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Co-Composting of Hop Bines and Wood-Based Biochar: Effects on Composting and Plant Growth in Copper-Contaminated Soils

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Abstract: Decades of intensive use of copper-based fungicides against downy mildew in hops has led to considerable accumulation of copper in topsoil, resulting in toxic effects on plants. Due to its high sorption capacity, the application of co-composted biochar compost might reduce copper toxicity, whereby a synergistic effect of the composting process is supposed to occur. Furthermore, biochar addition might improve the composting process itself. Therefore, hop bines were co-composted without as well as with 5 and 20 vol% biochar, respectively. During composting, the temperature and concentration of O_2, CO_2, H_2S, CH_4 and NH_3 in the compost heaps were regularly recorded. The biochar-free compost as well as the two composts with the biochar addition were characterized with regard to their plant-growing properties and were mixed into soils artificially spiked with different amounts of copper as well as into copper-polluted hop garden and apple orchard soils. The respective soil without the compost addition was used as the control, and further treatments with biochar alone and in combination with biochar-free compost were included in a plant response test with Chinese cabbage. The biochar addition increased the temperature within the compost heaps by about 30 °C and extended the duration of the thermophilic phase by almost 30 days, resulting in a higher degree of hygienization. Furthermore, the application of co-composted biochar composts significantly improved plant biomass by up to 148% and reduced the copper concentration, especially of roots, by up to 35%. However, no significant differences in the biochar-free compost were found in the artificially copper-spiked soils, and the effect of co-composted biochar compost did not differ from the effect of biochar alone and in combination with biochar-free compost. Nevertheless, the co-composting of hop bines with biochar is recommended to benefit from the positive side effect of improved sanitization in addition to reducing copper toxicity.

Keywords: phytotoxicity; immobilization; bioavailability; remediation; plant response test; composting process

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1. Introduction

Copper-based fungicides have been used in Germany for more than 150 years against fungal diseases such as downy mildew in grapes (*Plasmopara viticola*) and hops (*Pseudoperonospora humuli*), apple scab (*Venturia* spp.) and potato blight (*Phytophthora infestans*) [1]. Especially in hop cultivation, annual copper application rates up to 60 kg ha^{-1} were common until well into the 20th century. Since copper is strongly fixed by organic matter, phyllosilicates and carbonates, as well as hydrous oxides of iron, aluminum and manganese [2–4], the long-term application of large amounts of copper have led to considerable accumulation in topsoil. Unpolluted soils usually contain copper concentrations between 2 and 40 mg kg^{-1} [2], whereas topsoils from hop gardens can contain more than 120 mg kg^{-1} after 50 years of use [5]. This might cause toxic effects on plants. Besides the inhibition of germination, impairment of root growth and the occurrence of leaf chlorosis and necrosis are possible [6–8]. This leads to a reduction in nutrient uptake and photosynthetic activity and subsequently to a decrease in total biomass [9–13]. In hop cultivation, such symptoms of copper toxicity particularly occur in the first few years after replanting copper-contaminated hop gardens, as long as the root system is limited to the topsoil. Moreover, copper not only affects plants, but also soil micro-, meso- and macrofauna. Thus, the abundance of bacteria, fungi and earthworms in soils generally decreases with increasing copper contamination [14–16].

To face these numerous risks, an easy-to-apply and cost-effective solution is needed to restore the productivity and fertility of such contaminated soils and maintain them for the future. Mackie et al. [17] investigated the possibility of reducing the copper content of polluted vineyard soil via phytoextraction with various cover crops. However, the maximum annual removal rate of 33 g of copper per hectare was far too low for remediation in a reasonable time scale, which is a widely known problem for phytoextraction methods [18]. Thus, a more feasible approach might be a reduction in copper bioavailability. Ambrosini et al. [19,20] and Chatzistathis et al. [21] achieved this by raising the soil pH with lime. However, in order to avoid collateral problems such as deficiencies of other trace elements or phosphorus, as well as increased decomposition of soil organic carbon [22–24], the liming of copper-contaminated soils above the optimum pH range is not a reasonable alternative.

A more expedient approach to reduce the bioavailability of copper in soil might be the use of organic amendments such as compost and biochar made from pyrolysis [25,26]. Numerous studies, reviewed by Rizwan et al. [27], Wang et al. [28] and Wang et al. [29], have revealed that, biochar—alone and in combination with compost—has great potential for adsorbing heavy metals in soil, resulting in less toxic-induced stress for plants as well as soil organisms. Furthermore, the co-composting of biochar, especially if added directly at the beginning of the composting process, can further improve the ability to adsorb copper in soil by increasing the number of negatively charged and oxygen-containing functional groups on the biochar's surface [30], which are closely correlated to the copper sorption capacity [31]. Apart from this, the addition of biochar can accelerate the composting process by enhancing aeration and pile structure and serving as a habitat for microorganisms [32,33]. Both lead to higher microbial activity and thus a higher composting temperature.

Such an improvement in the composting process could be of particular interest to hop growers. Based on information provided by the German Hop Research Centre, in the Hallertau region—with about 17,000 ha, the world's largest hop growing region—230,000 t of hop bines arises annually as a by-product during the harvest [34]. The common practice of putting back chopped bines in hop gardens after the harvest season in late autumn bears phytopathological risks, especially the spread of hop wilt (*Verticilium albo-atrum)* [35]. Therefore, composting hop bines might be beneficial to killing pathogens [36,37]. However, as indicated by previous trials, the pile temperature during the sole composting of hop bines is not high enough for complete sanitization. This might be due to a high percentage of woody material and the coarse structure of chopped hop bines [34,38]. The addition of biochar may help to achieve complete hygienization of chopped hop bines by raising the composting temperature [39]. Thus, the use of biochar offers hop farmers the opportunity to produce a high-quality biochar compost from their organic residues, which may help to close local material flow, control hop wilt, reduce the toxic effects of high copper loads and improve soil quality by increasing soil organic carbon. However, previous research on the remediation of copper-contaminated soils has primarily focused on the use of biochar or the combination of biochar with mature compost. Compared to this, studies on the use of co-composted biochar are rather scarce [40]. Furthermore, in most cases, highly copper-contaminated mine and urban soils have been used [27,29,33]. In contrast, only few studies have dealt with arable soils, and—at least to our knowledge—none have dealt with soils from hop gardens.

To close this knowledge gap, chopped hop bines were composted together with different amounts of biochar. Subsequently, the potential of the biochar composts to reduce copper toxicity was evaluated in a plant response test using Chinese cabbage as a model plant. Arable soil spiked with copper as well as two soils from hop gardens and one from an apple orchard—all three with high copper loads due to the long-term application of copper-based fungicides—were used. The efficacy of the biochar composts was compared to that of pure biochar, biochar-free compost and a combination of both. We hypothesized that (i) the addition of biochar improves the composting process of hop bines, (ii) the application of biochar compost reduces the phyto-availability of copper and thus enhances plant growth and (iii) the biochar composts produced from co-composting have a higher efficacy than biochar alone as well as in combination with biochar-free compost.

2. Materials and Methods

2.1. Biochar, Hop Bines and Composting Procedure

The biochar (BC) used in the present trials was provided by a commercial producer (Carbuna AG, Memmingen, Germany). It was made from untreated wood from certified sustainable forestry at a temperature of 850 °C, and it complied with all thresholds of the European Biochar Certificate (EBC) for feed classification [41]. The most important physico-chemical properties are shown in Table 1.

