

# **Root-zone amendments of biochar-based fertilizers: yield increases of white cabbage in temperate climate**

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## **Supporting Information**

**Number of pages:14**

**Number of Figures: 6**

**Number of Tables: 6**

## Detailed description of the Vitamin-C (L-(+)-ascorbic acid) quantification

The content of vitamin C (L-(+)- ascorbic acid) in the cabbage head biomass was estimated by iodometric titration.(Spínola *et al.*, 2013) 10 g of shredded and frozen cabbage biomass were processed for 30 seconds in a 50 ml centrifuge tube with a laboratory mixer (Ultra-Turrax T18, IKA-Werke GmbH & Co. KG, Staufen, Germany) while adding 5 ml of distilled (DI) water. The blending stick was subsequently flushed with DI water to transfer all cabbage residues into the tubes. The tube was filled up to a volume of 35 ml with DI water and centrifuged at  $10.2^3 \times g$  for 6 minutes (Heraeus Megafuge 8, ThermoFisher Scientific Inc., Waltham, United States). The supernatant was transferred into a 100 ml Erlenmeyer flask and filled up to 50ml. 1 ml of self-prepared starch indicator solution (10g starch L<sup>-1</sup>, Carl Roth GmbH, Karlsruhe Germany) was added and the sample was titrated with a potassium iodide and iodine solution (0.005 molL<sup>-1</sup> iodine, Sigma Aldrich, St. Louis, USA) until the first glow of blue was visible and was stable for 60 seconds. The consumed amount of iodine solution was noted to determine the content of vitamin C in the cabbage biomass (1 mol of used iodine equals 1 mol of L-(+)- ascorbic acid). The titrations were performed under constant light conditions and by the same person for all samples. For each cabbage head, two biomass samples were prepared and titrated.

**Table S1. Biochar properties derived from Batch analysis according to the European Biochar Certificate.[1]**

Parameter	Unit	Value
organic Carbon (C <sub>org</sub> )	wt%	92.6
Hydrogen (H)	wt%	2
H/C <sub>org</sub>	mol/mol	0.18
Nitrogen (N)	wt%	0.52
Ash content (550 °C)	wt%	4
Potassium (K)	g kg <sup>-1</sup>	5
Phosphorus (P)	g kg <sup>-1</sup>	<1
Magnesium (Mg)	g kg <sup>-1</sup>	1
Calcium (Ca)	g kg <sup>-1</sup>	8
pH	1	8.4
BET <sup>a</sup> Specific Surface Area	m <sup>2</sup> g <sup>-1</sup>	366
Bulk Density	kg m <sup>-3</sup>	233
16 EPA PAH <sup>b</sup>	mg kg <sup>-1</sup>	0.76

<sup>a</sup>BET: Brunauer-Emmet-Teller

<sup>b</sup>16 EPA PAK: Sum of the 16 Polycyclic Aromatic Hydrocarbons (PAH) prioritized by the American Environmental Protection Agency (EPA).

