 1 defined by the International Biochar Initiative (IBI) Standard [55]. Additionally, the H/Corg molar ratio, which indicates biochar long-term stability, persistence in the soil and contribution to soil carbon sequestration, complied with the requirements of both the EBC and the IBI Standard ($H/Corg \leq 0.7$). Likewise, the O/Corg ratio, which allows us to differentiate biochar from other carbonization products [56], was found to meet the EBC and IBI-Standard requirements ($O/Corg \leq 0.4$). A similar N content of about 1% dw was detected in the two biochar types.

The organic fertilizer, i.e., the vermicompost from cattle manure, resulted in a mature and stable product, with a slightly alkaline pH, a Corg content of 8% dw and a N content of 1.5% dw. Other chemical properties of the two biochars and the organic fertilizer used in the experiment are shown in Table 2.

2.4. Plant Analysis

At each of the five leaf cuts performed during the experimental period (I, II, III, IV and V cut, respectively), growth parameters, such as leaf number (L_N), length (L_L) and weight (L_W), were determined. The leaves were counted, measured by length from the petiole base to the apex, cut and immediately weighed. After, they were dried in a ventilated oven at 70 °C until a steady weight to determine the dry weight. The dried leaf tissues were finally stored until analysis.

At the end of the experiment, productive parameters, such as total leaf number per plant (T_{LN}) and the total yield per plant (T_Y), were determined by cumulating the L_N and L_W values detected at each leaf cut. At the same time, the leaf dried tissues were grinded, homogeneously mixed and analyzed for the content in anions (NO_3^-, $P_2O_4^{3-}$, SO_4^{2-}), cations (NH_4^+, Na^+, K^+, Ca^{2+} and Mg^{2+}) and total nitrogen (total N). The anion and cation content was determined by ion exchange chromatography (Dionex ICS-5000, Dionex Corporation, Sunnyvale, CA, USA). More specifically, the anions were extracted from 0.5 g dried and ground samples, with 50 mL 3.5 mmol l^{-1} Na_2CO_3 and 1.0 mmol l^{-1} Na_2HCO_3, and were measured using an IonPac AG14 precolumn and an IonPac AS14 separation column. For the cations, 1.0 g dried and ground samples was used for the ash in a muffle furnace at 550 °C, and then digested in 20 mL 1.0 mol L^{-1} HCl in boiling water (99.5 ± 0.5 °C) for 30 min. The resulting solution was filtered, diluted and analyzed using an IonPac CG12A guard column and an IonPac CS12A analytical column. The data were expressed as mg 100 g^{-1} fresh weight (fw). The total N was determined by dry combustion, using a CHNS elemental analyzer (CHNS LECO 680). To this purpose, the dried and ground plant material was first weighed (2–5 g) and packed in tin foil capsules and then combusted in the automated CHNS analyzer.

2.5. Statistical Analysis

All the experimental data were tested for differences using analysis of variance (ANOVA) following a factorial randomized complete block design. According to the basic assumptions of ANOVA, the dataset was preliminary tested for a normal distribution and the common variance of the experimental error by applying the Shapiro–Wilk and Bartlett's tests, respectively. Data related to soil properties at the two soil sampling times (T1 and T2), leaf number, length and fresh weight over the five leaf cuts, total leaf number per plant, total yield per plant and leaf nutrient content at the end of the growing cycle were analyzed by two-way ANOVA to examine the effect of the factors: biochar (B, three levels: B0, Bw and Bv), fertilizer (F, three levels: F0, IF, OF) and their interaction (B × F). The statistical significance of the difference among the means was determined using Tukey's honest significance difference post hoc test at the 5% probability level. The ANOVA was performed using the JMP software package, version 15 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Soil Properties

The ANOVA performed on the data related to soil properties at the two soil sampling times, i.e., before plant transplanting (T1) and at the end of the growing cycle (T2), generally showed a significant effect of the experimental factors, biochar (B) and fertilizer (F), as well as their interaction, biochar × fertilizer (B × F) (Table 3).

Table 3. Statistical p values of two-way ANOVA comparing differences of soil pH and electrical conductivity (EC), extractable anion ($P_2O_4{}^{3-}$, $SO_4{}^{2-}$, $NO_3{}^{-}$), cation ($NH_4{}^{+}$, Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}), total nitrogen (total N), organic carbon (Corg) contents and C/N ratio, before plant transplanting (T1) and at the end of the growing cycle (T2).

	pH	**EC**	**$P_2O_4{}^{3-}$**	**$SO_4{}^{2-}$**	**$NO_3{}^{-}$**	**$NH_4{}^{+}$**	**Na^{+}**	**K^{+}**	**Ca^{2+}**	**Mg^{2+}**	**Total N**	**Corg**	**C/N**
							T1						
Biochar (B)	*	***	***	***	ns	***	*	***	ns	*	*	***	***
Fertilizer (F)	*	***	***	***	***	***	***	***	***	***	**	***	***
B × F	*	***	***	*	*	***	*	***	*	*	*	***	***
							T2						
Biochar (B)	*	***	***	ns	***	ns	***	***	***	***	*	***	***
Fertilizer (F)	**	***	***	***	***	ns	***	***	***	***	ns	***	ns
B × F	***	***	***	***	***	ns	***	***	***	***	**	***	**

ns, not significant; *, F test significant at $p \leq 0.05$; **, F test significant at $p \leq 0.01$; ***, F test significant at $p \leq 0.001$.