Table 1. Physico-chemical properties of biochar used for composting, as provided by the manufacturer (pH is indicated in $CaCl_2$; surface area (BET) is expressed in square meters per gram of dry matter and total N, P, K and C_{org} in grams per kilogram of dry matter).

pH ($CaCl_2$)	Surface Area (BET) $m^2\ g^{-1}$	N_{total} $g\ kg^{-1}$	P_{total} $g\ kg^{-1}$	K_{total} $g\ kg^{-1}$	C_{org} $g\ kg^{-1}$	Molar H/C_{org} Ratio
9.3	282	5	1	11	849	0.1

In addition to biochar, chopped hop bines (hop plants of the cultivar 'Mandarina Bavaria' harvested and processed according to common practice) were used for composting. Table 2 shows the content of total nitrogen (N_{total}), phosphorus (P_{total}), potassium (K_{total}) and carbon (C_{total}), as well as the ratio between total carbon and nitrogen in the chopped hop bines. The content of total carbon (VDLUFA Method No. A 4.1.3.2 [42]) and nitrogen (VDLUFA Method No. A 2.2.5 [42]) was analyzed after high-temperature combustion using an automated thermal elemental analyzer (Vario Max CN, Elementar, Langenselbold, Germany). For total phosphorus (P_{total}) and potassium (K_{total}), microwave-assisted digestion in HNO_3/H_2O_2 was performed (VDLUFA Method No. 2.1.1 [43]). Subsequently, the copper concentration in digestion solutions was analyzed via ICP-OES (iCAP 6000 DV, Thermo Fisher Scientific, Dreieich, Germany). All analyses were performed in duplicate, and the control samples (e.g., standard material) were part of each analytical run.

Table 2. Properties of chopped hop bines used for composting (total N, P, K and C in grams per kilogram of dry matter; C/N is the ratio between total C and N).

N_{total}	P_{total}	K_{total}	C_{total}	C/N
$g\ kg^{-1}$	$g\ kg^{-1}$	$g\ kg^{-1}$	$g\ kg^{-1}$	
18	2	13	23	24

Composting was performed on a hop farm in the Hallertau region (48°34′26″ N, 11°39′14″ E) about 50 km north of Munich over a period of 215 days from October 2021 to May 2022. Based on previous composting trials as well as information from the literature [44–47], the biochar was mixed into chopped hop bines at an application rate of 5 and 20% by volume (5 and 20 vol% BCC), corresponding to 17 and 46% dry weight. Additionally, chopped hop bines were composted without biochar (compost). Compost heaps of about 10 tons each (triangle windrows with a 3 m base width, 2 m height and about 20 m length) were piled up with the three materials. The composting process was controlled at least once a week by measuring the temperature and oxygen concentration in the compost heaps. If the oxygen concentration was less than 10% or the temperature was below 40 °C [48], the compost heaps were turned with a tractor-pulled compost turner (TG 303, Gujer Innotec AG, Illnau-Effretikon, Switzerland). After the fifth turning on the 65th day, the phase with high temperatures and thus the period of intensive rotting was finished. Subsequently, the heaps were covered with a compost fleece (AGRI plus, 200 g/m^2, dm-folien GmbH, Reutlingen, Germany) for a five-month maturing phase [49].

2.2. Plant Response Test

2.2.1. Experimental Setup

The plant response test with the mature composts consisted of three sub-experiments. The effect of the three composts was examined using an artificially copper-spiked soil (sub-experiment 1), as well as two soils from hop gardens and one from an apple orchard (sub-experiment 2). In the first sub-experiment, the impact of the composts on plant growth in relation to increasing copper loads was investigated. In contrast, the second sub-experiment focused on the transferability of these results to typical arable soils with long-term copper accumulation, as recently added copper may have a higher proportion of readily available fractions than aged copper found in hop garden or apple orchard soils [50]. In the third sub-experiment of the plant response test, the differences between co-composted biochar compost, biochar and the combination of biochar with biochar-free compost were assessed.

2.2.2. Origin of Test Soils

In the plant response test, topsoils from two hop gardens in the Hallertau region (hop garden 1 and 2) and from an apple orchard formerly used as a hop garden from near Lake Constance (apple orchard) were used. All three plantations had been treated with high rates of copper-based fungicides in the past and thus were highly contaminated with copper. Topsoils were excavated to a maximum depth of 20 cm, air-dried, homogenized and sieved using a 10 mm drum sieve. Furthermore, unpolluted soil from the tertiary hilly country north of Freising was included in the experiment (0 mg Cu). This soil was additionally spiked with copper at rates of 40, 140 and 240 mg kg^{-1} (40, 140 and 240 mg Cu) using copper sulfate ($CuSO_4 \cdot 5H_2O$; analytic grade; VWR Chemicals, Radnor, PA, USA) in order to achieve copper loads reflecting the contamination of hop gardens [5].

2.2.3. Soil Amendments and Soil Properties

As mentioned in the introduction section, copper toxicity in hop cultivation is of major concern in the first years after planting. Thus, the targeted application of biochar around the planting hole is likely more effective than spreading it evenly over the entire hop garden, as a planting-hole-specific application gives a high concentration of biochar in the root zone at reliable costs [51]. In the current study, the two co-composted biochar composts and the compost without biochar were mixed into each soil at an application rate of 24 g of dry matter per kg of dry soil, resulting in the same soil-to-compost ratio as that with a dry matter application of 2.5 kg around the planting hole (cuboid with an area of 0.4 m^2, a depth of 0.2 m and a bulk density of 1.3 kg of dry soil per liter). With 2000 to 2400 hop plants per hectare, this corresponds to a compost application of 5 to 6 tons per hectare on a dry matter basis. For the third sub-experiment, biochar alone (BC) as well as in combination with biochar-free compost (BC + compost) was mixed into the soil spiked with the highest copper load (240 mg Cu). Thus, the ratio of biochar and biochar-free compost to soil corresponded to the ratio in a mixture of soil and compost co-composted with a 20 vol% biochar addition. For all three sub-experiments, the respective soils without compost addition were used as controls.

Table 3 shows the soil type, pH, CAT- and $CaCl_2$-extractable copper and total copper contents of each treatment at the beginning of the plant response test. The soil type was determined via a finger test according to VDLUFA Method No. D 2.1 [42], and the pH was measured in a 0.01 M $CaCl_2$ solution using a soil-to-solution ratio of 1 + 2.5 (weight/volume;

VDLUFA Method No. A 5.1.1 [42]). For total copper (Cu_{total}), microwave-assisted digestion (Multiwave ECO, Anton Paar, Graz, Austria) in HNO_3/H_2O_2 was performed (VDLUFA Method No. 2.1.1 [43]). Furthermore, as indicators of bioavailable copper, soils were extracted with 0.01 M $CaCl_2$ as well as with CAT (0.01 M $CaCl_2$ plus 0.002 M DTPA) according to Houba et al. [52] and VDLUFA Method No. A 6.4.1 [42], respectively. The copper concentrations in extracts and digestion solutions were analyzed via ICP-OES (iCAP 6000 DV, Thermo Fisher Scientific, Dreieich, Germany). All analyses were performed in duplicate. To ensure analytical quality, control samples (e.g., standard material) were part of each analytical run.

Table 3. Properties of soils used in the plant response test (total, CAT- and $CaCl_2$-soluble copper in milligrams per kilogram of dry soil).

