



Article

Root-Zone Amendments of Biochar-Based Fertilizers: Yield Increases of White Cabbage in Temperate Climate

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Abstract: The use of biochar is an important tool to improve soil fertility, reduce the negative environmental impacts of agriculture, and build up terrestrial carbon sinks. However, crop yield increases by biochar amendment were not shown consistently for fertile soils under temperate climate. Recent studies show that biochar is more likely to increase crop yields when applied in combination with nutrients to prepare biochar-based fertilizers. Here, we focused on the root-zone amendment of biochar combined with mineral fertilizers in a greenhouse trial with white cabbage (*Brassica oleracea* convar. Capitata var. Alba) cultivated in a nutrient-rich silt loam soil originating from the temperate climate zone (Bavaria, Germany). Biochar was applied at a low dosage (1.3 t ha⁻¹). The biochar was placed either as a concentrated hotspot below the seedling or it was mixed into the soil in the root zone representing a mixture of biochar and soil in the planting basin. The nitrogen fertilizer (ammonium nitrate or urea) was either applied on the soil surface or loaded onto the biochar representing a nitrogen-enhanced biochar. On average, a 12% yield increase in dry cabbage heads was achieved with biochar plus fertilizer compared to the fertilized control without biochar. Most consistent positive yield responses were observed with a hotspot root-zone application of nitrogen-enhanced biochar, showing a maximum 21% dry cabbage-head yield increase. Belowground biomass and root-architecture suggested a decrease in the fine root content in these treatments compared to treatments without biochar and with soil-mixed biochar. We conclude that the hotspot amendment of a nitrogen-enhanced biochar in the root zone can optimize the growth of white cabbage by providing a nutrient depot in close proximity to the plant, enabling efficient nutrient supply. The amendment of low doses in the root zone of annual crops could become an economically interesting application option for biochar in the temperate climate zone.

Keywords: PyCCS; pyrogenic carbon capture and storage; nitrogen fertilizer; root architecture; Shovelomics



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1. Introduction

Biochar is the solid product of biomass pyrolysis. Over the past two decades, it has attracted scientific attention as a beneficial soil amendment and as a carbon sink [1,2]. In soil, biochar can, e.g., improve water and nitrogen (N) use efficiencies, the root growth of plants, and reduce soil-borne N₂O emissions, thus contributing to both climate change adaptation and mitigation [2,3]. While environmental benefits are desirable and adaptation strategies are desperately needed in agriculture, mainstreaming biochar application will only succeed at a sufficient pace when farmers experience direct benefits, e.g., increases in crop yield. Global meta-analyses show that biochar amendment with or without fertilizer can increase crop yields compared to the fertilized control by 10% on average or by up to 25% when biochar is combined with mineral fertilizer compared to the fertilized control [4,5]. Biochar

can also provide crop yield increases when applied to the soil at low application rates in the range of 1 t ha^{-1} as a carrier for nutrients, which is termed as a biochar-based fertilizer [6,7]. However, crop yield increases after the amendment of biochar or biochar based-fertilizers have only been systematically achieved in soils under tropical or subtropical climate but rarely in soils in the continental and humid-temperate climate zone with a mean annual temperature lower than $10 \text{ }^{\circ}\text{C}$ [4]. Still, to the best of our knowledge, there is no evidence that biochar-based fertilization in general cannot increase crop yields under such climatic conditions. Thus, further research is needed to study the effect of different application modes of biochar-based fertilizers in soils under temperate climate.

Despite the large number of studies on different formulations of biochar-based (slow-release) fertilizers [7–11], there are no studies to our knowledge that have compared different types of biochar positionings in the field/pot; thus, this factor could not be investigated so far in any meta-analysis. Mostly, biochar or the biochar-based fertilizer was spread homogeneously onto the field and incorporated into the soil by (minimal) tillage. However, this approach results in low biochar concentrations in close proximity to the plant, when economically feasible amounts (e.g., $<2 \text{ t ha}^{-1}$) are amended to the soil. As a contrast, the root-zone amendment of biochar-based fertilizers was proposed as a beneficial strategy, since the biochar is placed where most of the plant roots are present, which allows a targeted and effective fertilization (Figure 1) [6,12]. Studies that applied biochar-based fertilizers in the root zone of annual crops prepared them by physical mixing of the biochar with nutrient-dense liquids or solids, achieving crop-yield increases independent of the crop that was cultivated in tropical and temperate/alpine climate zones [6,12–16]. Still, to date, no study has systematically investigated the combined effects of different types of biochar root-zone amendments and different mineral N compounds on the impact of biochar-based fertilization on plant productivity and root growth response, which is the focus of this study. Furthermore, we test whether biochar-based fertilization based on a priori mixing of biochar with N fertilizer (N-enhanced biochar) is superior to the soil amendment of pure biochar combined with a regular soil-surface N fertilization. We therefore conducted a greenhouse trial with white cabbage, applying two different types of root-zone amendments of low-dosed biochar (1.3 t ha^{-1}) with a combination of the above-mentioned experimental factors and two different N fertilizers to study the transferability of the results to different N fertilizer compounds. The aboveground biomass yields were analyzed and the root architecture of the plants was studied with the REST Shovelomics software to observe a potential effect of different root-zone amendments and N fertilization methods on root growth [17]. The Shovelomics approach allows one to characterize the architecture of rootstocks, which was previously successively applied in a study to evaluate differences in the root architecture of maize plants following a root-zone amendment of biochar in a tropical soil [18]. With this study, we aim to contribute to the understanding of new strategies in biochar-based fertilization, focusing on fertile soils in the temperate climate zone and their effects on crop yields and root development.