Considering the effect of biochar (B), at both T1 and T2, the $P_2O_4{}^{3-}$, Na^{+}, K^{+} and total N contents were significantly higher ($p \leq 0.001$, except for Na^{+} at T1 and total N at T1 and T2 when $p \leq 0.05$) in the soil amended with the biochar from vineyard prunings (Bv) than the biochar from wood chips (Bw) and the soil not amended (B0) (Tables 3 and 4). The biochar addition did not affect the $NO_3{}^{-}$ and Ca^{2+} contents at T1, but the effect was the opposite at T2 when significantly higher ($p \leq 0.001$) $NO_3{}^{-}$ and Ca^{2+} contents were observed in the soil amended with Bw than Bv and B0. Moreover, the biochar addition affected the $SO_4{}^{2-}$ content at T1, with Bv-treated soil showing a significantly higher ($p \leq 0.001$) $SO_4{}^{2-}$ content than Bw and B0, but these differences were no longer significant at T2. On the contrary, the addition of biochar significantly decreased ($p \leq 0.001$) at T1 the NH_4^+ content from B0, both in the soil amended with Bw and Bv, by about 50% and 20%, respectively. No differences among the biochar treatments were observed at T2 due to a very high reduction in NH_4^+ soil content, whose amount reached values below the instrumental detection limit. At both T1 and T2, Mg^{2+} and Corg contents resulted in significantly higher ($p \leq 0.001$, except for Mg^{2+} at T1 when $p \leq 0.05$) values in soil amended with both Bw and Bv in comparison with B0. At T1, soil amendment with Bv significantly increased ($p \leq 0.05$) soil pH over Bw. However, at T2, the difference between the two biochar treatments was not significant, with Bv significantly ($p \leq 0.05$) lower than B0. Furthermore, at T1, the EC significantly decreased ($p \leq 0.001$) both in Bw- and Bv-treated soil in comparison to B0, by about 23 and 26% respectively, but at T2, an opposite effect was observed, with Bv showing a significant higher ($p \leq 0.001$) EC value than Bw and B0. Finally, at both soil sampling times, the addition of the two biochars significantly increased ($p \leq 0.001$) the soil C/N value over B0: at T1, the soil C/N was, on average, 300% higher in Bw and Bv than B0; at T2, the C/N value was 200% and 110% higher in Bw and Bv, respectively, than B0.

Table 4. Soil pH and electrical conductivity (EC, dSm^{-1}), extractable anion ($P_2O_4^{3-}$, SO_4^{2-}, NO_3^-, mg kg^{-1}), cation (NH_4^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, mg kg^{-1}), total nitrogen (total N, % dw), organic carbon (Corg, % dw) contents and C/N ratio, before plant transplanting (T1) and at the end of the growing cycle (T2).

Experimental Factor		**pH**	**EC**	**$P_2O_4^{3-}$**	**SO_4^{2-}**	**NO_3^-**	**NH_4^+**	**Na^+**	**K^+**	**Ca^{2+}**	**Mg^{2+}**	**Total N**	**Corg**	**C/N**
								T1						
Biochar (B)														
B0		7.6 ab	1.2 a	7.2 c	108.5 b	294.2	5.7 a	82.7 ab	75.1 b	325.3	22.7 b	0.2 a	0.7 c	4.4 b
Bw		7.6 b	1.0 b	8.9 b	96.4 c	275.5	3.0 c	77.4 b	77.4 b	332.8	24.2 ab	0.1 b	2.2 a	18.0 a
Bv		7.6 a	0.9 b	14.6 a	143.3 a	297.3	4.7 b	88.0 a	199.8 a	306.9	25.7 a	0.1 ab	2.1 b	17.6 a
Fertilizer (F)														
F0		7.6 a	0.6 c	10.5 b	88.0 b	31.3 c	0.0 b	72.6 b	82.2 c	258.6 c	18.6 b	0.1 b	1.4 c	12.3 b
IF		7.6 b	0.8 b	7.7 c	94.6 b	632.5 a	13.5 a	75.8 b	120.9 b	378.4 a	26.8 a	0.2 a	1.6 b	11.7 b
OF		7.6 ab	1.6 a	12.4 a	165.7 a	203.2 b	0.0 b	99.8 a	149.2 a	328.1 b	27.3 a	0.1 b	2.0 a	15.9 a
Biochar × Fertilizer														
B0	F0	7.6 ab	0.6 d	8.8 c	84.3 e	49.1 c	0.0 d	74.2 bc	62.9 ef	287.1 bc	19.9 cd	0.2 ab	0.6 d	3.9 c
	IF	7.6 ab	0.9 c	4.8 d	80.0 e	618.1 a	17.2 a	70.9 a	79.0 def	351.5 ab	22.9 bcd	0.2 a	0.7 d	3.6 c
	OF	7.6 ab	2.1 a	7.9 c	161.3 b	215.4 b	0.0 d	103.0 a	83.4 de	337.5 ab	25.4 abc	0.1 b	0.7 d	5.8 c
Bw	F0	7.7 ab	0.5 d	8.8 c	66.3 e	0.0 c	0.0 d	67.0 c	53.6 f	245.1 c	17.9 d	0.1 b	1.8 c	13.0 b
	IF	7.5 b	0.6 d	6.2 cd	79.4 e	649.2 a	9.0 c	72.6 bc	91.8 d	404.9 a	27.5 ab	0.1 b	2.5 b	21.0 a
	OF	7.5 b	1.8 b	11.7 b	143.4 bc	177.1 b	0.0 d	92.7 ab	86.7 de	348.5 ab	27.1 ab	0.1 b	2.4 b	19.9 a
Bv	F0	7.6 ab	0.6 d	13.9 b	113.3 d	44.9 c	0.0 d	76.7 bc	130.1 c	243.5 c	17.9 d	0.1 b	1.8 c	20.1 a
	IF	7.6 ab	1.0 c	12.1 b	124.3 cd	630.0 a	14.2 b	83.8 abc	192.0 b	378.8 a	29.9 a	0.2 a	1.7 c	10.6 b
	OF	7.7 a	1.0 c	17.7 a	192.2 a	217.1 b	0.0 d	103.6 a	277.4 a	298.2 bc	29.4 a	0.1 b	2.8 a	22.1 a
								T2						
Biochar (B)														
B0		7.9 a	0.5 b	14.0 b	75.1	77.6 b	0.5	53.1 a	47.1 c	234.6 b	17.2 c	0.1 ab	0.8 c	9.0 c
Bw		7.9 ab	0.5 b	11.4 c	74.1	90.4 a	0.5	47.1 b	50.3 b	251.9 a	19.1 a	0.1 b	2.2 b	29.0 a
Bv		7.9 b	1.0 a	19.6 a	73.4	9.3 c	0.5	53.0 a	118.2 a	209.0 c	17.5 b	0.1 a	2.3 a	19.0 b
Fertilizer (F)														
F0		7.9 a	0.7 a	13.5 b	71.8 b	1.3 b	0.5	60.6 a	74.9 a	222.8 b	17.6 c	0.1	1.6 c	17.0
IF		7.9 a	0.6 b	12.7 c	60.3 c	175.3 a	0.5	35.8 c	65.2 b	255.4 a	18.0 b	0.1	1.7 b	23.1
OF		7.9 b	0.7 a	18.8 a	90.5 a	0.7 b	0.5	56.9 b	75,6 a	217.1 c	18.2 a	0.1	1.9 a	16.9
Biochar × Fertilizer														
B0	F0	7.9 bcd	0.4 d	12.3 e	82.7 c	0.0 d	0.5	67.8 a	47.7 f	218.2 e	16.3 g	0.1 b	0.8 cd	6.4 c
	IF	8.0 ab	0.7 b	10.0 f	64.5 d	232.9 b	0.5	31.4 f	39.4 h	265.7 b	17.2 f	0.1 b	0.7 d	9.4 bc
	OF	7.9 abc	0.4 d	19.9 b	78.0 c	0.0 d	0.5	60.2 b	54.2 e	219.7 e	18.0 d	0.1 b	0.9 c	11.2 bc
Bw	F0	8.0 a	0.4 d	10.3 f	68.1 d	4.0 d	0.5	59.2 b	58.7 d	248.4 c	20.1 a	0.1 b	2.1 b	20.4 bc
	IF	7.8 cd	0.6 bc	9.7 f	53.8 e	265.2 a	0.5	30.6 f	43.9 g	281.9 a	19.4 b	0.0 b	2.1 b	45.7 a
	OF	7.8 d	0.5 cd	14.1 d	100.4 a	2.0 b 0.1 f	0.5	51.6 d	48.3 f	225.4 d	17.7 e	0.1 b	2.4 a	20.9 bc
Bv	F0	7.8 cd	1.2 a	17.9 c	64.6 d	0.0 d	0.5	54.7 c	118.2 b	201.9 g	16.3 g	0.1 b	2.0 b	24.1 b
	IF	7.9 abc	0.4 d	18.4 c	62.8 d	27.8 c	0.5	45.3 e	112.1 c	218.7 e	17.2 f	0.2 a	2.4 a	14.3 bc
	OF	7.9 bcd	1.3 a	22.4 a	93.0 b	0.0 d	0.5	59.0 b	124.2 a	206.2 f	18.9 c	0.1 b	2.4 a	18.6 bc