Soil	Copper Load	Soil Amendment	pH ($CaCl_2$)	Cu_{total} mg kg^{-1}	Cu (CAT) mg kg^{-1}	Cu ($CaCl_2$) mg kg^{-1}
unpolluted soil (silty sand)	0 mg Cu	-	6.2	30	1	0.05
		compost	6.5	35	3	0.05
		5 vol% BCC	6.5	34	3	0.05
		20 vol% BCC	6.7	37	2	0.04
	40 mg Cu	-	6.5	63	23	0.14
		compost	6.5	67	23	0.19
		5 vol% BCC	6.6	66	23	0.19
		20 vol% BCC	6.6	68	22	0.20
	140 mg Cu	-	6.4	160	79	0.34
		compost	6.5	147	71	0.45
		5 vol% BCC	6.6	147	72	0.39
		20 vol% BCC	6.7	150	74	0.35
	240 mg Cu	-	6.6	246	140	0.49
		compost	6.4	230	124	0.45
		5 vol% BCC	6.5	240	133	0.58
		20 vol% BCC	6.6	247	135	0.53
		BC	6.4	219	114	0.26
		BC + compost	6.8	244	137	0.35
hop garden 1 (silty loam)	-	-	6.9	202	85	0.46
		compost	7.0	202	80	0.67
		5 vol% BCC	7.1	203	88	0.55
		20 vol% BCC	7.2	198	85	0.40
hop garden 2 (silty loam)	-	-	7.1	246	120	0.53
		compost	7.2	247	110	0.57
		5 vol% BCC	7.2	247	111	0.62
		20 vol% BCC	7.3	248	103	0.45
apple orchard (silty loam)	-	-	6.5	283	115	0.41
		compost	6.4	289	110	0.42
		5 vol% BCC	6.6	271	104	0.52
		20 vol% BCC	6.7	280	106	0.37

2.2.4. Plant Cultivation

The plant response test with Chinese cabbage was conducted according to VDLUFA method No. A 10.2.1 [42]. After mixing in the treatment-specific amounts of copper sulfate and/or organic amendments, soils were moistened to 60% of their maximum water capacity and filled into Styrofoam trays (20 cm × 16 cm × 4 cm, approx. 1.3 L volume) according to the bulk density. For the control, each soil without any amendment was treated in the same way. Three trays per treatment were prepared and sown with 40 seeds of Chinese cabbage ('Richi F1', Sakata Seed Corporation, Morgan Hill, CA, USA).

The experiment started in July and ran for 21 days until August 2022. Trays were arranged in a randomized block design in a glass-sheltered greenhouse. The climate settings were adjusted to 22/20 °C (day/night) for heating and 25 °C for ventilation. To meet the nutrient demands of the plants, complete water-soluble fertilizer (15% N + 9%

P_2O_5 + 16% K_2O + 2% Mg + trace elements except copper; custom blend provided by Planta, Regenstauf, Germany) was applied via fertigation according to VDLUFA Method No. A 10.2.1 [42]. Irrigation was performed with deionized water, which was added by weight evenly on the soil's surface, maintaining the soil moisture between 60 and 80% of the maximum water capacity.

2.3. Data Collection

During the thermophilic phase of composting, the temperatures as well as concentrations of O_2, CO_2, H_2S, CH_4 and NH_3 in the center of the compost heaps were recorded regularly using a digital thermometer (TL253, Proster Trading Ltd., Hong Kong, China) and a portable gas analyzer (Dräger X-am 7000, Dräger Safety, Lübeck, Germany), both equipped with a piercing probe with a 1.25 m length. At each sampling date, six measurements were made for each compost along the entire length of the heap. Furthermore, dry matter loss during composting was calculated from the dry weight of the compost heaps at the beginning and the end of the composting process after 215 days. In addition, after the completion of composting, maturity was assessed via a self-heating test in Dewar flasks according to DIN EN 16087-2 [53]. pH was measured in a 0.01 M $CaCl_2$ solution (compost to solution ratio of 1 + 2.5 (volume/volume); VDLUFA Method No. A 5.1.1 [42]), and water-soluble salts were determined according to VDLUFA Method No. A 13.4.1 [42]. For total phosphorus (P_{total}) and potassium (K_{total}), microwave-assisted digestion in HNO_3/H_2O_2 was performed (VDLUFA Method No. 2.1.1 [43]). Furthermore, soluble fractions of P and K were determined via extraction with a calcium-acetate-lactate (CAL) solution (VDLUFA Method No. A 6.2.1.1 [42]). The concentrations of P and K in digestion solutions and extracts were analyzed via ICP-OES (iCAP 6000 DV, Thermo Fisher Scientific, Dreieich, Germany). After extraction with a 0.01 M $CaCl_2$ solution (VDLUFA Method No. A 6.1.4.1 [42]), the contents of soluble nitrate and ammonium were measured photometrically via a continuous flow analysis (AA500, SEAL Analytical, Norderstedt, Germany). Moreover, the content of total carbon (C_{total}; VDLUFA Method No. A 4.1.3.2 [42]) and nitrogen (N_{total}; VDLUFA Method No. A 2.2.5 [42]) was analyzed after high-temperature combustion using an automated thermal elemental analyzer (Vario Max CN, Elementar, Langenselbold, Germany). All analyses were performed in duplicate, and the control samples (e.g., standard material) were part of each analytical run.

At the end of the plant response test, plants were cut off just above the soil's surface, and the above-ground fresh biomass was recorded. The above-ground biomass was then dried at 60 °C in a forced-air oven until a constant weight and dry weight were determined. In addition, root growth was visually rated from 1 (no roots) to 7 (intensive rooting) after the Styrofoam trays had been removed. Subsequently, roots were picked out of the soil, washed with deionized water and dried at 60 °C until weight constancy. The copper concentrations of both tissues—the above-ground biomass (shoots) and roots—was analyzed as described for the analysis of total copper in soils after grinding in a centrifugal mill (ZM 200, Retsch, Haan, Germany).

2.4. Statistical Analysis

For each level of copper loading (0, 40, 140 and 240 mg Cu) in the first sub-experiment of the plant response test, above-ground fresh and dry biomasses, as well as the copper concentrations of shoots and roots in soils amended with different types of compost (compost, 5 vol% BCC and 20 vol% BCC) and in the soil without compost (control), were subjected to a one-way ANOVA. Subsequently, the significances of means were tested against each other within the same level of copper loading via a Tukey test at a significance level of 5%. Moreover, the same procedure was conducted on each copper-contaminated soil (40, 140 and 240 mg Cu) for the percentage deviation in fresh biomass between copper-spiked treatments and the corresponding treatments without copper addition, but with the same compost amendment (compost, 5 vol% BCC and 20 vol% BCC) or without compost (control). For the second sub-experiment with soils from two hop gardens and an apple orchard, above-ground fresh and dry biomasses, as well as the copper concentrations of shoots and roots, were also compared via a one-way ANOVA and a subsequent Tukey test with $p \leq 0.05$ in relation to the application of amendments. In the third sub-experiment, using only the highest copper-spiked soil (240 mg Cu), the benefit of the co-composting of biochar (20 vol% BCC) compared to the application of biochar (BC) and to the combined application of biochar plus biochar-free compost (BC + compost) was evaluated in the same way. For further comparison, treatments without any amendment (control) and with the amendment of biochar-free compost (compost) were used.

In addition to above-ground fresh and dry biomasses, as well as the copper concentrations of shoots and roots, the scores of the visual ratings of root growth were analyzed via a Kruskal–Wallis test ($p \leq 0.05$). In case of significant differences, ranks were tested pairwise (Nemenyi test, $p \leq 0.05$) to the respective control, which means to the same amendment in the unspiked soil (0 mg Cu) in the first sub-experiment, as well as to the same soil (hop garden 1 and 2, apple orchard, 0, 40, 140 and 240 mg Cu) without any amendment in the first, second and third sub-experiment, respectively. For data pre-processing and visualization, MS Excel 2016 (Microsoft Corporation, Redmond, WA, USA) was used, whereas statistical calculations were performed with Minitab V18 (Minitab Inc., State College, PA, USA).

3. Results

3.1. Composting

Figure 1a shows the time course of temperature in the heaps during the thermophilic phase of composting, as well as the ambient temperature at a 2 m height. After only three days, all compost heaps heated up to 60 to 70 °C, whereby temperatures were highest in the compost co-composted with 5 vol% biochar (5 vol% BCC) and lowest in the one without biochar (compost). Subsequently, temperature profiles were initially similar in all composts. However, after the third turning on day 37, they differed clearly; whereas the compost without biochar did not re-warm, the temperature rose again significantly in both heaps with biochar and was about 30 °C higher than that in the biochar-free heap, up to around day 60. Thus, the thermophilic phase, which is characterized by temperatures above 40 °C [48], was almost 30 days longer with the addition of biochar. Differences between the two co-composted biochar composts were less pronounced; whereas the lower biochar input of 5 vol% (5 vol% BCC) caused slightly higher temperatures until day 24, the opposite was found later between days 41 and 57, when the higher application rate of 20 vol% biochar (20 vol% BCC) resulted in substantially higher temperatures. After the fifth turning on day 65, none of the compost heated up again strongly. Thus, it was assumed that the end of the intensive phase of composting had been reached at this point.