**Table S2. Basic soil properties and nutrient contents of the soil used in the Greenhouse Trial.**

Parameter	Unit	Value	Method
Humus content	wt% DM	3.7	DIN ISO 10694: 1996-08
Total Organic Carbon	wt% DM	2.1	DIN ISO 10694: 1996-08
pH	1	7.1	VDLUFA Methodenbuch Band I, Kapitel 5.1.1, 7.Teillieferung, 2016
Electric Conductivity	$\mu\text{Scm}^{-1}$	79	VDLUFA Methodenbuch Band I, Kapitel 10.1.1, 1991
Salt content	$\text{mg}(100\text{g})^{-1}$ DM	42	VDLUFA Methodenbuch Band I, Kapitel 10.1.1, 1991
Kjeldahl-N	wt% DM	0.23	DIN 19684-4
NH <sub>4</sub> -N	$\text{mg}(100\text{g})^{-1}$ DM	<0.05	VDLUFA Methodenbuch Band I, 3. Teillieferung, Kapitel 6.1.4.1, 2002
NO <sub>3</sub> -N	$\text{mg}(100\text{g})^{-1}$ DM	1.9	VDLUFA Methodenbuch Band I, 3. Teillieferung, Kapitel 6.1.4.1, 2002
S <sub>min</sub>	$\text{mg}(100\text{g})^{-1}$ DM	0.51	VDLUFA Methodenbuch I, A 6.3.1 (2016), Extraktion mit 0,0125 M CaCl <sub>2</sub>
Potassium (K)	$\text{mg}(100\text{g})^{-1}$ DM	27.2	Calcium lactate extract, VDLUFA Methodenbuch Band I, 6.Teillieferung, Kapitel 6.2.1.1, 2012
Phosphorus (P)	$\text{mg}(100\text{g})^{-1}$ DM	35.3	Calcium lactate extract, VDLUFA Methodenbuch Band I, 6.Teillieferung, Kapitel 6.2.1.1, 2012
Magnesium (Mg)	$\text{mg}(100\text{g})^{-1}$ DM	29.8	Calcium lactate extract, VDLUFA Methodenbuch Band I, 6.Teillieferung, Kapitel 6.2.1.1, 2012
Boron (B)	$\text{mgkg}^{-1}$ DM	0.9	CAT extract, VDLUFA Methodenbuch Band I, 3. Teillieferung, Kapitel 6.4.1, 2002
Manganese (Mn)	$\text{mgkg}^{-1}$ DM	160	CAT extract, VDLUFA Methodenbuch Band I, 3. Teillieferung, Kapitel 6.4.1, 2002
Copper (Cu)	$\text{mgkg}^{-1}$ DM	4.2	CAT extract, VDLUFA Methodenbuch Band I, 3. Teillieferung, Kapitel 6.4.1, 2002
Zinc (Zn)	$\text{mgkg}^{-1}$ DM	10	CAT extract, VDLUFA Methodenbuch Band I, 3. Teillieferung, Kapitel 6.4.1, 2002



**Table S3. Cation exchange Capacity of the Soil used in the Greenhouse Trial.**

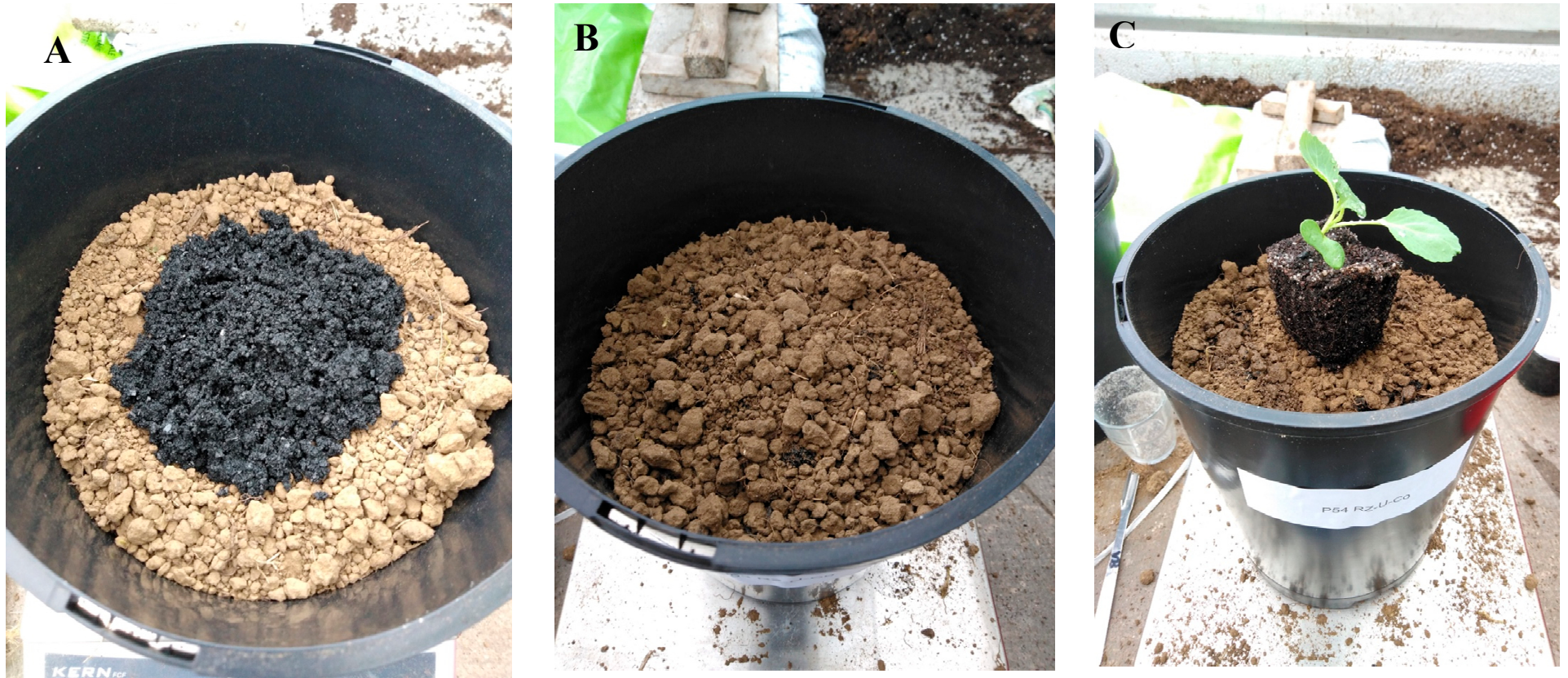
<b>Parameter</b>	<b>Unit</b>	<b>Value</b>	<b>Method</b>
Cation Exchange Capacity (eff. <sup>a</sup> )	cmol*kg <sup>-1</sup>	23.6	DIN EN ISO 11260:2011-09
Exchange Acidity	cmol*kg <sup>-1</sup>	<0.1	DIN EN ISO 11260:2011-09
Exchangable Mg (eff. <sup>a</sup> )	cmol*kg <sup>-1</sup>	4.1	DIN EN ISO 11260:2011-09
Exchangable Ca (eff. <sup>a</sup> )	cmol*kg <sup>-1</sup>	24.5	DIN EN ISO 11260:2011-09
Exchangable Na (eff. <sup>a</sup> )	cmol*kg <sup>-1</sup>	<0.1	DIN EN ISO 11260:2011-09
Exchangable K (eff. <sup>a</sup> )	cmol*kg <sup>-1</sup>	1.2	DIN EN ISO 11260:2011-09
Sum of exchangeable Cations (eff. <sup>a</sup> )	cmol*kg <sup>-1</sup>	29.8	DIN EN ISO 11260:2011-09
Cation Exchange Capacity (pot. <sup>b</sup> )	cmol*kg <sup>-1</sup>	22.2	DIN ISO 13536: 1997-04
Exchangable Mg (pot. <sup>b</sup> )	cmol*kg <sup>-1</sup>	2.4	DIN ISO 13536: 1997-04
Exchangable Ca (pot. <sup>b</sup> )	cmol*kg <sup>-1</sup>	17	DIN ISO 13536: 1997-04
Exchangable Na (pot. <sup>b</sup> )	cmol*kg <sup>-1</sup>	<0.1	DIN ISO 13536: 1997-04
Exchangable K (pot. <sup>b</sup> )	cmol*kg <sup>-1</sup>	0.9	DIN ISO 13536: 1997-04
Sum of exchangeable Cations (pot. <sup>b</sup> )	cmol*kg <sup>-1</sup>	20	DIN ISO 13536: 1997-04