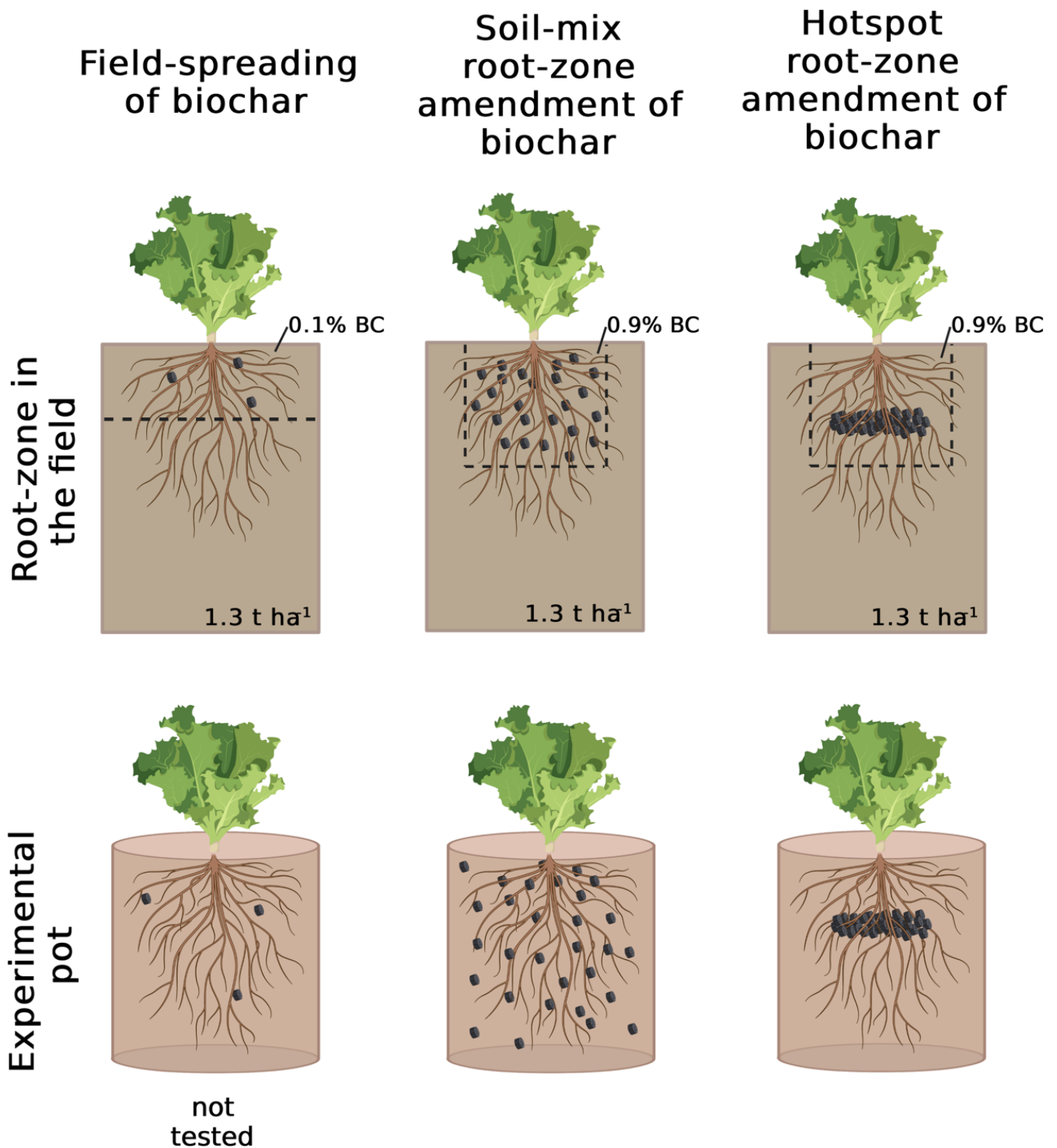


Figure 1. Illustrative comparison of the homogeneous field spreading of 1.3 tons of biochar (BC) per hectare (**left column**) with the two tested methods to prepare BC root-zone amendments in this study: soil-mix root-zone amendment (**middle column**) and hotspot root-zone amendment of BC (**right column**). For the homogeneous BC field spreading, the shown local BC weight concentration is based on an assumed soil bulk density of 1.3 t m⁻³ and an incorporation depth of biochar in the upper 10 cm. The local BC concentration in the soil-mix root-zone amendment of 0.9% was used in the greenhouse trial. The amount of BC applied per pot resulted from dividing the total amount of 1.3 t of BC applied per hectare by the number of plants growing per hectare (40,000). The graphic was created with BioRender.com (accessed on 4 March 2022).

2. Materials and Methods

2.1. Biochar Origin and Further Processing

Biochar was provided by Carbon Cycle GmbH & Co. KG (Rieden, Germany) and produced from untreated wood chips. The pyrolysis process was performed with a maximum temperature of 750 °C in an electrically heated vertical moving bed reactor (Carbon Technik Schuster GmbH, Dischingen, Germany). The biochar used was certified according to the European Biochar Certificate (Certification class AgroOrganic) [19], and a detailed analysis is provided in Table S1. After pyrolysis, biochar was milled with a hammer mill (Model HM420B with 11 kW and 12 mm sieve, Evertech GmbH, Rodgau, Germany) and stored in fabric bags until further processing.

2.2. Combining Biochar and N Fertilizer

All chemicals were of analytical grade (Carl Roth GmbH, Karlsruhe, Germany), and solutions were prepared with de-ionized (DI) water. To prepare N-enhanced biochar, 32 g of dry matter (DM) equivalent biochar (88% DM content) was mixed with 45 mL of ammonium nitrate or urea solution (54 g N L⁻¹) to achieve 80% of its water holding capacity (WHC = 1.8 mL g⁻¹) and an additional N content of 78 mg N (g char)⁻¹. The mixture was stored for 3 days at room temperature in a closed glass jar.

2.3. Greenhouse Trial

The pot trial was comprised of 11 treatments (Table 1) and was conducted for 70 days from 5th of July to 13th of September 2021 in a greenhouse with east–west orientation located in Offenburg, Germany. The treatment with ID11 had to be dismissed due to an error that occurred during fertilization (Table 1). A silt loam topsoil originating from Straßkirchen (Bavaria, Germany) was used, on which grass for hay harvest was grown and which received mineral fertilization in the years before. The soil contained high levels of available phosphorus (P), potassium (K), and magnesium (Mg), and a detailed soil analysis conducted by Eurofins Umwelt Ost GmbH (Jena, Germany) is provided in Tables S2–S4. White cabbage (variety Sunta F1, Bruno Nebelung GmbH, Everswinkel, Germany) was preplanted for 20 days in growing media. Five replicate pots were prepared for each treatment and arranged into a randomized complete block design. The pots were repositioned at random within each block on a weekly basis. The biochar and fertilizer dosages were calculated per pot, assuming a biochar application rate of 1.3 t ha⁻¹ (32 g biochar plant⁻¹) and a N fertilization rate of 100 kg N ha⁻¹ (2.5 g N plant⁻¹) with an assumed plant density of 40,000 plants ha⁻¹.

Table 1. Treatments prepared for the greenhouse trial with a three-factorial experimental setup: type of nitrogen (N) fertilizer, type of biochar (BC) root-zone amendment (none, hotspot, or soil-mix application), and the type of N fertilization method (N fertilization via N-enhanced biochar or soil-surface N fertilization). n.a.: not applicable.

Treatment ID	Nitrogen Fertilizer	Type of Biochar Root-Zone Amendment	Nitrogen Fertilization Method
1	none	none	n.a.
2	NH ₄ NO ₃	none	soil-surface fertilization
3	NH ₄ NO ₃	Hotspot	N-enhanced biochar
4	NH ₄ NO ₃	Hotspot	soil-surface fertilization
5	NH ₄ NO ₃	Soil-Mix	N-enhanced biochar
6	NH ₄ NO ₃	Soil-Mix	soil-surface fertilization
7	Urea	none	soil-surface fertilization
8	Urea	Hotspot	N-enhanced biochar
9	Urea	Hotspot	soil-surface fertilization
10	Urea	Soil-Mix	N-enhanced biochar
11 ^a	Urea	Soil-Mix	soil-surface fertilization

^a This treatment had to be dismissed due to organizational issues.