The time course of oxygen concentration in the compost heaps (Figure 1b) was more or less the opposite of the temperature curves; after an initial phase with quite low values between days 3 and 9, especially in the heaps without or with the 5 vol% biochar addition, the oxygen concentration rose again in all heaps. In the biochar-free compost, the oxygen concentration almost reached the atmospheric level on day 13 and remained there during further composting. Only during the initial phase (until day 13), the lower application rate of 5 vol% biochar caused a larger decrease in the oxygen concentration compared to 20 vol% biochar. In contrast, from day 19 onward, the time course of oxygen concentration in the biochar-containing heaps was quite similar. The oxygen concentration ranged between 15 and 20% and always showed clear downward peaks, whenever the heap temperatures increased after turning the compost. The carbon dioxide concentration in the compost heaps was closely negatively correlated with the oxygen concentration ($R^2 > 0.98$) and never exceeded 13%. Furthermore, critical levels of methane, hydrogen sulfide and ammonia were not detected at any time during composting.

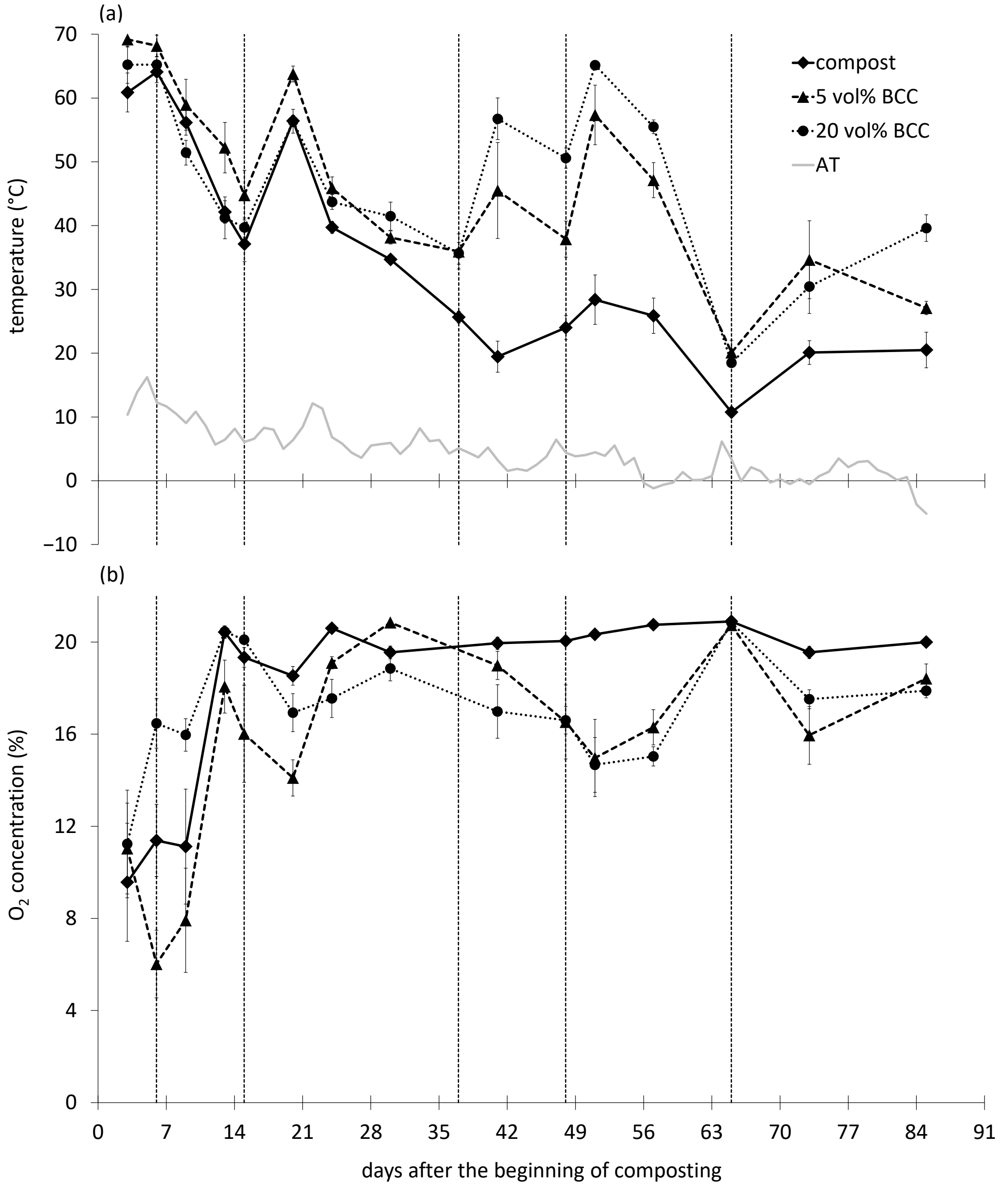

Figure 1. Temperature (**a**) and oxygen concentration (**b**) in the heaps during the thermophilic phase of hop bine composting without biochar (compost) as well as with 5 and 20 vol% biochar additions (5 and 20 vol% BCC), respectively. The gray line shows the ambient temperature (AT). Vertical lines indicate compost turning dates. Error bars represent the standard error of the mean (n = 6).

Whereas dry matter loss during the composting of hop bines without biochar was 46%, it was 44 and 31% with the 5 and 20 vol% biochar additions, respectively (Table 4). Assuming that carbon derived from biochar is largely stable in relation to microbial decomposition [54] and that dry matter loss is therefore almost exclusively due to the degradation of the hop bines, the addition of 5 or 20 vol% biochar at the start of composting led to dry matter losses in hop bines of 53 and 58%, respectively.

The self-heating test after 215 days of composting confirmed the maturity of all composts, as temperatures did not exceed 30 °C during the eight-day test. Although the pH was the same in all composts, water-soluble salts as well as $CaCl_2$-soluble nitrogen and CAL-soluble phosphorus and potassium were highest in the compost co-composted with 5 vol% biochar (5 vol% BCC; Table 5). The lowest values were found in the compost co-composted with 20 vol% biochar (20 vol% BCC) for $CaCl_2$-soluble nitrogen and in the biochar-free compost (compost) for CAL-soluble phosphorus and potassium. In all three composts, $CaCl_2$-soluble nitrogen was mainly present in form of nitrate (≥98%), which confirms the maturity of the composts [55]. In terms of total contents, the highest concentrations were recorded in the compost without biochar for nitrogen and phosphorus and in the compost co-composted with 5 vol% biochar for potassium, whereas the compost co-composted with 20 vol% biochar showed the lowest levels of all three macronutrients. Finally, the total carbon content and the C/N ratio increased with increasing percentages of biochar addition at the beginning of composting.

Table 4. Dry weight of hop bines and biochar at the beginning (separately) and end of composting after 215 days (total), as well as dry matter loss of the total and of hop bines (dry weight in kilograms, and dry matter loss in percent).

Soil Amendment	Beginning of Composting		End of Composting	Dry Matter Loss	
	Hop Bines kg	Biochar kg	Total kg	Total %	Hop Bines [1] %
Compost	3181	-	1720	46	46
5 vol% BCC	2235	443	1488	44	53
20 vol% BCC	2132	1811	2707	31	58

[1] dry matter loss assumed exclusively due to the degradation of hop bines.

Table 5. Properties of the mature composts used as soil amendments (pH is indicated in $CaCl_2$; water-soluble salts are expressed in grams of KCl per liter, whereas $CaCl_2$-soluble NH_4-N, NO_3-N and N, as well as CAL-soluble P and K, are expressed in milligrams per liter; total N, P, K and C are expressed in grams per kilogram of dry matter (DM); C/N is the ratio between total C and N).