B0, no biochar; F0, no fertilizer; Bw, biochar from wood chips; Bv, biochar from vineyard prunings; IF, inorganic fertilizer (ammonium nitrate); OF, organic fertilizer (vermicompost from cattle manure). Values are means (n = 4). In columns, means followed by different letters are significantly different ($p \leq 0.05$; Tukey's test).

Relative to the fertilizer (F), at both T1 and T2, the application of the organic fertilizer, namely vermicompost (OF), significantly increased ($p \leq 0.001$) the soil EC value, as well as the contents of $P_2O_4{}^{3-}$, $SO_4{}^{2-}$, Na+, K+ and Corg over the application of the inorganic fertilizer, namely ammonium nitrate (IF) and the soil not fertilized (F0) (Tables 3 and 4). On the contrary, soil fertilization with IF significantly increased ($p \leq 0.001$, except for total N when $p \leq 0.01$) the contents of Ca^{2+}, total N and the two nitrogen inorganic forms, $NO_3{}^-$ and $NH_4{}^+$, in comparison with OF and F0 at T1 (Tables 3 and 4). At T2, the effect of IF still resulted in significant effects ($p \leq 0.001$) on $NO_3{}^-$ and Ca^{2+}, but not on $NH_4{}^+$ and total N contents. In particular, as above reported for biochar, in both fertilized and unfertilized soil, the $NH_4{}^+$ content reached values below the instrumental detection limit. The fertilization also affected the soil pH, with a lower value in IF than F0 at T1 ($p \leq 0.05$) and in OF than F0-treated soil at T2 ($p \leq 0.01$). Moreover, at both T1 and T2, the application of IF and OF significantly increased ($p \leq 0.001$) soil Mg^{2+} content over F0. The C/N value was not affected by fertilizer application at T2, although it showed significant ($p \leq 0.001$) differences among the considered treatments at T1, when soil fertilization with OF increased the C/N value over IF and F0, by 36 and 30%, respectively.

All considered soil properties significantly varied also in relation to the co-application of biochar and fertilizer (B × F) (Tables 3 and 4). At both T1 and T2, significant increases ($p \leq 0.001$) of anion and cation contents were observed following the co-application of both Bw and Bv with OF than IF, except for $NO_3{}^-$, $NH_4{}^+$, which showed the opposite behavior. Indeed, they were significantly higher ($p \leq 0.001$) when both the biochars were co-applied with the inorganic fertilizer than the organic one. However, at both soil sampling times, significant higher ($p \leq 0.001$) EC values and Corg contents were found when Bw and Bv were co-applied with OF than IF. Moreover, total N content was significantly increased ($p \leq 0.01$) by the co-application of Bv with IF than OF, at both T1 and T2. The pH showed a higher value ($p \leq 0.05$) in Bv-OF than Bw-OF-treated soil at T1, while the co-application of the two biochars with both IF and OF did not result in statistically different pH values at T2 (Table 4). Finally, the C/N ratio reached the highest value ($p \leq 0.01$) at T2, following the co-application of Bw with IF (Table 4).