Pots with a volume of 4 L (21 cm height, 17 cm inner diameter at the top) were initially filled with 2590 g of DM equivalent soil (88% DM content at trial setup) and compacted by knocking on a table three times. For the treatments without biochar amendments, one seedling per pot was placed onto this amount of soil, which was properly enclosed by another 890 g of DM equivalent of soil. Two different root-zone amendments of biochar were conducted: a biochar–soil mix and the concentrated hotspot biochar root-zone amendment (Figure 1). In the pots for the soil-mix root-zone amendment of biochar, 3480 g of DM equivalent of soil was mixed with 32 g of DM equivalent of N-enhanced or pure biochar, and the above-described planting procedure was conducted in the same way. The soil-mix root-zone amendment represented a mixture of biochar and the soil in the planting basin in the field (Figure 1). In the treatments with hotspot biochar amendment, a cylinder with 8 cm diameter and 1 cm height was formed in the middle of the first portion of soil in which the biochar was subsequently placed (10 cm below soil surface). The biochar was then covered with a 1 cm soil layer of the remaining soil before the seedling was planted (Figure S1). Pots were stepwise (50 mL) watered to 65% of their WHC with well water, according to previous quantification of soil and biochar WHC. During the experiment, pots were watered daily with a fixed amount of water through a drip line (Figure S2). The daily water supply was adapted, if necessary, once a week after all pots were manually re-watered to 65% of maximum WHC. The soil in each pot was covered with 50 g of quartz sand to allow a homogeneous distribution of water from the drip line during the experiment (Figure S2).

Three days after planting, 100 mL of a nutrient solution was injected by means of a medical syringe in 2 cm soil depth at 8 different spots in the pot. Each plant received 2.5 g of N as ammonium nitrate or urea, 0.9 g of potassium as tri-potassium citrate, 1.0 g of sodium and 0.7 g sulfur as sodium sulfate, and 1.0 g of calcium as calcium acetate. The solution was prepared without additional N for the treatments with N-enhanced biochar. Phosphorus was not fertilized, since the soil already contained 35 mg of available P (100 g)^{−1} (Table S2).

2.4. Aboveground Biomass Harvest and Data Collection

After 69 days, dimensionless chlorophyll content was determined non-destructively with a handheld spectrophotometer (SPAD-502Plus, Konica Minolta, Marunouchi, Japan) on the three outer leaves of each plant. On the next day, total aboveground biomass was measured after cutting the stem at the lowest leaf base. The cabbage head was weighed after separating the outer protruding, nonmarketable leaves with a knife (Figure S3). The diameter of the cabbage head was measured by means of a caliper with 1 mm accuracy to calculate the head volume by assuming the geometry of an ellipsoid. Subsequently, the whole cabbage heads were manually rasped and homogenized manually to allow for better drying conditions and preparation of representative subsamples. An aliquot (40 g) was dried at 105 °C to a constant weight to calculate the dry matter content of aboveground biomass. A 20 g aliquot was immediately stored at −18 °C for the analysis of vitamin C content.

2.5. Belowground Biomass Analysis

Pots were overthrown into a cold-water bath and left for five minutes to enable an easier removal of soil particles from the roots. The soil was gently removed from the rootstocks by hand and shaking the rootstock inside the water bath to further pre-clean the roots. Each rootstock was separated into two halves by cutting the stem lengthwise with a knife. Each pre-cleaned half was washed under a stream of tap water for another 15 min, and all visible soil particles were removed. The rootstocks were stored in water to keep them moistened until photographs were taken from one half of the rootstock per plant within two days after washing.

Rootstocks were fixed in a sample holder in front of a black background in order to generate high-contrast images (Figure S4). The pictures were taken in a completely darkened room with two soft boxes to uniformly illuminate the samples from the left and right side, respectively. A Canon EOS 70D with an EF-S 18–55 mm objective (Canon AG,

Tokyo, Japan) was positioned on a tripod and used at a focal length of 35 mm, 0.8 s exposure time, and an *f*-number of *f*/6.3 in self-timer mode to avoid wriggling of the images. All camera settings, positions of lighting equipment, and distances of the samples relative to the camera and the black background were kept constant for all images. Unprocessed images were evaluated with the open-source software “Root Estimator for Shovelomics Traits” (REST), designed and presented in detail by Colombi and colleagues [17]. The root area, total projected structure lengths, and root fill factors were evaluated.

2.6. Vitamin-C Quantification

The content of vitamin C (L-(+)-ascorbic acid) in the cabbage-head biomass was estimated by iodometric titration [20]. In short, a subsample of the frozen cabbage head was extracted with DI water, and the extract was titrated with a potassium iodide and iodine solution by using a starch indicator solution. A detailed description of the procedure is provided in the Supporting Information (SI).

2.7. Data Analysis

The effects of all three factors (type of biochar root-zone amendment, N-fertilizer type, and N-fertilization method) were determined using three-way analysis of variance (ANOVA). When including the controls without biochar amendments, the interaction of the type of root-zone amendment and the N-fertilization method was not evaluable, since N fertilization via a N-enhanced biochar could not be performed without a biochar amendment. Therefore, ANOVA was also conducted for the biochar treatments alone to estimate the effect of this interaction. Means for different factor levels were compared at $p < 0.05$ using the Tukey HSD test or Student’s *t*-test (for comparison of only two means). Due to organizational issues, the treatment with soil-mixed biochar root-zone amendment and soil-surface urea fertilization had to be dismissed (treatment ID11, Table 1). Therefore, the pot trial represents a part-factorial experimental setup. To correct the unbalance in the experimental design, data are shown as least square means when data were averaged over a specific experimental factor. All statistical analysis was performed using JMP 10 (SAS Institute Inc., Cary, NC, USA).

3. Results and Discussion

3.1. Aboveground Biomass Yields

Fresh cabbage-head yields ranged within 200 and 270 g plant⁻¹ for the different treatments (Table 2). These rather low cabbage-head yields may be explained by the rather low N-fertilization rate (2.5 g N plant⁻¹) and the circumstance that the plants were grown in pots and not in the field with more space for root development. In general, the presence of a N fertilizer significantly increased aboveground biomass yields, which was reflected by the control without N fertilization, which yielded the lowest aboveground biomass and did not produce a cabbage head (Table 2). The increase in dry cabbage-head biomass averaged over all biochar amendments compared to the fertilized control without biochar was 12% ($p = 0.09$). The highest yield increase of dry cabbage-head biomass compared to the respective control was achieved with the NH₄NO₃ fertilizer with a hotspot pure-biochar amendment and additional soil-surface N fertilization (24% head yield increase) and for urea when the N-enhanced biochar was amended as a hotspot (14% head yield increase, Figure 2B). Still, for both N fertilizers, the amendment of a N-enhanced biochar as a hotspot in the root zone consistently provided high cabbage-head yield increases compared to the equally fertilized control without biochar (Figure 2). While the application of the N fertilizer loaded onto biochar provided head yield increases in all tested treatments, soil-surface fertilization with urea combined with a hotspot pure-biochar amendment had no effect on head yield increases compared to the urea fertilized no-biochar control (Figure 2).