<sup>a</sup>eff.: effective

<sup>b</sup>pot.: potential

**Table S4. Particle Size Distribution of the soil used in the Greenhouse Trial.**

<b>Parameter</b>	<b>Unit</b>	<b>Value</b>	<b>Method</b>
Clay (<2µm)	wt%	28	DIN ISO 11277:2002:08
Coarse Sand (0.63 - 2mm)	wt%	2	DIN ISO 11277:2002:08
Medium Sand (0.2 - 0.63mm)	wt%	2	DIN ISO 11277:2002:08
Fine Sand (0.063 - 0.2mm)	wt%	5	DIN ISO 11277:2002:08
Coarse Silt (20-63 µm)	wt%	31	DIN ISO 11277:2002:08
Medium Silt (6.3 - 20 µm)	wt%	24	DIN ISO 11277:2002:08
Fine Silt	wt%	24	DIN ISO 11277:2002:08
Gravel (>2 mm)	wt%	<1	DIN ISO 11277:2002:08
Coarse Soil (>2mm)	wt%	<1	DIN ISO 11277:2002:08



**Figure S1.** (A) Hotspot root-zone amendment of biochar enriched with mineral nitrogen solution at a depth of 5 cm below the soil level, (B) covering of the hotspot biochar amendment with a 1cm soil layer and (C) positioning of a cabbage seedling onto the hotspot biochar amendment. Differences in soil visualization in panel (A) compared to (B) and (C) were caused by different white balances on the images due to varying light conditions in the greenhouse.