Generally, among the considered soil properties, $P_2O_4{}^{3-}$ and Corg contents, as well as pH and C/N values increased at the end of the growing cycle, whereas the electrical conductivity (EC) value and, expectedly, the contents of extractable anions ($SO_4{}^{2-}$, $NO_3{}^-$), cations ($NH_4{}^+$, Na^+, K^+, Ca^{2+}, Mg^{2+}) and total nitrogen (total N) showed lower values.

3.2. Plant Growth Parameters

The growth parameters of Swiss chard, such as leaf number (L_N), length (L_L) and fresh weight (L_{FW}), measured at each of the five leaf cuts performed during the whole experimental period, are reported in Table 5.

With reference to the I and II cuts, the considered experimental factors affected plant growth response differently. Indeed, at the I cut, the ANOVA revealed that neither the two factors, biochar and fertilizer, nor their interaction, biochar × fertilizer, influenced L_N. Therefore, a similar value of this parameter was observed in all considered treatments. However, L_L was affected by fertilizer ($p \leq 0.05$) and L_{FW} by both biochar and fertilizer ($p \leq 0.01$), as well as by their interaction ($p \leq 0.01$). In particular, plants growing on the soil fertilized with OF resulted in 17 and 45% higher L_L and L_{FW} values, respectively, than the soil not fertilized (F0), while plants growing on the soil amended with Bv showed a 20% lower L_{FW} value than the soil not amended (B0), which in turn did not differ from the soil amended with Bw. Futhemore, among the considered experimental treatments, B0-OF showed the highest L_{FW} value (13.7 g), accounting for a 86% increase in comparison with the other treatments (on average, 7.2 g). At the II cut, L_N and L_L did not differ among the tested biochars and fertilizers, as well as in relation to the biochar × fertilizer interaction, while L_{FW} was significantly affected by the fertilizer ($p \leq 0.001$). More specifically, both plants fertilized with OF and plants fertilized with IF respectively showed 75 and 90% higher L_{FW} values than plants not fertilized (F0). At the following III, IV and V cuts, the three considered growth parameters showed a similar response to the experimental factors. Relative to the effects of both biochar and biochar × fertilizer interaction, L_N, L_L and L_{FW} never showed significant differences. On the contrary, they were statistically different among the fertilizers ($p \leq 0.001$, except for L_N at III cut when $p \leq 0.05$, and L_{FW} at V cut when $p \leq 0.01$). Both the plants fertilized with IF and the plants fertilized with OF showed significantly higher L_N, L_L and L_{FW} values than the plants not fertilized (F0). Particularly L_{FW} highly increased, with 300, 240 and 120% higher values (as averages of IF and OF) in fertilized than unfertilized plants, at the III, IV and V cuts, respectively.

Table 5. Leaf number (L_N, n°), length (L_L, cm) and fresh weight (L_{FW}, g), at the five leaf cuts performed during the growing cycle of Swiss chard.

Experimental		I Cut			II Cut			III Cut			IV Cut			V Cut		
Factor		L_N	L_L	L_{FW}	L_N	L_L	L_{FW}	L_N	L_L	L_{FW}	L_N	L_L	L_{FW}	L_N	L_L	L_{FW}
Biochar (B)																
B0		4.4 a	10.5	9.4 a	1.9	14.2	7.3	2.0	13.9	7.9	2.6	14.0	6.7	2.8	11.4	5.2
Bw		4.2 a	9.4	6.7 b	1.8	14.3	6.2	2.1	13.6	7.8	2.7	13.0	4.8	2.4	10.0	3.4
Bw		4.1	10.2	7.6 ab	2.0	15.7	8.6	2.3	15.7	9.8	2.7	12.8	5.4	3.0	10.8	4.3
Fertilizer (F)																
F0		4.2	9.3 b	6.4 b	1.8	13.3	4.7 b	1.5 b	10.2 b	2.8 b	2.0 b	8.9 c	2.2 b	2.1 b	8.1 b	2.4 b
IF		4.4	9.8 ab	8.0 ab	1.9	15.2	8.2 a	2.5 a	16.5 a	11.5 a	3.1 a	17.1 a	9.0 a	3.3 a	12.1 a	5.6 a
OF		4.2	10.9 a	9.3 a	1.9	15.3	8.9 a	2.4 ab	16.2 a	11.1 a	2.8 a	13.9 b	5.8 a	2.8 a	11.9 a	4.9 a
Biochar × Fertilizer																
B0	F0	4.3	9.5	6.1 b	4.4	13.0	4.4	1.0	9.1	2.3	2.0	8.5	2.4	2.5	8.6	2.9
	IF	4.3	9.7	8.7 b	8.2	14.9	8.2	2.5	17.0	11.5	3.0	20.0	11.6	3.0	13.8	7.2
	OF	4.8	12.5	13.4 a	9.2	14.6	9.2	2.5	15.8	9.9	2.8	13.6	6.2	3.0	11.8	5.6
Bw	F0	4.3	8.8	6.1 b	4.3	12.7	4.3	1.8	10.7	3.3	2.0	8.8	2.1	1.8	7.8	2.1
	IF	4.5	9.6	7.5 b	6.5	15.0	6.5	2.3	14.8	8.8	3.3	15.6	7.7	3.0	10.9	4.5
	OF	3.8	9.8	6.6 b	7.8	15.1	7.8	2.3	15.3	12.5	2.8	14.5	4.7	2.5	11.3	3.6
Bv	F0	4.0	9.7	7.2 b	5.5	14.5	5.5	1.7	10.9	3.0	2.0	9.6	2.0	2.0	8.0	2.2
	IF	4.3	10.4	8.0 b	10.3	16.0	10.3	3.0	18.3	14.9	3.0	15.1	7.3	4.0	11.6	4.8
	OF	4.0	10.5	7.5 b	9.9	16.7	9.9	2.3	18.0	11.4	3.0	13.8	6.8	3.0	12.9	5.9
Significance																
B		ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
F		ns	*	**	ns	ns	***	*	***	***	***	***	***	***	***	**
B × F		ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