Table 2. Aboveground biomass yields and parameters based on fresh matter (FM) and dry matter (DM) of cabbage plants for all treatments. The cabbage-head ratio presents the mass ratio of the cabbage head compared to the total aboveground biomass including the non-marketable part of the plant (based on DM). SPAD values present a proxy for the chlorophyll content in the plant tissue. Treatment identifiers refer to the explanations in Table 1. Errors are presented as standard errors of the mean ($n = 5$). Different letters within a column indicate a statistically significant difference between individual treatments (at $p < 0.05$, Tukey HSD post hoc test). n.a.: not applicable.

Treatment	Aboveground Biomass (FM)	Cabbage Heads (FM)	Aboveground Biomass (DM)	Cabbage-Head Ratio (DM)	Cabbage-Head Volume	SPAD	Vitamin C Content in Head Biomass	Dry Root Biomass
	g	g	g	wt%	cm ³		(mg(100 g) ⁻¹)	g
1: No N-Fert., no BC NH₄NO₃-Fertilizer	106.1 ± 6.9 b	n.a.	17.5 ± 1.5 b	n.a.	n.a.	48.0 ± 1.7 a	n.a.	1.4 ± 0.1 a
2: no BC-Control	430.9 ± 34.5 a	206.8 ± 32.5 a	29.4 ± 0.8 a	47.0 ± 4.0 b	2270.5 ± 313.1 a	53.6 ± 1.9 a	7.1 ± 0.2 a	1.4 ± 0.3 a
3: BC-Hotspot, N-enhanced BC	465.5 ± 5.4 a	271.7 ± 7.0 a	28.5 ± 0.9 a	58.4 ± 1.3 a	2874.1 ± 133.2 a	54.0 ± 1.7 a	7.4 ± 0.7 a	1.3 ± 0.1 a
4: BC-Hotspot, pure BC	470.9 ± 15.3 a	263.6 ± 8.1 a	30.8 ± 0.7 a	56.0 ± 0.9 ab	2803.5 ± 134.3 a	54.8 ± 1.7 a	8.5 ± 0.3 a	1.5 ± 0.1 a
5: BC-mixed, N-enhanced BC	436.9 ± 9.5 a	227.5 ± 7.0 a	29.2 ± 0.5 a	52.1 ± 1.1 ab	2420.3 ± 81.5 a	54.3 ± 1.7 a	7.0 ± 0.1 a	1.6 ± 0.2 a
6: BC-mixed, pure BC Urea-Fertilizer	465.1 ± 21.3 a	246.1 ± 12.6 a	30.9 ± 1.5 a	53.1 ± 2.3 ab	2464 ± 142.2 a	53.9 ± 1.7 a	8.1 ± 0.9 a	1.6 ± 0.2 a
7: no BC-Control	452.1 ± 10.5 a	236.8 ± 5.8 a	29.2 ± 0.6 a	52.4 ± 1.3 ab	2401.1 ± 106.5 a	54.5 ± 2.8 a	7.8 ± 0.4 a	1.4 ± 0.1 a
8: BC-Hotspot, N-enhanced BC	447.5 ± 4.6 a	258.9 ± 8.7 a	30.04 ± 0.9 a	57.8 ± 1.4 a	2691.8 ± 88.8 a	56.9 ± 2.8 a	8.0 ± 0.2 a	1.5 ± 0.1 a
9: BC-Hotspot, pure BC	433.7 ± 21.6 a	234.1 ± 21.5 a	27.9 ± 1.2 a	53.8 ± 3.5 ab	2557.3 ± 296.6 a	54.0 ± 1.2 a	7.7 ± 0.5 a	1.4 ± 0.1 a
10: BC-mixed, N-enhanced BC	439.5 ± 7.7 a	259.3 ± 7.9 a	27.9 ± 1.1 a	59.0 ± 1.1 a	2615.0 ± 195.3 a	56.7 ± 2.4 a	7.2 ± 0.2 a	1.5 ± 0.1 a
<i>p</i> -Value (ANOVA)	<0.0001	0.06	<0.0001	0.01	0.33	0.18	0.38	0.85

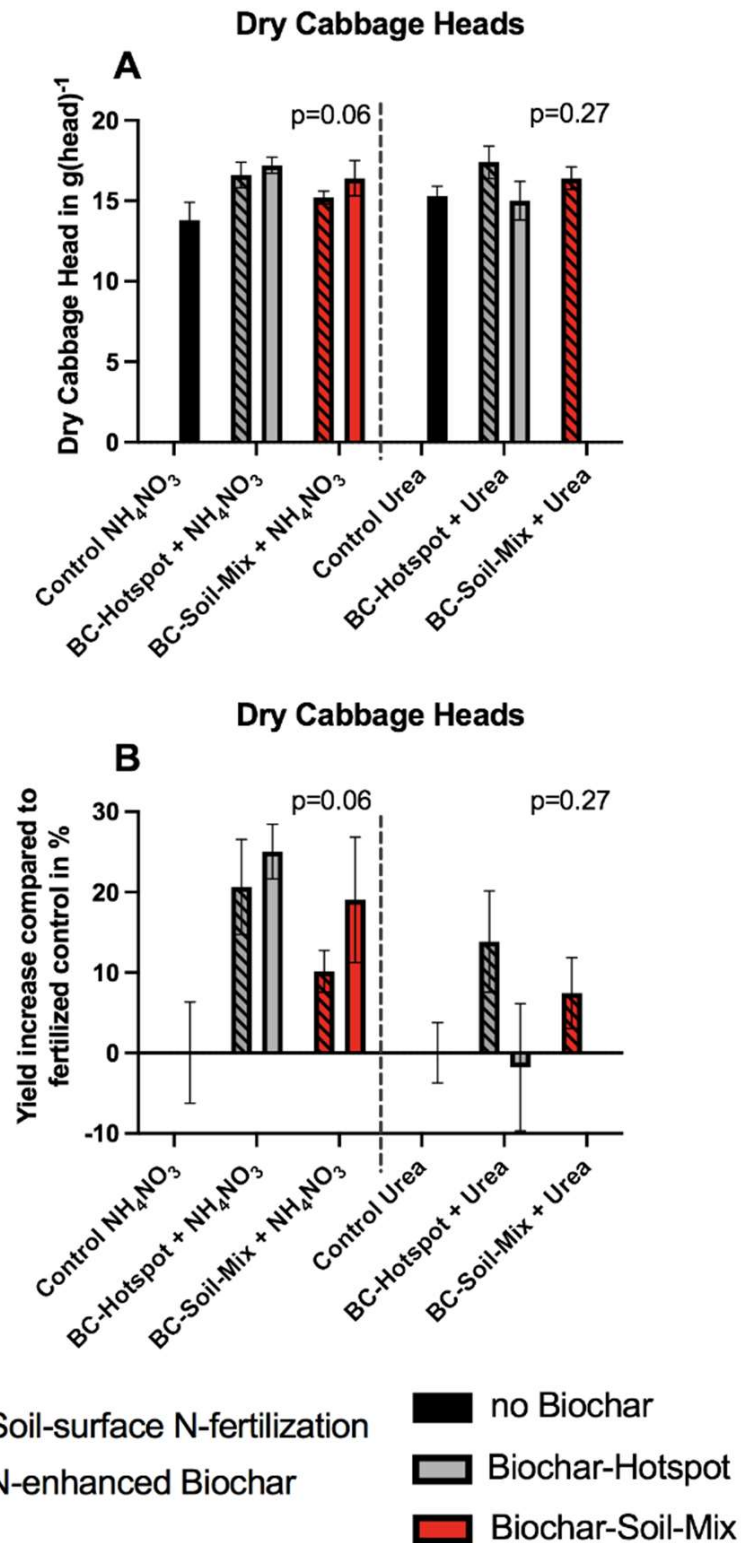


Figure 2. Dry cabbage-head weights (A) and yield increases of dry cabbage-head biomass (B) compared to the respective nitrogen (N) fertilized control (NH₄NO₃ or urea) for all biochar (BC) treatments, depending on the type of BC root-zone amendment (hotspot or soil-mix) and the N fertilization method (N-enhanced biochar or soil-surface N fertilization). Error bars indicate the standard error of the mean of replicated (*n* = 5) planting experiments. The *p*-values were derived from one-way ANOVA comparing all treatments fertilized with the same N compound.