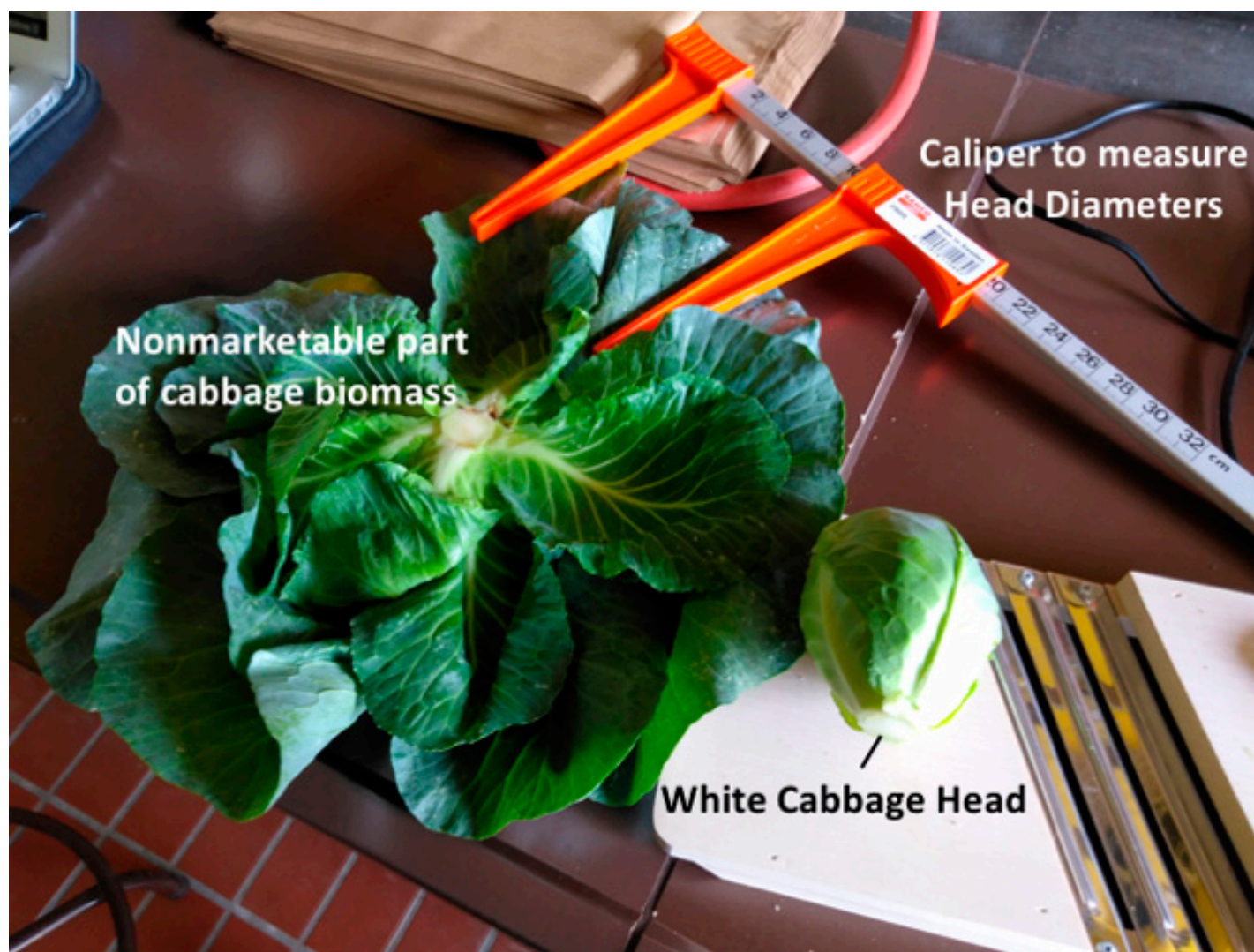


**Figure S2.** Fixation of the drip-line in the pot which provided automated water supply during the experiment.



**Figure S3.** Setup for the imaging of the cleaned and halved cabbage rootstocks for the Shovelomics analysis: Two softboxes for homogeneous illumination of the mounted, cleaned and halved rootstock, black backscreen to generate high contrast images, and a digital camera (Canon EOS 70D) to take high-resolution photographs.