B0, no biochar; F0, no fertilizer; Bw, biochar from wood chips; Bv, biochar from vineyard prunings; IF, inorganic fertilizer (ammonium nitrate); OF, organic fertilizer (vermicompost from cattle manure). Values are means (n = 4). In columns, means followed by different letters are significantly different ($p \leq 0.05$; Tukey's test). ns, not significant; *, F test significant at $p \leq 0.05$; **, F test significant at $p \leq 0.01$; ***, F test significant at $p \leq 0.001$.

Considering the five leaf cuts as a whole, both in terms of total leaf number per plant (T_{LN}) and total yield per plant (T_Y) (Figure 1), both biochar and the biochar × fertilizer interaction did not show statistically significant effects, while the addition of fertilizer highly affected ($p \leq 0.001$) these two parameters. IF and OF similarly influenced plant response, both being effective for Swiss chard growth and yield. Indeed, soil fertilization with IF and OF respectively increased T_{LN} value by 31 and 22% in comparison with F0 (Figure 1a). Similarly, IF- and OF-treated plants respectively showed 133 and 116% higher T_Y values than F0 (Figure 1b).

3.3. Leaf Nutrient Content

The content of anions ($P_2O_4^{3-}$, SO_4^{2-}, NO_3^{-}), cations (NH_4^+, Na^+, K^+, Ca^{2+}, Mg^{2+}) and total nitrogen (total N), determined in leaf tissues (Table 6) was highly affected by the two experimental factors, biochar and fertilizer, as well as by their interaction ($p \leq 0.001$).

Relative to the biochar, a significantly higher ($p \leq 0.001$) content of all considered nutrients was observed in the plants growing on soil amended with Bw than Bv, except for SO_4^{2-} and total N. The former was higher in plants growing on soil not amended with biochar (B0); the latter was higher in plants treated with Bv.

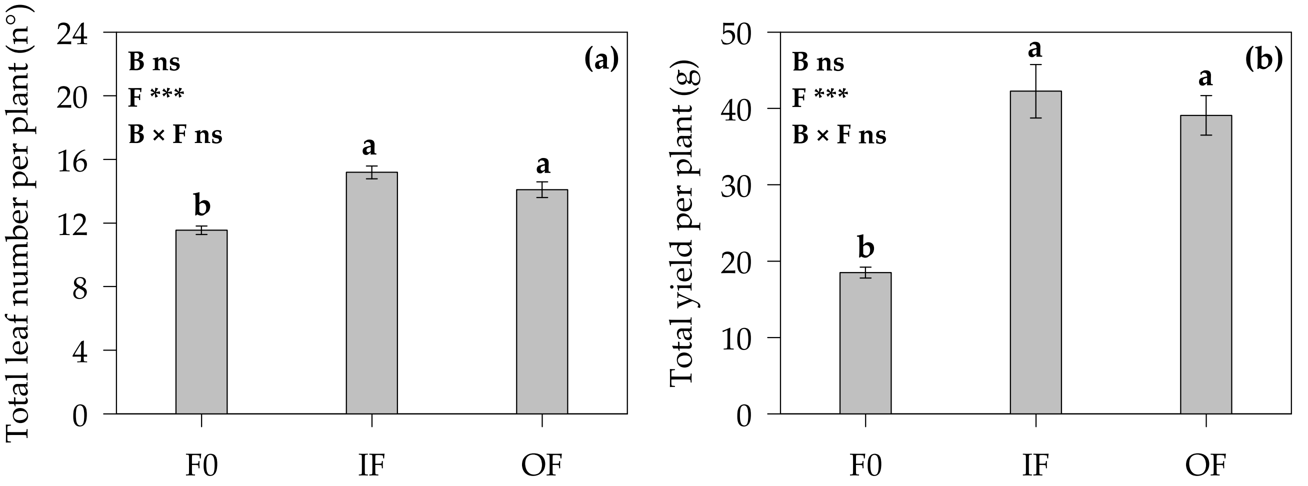

Figure 1. Main effect of fertilizer on total leaf number per plant (**a**) and total yield per plant (**b**) of Swiss chard. B, biochar; F, fertilizer. F0, no fertilizer; IF, inorganic fertilizer or ammonium nitrate; OF, organic fertilizer or vermicompost from cattle manure. Values are means (n = 4) ± standard errors. Different letters above histograms indicate significant differences among treatments ($p \leq 0.05$; Tukey's test). ns, not significant; ***, F test significant at $p \leq 0.001$.

Table 6. Content of anions ($P_2O_4^{3-}$, SO_4^{2-}, NO_3^-, mg kg^{-1} fw), cations (Na^+, Ca^{2+}, Mg^{2+}, mg kg^{-1} fw) and total nitrogen (total N, % dw) in Swiss chard leaves.