Since the method of N fertilization (N-enhanced biochar vs. soil-surface N fertilization) did not change aboveground biomass parameters significantly (Table S5), it is appropriate to present data averaged over this experimental factor (Figure 3) [21]. Averaged over both approaches, the increases in dry cabbage-head biomass compared to the N fertilized no-biochar control were significantly intensified for the NH_4NO_3 -fertilized plants when biochar was amended as a hotspot in the root zone (22% yield increase with a hotspot and 14% with a soil-mix biochar amendment, Figure 3A). For the urea fertilizer, a biochar amendment in the root zone only showed a slight tendency (not significant, $p = 0.64$) to increase dry cabbage-head biomass compared to the no-biochar control (5% for hotspot and 7% for soil-mix root-zone biochar amendment, averaged over the N-fertilization method) (Figure 3A).

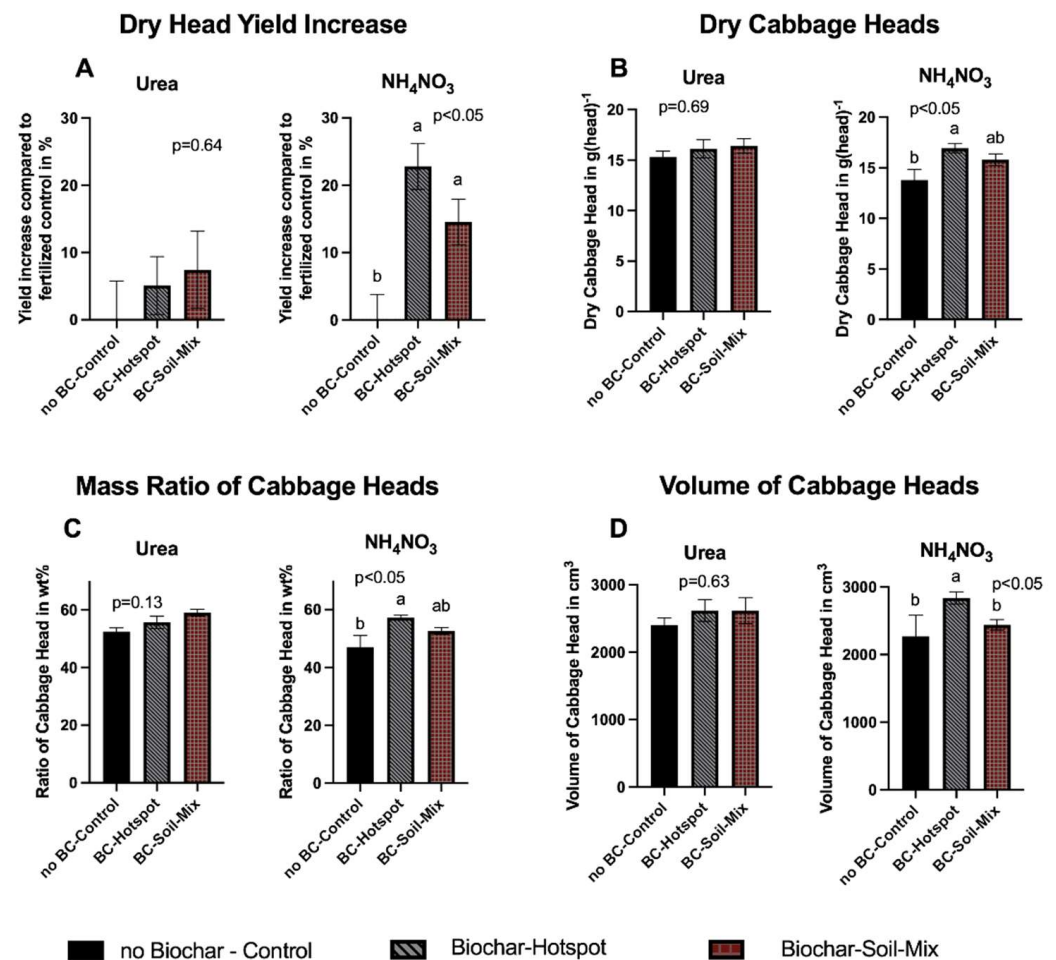


Figure 3. Aboveground biomass parameters averaged over the nitrogen (N) fertilization method and separated by the N-fertilizer type evaluated for fertilized plants without biochar amendment (no BC-Control) or biochar root-zone amendments (BC-Hotspot or BC-Soil-Mix) presented as least square means (processed with JMP10): (A) fresh cabbage-head biomass, (B) dry cabbage-head biomass, (C) mass ratio of cabbage heads (dry head biomass divided by total dry aboveground biomass), and (D) volume of cabbage heads. The error bars represent one standard error in each direction ($n = 10$ for no BC-control, $n = 20$ for BC-Hotspot and $n = 15$ for BC-Soil-Mix). Different letters above error bars indicate significant differences among the types of biochar root-zone amendments treated with the same N fertilizer ($p < 0.05$, Tukey HSD post hoc test).

This lower averaged increase in cabbage-head yield for the urea-fertilized biochar treatments can be explained by a better performance of the urea-fertilized control compared to the NH_4NO_3 -fertilized control (Table 2, Figure 3B); therefore, the type of N fertilizer

significantly influenced the extent of head yield increases provided by the different biochar root-zone amendments (Table S6, Figure 2).

Total aboveground biomass yields were not affected by biochar amendment (Tables 2 and S5). However, the mass ratio of cabbage heads related to the total biomass in presence of biochar increased significantly due to increases in the mass of cabbage heads ($p = 0.03$, Table S5 and Figure 3C). For the NH_4NO_3 -fertilized treatments, this ratio increased from 47% (no biochar) to 57% (hotspot biochar amendment), while for the urea fertilizer, the soil-mix biochar root-zone amendment maximized this ratio from 52% to 59% (Table 2 and Figure 3C). With that, the marketable part of the cabbage plant was increased in presence of biochar. Focusing on the biochar treatments, no significant difference was observed for this ratio for the type of root-zone amendment, the N fertilizer type, or the N-fertilization method (Table S6).

