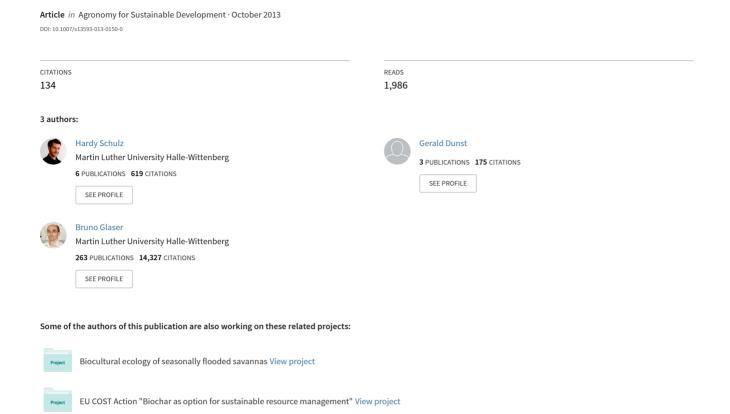
#### Positive effects of composted biochar on plant growth and soil fertility



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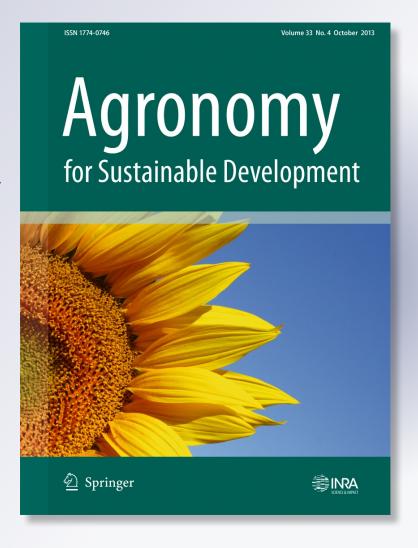
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#### RESEARCH ARTICLE

### Positive effects of composted biochar on plant growth and soil fertility

Hardy Schulz · Gerald Dunst · Bruno Glaser

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**Abstract** Mankind is actually facing serious issues due to the overexploitation of fossil fuels, biomass, soils, nitrogen, and phosphorus. It is claimed that biochar addition to soil improves C sequestration to prevent CO<sub>2</sub> from atmospheric cycling. Biochar addition should also increase soil fertility in a similar way as anthropogenic dark earths of Central Amazonia. Previous studies have shown that biochar stimulates plant growth and increase fertilizer efficiency, especially when biochar is combined with organic fertilizers such as compost. However, little is known about optimum addition amounts and mixture ratios of biochar and compost. Indeed most experiments to mimic Terra preta de Indio focused on biochar alone or biochar in combination with mineral fertilizers. Therefore, we studied optimum biochar and compost amounts and mixture ratios with respect to plant response and soil fertility. We tested the effect of total amount from 0 to 200 Mg/ha, and biochar proportion from 0 % to 50 % biochar, of 18 different compost mixtures on growth of oat (Avena sativa L.) and soil properties in a fully randomized greenhouse study with sandy and loamy soil substrates. We sampled soil substrates before and after plant growth and analyzed plant growth and yield, total organic carbon (TOC), total nitrogen (TN), mineralized nitrogen (Nmin), soil reaction (pH), and electrical conductivity (EC) applying standard procedures. Results show that biomass production was increased with rising biochar and compost amounts. Oat plant height and seed weight was improved only with rising biochar amounts,

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but not with compost amounts. This could be explained by increase of total organic C and total N but not by plant-available ammonium and nitrate. The positive influence of composted biochar on plant growth and soil properties suggests that composting is a good way to overcome biochar's inherent nutrient deficiency, making it a suitable technique helping to refine farm-scale nutrient cycles.

**Keywords** Climate change mitigation  $\cdot$  Soil amendment  $\cdot$  Soil fertility  $\cdot$  *Terra preta*  $\cdot$  C sequestration

#### 1 Introduction

Soil organic matter (SOM) improves soil fertility by providing nutrients through mineralization, nutrient, and water holding capacity and acts as habitat for soil micro-organisms (Fischer and Glaser 2012). However, current land management options including no tillage and organic farming contribute only little to increase SOM contents in agricultural soils due to increasing agricultural intensification (Lal 2009). The addition of animal manure, green manure, and compost significantly contributed to C sequestration in soil (Spaccini et al. 2002). Lynch et al. (2005) observed a stabilization of about 89 % initial carbon from corn silage compost 2 years after application and 93 % C sequestration 1 year after sewage sludge compost amendments on the same field. Gerzabek et al. (1997) calculated a half life of about 7 years for organic carbon of fieldapplied composted animal manure. On the other hand, biochar is reported to be stable for hundreds or even thousands of years, being one of the most recalcitrant forms of carbon from organic matter (Kuzyakov et al. 2009).

Biochar is claimed to achieve several sustainability goals including C sequestration, soil and plant growth improvements, and energy production (Marris 2006). Biochar production yields several beneficial co-products like hydrogen,





bio-oil, syngas, process heat, and liquid smoke via the pyrolysis process while the implication of small stoves for food processing in developing countries could help to reduce respiratory diseases and decrease the pressure on forests (Barrow 2011, Mankasingh et al. 2011).