**Figure S4.** Separation of cabbage heads from residual, nonmarketable aboveground biomass.

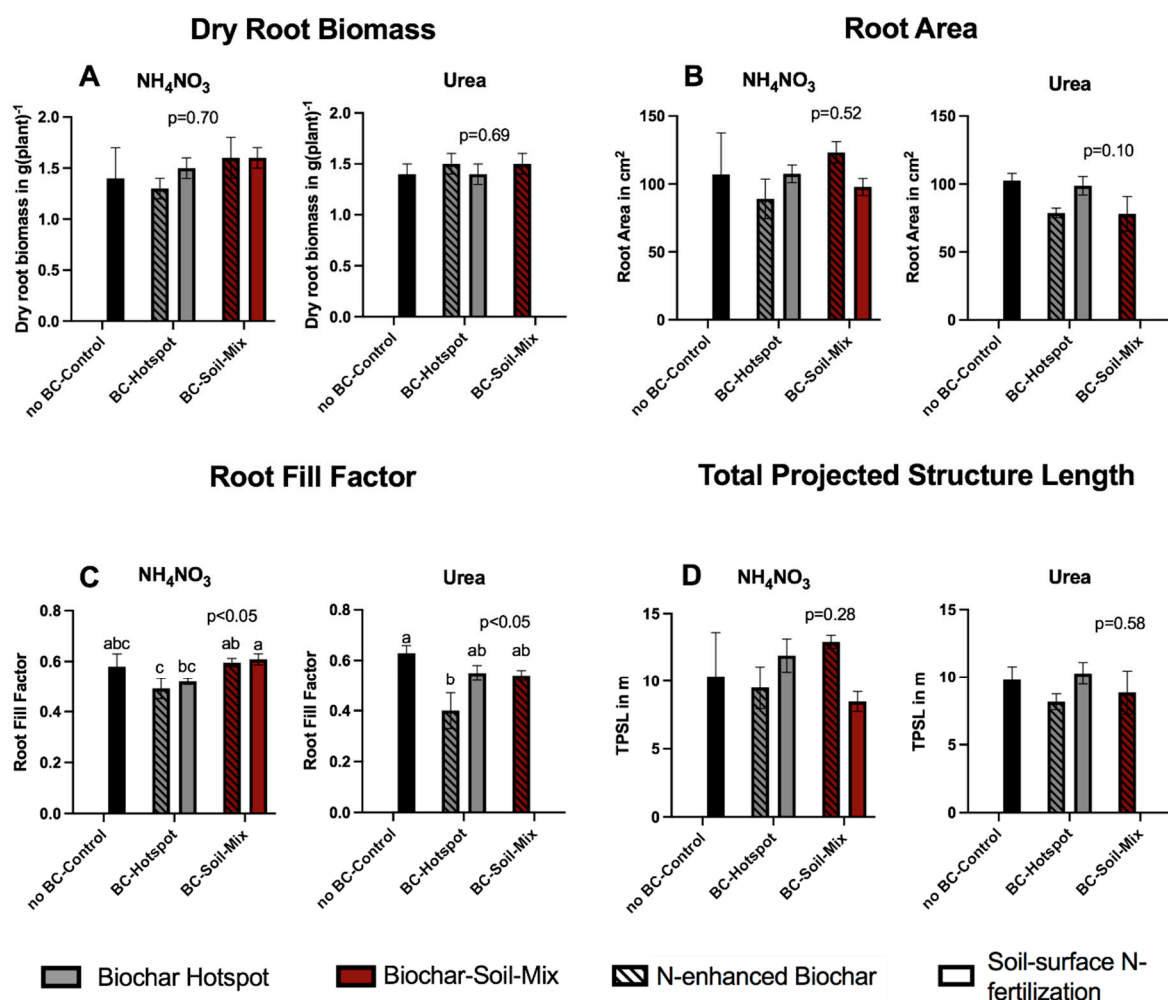
**Table S5.** Analysis of Variance for all Treatments (including N fertilized controls, excluding the control without N fertilizer).