Within the biochar treatments, absolute cabbage-head yields were not significantly influenced neither by the type of root-zone amendment (hotspot vs. soil-mix root-zone application), the type of N fertilizer (ammonium nitrate vs. urea), nor the N-fertilization method (N-enhanced biochar vs. soil-surface N fertilization) (Table S6). Still, the interaction of the N-fertilization method and type of N fertilizer was almost significant ($p = 0.06$, Table S6), when plants were grown with biochar. This was reflected by the low cabbage-head yield in the treatment with hotspot biochar amendment with urea used as the soil-surface fertilizer, while in the complementary treatment fertilized with NH_4NO_3 , the overall highest dry head biomass was obtained (Table 2 and Figure 2). However, this contrast cannot be directly explained by the data we collected.

With higher cabbage-head yields in presence of the biochar amendments, the volume of cabbage heads was increased as well (Figure 3D); therefore, cabbage-head density was not altered by biochar amendment.

Vitamin C contents in frozen cabbage-head biomass (stored for two months at $-18\text{ }^\circ\text{C}$) were not altered by any factor applied in this study (Table 2). In general, vitamin C contents were within 7.0 and 8.5 mg $(100\text{ g})^{-1}$ for the different treatments and are in the lower range of reported vitamin C contents for white cabbage, which are typically within 5–30 mg $(100\text{ g})^{-1}$ but can reach up to 70 mg $(100\text{ g})^{-1}$ [22,23]. SPAD values of aboveground biomass, which represent a proxy for the chlorophyll content in plant tissues, were also not significantly different for the varying treatments (Table 2). Only in the control plants that did not receive any N fertilizer (no N-Fert., no BC) were the SPAD values slightly lower (Table 2). The influence of the different biochar root-zone amendments on the nutrient contents in plant tissues and the nutrient use efficiency should be conducted in future research.

In summary, the amendment of biochar in the root zone increased cabbage-head yields independent of the type of N fertilizer applied in this study but to a higher relative extent when fertilized with NH_4NO_3 . The hotspot amendment of a N-enhanced biochar in the root zone showed the most consistent positive impacts on cabbage-head yields for both N fertilizers (Figure 2). In this case, the biochar may act as a nutrient depot within close proximity to the root system, which may ease nutrient supply for the plant. This approach to obtain a biochar root-zone amendment in the field could be achieved by applying a biochar layer in the planting/seeding row. In contrast, the soil-mix root-zone amendment of biochar can be translated to field conditions as a mixture of biochar and soil in the planting basin of the cabbage plant and should not be confused with a homogeneous spreading of the biochar onto the whole field (Figure 1). For the homogeneous amendment of 1.3 t ha^{-1} of a biochar-based fertilizer onto the whole cabbage field, we would expect a less intense improvement in cabbage-head yield, since this would result in a biochar concentration of only 0.1–0.03 % (w/w) when biochar is incorporated to a soil depth of 10–30 cm (assuming a soil-bulk density of 1.3 t m^{-3}), which is much lower than the biochar concentration that would result from the soil-mix root-zone amendment (0.9 % w/w in our study, Figure 1).

The average yield increases in fresh cabbage-head biomass of 15% with biochar amendment and the maximum increase of 31% compared to the fertilized control are rather un-

usual when compared with meta-analyses that report no significant effect in crop yield increase when biochar is amended to soils in the humid-temperate climate zone [4]. Such a climate-dependent effect was not observed in a recently published meta-analysis when biochar was used as a fertilizer carrier at low biochar application rates (in the range of 1 t ha^{-1}), but still, biochar-based fertilizers only increased crop yields when applied to highly weathered or weakly developed soils and not when applied to already fertile soils, as was carried out in our study [7]. However, significant yield increases were reported before for various vegetable crops following the root-zone amendment of biochar that was enriched with a mineral NPK-fertilizer solution or cow urine as a nutrient source when added to fertile silt loam soils under temperate/alpine climate conditions in Nepal [6]. Thereby, the liquid nutrient enrichment of biochar with mineral NPK fertilizers provided lower crop yield increases (<50% increase compared to fertilized control) than biochar enriched with an organic nutrient source (i.e., cow urine with yield increases of up to 300%) [6]. The crop yield increases of up to 30% provided by the N-enriched biochar amended in the root zone in our study can therefore be seen in line with these results, and they indicate that a simple liquid nutrient enrichment of biochar positioned in the root zone is a promising strategy for biochar-based fertilization, as well as in fertile soils in the temperate climate zone. This may be due to a higher use efficiency of fertilized nutrients by the plants, which was also reported for conventional mineral fertilizer granules without biochar, when applied to the root zone of annual plants [24–26]. However, not only the nutrient-enriched biochar in our study promoted the growth of cabbage heads but the pure biochar along with soil-surface N fertilization as well, in most of the treatments. This is in contrast to a previous greenhouse pot study on quinoa where the addition of a pristine biochar (2% *w/w*) along with two different mineral N-fertilization levels to a nutrient poor sandy soil decreased aboveground biomass yields compared to the equivalently fertilized control without biochar [27]. However, Kammann and colleagues applied mineral fertilizer as a top-dress on average every nine days (nine doses within 82 days of cultivation), which could already be interpreted as a fertigation practice with probably nearly ideal nutrient supply. In contrast, we applied the soil-surface N fertilizer only once at the beginning of the cultivation; thus, the plants grown with biochar could take advantage of a nutrient depot when compared to the no-biochar controls.

Assuming the average fresh head yield increase of 20% provided by the N-enhanced biochar applied as a hotspot below the seedling in our study, a typical yield (fresh matter) of white cabbage heads in Germany of 90 t ha^{-1} would be increased to 108 t ha^{-1} . This would generate an approximate additional income of EUR 1200 ha^{-1} for the farmer, assuming a producer price of EUR 68.9 t^{-1} [28,29]. This additional income would cover the cost for the biochar at an application rate of 1.3 t ha^{-1} , and there would be a little but not significant added financial profit for the farmer within one season, assuming a commercial biochar price of EUR 500–1000 t^{-1} . Therefore, the biochar application rate and mode would have to be further optimized to create a higher economic incentive to apply this specific biochar-based fertilizer. However, it has to be considered that possible yield increases under field conditions may be different than in our greenhouse study, e.g., since we did not grow the cabbages to typical plant-specific head weights, which may be a result of the limited soil volume in our experimental pots (<0.5 kg head^{-1} in our study compared to commercial weights of approximately 1 kg head^{-1}).