Biochar effects on plant growth are well studied (Waters et al. 2011, Sohi et al. 2010; Glaser et al. 2002). A recent meta-analysis of Jeffery et al. (2011) comprising of 177 individual studies revealed that beneficial effects of biochar incorporation into soils outbalance negative (only one study showed negative effects) and neutral effects. They found an average increase of crop productivity of around 10 % as an effect of biochar amendments. This appears that a small increase which might be due to the wide range of types of biochar and substrates in strongly varying conditions.

Another explanation for this relatively low overall biochar effect might be the fact that pure biochar does not directly enrich the soil with nutrients (Lehmann and Joseph 2009). Instead, elevating the C/N ratio causes the threat of nitrogen immobilization (Lehmann et al. 2003), and the soils must be fertilized additionally to produce conditions that are agriculturally valuable. Regarding increasing costs for artificial fertilization and even deteriorating prospects with respect to the limited stock of phosphorus and other elemental supplements, a beneficial combination of biochar and compost has already been proven in recent studies (Schulz and Glaser 2012; Fischer and Glaser 2012; Prost et al. 2013). Therefore, the aim of this study was to find optimum biochar and compost amounts and mixture ratios with respect to plant response and soil fertility. For this purpose, biochar was composted using same feedstock but varying biochar/compost ratios. These composted biochar substrates were used subsequently in a pot trial with two different soil substrates (sand, loam) growing oat (Avena sativa L.) in order to find optimum amounts and biochar/ compost mixtures (Fig. 1).

#### 2 Material and methods

#### 2.1 Soil substrates

We used two different types of soil substrates, washed sand (Kiesgrube ZAPF, Weidenberg, Germany) and a loamy soil (Ökologisch Botanischer Garten, University of Bayreuth, Germany). Basic properties of these substrates are given in Table 1. Both substrates were not used for agriculture nor were they fertilized prior to the experiment.

#### 2.2 Composted biochar

Biochar was mixed with fresh organic material, then treated at a professional compost production facility (Sonnenerde<sup>©</sup>,







Fig. 1 Image of our greenhouse experiment using 18 treatments with composted biochar. Photo: Hardy Schulz

Oberwarterstraße 100, A-7422 Riedlingsdorf, Austria). Biochar was produced traditionally in a charcoal kiln from beech wood (350-450 °C for 6 days) and then crushed to particle size below 3 cm using a vibrating roll. Organic material for the compost was 50 % sewage sludge (comprising 25 % dry matter), 25 % freshly chaffed lop (high percentage of fine material like grass, leaves and twigs), and 25 % sieve leftovers of earlier composting (50 % soil and 50 % branches and not decayed composting leftovers). Six different amounts of biochar were blended into the composting material, resulting in 0-50 wt.% of biochar in the final product (Table 1). The composting process lasted for 8 weeks, and the piles were turned over three times per week (triangular cross section 2.5 m broad and 1.3 m high, humidity between 50 % and 60 %), resulting in composted biochars with a degree of maturity of 5 and properties given in Table 1. As this composted biochar belongs to a series of biochar composting experiments, we will refer it to the B series composted biochar throughout the manuscript, which is indicated by their name (Table 1).

#### 2.3 Plant growth study

The study was set up in a greenhouse at average temperature around 22 °C, 200 ml water irrigation every other day, and constant light conditions (400 W sodium discharge lamp, 8 h per day). The treatment matrix comprised gradually increasing biochar and compost levels obtained by using the six different composted biochars (Table 1) applied at three different application amounts (50–100–200 Mg ha<sup>-1</sup>)

[able 1 Properties of soil substrates prior to (t0) and after the experiment (t1) and of the applied composted biochars