Soil Amendment	pH ($CaCl_2$)	Salts (H_2O) g L^{-1}	NH_4-N ($CaCl_2$) mg L^{-1}	NO_3-N ($CaCl_2$) mg L^{-1}	N ($CaCl_2$) mg L^{-1}	P (CAL) mg L^{-1}	K (CAL) mg L^{-1}	N_{total} g kg^{-1}	P_{total} g kg^{-1}	K_{total} g kg^{-1}	C_{total} g kg^{-1}	C/N
Compost	8.0	2.3	4	164	168	107	1487	27	2.5	17	376	14
5 vol% BCC	8.0	3.5	4	285	289	234	2462	25	2.2	19	466	19
20 vol% BCC	8.0	2.7	1	138	139	137	2134	13	1.6	14	629	48

3.2. Plant Response Test

3.2.1. Effect of Composts on Artificially Copper-Spiked Soils

Irrespective of the copper level in the soil, the application of all composts significantly increased the above-ground fresh and dry biomass of Chinese cabbage compared to the same soil without compost (Figure 2a). In addition, leaf chloroses, which occurred at the end of the experiment in the unamended soil spiked with 240 mg kg^{-1} copper, were also prevented by each of the three composts. Although in most cases the three composts did not differ significantly in their growth-promoting effects, the highest biomass was consistently found in treatments where compost co-composted with 5 vol% biochar was applied. In addition to the effect on the above-ground biomass, the compost application reduced the copper concentration of shoots in copper-contaminated soils significantly by up to 30% compared to the respective control soil (Figure 2b). However, as with the above-ground biomass, the copper concentrations of shoots did not significantly differ between the three composts. This was also true for the copper concentrations of the roots in the most copper-spiked soil (240 mg Cu; Figure 2c). In contrast, at the medium level of contamination (140 mg Cu), the copper concentration of roots was only reduced by the two co-composted biochar composts (5 and 20 vol% BCC) but not by the biochar-free compost (compost), whereas for at the lowest level of contamination (40 mg Cu), only the biochar-free compost led to a significant reduction in the copper concentration of the roots. The evaluation of root ratings showed that, regardless of the amendment type, root growth was significantly reduced by copper loadings of 140 and 240 mg kg^{-1}. However, compared to the unamended soil, the application of all composts significantly enhanced root growth, irrespective of the copper level in the soil.

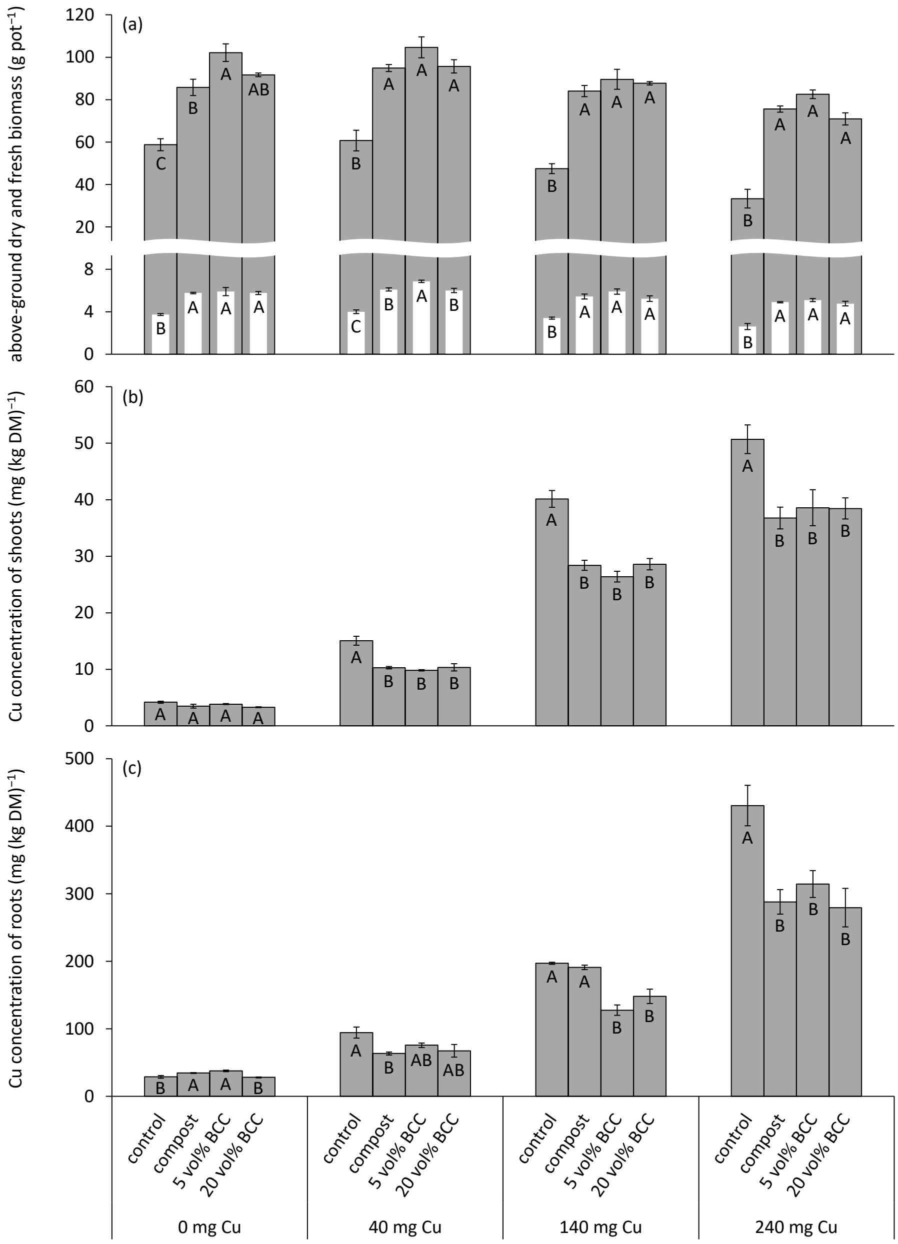

Figure 2. Above-ground fresh ((**a**), gray columns) and dry ((**a**), white columns) biomass, as well as copper (Cu) concentrations of shoots (**b**) and roots (**c**) of Chinese cabbage cultivated in unpolluted soil (0 mg Cu) and in the same soil spiked with different amounts of copper (40, 140 and 240 mg Cu), each either amended with different types of compost (compost = compost without biochar; 5 vol% BCC = compost co-composted with 5 vol% biochar; 20 vol% BCC = compost co-composted with 20 vol% biochar) or without compost amendment (control). Treatments with the same letter within a copper level do not differ significantly (Tukey test with $p \leq 0.05$). Error bars represent the standard error of the mean ($n = 3$).

Figure 3 shows the percentage difference in above-ground fresh biomass between soils without and with copper spiking. At the lowest level of 40 mg kg^{-1}, even a small enhancement in fresh biomass compared to the respective unpolluted soil was observed for all soil amendments, whereas copper additions of 140 and especially 240 mg kg^{-1} resulted in fresh biomass losses. The extent of the fresh biomass loss depended on the type of soil amendment. When applying the biochar-free compost (0% BCC) to the soil contaminated with 140 mg kg^{-1} copper, fresh biomass loss was significantly reduced by 17 percentage points compared to the control soil without compost. An even higher reduction in fresh biomass loss of 31 percentage points was observed at the highest level of copper contamination, where both co-composted biochar composts also reduced fresh biomass losses significantly (5 vol% BCC: 24 points; 20 vol% BCC: 21 points). However, among the three composts, no significant differences were found at any copper level.