3.2. Belowground Biomass Yields and Root Architecture

The different factors applied in the experiment had no impact on the absolute root biomass weights of white cabbage plants (Table 2) but significant impacts on the root architecture, including the root fill factor, area, and total projected structure length (Table S5, Figures 4 and S5). It should be noted that variations among the treatments compared to the head biomass cannot be resolved for the root biomass because of the low absolute root weights (<2 g plant^{-1} , Table 2). The optical-based analysis of the root structure and total root area may be more sensitive and is an important additional tool to evaluate the root

growth under different biochar treatments. These root architecture-related parameters were evaluated with the REST Shovelomics software [17], which is based on processing high-contrast images from the washed rootstocks (Figure 5). The root-fill factor was most impacted by the type of root-zone amendment and the N-fertilization method ($p < 0.05$, Tables S5 and S6, Figure 4A). Lower fill factors were observed with the hotspot biochar amendment compared to both the fertilized no-biochar control and the soil-mix root-zone biochar amendment, especially for hotspot amendments of a N-enhanced biochar (Figures 4A and S5). The root fill factor in general is described as the number of root-derived pixels divided by the total number of pixels in the area of the photograph, in which 90% of root-derived pixels were registered. Therefore, the root fill factor is a measure for the root-hair density: low fill factors indicate a low root-hair density and with that fewer root hairs and more room between individual root hairs and vice versa. In a Shovelomics study on maize grown under field conditions, biochar mixed with soil in the planting basin resulted in a significant increase in root fill factor compared to the no-biochar control, which was not the case in our study for the comparable soil-mix biochar amendments (Figure 4A) [18]. However, biochar amendment in their (tropical) study (aeolian acidic sandy soil and sandy loam soil) also tended to increase both the above and belowground biomass compared to fertilized controls.

The area of the roots was not altered with the biochar amendments compared to the fertilized no-biochar control (Table S5, Figure S5), but within the biochar treatments, the interaction of the N-fertilization method and the type of biochar root-zone amendment significantly influenced root areas (Table S6, Figure 4B). For the hotspot biochar amendment, the application of a N-enhanced biochar led to lower root areas than for the soil-surface N fertilization (84 vs. 103 cm², Figure 4B). The exact opposite was registered for the soil-mix biochar amendments, where the N-enhanced biochar maximized the root area (Figure 4B). The same pattern as for the root areas was observed for the biochar treatments regarding the total projected structure lengths of the roots (Figure 4C). Furthermore, biochar-amended plants that received a fertilization with NH₄NO₃ had significantly higher root areas and total projected structure lengths than the plants fertilized with urea (Figure S6). As no difference in absolute root biomass production was registered among the different treatments (Table 2), a change in the root area is interpreted as a change in the total amount of root hairs and with that, the content of fine roots. Thereby, the results for the root area and projected structure lengths are consistent with the observed root fill factors, which all indicate that the content of fine roots was lower with hotspot N-enhanced biochar amendments compared to the mixed-soil amendment of the N-enhanced biochar.

The hotspot amendment of the N-enhanced biochar may act as a nutrient depot in close proximity to the plant, which in turn requires fewer fine roots for nutrient supply. In contrast, a soil-mix root-zone application of the N-enhanced biochar may require the development of more root hairs to reach the finely distributed N-carrying biochar particles in the pot. Furthermore, NH₄NO₃ is more mobile within the soil than urea and might spread more widely across the pot, which might explain the higher root area in the NH₄NO₃-fertilized treatments (Figure S6).

Data from a meta-analysis showed that root growth, especially for annual plants, is typically stimulated by large biochar amendments to soil (>10 t ha⁻¹), which was not the case for the low biochar amendments (1.3 t ha⁻¹) in our study [30]. However, our data show that biochar root-zone amendments and the N-fertilization method can influence the root architecture in a way that was not reported before. In summary, the reduced fine root development paired with higher aboveground biomass production in the treatments with N-enhanced biochar applied as a hotspot strongly indicated an improved plant nutrition of white cabbage plants. This should be studied under real field conditions to examine the potential positive or negative changes of crop nutrition and water supply of the plant through potential changes in its fine root biomass development when biochar is amended as a hotspot in the root zone.