Experimental Factor	$P_2O_4^{3-}$	SO_4^{2-}	NO_3^-	NH_4^+	Na^+	K^+	Ca^{2+}	Mg^{2+}	Total N
Biochar (B)									
B0	1143.5 a	378.2 a	1723.8 a	92.5 b	2247.9 a	4414.0 b	1.6 c	156.3 c	4.9 b
Bw	1149.3 a	339.1 b	1696.5 a	104.9 a	2187.6 a	4754.2 a	3.1 a	184.5 a	4.8 c
Bv	761.5 b	291.6 c	859.4 b	77.5 c	1743.7 b	4054.6 c	2.0 b	172.1 b	5.1 a
Fertilizer (F)									
F0	1500.1 a	562.5 a	0.0 c	132.7 a	2306.2 a	4253.7 a	3.2 a	164.3 b	4.0 b
IF	788.4 b	163.4 b	3612.7 a	85.1 b	2088.6 b	5229.6 b	1.7 b	188.8 a	5.8 a
OF	765.7 b	283.0 c	667.0 b	57.2 c	1784.3 c	3739.4 c	1.8 b	159.9 b	5.0 c
Biochar × Fertilizer									
B0-F0	1594.4 a	673.0 a	0.0 g	137.2 a	2360.5 b	4357.8 cd	2.3 b	155.0 cd	3.7 f
B0-IF	810.7 de	98.9 h	4568.0 a	62.0 d	2180.6 ac	4835.4 b	1.0 d	155.7 cd	6.0 a
B0-OF	1025.4 c	362.7 d	603.5 e	78.3 cd	2202.8 ac	4048.9 d	1.4 c	158.4 c	5.1 c
Bw-F0	1684.4 a	541.8 b	0.0 g	138.0 a	2385.3 a	4054.8 d	5.6 a	179.6 b	3.7 f
Bw-IF	840.9 de	141.5 g	3923.5 b	106.8 b	2012.6 c	5503.3 a	2.1 b	193.0 b	5.8 ab
Bw-OF	922.5 cd	334.1 e	1166.1 d	70.0 cd	2164.8 ac	4704.4 bc	1.6 c	180.8 b	4.8 d
Bv- F0	1221.6 b	472.7 c	0.0 g	122.8 ab	2172.9 ac	4348.4 cd	1.7 c	158.2 c	4.6 e
Bv-IF	713.5 e	249.9 f	2346.7 c	86.4 c	2072.7 bc	5350.3 a	2.0 b	217.6 a	5.7 b
Bv-OF	349.3 f	152.2 g	231.4 f	23.4 e	985.5 d	2465.0 e	2.3 b	140.5 d	5.1 c
Significance									
B	***	***	***	***	***	***	***	***	***
F	***	***	***	***	***	***	***	***	***
B × F	***	***	***	***	***	***	***	***	***

B0, no biochar; F0, no fertilizer; Bw, biochar from wood chips; Bv, biochar from vineyard prunings; IF, inorganic fertilizer (ammonium nitrate); OF, organic fertilizer (vermicompost from cattle manure). Values are means (n = 4). In columns, means followed by different letters are significantly different ($p \leq 0.05$; Tukey's test). ***, F test significant at $p \leq 0.001$.

Among the fertilizers, a highly significant effect ($p \leq 0.001$) of IF on NO_3^-, total N and Mg^{2+} leaf content was detected. In particular, the NO_3^- leaf content of IF fertilized plants accounted for a very high increase that was equal to more than five times the NO_3^-

content of OF-fertilized plants. On the contrary, the contents of NH_4^+ and the remaining nutrients were significantly higher in the plants not fertilized (F0).

Considering the biochar × fertilizer interaction, the co-application of both Bw and Bv with IF resulted in higher leaf NO_3^- and total N content than their co-application with OF. More specifically, Bw-IF and Bv-IF-treated plants resulted in about three and ten times higher NO_3^- and total N contents than Bw-OF and Bv-OF treated plants, respectively. In contrast, the NH_4^+ content was significantly lower following the co-application of the two biochars with the two fertilizers (Bw-IF, Bw-OF and Bv-OF, Bv-OF, respectively) showing higher values in plants only treated with the two biochars. Indeed, Bw-F0 and Bv-F0-treated plants showed increases in leaf NH_4^+ content of about 30 and 40% in comparison with Bw-IF and Bv-IF, and 100 and 400% in comparison with Bw-OF and Bv-OF, respectively. Additionally, the contents of $P_2O_4^{3-}$ and SO_4^{2-}, Na^+ and Ca^{2+} showed a similar trend, while K^+ and Mg^{2+} were generally higher following the co-application of the two biochars with IF (Bw-IF and Bv-IF, respectively).

4. Discussion

4.1. Soil Properties

The co-application of biochar with inorganic and/or organic fertilizers is reported as a sustainable and environmentally friendly solution to overcome the limitation of biochar arising from an insufficient amount of nutrients contained in them and to improve soil fertility, plant nutrient availability and crop yield [41]. Moreover, in aim of the widespread adoption and integration of biochar with farming operations, formulations that combine biochar with inorganic and/or organic fertilizers are likely to have high nutrient-use efficiency and to be the most cost-effective [28].