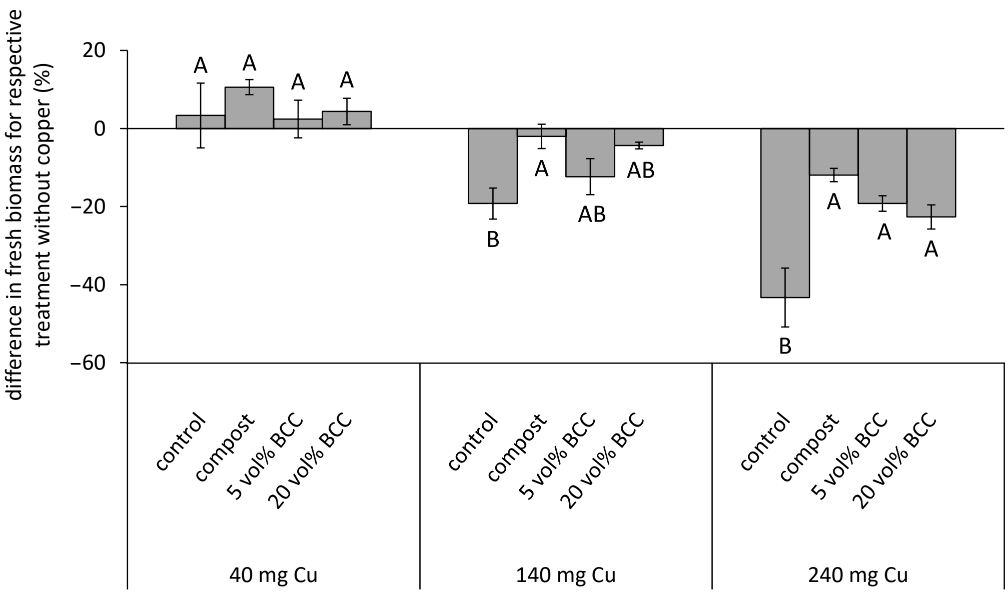

Figure 3. Percentage difference in above-ground fresh biomass of Chinese cabbage cultivated in soil spiked with different amounts of copper (40, 140 and 240 mg Cu) compared to the respective treatment without copper addition. Each soil was either amended with different types of compost (compost = compost without biochar; 5 vol% BCC = compost co-composted with 5 vol% biochar; 20 vol% BCC = compost co-composted with 20 vol% biochar) or was not amended with compost (control). Treatments with the same letter within a copper level do not differ significantly (Tukey test with $p \leq 0.05$). Error bars represent the standard error of the mean ($n = 3$).

3.2.2. Effect of Composts on Hop Garden and Apple Orchard Soils

In contrast to the copper-spiked soils, clear differences in above-ground fresh biomass were observed among the three composts in soils from plantations (Figure 4a). For all three soils, the highest fresh biomass was found in treatments where the compost co-composted with 5 vol% biochar (5 vol% BCC) was applied, followed by the compost co-composted with 20 vol% BC (20 vol% BCC). On the other hand, significant enhancement of fresh biomass by the biochar-free compost (compost) was only observed for the soil from hop garden 2. In contrast to fresh biomass, no significant differences were recorded for dry biomass. Furthermore, only the compost without biochar significantly reduced the copper concentration of shoots in one soil (hop garden 1; Figure 4b). Apart from that, the copper concentration of roots was generally reduced by compost applications (Figure 4c). However, only the reduction in the soil from the apple orchard was significant for all three composts, with an average of 23%. Although the copper concentration of roots was also reduced in both soils from hop gardens, a significant reduction was only observed when using compost co-composted with 20% biochar (20 vol% BCC) in the soil from hop garden 2. Root growth was significantly enhanced by all three composts in the soil from hop garden 1. In contrast, no effect was found for the soils from hop garden 2 and the apple orchard.

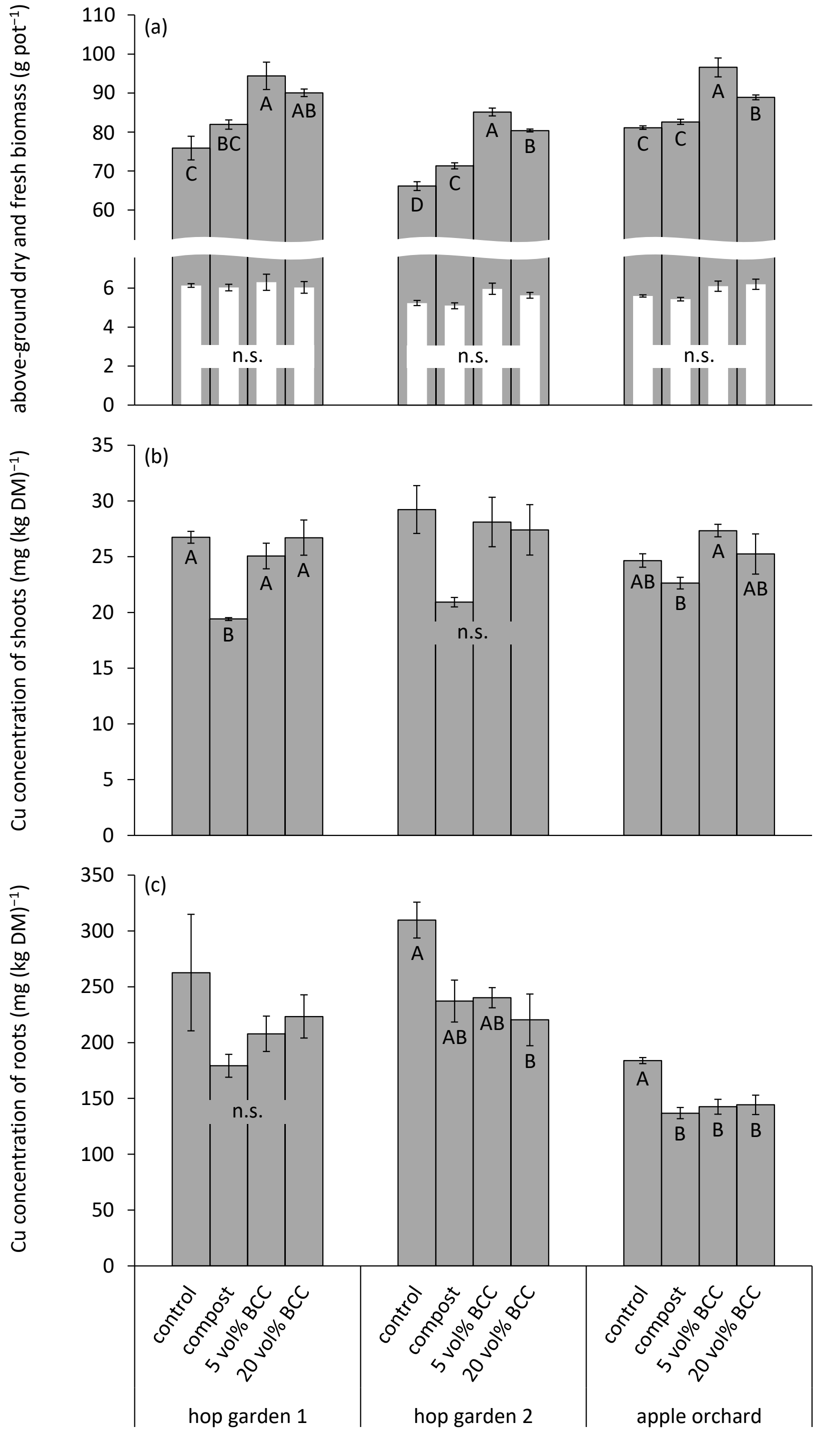

Figure 4. Above-ground fresh ((**a**), gray columns) and dry ((**a**), white columns) biomass, as well as copper (Cu) concentration of shoots (**b**) and roots (**c**), of Chinese cabbage cultivated in copper-contaminated soils from two hop gardens and an apple orchard, each either with amendments of different types of compost (compost = compost without biochar; 5 vol% BCC = compost co-composted with 5 vol% biochar; 20 vol% BCC = compost composted with 20 vol% biochar) or without compost amendment (control). Treatments with the same letter within a soil origin do not differ significantly (Tukey test with $p \leq 0.05$; n.s. = no significant effect in the ANOVA). Error bars represent the standard error of the mean (n = 3).