Substrates t TOC (g kg	$t$ TOC IC TN $(g \text{ kg}^{-1})$ $(g \text{ kg}^{-1})$ $(g \text{ kg}^{-1})$	IC (g kg <sup>-1</sup> )	TN (g kg <sup>-1</sup> )	C/N	$NO_3$ (mg kg <sup>-1</sup> )	$\mathrm{NH_4}$ (mg kg <sup>-1</sup> )	$NH_4 \qquad AI \qquad Ca \qquad K \qquad Mg \qquad Na \qquad P \\ kg^{-1})  (mg \ kg^{-1})$	Ca (mg kg <sup>-1</sup> )	$K \\ (\text{mg kg}^{-1})$	$\begin{array}{c} \rm Mg \\ \rm (mg~kg^{-1}) \end{array}$	Na (mg kg <sup>-1</sup> )	P (mg kg <sup>-1</sup> )	pH <sub>CaCI2</sub> I	EC Bic (µS/cm) (kg	Biochar (kg mg <sup>-1</sup> )	Biochar Black (kg mg <sup>-1</sup> ) carbon(kg mg <sup>-1</sup> )
Sand	0 0.24±0.02 n.a.	n.a.	0.00±0.00	$0.00\pm0.00$ $0.00\pm0.10$ $1.90\pm0.30$ $4.36\pm0.43$	1.90±0.30		n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	$6.3\pm0.1$ $13.5\pm2.1$	13.5±2.1 0		0
Loam	0 15.60±0.39 n.a.	n.a.	$1.42\!\pm\!0.07$	$1.42\pm0.07$ $11.12\pm0.65$ $8.71\pm0.47$ $9.1\pm0.69$	$8.71 \!\pm\! 0.47$		n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	6.4±0.0 8	81.3±1.3 0		0
Sand	1 0.44±0.03 n.a.	n.a.	$0.04\!\pm\!0.01$	$0.04{\pm}0.01  19.29{\pm}7.70  1.07{\pm}0.40  7.07{\pm}0.77$	$1.07\!\pm\!0.40$		n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	6.7±0.1 2	21.5±1.5 0		0
Loam	1 15.27±0.22 n.a.	n.a.	$1.51\!\pm\!0.03$	$1.51 {\pm} 0.03  10.09 {\pm} 0.08  7.59 {\pm} 1.43  9.52 {\pm} 0.49$	$7.59 \pm 1.43$		n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	6.4±0.4 9	$91.8\pm5.2$ 0		0
Amendments	nts															
B0	0 121.5	9.5	10.9	11	10.8	0.0	10	2,269	135	26	38	32	7.3	0		0
B25	0 139.1	8.9	10	14	7.0	0.3	17	1,938	135	92	38	48	7.2	44.9		33.5
B50	0 166.8	8.3	10.4	16	11.5	0.1	31	758		38	38	35	9.7	80.8		58.2
B100	0 227.2	8.8	11.2	20	11.0	0.4	6	2,462	132	108	33	29	7.4	148.1		107.3
B250	0 335.5	5.2	10.1	33	9.5	6.0	23	1,785	132	102	32	48	7.3	239	239.7	191.4
B500	0 379.2	4.6	10.2	37	12.3	0.3	53	1,208	100	68	25	72	7.2	468	∞	251.3
																Ì

Name refers to the B series composts (B) followed by a number resembling the approximate fraction of biochar in the composted biochar (pre-composting) mixture in kg biochar per mg. Depicted inorganic carbon, TN total nitrogen,  $NH_d$  ammonium, EC electrical conductivity, n.a. not available,  $\pm$  standard error of the mean (n=4)contents of biochar and black carbon are post-composting, e.g., from the products applied in this study TOC total organic carbon,

each plus one control (no amendment) resulting in 19 different treatments (marked with crosses in result graphs Figs. 2, 3, 4 and 5) which were replicated four times each.

Soil substrates were sieved (<2 mm) and homogenized for 10 min using a standard drilling machine with a stirring staff. Then, the null treatments (control) for each soil substrate were separated. The different application amounts (50–100–200 Mg ha<sup>-1</sup>) of each of the 6 different composted biochars (Table 1) were added to the substrates, stirred for another 5 min before separating the weighed single pot fills (4 replicates). Common plant nursery bags were used as pot substitutes. The nursery bags (15×15×20 cm) contained 3 kg dry matter of the soil substrates plus the amendments. Bags were equipped with fleece bottoms and placed on a plastic mat of two cm height to allow for drainage but to prevent the pots to take up the drainage of the surrounding pots.

We grew oats (*Avena sativa ssp. nuda*), for being a common cereal grain plant cultivated worldwide and not sensitive to soil quality and climatic conditions, though water demanding. Sowing was done using the equivalent of 120 to 160 kg ha<sup>-1</sup> (equal to 250 to 350 seeds per m<sup>2</sup> or 10 seeds per pot). All pots were arranged in a completely randomized block design. Plants were harvested after 5 months when fully mature.

#### 2.4 Soil and plant analyses

After 5 months of growth, above-ground biomass was harvested and plant height was recorded. Weights of fresh biomass and harvested seeds were determined. Plant biomass was dried in the laboratory at 65 °C, and then weighted again. Results were scaled up to Mg ha<sup>-1</sup> using the pot surface area (225 cm<sup>2</sup> per pot).

Soil was sampled prior to the experiment (only sandy and loamy substrate), after composted biochar addition (t0) and after harvest (t1). Soil pH was measured in distilled water and in 0.01 M CaCl<sub>2</sub> (1:2.5 w/v); the suspension in distilled water was also used to determine the electrical conductivity (EC). Total organic carbon (TOC) and total nitrogen (N) were measured by dry combustion with a VARIOMAX CNS elemental analyzer (Elementar, Hanau, Germany). Plant-available ammonium and nitrate were determined colourimetrically using an automated photometer (SAN plus, Skalar Anayltical B.V., Breda, The Netherlands), after shaking 8 g soil with 40 ml 1 M KCl for 60 min and filtration according to Thun et al. (1991).

Other plant-available nutrients were extracted by Mehlich-III extraction (Mehlich 1984). To do so, 2.5 g of soil were passed through a 2-mm sieve into 125 ml Erlenmeyer flasks, and 30 ml of Mehlich-III extractant (0.2 M CH<sub>3</sub>COOH, 0.25 M NH<sub>4</sub>NO<sub>3</sub>, 0.015 M NH<sub>4</sub>F, 0.013 M HNO<sub>3</sub>, and 0.001 M EDTA) added. The suspension was shaken for





5 min on a rotating shaker with 120 rpm. After filtrating through No. 42 Whatman filter paper, filtrates were analyzed by ICP-OES (BayCEER, University of Bayreuth).