The findings of the current study showed an overall positive effect of co-application of the two biochar types and fertilizing sources on the considered soil properties. More specifically, an increase in the anion ($P_2O_4^{3-}$ and SO_4^{2-}) and cation (Na^+, K^+ and Mg^{2+}) contents was detected in the soil following the co-application of both Bw and Bv with OF. The rich content of these nutrients in the vermicompost likely accounted for this result, as also reported by other authors [57,58]. This assumption is supported by the higher $P_2O_4^{3-}$, SO_4^{2-}, Na^+, K^+ and Mg^{2+} contents detected in the soil treated with organic fertilizer alone than in the soil treated with inorganic fertilizer alone and the untreated control (Table 4). Moreover, these findings agreed with results from previous studies that found increased nutrient contents, particularly of phosphorus and potassium, in soil amended with a biochar–vermicompost mixture [59]. Additionally, the EC value of the soil was increased by the co-application of the two biochars, and particularly Bw, with OF, especially at the beginning of the experiment. Moreover, this result was likely due to the high anion and cation content of the vermicompost that was reflected in its high EC. The electrical conductivity of vermicompost depends on the raw materials used for vermicomposting and is also related to their ion concentration [60]. Vermicompost requires careful and controlled use in terms of soil salinity, particularly when applied in high doses [61]. In this regard, Fernández-Gómez et al. [62] suggested that vermicompost with EC values lower than 4.0 dS m^{-1} is suitable for soil application. The EC of vermicompost used in this study was in accordance with this limit and, despite the EC increase in soil in which biochar and vermicompost were co-applied, the EC value remained well below the indicative threshold (4.0 dS m^{-1}) beyond which a soil is defined as saline [63]. The current results also showed a positive effect of the biochar and vermicompost co-application on soil Corg content, directly as a result of the large amount of carbon in the two biochars but also the supply of organic matter from vermicompost [64]. The vermicompost's contribution to soil Corg content is clearly evidenced by the higher Corg in soil in which the two biochars were respectively co-applied with vermicompost than applied alone (Table 4). The application of vermicompost likely boosted the Corg of the soil by providing organic matter in higher mineralizable form than that of the recalcitrant biochar, as reported by Sarma et al. [65]. In the current study, the content of total N and the two inorganic N forms, NO_3^- and

NH_4^+, were also enhanced in the soil by the co-application of biochar and fertilizer, with the total N higher when Bv was co-applied with IF and the NO_3^- and NH_4^+ were higher when both tested biochars, Bw and Bv, were applied together with IF. The current results agreed with the observation of Oladele et al. [41] who reported that the combination of biochar and N fertilizer induced a significant increase in total N, in particular within the top 10 cm depth of the soil. Additionally, Khan et al. [66] observed that biochar soil application, in combination with nitrogen fertilizer, significantly affected NH_4^+ and NO_3^- content, according to an increasing trend with the increase in biochar and nitrogen amounts. Based on the observed data from the current experiment, the higher NH_4^+ and NO_3^- contents in the soil in which biochar and inorganic fertilizer were co-applied than the soil treated with only biochar clearly showed that biochar did not have any fertilizing effect and did not serve as a nitrogen source, but likely enabled the retention of the two inorganic nitrogen forms deriving from the co-applied inorganic fertilizer [21–23,67,68]. The mechanisms responsible for increased retention of NH_4^+ and NO_3^- may be related to the intrinsic properties of biochar, such as its high charged surface area, porous structure, and strong ion exchange capacity [69]. The biochar tested in the current experiment likely provided micro-pores and high surface charge area that increased the retention of NH_4^+ and NO_3^- [70,71]. Still considering the current findings, the biochar from vineyard prunings (Bv) led to a higher soil pH than the biochar from wood chips (Bw), particularly when co-applied with OF, likely due to its higher ash content [72]. Nevertheless, this soil pH increase was observed only at the beginning of the experiment and was negligible at the end when the sole Bw application determined the soil pH increase. The effect of biochar on soil pH, widely reported in literature [73,74], was also confirmed in the current experiment, although the increase in pH units was very low and occurred in a soil with a near neutral pH. This leads us to suppose there were no effects on soil nutrients' availability [75]. contrary to acidic soils, in which biochar application can have a liming effect, which is often associated with increased nutrient availability. Consistent with our expectation, the application of the two biochars increased soil C/N, reflecting their high carbon content (>65%) (Table 2). This result was in agreement with a previous study [76]. It is well known that the C/N value is a key factor controlling N mineralization or N immobilization occurring at the same time in the soil: a value > 25 indicates the probability of N immobilization, while a value < 25:1 indicates the probability of N mineralization [77]. Therefore, controlling this soil parameter is important to avoid limitations in nutrient supply and hence plant growth following soil biochar application.

4.2. Plant Growth and Yield

In regards to plant growth and productivity, the addition of both inorganic and organic fertilizers enhanced Swiss chard growth and yield response. The positive effect of the two fertilizing sources was observed from the first leaf cut, although it was clearer starting from the third until the fifth leaf cut, when an increase in leaf number, length and fresh weight was observed. Similarly, at the end of the growth cycle, both total leaf number per plant and total yield per plant clearly showed a significant increase as a response to soil addition with both inorganic and organic fertilizers. On the contrary, the two biochar types did not affect any of the considered plant growth and productivity parameters, and showed similar effects, although they derived from different feedstock. These findings confirmed previous results [49,50] showing a lower Swiss chard growth following soil addition with biochar, both applied alone and in a mixture with composts. Similar to these findings, in a pot experiment, soil amendment with biochar had no significant effect on the shoot growth of sweet pepper, geranium and basil, while it increased coriander shoot weight and decreased the weight of lettuce plants [78]. No significant impact of sole corncob biochar application on most of the growth parameters of red pepper was also reported in a pot experiment by Ali Jaaf et al. [79]. The current results clearly showed a non-beneficial effect of both biochar types tested on Swiss chard growth and productivity performance, suggesting that, at least in the current experimental conditions, biochar cannot benefit and sustain plant growth and yield. The decrease in plant growth in biochar applications is mainly attributed to reduced nutrient availability [80]. In this regard, several authors reported that the usually high biochar C/N value could lead to an immobilization of N [44], and particularly of NO_3^--N [45], in the soils amended with biochar. In these conditions, N availability for plant uptake is limited and plant growth and yield are reduced. Due to the high C/N ratio of both of the biochars used in the current experiment (Table 2), N immobilization likely occurred in the soil, as evidenced by the zero NO_3^- leaf concentration of plants growing in the soil only amended with the two biochars and their NH_4^+ concentration similar to that of plants growing in the control soil (Table 6). As above reported, the addition of inorganic and organic fertilizers enhanced the growth and productive response of the Swiss chard. More specifically, both of the fertilizers were similarly effective in increasing the total leaf number per plant and total yield per plant. This is an interesting result, further confirming the fertilizing value of vermicompost, which has already been observed in a previous study [50]. This result also allows us to hypothesize the possibility of using vermicompost as a substitute of chemical fertilizers, in aim of a more sustainable cropping practice. In this regard, the current findings agreed with those of a number of studies, as documented in several review papers [81,82]. Indeed, vermicompost is reported to be an ideal organic fertilizer for better growth and yield of many crops due to its plant-available nutrients (nitrates, phosphates, calcium and potassium) and plant growth regulators' contents, as well as its high porosity, aeration, drainage and water-holding capacity [81].