Parameter	Type of biochar root-zone amendment				N fertilizer type				N fertilization method				N fertilizer type * type of biochar root-zone amendment				N fertilization method * N fertilizer type			
	DF	Sum of Squares	F Ratio	p-Value	DF	Sum of Squares	F Ratio	p-Value	DF	Sum of Squares	F Ratio	p-Value	DF	Sum of Squares	F Ratio	p-Value	DF	Sum of Squares	F Ratio	p-Value
Aboveground Biomass weights (FM)	2	1.5E+03	0.6	<b>0.553</b>	1	1.3E+02	0.1	<b>0.742</b>	1	3.2E+01	0.0	<b>0.872</b>	2	4.9E+03	2.0	<b>0.152</b>	1	1.3E+03	1.0	<b>0.314</b>
Cabbage Head weights (FM)	2	5.1E+03	3.1	<b>0.061</b>	1	1.0E+03	1.2	<b>0.281</b>	1	5.8E+02	0.7	<b>0.411</b>	2	6.0E+03	3.6	<b>0.040 *</b>	1	1.4E+03	1.7	<b>0.208</b>
Aboveground Biomass weights (DM)	2	5.3E+00	0.7	<b>0.512</b>	1	4.5E+00	1.2	<b>0.291</b>	1	7.0E-02	0.0	<b>0.894</b>	2	2.0E+01	2.6	<b>0.089</b>	1	2.8E+01	7.1	<b>0.010 *</b>
Cabbage Head weights (DM)	2	1.7E+01	3.0	<b>0.065</b>	1	3.1E+00	1.1	<b>0.311</b>	1	3.9E+00	1.3	<b>0.257</b>	2	1.8E+01	3.0	<b>0.063</b>	1	1.8E+01	6.1	<b>0.020 *</b>
Increase in cabbage head weights (FM)	2	1.3E+03	6.0	<b>0.006*</b>	1	8.3E+02	7.8	<b>0.009*</b>	1	9.7E+01	0.9	<b>0.35</b>	2	1.5E+03	6.8	<b>0.003*</b>	1	2.2E+01	2.4	<b>0.127</b>
Increase in cabbage head weights (DM)	2	9.5E+02	3.8	<b>0.033*</b>	1	4.2E+02	3.3	<b>0.076</b>	1	1.2E+02	1.0	<b>0.33</b>	2	9.8E+02	3.9	<b>0.029*</b>	1	7.6E+02	6.1	<b>0.019*</b>
Cabbage Head Mass Ratio (DM)	2	1.6E+02	3.9	<b>0.032 *</b>	1	9.6E+01	4.8	<b>0.036 *</b>	1	3.7E+01	1.9	<b>0.183</b>	2	1.2E+02	2.9	<b>0.070</b>	1	2.0E+01	1.0	<b>0.325</b>
Cabbage Head Volume	2	7.9E+05	2.8	<b>0.075</b>	1	1.0E+03	0.0	<b>0.932</b>	1	3.0E+04	0.2	<b>0.646</b>	2	2.8E+05	1.0	<b>0.371</b>	1	2.0E+04	0.1	<b>0.710</b>
Root Biomass	2	7.3E-02	0.5	<b>0.637</b>	1	1.9E-04	0.0	<b>0.961</b>	1	1.9E-05	0.0	<b>0.988</b>	2	3.3E-02	0.2	<b>0.816</b>	1	4.6E-02	0.6	<b>0.454</b>
Root Area	2	4.1E+02	0.3	<b>0.754</b>	1	2.3E+03	3.1	<b>0.086</b>	1	4.3E+02	0.6	<b>0.442</b>	2	2.6E+02	0.2	<b>0.833</b>	1	8.7E+02	1.2	<b>0.277</b>
Root Fill Factor	2	9.8E-02	9.7	<b>&lt;0.001 *</b>	1	9.5E-04	0.2	<b>0.668</b>	1	4.5E-02	8.9	<b>0.005 *</b>	2	2.5E-03	0.3	<b>0.780</b>	1	2.6E-02	5.2	<b>0.030 *</b>
Total Projected Structure Length	2	1.2E+00	0.1	<b>0.942</b>	1	1.8E+01	1.7	<b>0.197</b>	1	2.0E+00	0.2	<b>0.666</b>	2	1.4E+00	0.1	<b>0.936</b>	1	1.6E+01	1.5	<b>0.232</b>