#### 2.5 Statistical analyses

Multiple linear regressions (MLR) were performed to distinguish between influences of the main factors "biochar per hectare" and "compost per hectare" (hereafter called "biochar effect" and "compost effect" when calculated as single factor or "biochar and compost effect" if calculated in combination). As a precondition, both factors biochar and compost are required to be applied in increasing amounts and independent from each other, which was proven with a linear regression of the respective compost or biochar amounts in our different amendments (R=0.135, ns). To further explain biochar and compost effects on plant growth, stepwise multiple regression (SMR) between plant growth and all measured soil properties at t0 was conducted. This technique is based on the assumption that if biochar had a stronger influence on TOC than compost, the effect of TOC on plant growth could largely be attributed to the biochar amendment. The correlation of choice was Pearson (two-tailed).

Asterisks \*, \*\*, and \*\*\* indicate p < 0.05, p < 0.01, and p < 0.001, respectively. The values behind "±"symbols in the text and in the tables represent one standard error of the mean (n=4). All analyses were performed with SPSS Statistics 17 (IBM).

#### 2.6 Graphic implementation

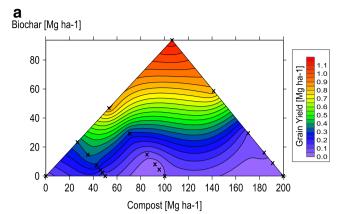
Original data matrix is three-dimensional (biochar amount, compost amount and response variable) and rather complex (19 different biochar and compost combinations see marks in Figs. 2, 3, 4 and 5). In addition, as there is a rather regular gradual increase of both biochar and compost amounts, interpolation between biochar and compost amounts is possible. Therefore, for efficient presentation of original data we choose contour plots obtained by Kriging (*x* and *y* axis plus colors). Graphs were prepared using Surfer 10 (Golden Software Inc. 2011, Colorado, USA).

#### 3 Results and discussion

#### 3.1 Plant growth

Control plant height was about 33 % higher on loamy compared to sandy substrate. Plant growth was generally higher on loamy soil substrate (85–100 cm) compared to sandy soil substrate (55–90 cm; Fig. 2).

Addition of composted biochar significantly increased plant height. Interestingly, the relative contribution of



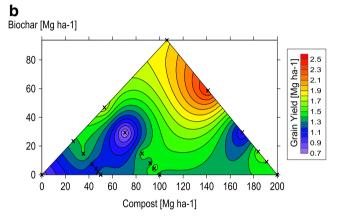


Fig. 2 Seed weight of oat (*Avena sativa* L.) after 5 months of growth in a greenhouse on sandy (**a**) or loamy substrate (**b**) as function of biochar and compost amount added as composted biochar of 18 different treatments, indicated by x (n=4). Interpolation was done by Kriging

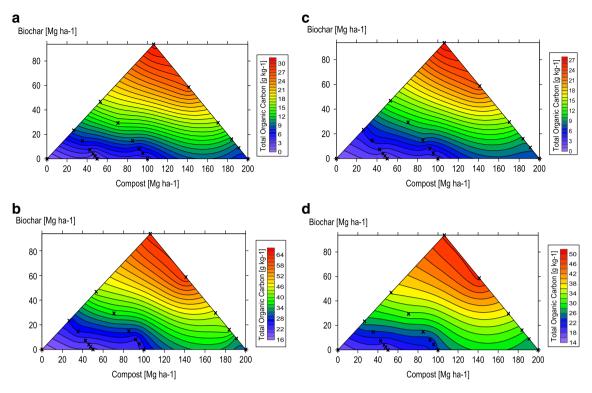
biochar to improved plant growth in the sandy soil substrate was significantly higher than in the loamy soil substrate indicated by a steeper slope of the multiple linear regressions. In the sandy soil substrate even no significant correlation between plant height and compost amount was detected. (Table 2)

On pure soil substrate, above-ground biomass was about two times higher on loam compared to sand (indicated by the intercept of the regression equation, Table 2). Total plant biomass was higher on loam (4–8 Mg ha<sup>-1</sup>) compared to sand (2–6 Mg ha<sup>-1</sup>). Composted biochar addition significantly increased above-ground biomass (MLR, p<0.01) but the effect of biochar was at least one order of magnitude higher than that of compost for both sandy and loamy soil substrates indicated by the slope of the regression equation (Table 2).

On pure soil substrate, grain yield was about six times higher on loamy compared to sandy soil substrate, i.e., 1.2–2.5 and 0.1–1.0 Mg ha<sup>-1</sup>, respectively. Composted biochar addition significantly (p<0.001) increased grain yield which was only due to the addition of biochar while compost did not significantly contribute to this effect (Table 2).