3.2.3. Effect of Pure Biochar Alone and in Combination with Biochar-Free Compost

As shown in Figure 5 for the most copper-spiked soil, there were no significant differences, neither in fresh or dry biomass nor in the copper concentrations of the shoots and roots of Chinese cabbage between the biochar (BC), the combination of biochar with biochar-free compost (BC + compost) and the compost without biochar (compost), as well as that co-composted with 20 vol% biochar (20 vol% BCC). However, compared to the unamended soil, despite having a higher fresh and dry biomass, there was no significant reduction in the copper concentration of shoots by the combination of biochar with biochar-free compost, and there was no significant reduction in the copper concentration of roots by biochar alone and in combination with biochar-free compost. In the same way as compost amendments without biochar or co-composted with 20 vol% biochar, biochar alone and in combination with biochar-free compost significantly improved root growth compared to the unamended control soil.

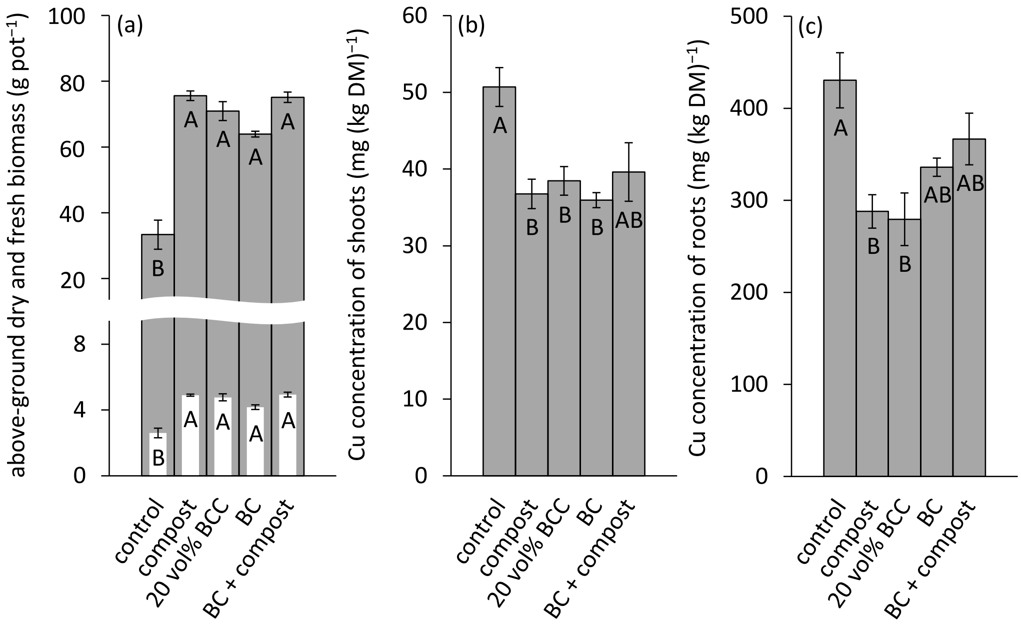

Figure 5. Above-ground fresh ((**a**), gray columns) and dry biomass ((**a**), white columns), as well as copper (Cu) concentrations of shoots (**b**) and roots (**c**), of Chinese cabbage cultivated in the highest copper-spiked soil (240 mg kg^{-1}), amended on the one hand with compost either without biochar (compost) or co-composted with 20 vol% biochar (20 vol% BCC) and on the other hand with biochar either alone (BC) or in combination with biochar-free compost (BC + compost) compared to the soil without amendment (control). Treatments with the same letter do not differ significantly (Tukey test with $p \leq 0.05$). Error bars represent the standard error of the mean ($n = 3$).

4. Discussion

4.1. Effect of Biochar on Composting

Both the low and high biochar application resulted in longer-lasting higher temperatures during composting compared to the compost without biochar addition (Figure 1a). Similar results were obtained by Wei et al. [56], when composting tomato stalks with chicken manure, and by Jain et al. [57] in batch composting nitrogen-rich organic waste. However, such an enhancement of the composting process via biochar addition was observed not only in combination with nutrient-rich organic material, but also with nutrient-poor and structurally rich organic material, as used in the present experiment [39]. Since the heating of compost piles is the result of metabolic activity during the microbial decomposition of organic matter, biochar addition seems to enhance microbial activity. This assumption is further confirmed by the higher consumption of oxygen (Figure 1b) and the simultaneous higher production of carbon dioxide, which is a sign of higher microbial activity [58,59]. This promoting effect of biochar on microorganisms might be due to the optimization of moisture, aeration and nutrient conditions in the heaps and the provision of a suitable habitat for microorganisms due to the large surface area and high microporosity of the biochar particles [60,61]. In addition to increasing microbial activity, the reduction in heat losses by filling free spaces in the compost pile with biochar particles may have contributed to the prolongation of the thermophilic phase [62]. Higher absorption of sunlight by the darker color of the compost piles with biochar [63] is rather unlikely, as composting was performed in late autumn.

From a phytopathological point of view, biochar addition has phytosanitary effects because, the longer that high temperatures are maintained during the composting process, the more likely that all pathogens—in hop production, particularly *Verticillium*—are killed [64]. Since, according to Talboys [37], a temperature of 40 °C over a period of 7 days is sufficient to kill *Verticillium*, even the compost without biochar addition (compost) should be free of *Verticillium* (Figure 1a). However, these are the minimum requirements that must be achieved not only in the center, but also at the edge of the compost heap. Thus, frequent turning of piles followed by a repeated temperature increase is essential for complete sanitization [65,66]. Due to the short thermophilic phase, the biochar-free compost (compost) was only turned twice during this phase, whereas the composts co-composted with biochar (5 and 20 vol% BCC) were turned four times (Figure 1a). This more frequent turning significantly reduces the risk of *Verticillium* survival. Especially in view of the potential damage, which might be caused by spreading this soil-borne pathogen in hop gardens [67], the addition of biochar to composting is therefore highly recommended.

In addition to the enhanced time–temperature profile during composting, the promotion of microbial activity by adding biochar also led to an increase in the dry matter loss of hop bines—at least, if biochar is assumed to be recalcitrant against microbial degradation (Table 4). This assumption is supported by the high C/N ratios of 19 (5 vol% BCC; Table 5) and 48 (20 vol% BCC; Table 5), which are quite typical for biochar-containing composts [68]. Furthermore, apart from less leaching of nutrients by the addition of biochar [32,57], enhanced dry matter loss could be another reason for the mostly higher contents of soluble nitrogen, phosphorus and potassium in the biochar composts compared to the biochar-free one (Table 5). However, due to the low contents of these nutrients in the biochar (Table 1), total nitrogen, phosphorus and potassium were lowest in the compost co-composted with the highest biochar addition of 20 vol% (Table 5). Nevertheless, according to the guidelines of the German Federal Compost Quality Association [69], all composts were of high quality and, in terms of their salt and nutrient contents, met the minimum requirements for use as a growing medium constituent up to 20 vol%. For this reason and in view of the increased sanitization, it can be concluded that the addition of biochar improves the composting process of hop bines, which confirms our first hypothesis.