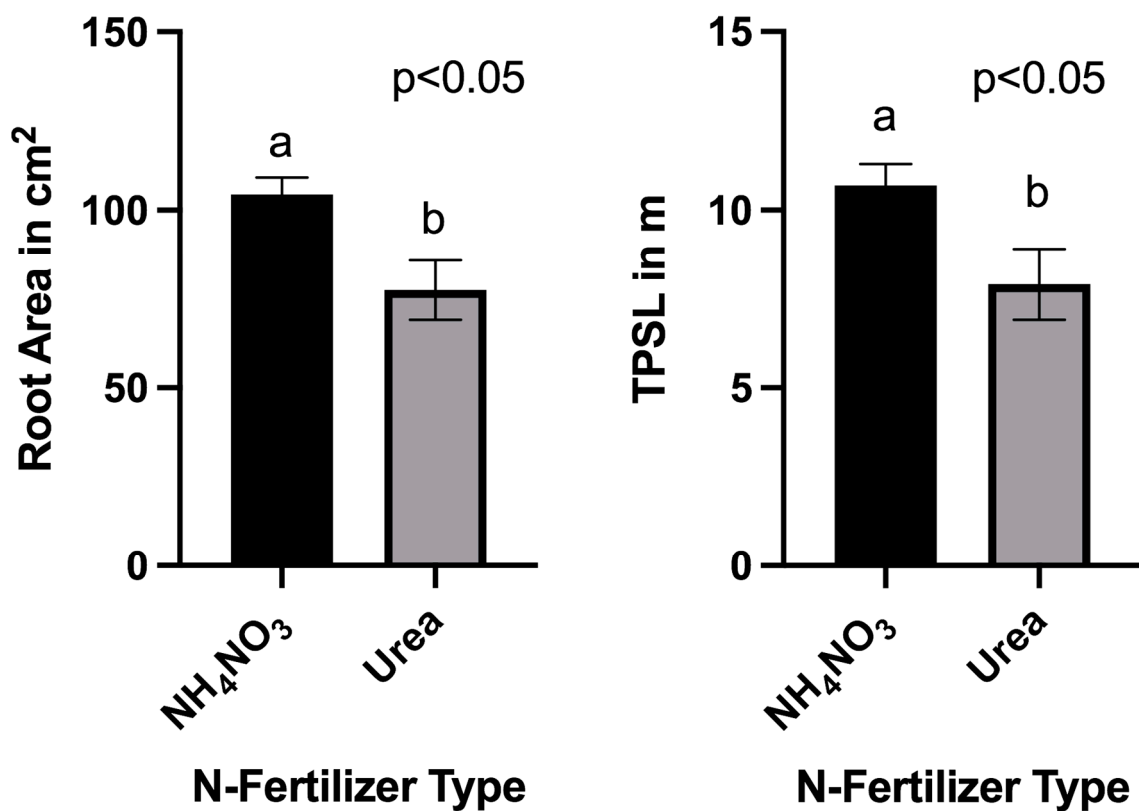
**Table S6.** Analysis of Variance for all Biochar Treatments.