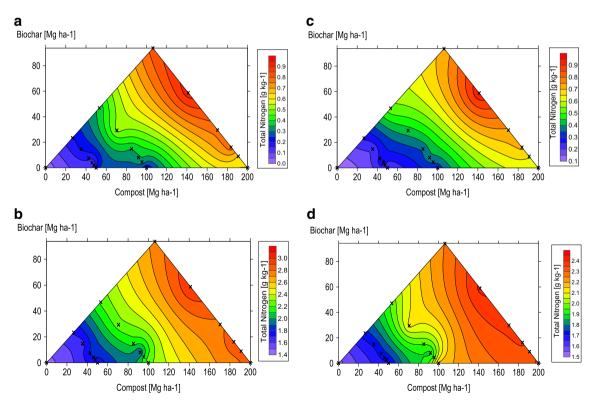






**Fig. 3** Total organic carbon (TOC) in sandy and loamy substrate before (**a** and **b**, respectively) and after (**c** and **d**, respectively) 5 months of plant growth in a greenhouse as function of biochar

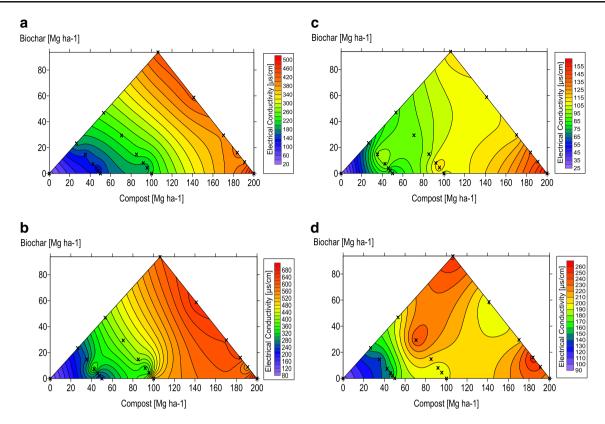
and compost amount added as composted biochar of 18 different treatments, indicated by x (n=4). Interpolation was done by Kriging



**Fig. 4** Total nitrogen content in sandy and loamy substrate before (a and b, respectively) and after (c and d, respectively) 5 months of plant growth in a greenhouse as function of biochar and

compost amount added as composted biochar of 18 different treatments, indicated by x (n=4). Interpolation was done by Kriging





**Fig. 5** Electrical conductivity in sandy and loamy substrate before (a and b, respectively) and after (c and d, respectively) 5 months of plant growth in a greenhouse as function of biochar and

compost amount added as composted biochar of 18 different treatments, indicated by x (n=4). Interpolation was done by Kriging

#### 3.2 Total organic carbon

In pure soil substrate, TOC content was about 60 times higher in loamy (15.60±0.39 g kg<sup>-1</sup>) compared to sandy soil substrate (0.25±0.02 g kg<sup>-1</sup>; Fig. 3). Composted biochar addition significantly (p < 0.001) increased TOC content. The contribution of biochar to TOC was about one order of magnitude higher compared to compost (Table 2). Surprisingly, the effect of biochar and compost on TOC increase was similar for sandy and loamy soils as indicated by a similar slope of the multiple linear regressions (Table 2). Generally, both biochar and compost TOC contents decreased during one growth period (Table 2). From these data (slopes of respective MLR, Table 2) no obvious trend in decomposition rates could be observed, although it appears that higher application amounts show faster decomposition rates on loamy but lower decomposition in sandy substrates.

To identify the soil properties responsible for the increased plant growth, we conducted a stepwise multiple regression (SMR) using soil properties as predictors for plant growth to calculate the influence of TOC, TN, C/N, NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, pH, and EC on plant growth parameters seed weight, plant weight and plant heights. From these results, it is obvious that TOC increase is most importantly improving

all plant responses except plant height on loam. The model predictor  $R^2$  values reveal that seed weight, plant weight, and plant height are better described by TOC on sandy (0.304, 0.580, and 0.177) than on loamy substrate (0.135, 0.359, and 0.156).

However, the question now remains why TOC is the dominant factor for plant growth stimulation in our composted biochar-amended soil substrates and not for instance plant-available nutrients (NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>) or soluble salts (EC). A possible explanation for this might be the fact that increasing TOC by composted biochar additions increased reactive surfaces and stimulated thus microbial growth leading to a short-term immobilization of plant-available nutrients. In the course of plant growth, these nutrients could be released by mineralization of compost and dead micro-organisms thus leading to better plant growth.

Following the ideas of Schulz and Glaser (2012) we hypothesized that composting biochar would have stabilizing effects especially on easily degradable components of the compost. As expected, in all experiments there was a highly significant correlation between both TOC content and fractions of added biochar as well as added compost. Apparently, the slope of the linear regression was always much higher for the effect of biochar compared to the effect of compost





Table 2 Results of multiple linear regressions (MLR) of soil properties and plant growth as function of biochar ("bc") and compost ("co") amendments