4.2. Effect of Co-Composted Biochar Compost on Plant Growth and Phyto-Availability of Copper

In the current experiment, the application of compost—whether without biochar or co-composted with biochar—significantly enhanced the above-ground fresh and dry biomass and improved the root growth of Chinese cabbage in the artificially copper-spiked soils (Figure 2a). Similar growth-promoting effects of organic amendments, including biochar and compost, in copper-contaminated soils have also been observed in previous studies [70–72]. However, plant growth was also improved in the uncontaminated soil, which can be attributed to the positive effects of the compost amendment on the soil's properties. These include the increase in soil organic carbon, water retention and holding capacity, as well as available nutrients and improvements in soil aggregation and stability, cation exchange capacity, soil pH and microbial activity [73–79]. However, due to the consistent maintenance of soil moisture within the range of 60 to 80% of the maximum water capacity and the continuous application of a water-soluble fertilizer, the significance of enhanced water retention and holding capacity, as well as nutrient supply, should be negligible in the current experimental setup. The same applies to soil pH, which was already in the optimum range for the used soil [80] and was not significantly altered by the composts (Table 3).

In addition to the positive effects on soil properties, the reduction in copper phyto-availability also seems to have contributed to the improved plant performance, as the copper-induced loss in fresh biomass of plants was consistently reduced by compost amendments (Figure 3). This assumption is further confirmed by the copper concentration of the shoots of Chinese cabbage (Figure 2b), which exceeded the toxic limit of 15 to 20 mg kg^{-1} [81–84] with copper additions of 140 and 240 mg kg^{-1}, respectively, irrespective of the compost amendment, but it was significantly reduced by all composts to the same extent. Furthermore, the prevention of leaf chlorosis in the compost-amended soils indicates a reduction in copper phyto-availability. In contrast to the shoots, the accumulation of copper was consistently more pronounced in the roots (Figure 2c), where copper levels were seven times higher due to species-specific limited translocation [85,86]. However, these elevated levels were also significantly reduced by the composts. Possible mechanisms underlying such a reduction in copper phyto-availability by organic amendments include ion exchange, electrostatic interaction, complexation, precipitation and physical adsorption [27–29,87]. In contrast to the copper concentration in plant biomass, the concentration of $CaCl_{2-}$ and CAT-soluble copper in the contaminated soils was not consistently reduced by compost application (Table 3). This seems to contradict the assumption of a reduction in copper phyto-availability. However, Soja et al. [88] postulated that the analysis of extractable copper is not suitable for assessing the ecotoxicological potential of copper in soil. They calculated that, despite an increase in the extractable fraction, the content of more toxic divalent copper in soil was significantly reduced by organic amendments.

Similar to the artificially copper-spiked soils, the application of compost—especially co-composted biochar compost—increased the above-ground fresh biomass of Chinese cabbage in the hop garden and apple orchard soils (Figure 4a). In contrast to the findings of Schulz et al. [89], who found that plant growth proportionally improves with increasing amounts of biochar in co-composted composts, the highest biomass was consistently achieved with an initial addition of 5 vol% biochar. However, the increase in fresh biomass due to compost application in the soils with long-term copper accumulation was only about 15%, which is considerably lower than 83% observed in the artificially copper-spiked soils. In addition, the enhancement of root growth by compost amendments was significantly less pronounced in the plantation soils. The stronger growth-promoting effect of compost amendments in the artificially copper-spiked soils might be related to their poor soil structure (silty sand), which benefits more from positive effects on the soil properties of organic inputs compared to the loamy soils from plantations [90,91]. Thus, the main compost effect in the hop garden and apple orchard soils was likely the reduction in copper phyto-availability, which is supported by the decline in the copper concentration of roots after compost application (Figure 4c). However, despite comparable contents of total copper in the hop garden and apple orchard soils in relation to the most copper-spiked soil (240 mg Cu; Table 3), the copper concentrations of the shoots and roots of Chinese cabbage were quite lower in the plantation soils (Figure 4b,c). Thus, the minimum concentration of copper in the above-ground biomass required to trigger toxic reactions (15 to 20 mg kg^{-1}) was exceeded (Figure 4b) to a lesser degree, which might explain the absence of leaf chloroses compared to the most copper-spiked soil. This discrepancy between soils with comparable contents of total copper aligns with the findings of Chigbo and Batty [92], who reported a higher concentration and accumulation of copper in *Brassica juncea* in freshly copper-spiked soil compared to soil with aged copper contamination. Considering the growth-promoting effect and the reduction in copper phyto-availability due to the incorporation of co-composted biochar composts into copper-contaminated soils from the hop gardens and apple orchard, our second hypothesis is confirmed. However, for soils artificially spiked with copper, the hypothesis must be rejected due to the absence of differences compared to the biochar-free compost.

4.3. Difference in Efficacy of Co-Composted Biochar Compost Compared to Biochar Alone and in Combination with Biochar-Free Compost

Like compost amendments without biochar (compost) or co-composted with the 20 vol% biochar addition (20 vol% BCC), both biochar alone (BC) and in combination with biochar-free compost (BC + compost) significantly improved root growth and increased the above-ground biomass of Chinese cabbage in the most copper-spiked soil (Figure 5a). These findings are consistent with the results of a field trial with peanut plants conducted by Agegnehu et al. [44], where no differences were observed in seed yield, total pod yield and chlorophyll content between biochar, compost, the combination of both and co-composted biochar compost. In addition to the similar efficacy of the organic amendments on plant growth, a comparable reduction in copper phyto-availability (Figure 5b,c) was found. Thus, even the compost without biochar already exhibited high potential for reducing the phyto-availability of copper in soil, which is in line with the findings of others [93–95]. Moreover, no synergistic effects were observed by combining biochar with biochar-free compost, which confirms the study by Seehausen et al. [96], but it is contrary to previous findings [97,98]. Nevertheless, the combination of compost with biochar is generally recommended, as the application of biochar-free compost provides shorter longevity in soil due to faster degradation compared to the combination with biochar [99]. Furthermore, the application of biochar without compost can affect plant growth by immobilizing nutrients in the soil, mainly due to the adsorption of mineral nitrogen and dissolved organic carbon [100–102]. Although this effect did not occur in the current experiment, which is likely due to the continuous supply of water-soluble nutrients, the combination of pure biochar with organic amendments such as compost is suggested to avoid any problems [75,103]. However, without co-composting, the beneficial effect of biochar on the composting process, including improved sanitization, would be missing. Finally, in view of reducing the phyto-availability of copper and improving plant performance without positive side effects, our third hypothesis has to be rejected.

5. Conclusions

The addition of biochar to chopped hop bines improved the composting process by increasing the temperatures in the compost heaps and extending the thermophilic phase. Thus, the co-composting of biochar and hop bines ensures better hygienization, which reduces the risk of *Verticillium* survival. However, the optimum mixing ratio between hop bines and biochar should be a part of future research, as even smaller amounts of biochar, as used in the current experiment, might already be sufficient. This would increase the practical suitability of biochar application, which is often limited by economic reasons due to the high prices of biochar. In contrast to the composting process, no clear benefits of biochar addition were found in the plant response test. This was true for both the application of co-composted biochar composts as well as the application of biochar either alone or in combination with biochar-free compost. Only in the soils from the hop gardens and orchard but not in the artificially copper-spiked soils, a slight positive effect of co-composted biochar was found; this means a significant increase in plant growth compared to the application of biochar-free compost was observed. Thus, from an agronomic point of view, the application of biochar to mitigate copper-induced growth reduction seems to be unnecessary, as the same effect could be obtained with more cost-effective biochar-free compost. However, there are limitations to the current study that need to be considered. The plant response test in the present experiment was conducted under controlled environmental conditions with an optimum supply of water and nutrients and lasted only 21 days. Thus, the results cannot directly be transferred to the replanting of hop gardens, where symptoms of copper toxicity are often observed over the first years after planting, and plants might suffer from a temporary lack of water or nutrients. Therefore, future research should focus on validating the mitigation effects of biochar-free as well as co-composted biochar compost under field conditions, especially over a longer period and under water- as well as nutrient-limited conditions. Furthermore, the possible side effects of biochar application should be considered. This includes the increase in soil organic carbon, cation exchange capacity, water retention and holding capacity, as well as in the nutrient and water supply of plants. In addition, biochar application might improve soil aggregation, which reduces soil loss by erosion, and promote microbial activity, all together leading to improvements in soil fertility.

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