The results of the present study allow us to speculate that Swiss chard cultivation should be oriented towards a more sustainable cultivation practice. This should involve the use of only organic amendments, and among these particularly vermicompost, in order to replace chemical fertilizers and achieve a better environmental performance of the crop production process. Vermicompost is gaining interest as it can be a greener alternative or it can be integrated with chemical fertilizers to maintain and further improve soil quality and crop production, avoiding the excessive use of inorganic fertilizers that deteriorates the physical and chemical soil properties and causes the leaching of nutrients and pollution of the environment [83]. The application of vermicompost can positively affect the biological and physico-chemical fertility of agricultural soil, which is advantageous to the development of plants. In addition, the vermicomposting process is a suitable option for the recycling of organic waste residues from agriculture, municipal and industrial wastes, avoiding their treatment or disposal and providing nutrient-rich products as valuable organic fertilizers, particularly for horticultural purposes [83].

4.3. Leaf Nutrient Content

Considering the current results related to the nutrient content of Swiss chard, the two biochars particularly increased the content of K^+, Ca^{2+} and Mg^{2+}, which are among the major essential elements for normal human health. In particular, K^+ was found to have the highest concentration and its value was in agreement with a previous study [84]. As expected, the inorganic fertilizer affected the NO_3^- and total N content of plants, according to the results of Ivanovic et al. [84] and Santamaria et al. [85], who reported that the nitrogen content of the Swiss chard is strongly affected by fertilization. The co-application of the two biochar and two fertilizer types determined a lower content of NO_3^-, NH_4^+, $P_2O_4^{3-}$ and SO_4^{2-}, Na^+ and Ca^{2+} in Swiss chard leaves, although it increased their content in the soil. This was likely due to the occurrence of nutrient retention and sorption by biochars, which reduced the nutrient availability for plant uptake. Several authors reported that biochar is effective in retaining anions and cations, such as NH_4^+, $P_2O_4^{3-}$ and NO_3^-, and attributed this ability to the high temperature (>600 °C) at which the biochar is obtained, providing micro-pores and a high surface charge area to increase retention [70,71,86,87]. Among the considered nutrients, the nitrate content of Swiss chard leaves was within the maximum levels (<3000 mg k^{-1} Fw for lettuce and similar leafy vegetables) set by the European Commission (Regulations No. 1881/2006 and 1258/2011) to avoid harmful effects of raw vegetable consumption on human health, although leaf NO_3^- content resulted in much lower values when vermicompost was applied to the soil, both alone or co-applied with the two biochar types. In this regard, the current results are in agreement with the study of Herencia et al. [88], who reported a lower nitrate content in Swiss chard under organic (from 546 to 1274 mg kg^{-1} fw) than mineral fertilization (from 780 to 2113 mg kg^{-1} fw). This result further suggests and strengthens the hypothesis that vermicompost has the potential to become a good substitute for inorganic fertilizer in Swiss chard cultivation. Its use could be a viable option not only for crop production, but also for its interesting effect of limiting the leaf accumulation of nitrates. In this regard, it is worth noting that leafy vegetables are characterized by a higher nitrate content than root or fruit vegetables [89]. Particularly, Swiss chard tends to accumulate more NO_3^- than other species, contributing highly to nitrate daily intake. When eaten, nitrate may be converted to nitrite causing several health diseases, such as methaemoglobinaemia, carcinogenic nitrosamines and even teratogenesis [90].

5. Conclusions

The results of the current study showed that the co-application of the two tested biochars, respectively deriving from vineyard prunings and wood chips, with the inorganic fertilizer, i.e., ammonium nitrate, and organic fertilizer, i.e., vermicompost, enhanced the soil properties, such as anion and cation, total nitrogen, nitrate, ammonium and carbon contents, than the sole application of biochar. The two fertilizing sources significantly improved the effect of the two biochar types, but these improvements were mainly attributable to the nutritional characteristics of the two fertilizers and, in particular, to vermicompost.

The fertilizer addition positively affected the considered plant growth and productivity parameters, with vermicompost showing a positive effect similar to that of ammonium nitrate. This suggests the effectiveness of this organic fertilizer to stimulate Swiss chard growth and its huge potential to replace inorganic fertilizer, in the context of sustainable crop production. However, the two tested biochars both resulted in lower plant growth and yield without significant differences from the untreated plants, although deriving from different feedstock.

Furthermore, the co-application of the two biochars with the two fertilizing sources increased the nutrient content of the Swiss chard leaves, particularly $P_2O_4^{3-}$, SO_4^{2-}, Na^+, $Ca^{2,}$ K^+ and Mg^{2+}, which are the major macro-elements essential for normal human health. Interestingly, vermicompost, both applied alone and co-applied with the biochars, resulted in a lower NO_3^- leaf content, suggesting that organic fertilization has the potential to reduce nitrate accumulation in the edible part of Swiss chard, resulting in a healthier and safer plant product.

Especially nowadays, the use of organic fertilizer is gaining interest and popularity in sustainable crop production and soil nutrient management. Moreover, considering that Swiss chard has value both commercially and on smaller scales, determining organic fertilizers that can maximize crop yield while preserving its quality would be beneficial to vegetable producers. The current study showed that the co-application of biochar and vermicompost is a better option for the sustainability of Swiss chard production, which can guarantee food security and safety.

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