Substrate	Variable	Unit	Time	Multiple regression	R	p (bc)	p (co)
Sand	Total organic carbon	g kg <sup>-1</sup>	t0	$0.646+(0.319\times bc)+(0.035\times co)$	0.978***	< 0.001	< 0.001
	Total nitrogen	$g kg^{-1}$		$-0.043+(0.006\times bc)+(0.004\times co)$	0.907***	< 0.001	< 0.001
	C/N			29.329+(0.297×bc)-(0.098×co)	0.562***	< 0.001	< 0.001
	Ammonium	$mg \ kg^{-1}$		$6.885 - (0.049 \times bc) - (0.019 \times co)$	0.455***	0.01	0.01
	Nitrate	$mg \ kg^{-1}$		$12.586 + (0.016 \times bc) + (0.000 \times co)$	0.063	0.59	0.96
	рН Н2О			$8.308+(0.000\times bc)+(0.000\times co)$	0.052	0.68	0.85
	pH CaCl2			$7.229 + (0.001 \times bc) + (0.001 \times co)$	0.135	0.76	0.29
	Electrical conductivity	$\mu S/cm$		25.850+(2.224×bc)+(1.964×co)	0.879***	< 0.001	0.04
	Total organic carbon	$g kg^{-1}$	t1	$-0.416+(0.271\times bc)-(0.037\times co)$	0.971***	< 0.001	< 0.001
	Total nitrogen	$g kg^{-1}$		$0.013+(0.005\times bc)+(0.003\times co)$	0.826***	< 0.001	< 0.001
	C/N			21.216+(0.321×bc)-(0.064×co)	0.726***	< 0.001	< 0.001
	Ammonium	${\rm mg~kg}^{-1}$		$7.157 + (0.012 \times bc) + (0.012 \times co)$	0.266	0.40	0.04
	Nitrate	${\rm mg~kg}^{-1}$		$9.319 - (0.084 \times bc) + (0.017 \times co)$	0.261	0.03	0.27
	рН Н2О			$7.361 + (0.000 \times bc) + (0.001 \times co)$	0.461***	0.80	< 0.001
	pH CaCl2			$7.332+(0.001\times bc)+(0.000\times co)$	0.085	0.69	0.59
	Electrical conductivity			$60.017 - (0.017 \times bc) + (0.448 \times co)$	0.796***	0.86	< 0.001
	Seed weight	${\rm Mg~ha}^{-1}$	t1	$0.139 + (0.013 \times bc) - (0.001 \times co)$	0.681***	< 0.001	0.04
	Plant weight	${\rm Mg~ha}^{-1}$		$2.490+(0.039\times bc)+(0.006\times co)$	0.787***	< 0.001	< 0.001
	Plant height	Cm		$65.05 + (0.224 \times bc) + (0.067 \times co)$	0.47***	0.00	0.15
Loam	Total organic carbon	$\rm g \ kg^{-1}$	t0	$14.447 + (0.490 \times bc) + (0.092 \times co)$	0.947***	< 0.001	< 0.001
	Total nitrogen	$\rm g~kg^{-1}$		$1.369 + (0.008 \times bc) + (0.008 \times co)$	0.901***	< 0.001	< 0.001
	C/N			$11.809 + (0.148 \times bc) - (0.004 \times co)$	0.945***	< 0.001	0.16
	Ammonium	${\rm mg~kg}^{-1}$		$9.726 - (0.059 \times bc) - (0.034 \times co)$	0.689***	< 0.001	< 0.001
	Nitrate	${\rm mg~kg}^{-1}$		$16.541 - (0.004 \times bc) + (0.010 \times co)$	0.095	0.89	0.42
	рН Н2О			$7.361 + (0.000 \times bc) + (0.001 \times co)$	0.461***	0.80	< 0.001
	pH CaCl2			$6.771 + (0.001 \times bc) + (0.002 \times co)$	0.560***	0.32	< 0.001
	Electrical conductivity	μS/cm		$139.200 + (2.283 \times bc) + (2.548 \times co)$	0.891***	< 0.001	< 0.001
	Total organic carbon	$g kg^{-1}$	t1	$16.184 + (0.348 \times bc) + (0.053 \times co)$	0.922***	< 0.001	< 0.001
	Total nitrogen	$g kg^{-1}$		$1.519 + (0.004 \times bc) + (0.004 \times co)$	0.812***	< 0.001	< 0.001
	C/N			$92.350+(0.541\times bc)-(0.022\times co)$	0.944***	< 0.001	0.54
	Ammonium	${\rm mg~kg}^{-1}$		$11.487 + (0.029 \times bc) + (0.029 \times co)$	0.238	0.46	0.07
	Nitrate	${\rm mg~kg}^{-1}$		$4.701 - (0.009 \times bc) - (0.015 \times co)$	0.210	0.66	0.09
	рН Н2О			$7.396 + (0.002 \times bc) + (0.002 \times co)$	0.623***	0.06	< 0.001
	pH CaCl2			$6.746 + (0.003 \times bc) + (0.003 \times co)$	0.581***	0.02	< 0.001
	Electrical conductivity	μS/cm		$130.150 + (0.564 \times bc) + (0.544 \times co)$	0.637***	0.01	< 0.001
	Seed weight	Mg ha <sup>-1</sup>	t1	$1.055 + (0.009 \times bc) + (0.002 \times co)$	0.387***	0.00	0.13
	Plant weight	Mg ha <sup>-1</sup>		$4.597 + (0.036 \times bc) + (0.008 \times co)$	0.590***	< 0.001	0.01
	Plant height	cm		$86.617 + (0.097 \times bc) + (0.051 \times co)$	0.42***	0.03	0.00

<sup>&</sup>quot;R" depicts the correlation coefficient of the MLR, "p" is the significance level of the respective amendment, "t0" is prior to plant growth, and "t1" is after harvest

(Table 2). Obviously, compost could not be stabilized in sandy soil over one vegetation period as indicated by a negative slope of compost amount while in loamy soil, compost was stable for at least one vegetation period indicated by more or less the same effect on TOC (Table 2). This difference is most probably due to organo–mineral stabilization of compost-derived organic matter in the loamy soil. Interestingly, also

the impact of biochar on TOC indicated by the slope of the regression equation in Table 2 decreased during one vegetation period which might be due to mineralization of the labile pool of biochar organic matter.