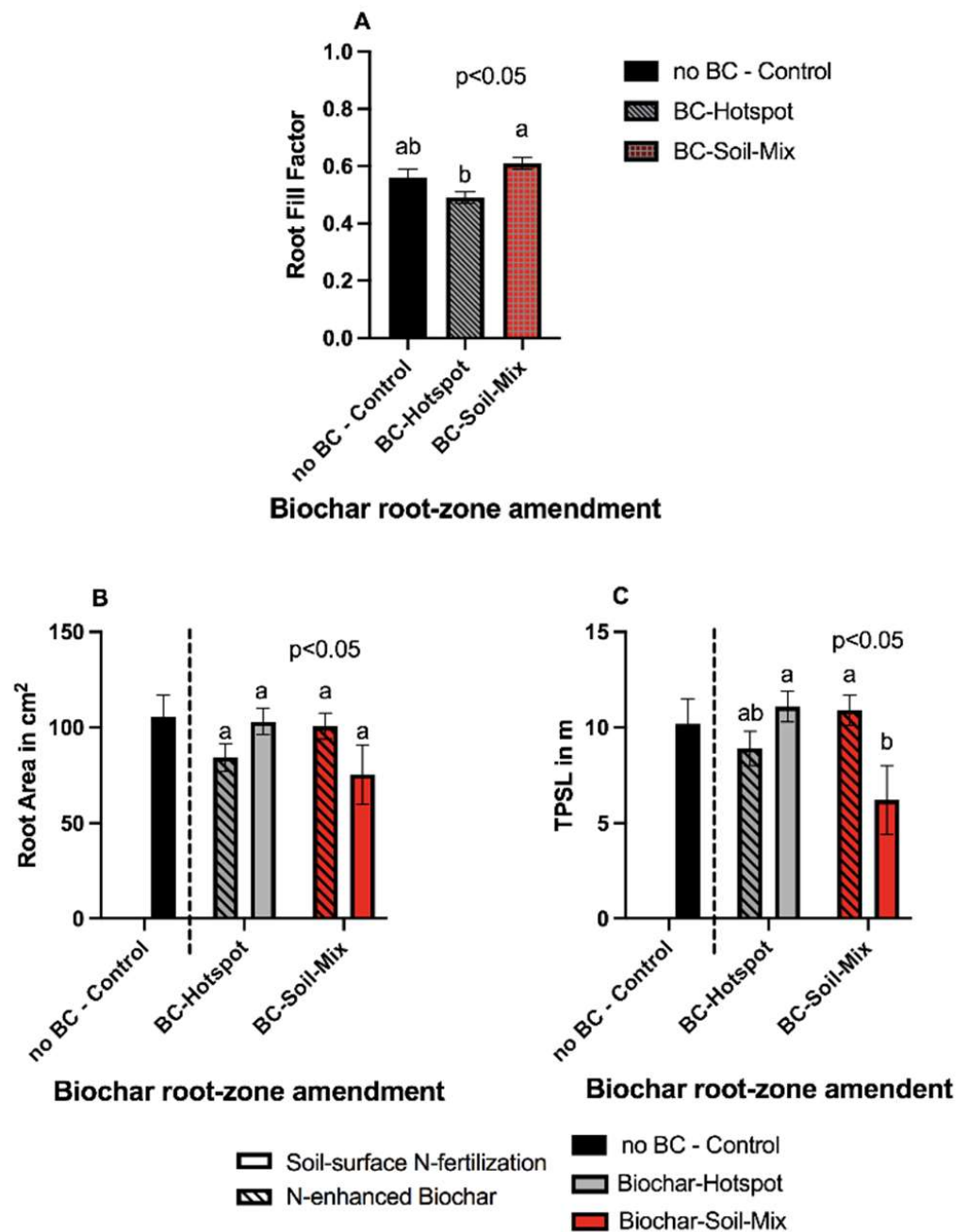


Figure 4. (A) Root fill factor depending on the biochar root-zone amendment (no-biochar (BC)-Control, BC hotspot in the root zone (BC-Hotspot), or BC mixed with soil (BC-Soil-Mix)) averaged over the type of nitrogen (N) fertilizer and the N-fertilization method. Data are provided as least square means (LSM), and the error bars represent one standard error in each direction calculated with the LSM method using JMP10 ($n = 10$ for no BC-Control, $n = 20$ for BC-Hotspot, and $n = 15$ for BC-Soil-Mix). (B,C) Root area and total projected structure lengths (TPSL) of the roots are averaged over the N-fertilizer type and separated by the N-fertilization method ($n = 10$). Parameters were determined from photographs of the rootstocks with the REST software (Colombi et al., 2015). Different letters above error bars indicate significant differences among the different variants (at $p < 0.05$, Tukey HSD post hoc test). The analysis of variance and the post hoc test in panel B and C only include the biochar treatments to examine the interactive effect of the type of root-zone amendment and the N-fertilization method. The no-BC-Control is only presented to ease interpretation of the results.

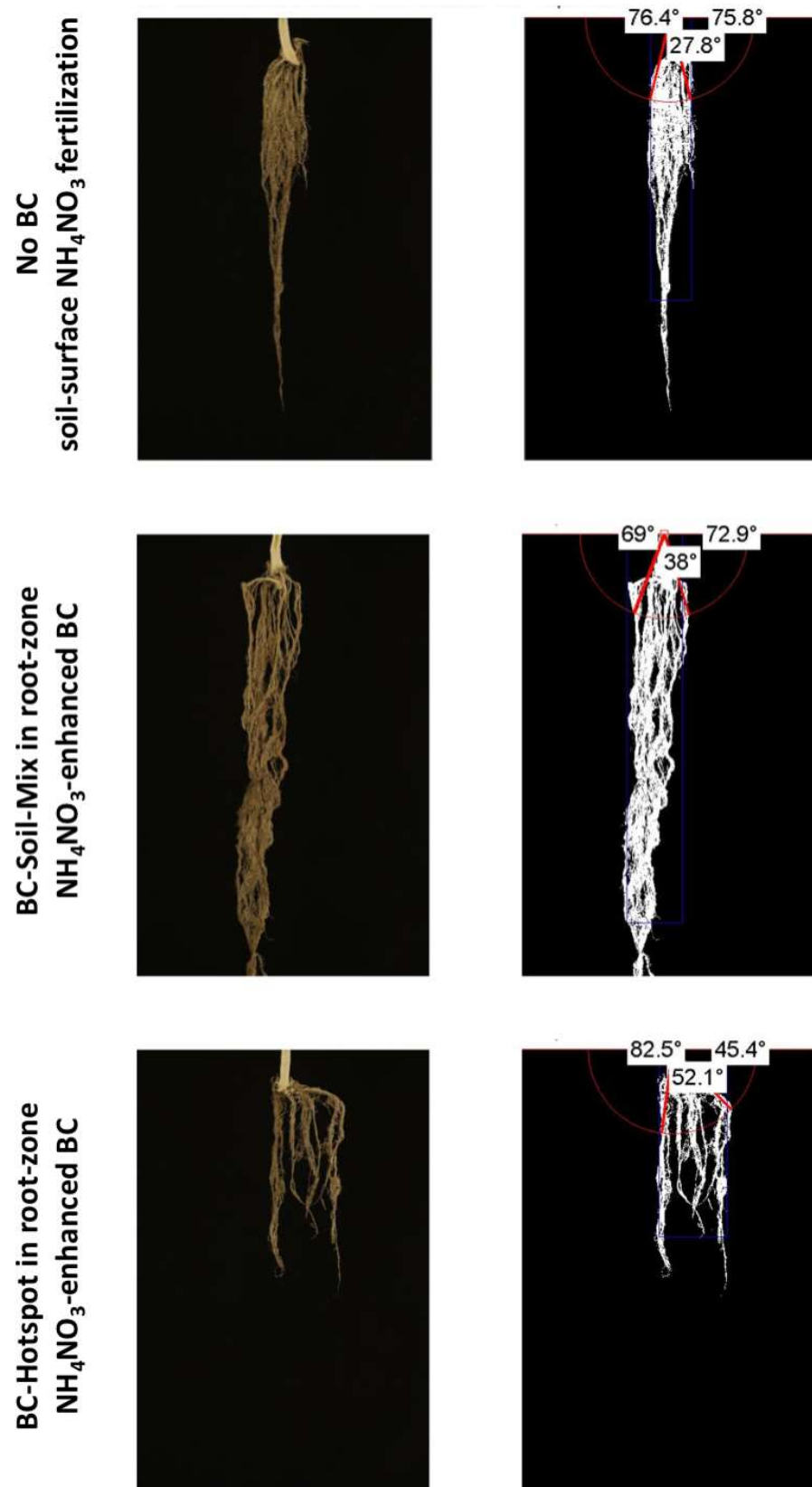


Figure 5. Photographs taken from the rootstock (**left image**) and the corresponding contrast image processed by the Shovelomics software REST [17] (**right image**) for selected plants fertilized with NH_4NO_3 and grown without biochar (BC) (top) and with root-zone-amended, NH_4NO_3 -enhanced biochar as soil mix (**middle**) or as hotspot (**bottom**).

4. Conclusions

Our results indicate that biochar-based fertilizers prepared by simple liquid nutrient enrichment positioned in the root zone of annual plants may also provide crop yield increases in fertile soils in the continental and humid-temperate climate zone. The use of the new method could provide an economic benefit for farmers, which was shown for white cabbage in our study. Field studies with low-dose root-zone amendments of biochar-based fertilizers are needed in temperate climates and should be conducted also with other cultivars to validate our results under field conditions and to find optimal biochar dosages and positions relative to the seedling. Technologies need to be further developed to perform such biochar root-zone amendments on a field scale. Future research is needed to understand the response of root growth and root architecture to the amendment of biochar-based fertilizers in the root zone of annual plants and its linkage with aboveground crop yields.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/horticulturae8040307/s1>, Detailed description of vitamin C quantification; Figure S1: Photograph of the concentrated biochar amendment in the pot; Figure S2: Fixation of the drip line in the pot; Figure S3: Separation of the cabbage heads from residual, nonmarketable aboveground biomass; Figure S4: Photographic equipment for the Shovelomics analysis; Figure S5: Root biomass parameters for all fertilized treatments; Figure S6: Root area and total projected structure lengths of the rootstocks averaged over all plants that received a biochar amendment separated by the fertilizer type; Table S1: Biochar properties derived from batch analysis according to the EBC; Table S2: Basic soil properties and nutrient contents of the soil used in the greenhouse trial; Table S3: Cation exchange capacity of the soil used in the greenhouse trial; Table S4: Particle size distribution of the soil used in the greenhouse trial; Table S5: Analysis of variance for all treatments; Table S6: Analysis of variance for all biochar treatments.

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