Parameter	Type of biochar root-zone amendment				N fertilizer type				N fertilization method				N fertilizer type * Type of biochar root-zone amendment				N fertilization method * Type of biochar root-zone amendment				N fertilization method*N fertilizer type			
	DF	Sum of Squares	F Ratio	p-Value	DF	Sum of Squares	F Ratio	p-Value	DF	Sum of Squares	F Ratio	p-Value	DF	Sum of Squares	F Ratio	p-Value	DF	Sum of Squares	F Ratio	p-Value	DF	Sum of Squares	F Ratio	p-Value
Aboveground Biomass weights (FM)	1	1.8E+02	0.2	<b>0.638</b>	1	1.5E+03	1.8	<b>0.189</b>	1	2.8E+02	0.3	<b>0.562</b>	1	5.6E+02	0.7	<b>0.417</b>	1	6.5E+02	0.8	<b>0.379</b>	1	3.8E+02	0.5	<b>0.503</b>
Cabbage Head weights (FM)	1	3.6E+02	0.7	<b>0.423</b>	1	6.5E+00	0.0	<b>0.914</b>	1	5.1E+01	0.1	<b>0.761</b>	1	2.3E+03	4.2	<b>0.053</b>	1	8.9E+02	1.6	<b>0.212</b>	1	3.5E+02	0.6	<b>0.433</b>
Aboveground Biomass weights (DM)	1	5.5E+00	1.2	<b>0.292</b>	1	2.2E+01	4.7	<b>0.0415 *</b>	1	3.8E-01	0.1	<b>0.779</b>	1	1.1E+01	2.3	<b>0.144</b>	1	4.1E-01	0.1	<b>0.769</b>	1	2.5E+01	5.3	<b>4 *</b>
Cabbage Head weights (DM)	1	4.5E+00	1.5	<b>0.234</b>	1	1.2E+00	0.4	<b>0.532</b>	1	2.1E+00	0.7	<b>0.416</b>	1	1.1E-01	0.0	<b>0.849</b>	1	4.8E-01	0.2	<b>0.692</b>	1	1.2E+01	3.9	<b>0.062</b>
Increase in cabbage head weights (FM)	1	8.1E+01	0.6	<b>0.438</b>	1	1.1E+03	8.1	<b>0.009*</b>	1	2.5E+00	0.0	<b>0.891</b>	1	5.4E+02	4.1	<b>0.051</b>	1	2.1E+02	1.6	<b>0.218</b>	1	5.1E+01	0.4	<b>0.538</b>
Increase in cabbage head weights (DM)	1	1.8E+02	1.1	<b>0.301</b>	1	1.1E+03	6.7	<b>0.015*</b>	1	5.4E+01	0.3	<b>0.569</b>	1	2.0E+01	0.1	<b>0.727</b>	1	2.5E+01	0.2	<b>0.690</b>	1	4.7E+02	2.9	<b>0.099</b>
Cabbage Head Mass Ratio (DM)	1	4.9E+00	0.3	<b>0.613</b>	1	2.7E+01	1.4	<b>0.245</b>	1	1.3E+01	0.7	<b>0.420</b>	1	6.0E+01	3.2	<b>0.087</b>	1	1.4E+01	0.8	<b>0.395</b>	1	4.6E+00	0.2	<b>0.626</b>
Cabbage Head Volume	1	2.0E+05	1.4	<b>0.248</b>	1	3.3E+03	0.0	<b>0.879</b>	1	8.5E+03	0.1	<b>0.807</b>	1	1.7E+05	1.2	<b>0.281</b>	1	1.6E+04	0.1	<b>0.736</b>	1	4.0E+03	0.0	<b>0.867</b>
Root Biomass	1	4.9E-03	0.1	<b>0.775</b>	1	5.3E-02	0.9	<b>0.353</b>	1	2.7E-02	0.5	<b>0.504</b>	1	8.9E-02	1.5	<b>0.231</b>	1	8.8E-02	1.5	<b>0.233</b>	1	1.2E-01	2.1	<b>0.165</b>
Root Area	1	1.5E+02	0.3	<b>0.574</b>	1	3.6E+03	7.8	<b>0.0103 *</b>	1	5.1E+01	0.1	<b>0.743</b>	1	1.5E+03	3.2	<b>0.085</b>	1	2.4E+03	5.2	<b>0.0324 *</b>	1	2.2E-01	0.0	<b>0.983</b>
Root Fill Factor	1	6.0E-02	12.2	<b>0.0019 *</b>	1	6.4E-04	0.1	<b>0.723</b>	1	3.2E-02	6.5	<b>2 *</b>	1	1.3E-03	0.3	<b>0.616</b>	1	2.6E-04	0.1	<b>0.819</b>	1	1.8E-02	3.6	<b>0.069</b>
Total Projected Structure Length	1	1.0E+01	1.5	<b>0.231</b>	1	4.0E+01	6.1	<b>0.0209 *</b>	1	7.4E+00	1.1	<b>0.301</b>	1	9.8E+00	1.5	<b>0.234</b>	1	5.8E+01	8.9	<b>0.0067 *</b>	1	2.4E-01	0.0	<b>0.849</b>





**Figure S5.** Root biomass parameters for all fertilized treatments including treatments without biochar (Control), with hotspot biochar (BC-Hotspot) and mixed soil (BC-Soil-Mix) biochar root-zone amendments, separated by the type of nitrogen fertilizer and the nitrogen fertilization method. Parameters were determined from photographs of the rootstocks with the REST-software.[2] The error bars represent one standard error in each direction (n=5). Different letters above error bars indicate significant differences among the different variants within a panel ( $p<0.05$ , Tukey-HSD post-hoc test).



**Figure S6.** Root area and total projected structure lengths (TPSL) of the rootstocks averaged over all plants that received a biochar root-zone amendment separated by the N fertilizer type. Parameters were determined from photographs of the rootstocks with the REST-software [2]. The error bars represent one standard error in each direction ( $n=20$  for  $\text{NH}_4\text{NO}_3$  and  $n=15$  for urea). Different letters above error bars indicate significant differences among the different variants within a panel ( $p < 0.05$ , student t-test).

- [1] EBC (2012–2022) “European Biochar Certificate—Guidelines for a Sustainable Production of Biochar.” European Biochar Foundation (EBC), Arbaz, Switzerland. Available online: <http://european-biochar.org> (accessed on 10 January 2022).
- [2] Colombi, T.; Kirchgessner, N.; Le Marié, C.A.; York, L.M.; Lynch, J.P.; Hund, A. Next Generation Shovelomics: Set up a Tent and REST. *Plant. Soil* 2015, 388, 1–20.