It was expected that in loamy substrate there should be a higher stabilization of organic matter via organo-mineral interaction. However, microbial decomposition differed





significantly between the two soil substrates. In loamy substrate, the easily available organic carbon added with our composted biochar seems to be more stable than in the sandy substrate with its initially extremely low TOC content. Especially the loamy substrate seemed preventing composted biochar with high biochar contents from being mineralized significantly during the period of our study.

A higher TOC stabilization could be monitored as a function of rising C/N ratios compared to rising biochar fractions in the pots over time, which is acceding to results of an earlier study (Sohi et al. 2010) where the more biochar was added the more initial (non-biochar) carbon was stored.

The differences between the substrate types in our study are contradicting the findings of Kimetu and Lehmann (2010) who found soils initially containing low carbon to be more efficient in stabilizing soil organic carbon after biochar additions than soils initially richer in carbon. An explanation for the higher mineralization rate in our sandy low-carbon substrate could be found in biochar-induced respiration (priming), which supposes that labile fractions on biochar surfaces and inside the pores (oils, etc.) could stimulate bacterial consumptions of initial soil organic carbon. We hypothesize that the microbial community that feeds on carbon was stimulated stronger in the sandy substrate, which was nearly free of (non-biochar) soil organic carbon and so there could have been an increased pressure on the added carbon; imagining a competition on limited resources. Another explanation could be found in the results of Kuzyakov et al. (2009) who explained the absence of priming in their study with limited nutrient availability and thereby limited microbial activity after their biochar amendments in a laboratory incubation experiment with Loess and Haplic Luvisol. A mechanism of positive priming after biochar addition was already reported by Wardle et al. (2008) in a forest humus litter bag experiment, who found losses of soil organic matter after biochar amendments, which he attributed to promoted microbial carbon decomposition. Eventually, the environment of Wardle's experiment was acidic whereas substrates of our experiment were nearly neutral. Another study revealed a possible positive feedback loop where higher contents of black carbon caused further soil micro-aggregate stabilization and stabilization of soil organic matter (Brodowski et al. 2006). Another reason for the stability of the measured TOC could be the formation of organo-mineral complexes with polyaromatic backbones that Glaser et al. (2000) discussed as a possible reason for organic matter stability in the original terra preta soils. Also Liang et al. (2010) found no positive priming effect after addition of organic material to a black carbon-rich Anthrosol from Brazil, rather they found an increased stabilization of the added organic carbon. In this study, organic matter increased by 3-8 % compared to the material added prior to the incubation in the black carbon-rich soils which is

exceeding our results. Yet the stability that comparisons of our TOC values before and after the growth period suggests that our composted biochar (biochar–compost mixtures) might show comparable stability like that from original *terra preta*.

Multiple regression analysis revealed a higher biochar effect on TOC content and thus a short-term C sequestration potential in loamy compared to sandy soil as indicated by the slope of the regression equation (Table 2). This is contradictory to the general paradigm that C sequestration upon addition of organic material (e.g., as composted biochar) is the higher, the lower the initial TOC content is.

#### 3.3 Total nitrogen

Initial total N content was not detectable in sandy (0.0±  $0.0 \text{ g kg}^{-1}$ ) and low in loamy soil substrate (1.42± 0.07 g kg<sup>-1</sup>; Fig. 4). Composted biochar addition significantly (p<0.001) increased total N contents (Fig. 4) with about equal significant contributions of both biochar (p<0.001) and compost (p<0.001) as indicated by about equal slopes of MLR's (Table 2). On both substrate types, the correlation coefficients were highly significant between total N and plant weights or plant heights respectively (sandy and loamy substrate at t0 and t1, p < 0.01). Seed weights correlated only by tendency (sandy substrate p=0.06 and p=0.05, loamy substrate p=0.06 and p=0.05 for t0 and t1, respectively). Total N content in soil substrate decreased during the growth period but still both biochar and compost significantly contributed to higher total N content after harvest when compared to the control (Table 2).

Changes in total N contents were especially noticeable regarding the higher amendments of composted biochar. The nitrogen losses after one growth period were larger with higher total amounts of amendments in loam and sand pots (Fig. 4). Because we always applied rising carbon amounts and nitrogen amounts combined in our composted biochar mixtures, a reason for TN decrease might be a growing need for proteins by the soil bacteria with a bigger amount of food sources offered. Also plant growth was stimulated with higher application amounts and higher nitrogen consumption could simply be the cause and effect of that.

Despite the absence of nitrogen or the very low nitrogen content of biochar generally, there are already proven stimulations of net nitrification from biochar additions to soils (Berglund et al. 2004, DeLuca et al. 2002). On the other hand, also absent and even negative effects on nitrification were reported, mainly depending on soil pH (Yao et al. 2011). In our study there was no influence of the soil pH values on nitrogen cycling because all treatments had a pH value close to neutral.





#### 3.4 C-to-N ratio (C/N)

Initial C/N ratio was  $24.8\pm0.0$  in sandy and  $11.2\pm0.7$  in loamy soil substrate. For both soil substrates, only biochar significantly (p<0.001) increased C/N ratio, while compost had a significantly (p<0.001; sand t0) negative or no significant effect on C/N ratio (Table 2). There was no significant difference in C/N ratio between t0 and t1 (Table 2) indicating that the composted biochar quality did not change during the experiment.

Even though C/N ratio increased up to 50 and 25 in sand and loam, respectively, plant growth was not negatively influenced (Table 2). As expected, only biochar contributed to an increase of C/N ratio (Table 2). On the other hand, compost had no or a negative effect on C/N ratio due to its higher N content (Table 2) corroborating the assumption that biochar would add especially organic C to soil and compost would provide especially N. Interestingly, the ratio of  $TOC_{loss}$  to  $TN_{loss}$  was the larger the higher the biochar amount was, implicating that more nitrogen was retained compared to carbon leading to the assumption that an amount of nitrogen might have been retained either physically by biochar or biologically by soil micro-organisms. Generally, higher C/N ratios coincided with lower TOC<sub>loss</sub> and TN<sub>loss</sub> which confirms results from Nguyen and Lehmann (2009) who related a higher biochar decomposition rate to higher O/C values and lower aryl C contents.

#### 3.5 Ammonium (NH<sub>4</sub><sup>+</sup>)

Initial NH<sub>4</sub><sup>+</sup> content was 4.36±0.43 mg kg<sup>-1</sup> in sandy and 9.71±0.69 mg kg<sup>-1</sup> in loamy soil substrate. Composted biochar addition had a significantly negative effect on NH<sub>4</sub><sup>+</sup> content in both soil substrates, with both amendments showing significant influence (Table 2). The negative impact of biochar on NH<sub>4</sub><sup>+</sup> content was two times as large as compost (Table 2). NH<sub>4</sub><sup>+</sup> content did not significantly correlate with plant growth on sandy substrate, but correlated negatively with plant weight and plant height on loamy substrate (p<0.01). NH<sub>4</sub><sup>+</sup> content increased in both soil substrates during the growth period but there were no significant effects neither of biochar nor of compost in the respective MLR (Table 2). There was no correlation between added biochar or compost amounts and soil NH<sub>4</sub><sup>+</sup> concentrations both for sandy and loamy substrates after plant growth (Table 2) which was also the case in a field study from Jones et al. (2011). Jones et al. (2011) showed that ammonium sorption under field conditions was significantly higher in agriculture soil compared to the concentration in the applied biochar (when calculating on weight basis). Still their results were opposite when calculated on volumetric contents, indicating that biochar particles do have the ability to adsorb NH<sub>4</sub><sup>+</sup> which was also suggested by Atkinson et al. (2010).

#### 3.6 Nitrate (NO<sub>3</sub><sup>-</sup>)

Initial NO<sub>3</sub><sup>-</sup> content was 1.90±0.30 mg kg<sup>-1</sup> in sandy and 8.71±0.46 mg kg<sup>-1</sup> in loamy soil substrate. Composted biochar addition did not significantly alter soil NO<sub>3</sub> content in both sandy and loamy soil substrate (Table 2). After one vegetation period, biochar had a negative effect on NO<sub>3</sub> content (p=0.03) while compost had no significant effect on NO<sub>3</sub> content in sandy soil substrate (Table 2). Nitrate concentration correlated negatively with grain yield at t1 and positively with plant heights at t0 on sandy substrate (both with p < 0.05). On loamy soil substrate, no significant biochar effect (t0, t1) but a negative compost effect on NO<sub>3</sub> content was observed at t1 (yet only by tendency, p=0.09; Table 2). The NO<sub>3</sub><sup>-</sup> content did not correlate with plant response on loamy substrate. The tendency of opposed nitrogen contents between t0 and t1 (see the "Ammonium (NH<sub>4</sub><sup>+</sup>)" section) was statistically proven only for loamy substrate, where to NO<sub>3</sub> content correlates negatively with t1  $NO_3^-$  content (p < 0.001).

Interestingly, nitrogen contents had only minor (total N and  $NO_3^-$ ) or no  $(NH_4^+)$  influence on plant response applying stepwise multiple regression (t0; SMR data not shown).

After one vegetation period (t1), soil NO<sub>3</sub><sup>-</sup> concentration was generally lower compared to t0 in both soil substrates which could not be correlated to increased plant growth (neither negatively nor positively). Larger fractions of biochar in the composted biochar decreased soil NO<sub>3</sub><sup>-</sup> concentration during plant growth on sandy soil substrate significantly while on loamy soil substrate increasing compost fractions in the composted biochar significantly increased soil NO<sub>3</sub><sup>-</sup> concentration (Table 2). These results could largely be attributed to elevated nitrate consumption due to improved plant growth, which is in-line with the effects that biochar or compost fractions had on plant growth as discussed above.

In a study of Jones et al. (2011), almost no nitrate was found on biochar particles after 3 years in a field trial and their increased yields could be correlated to biochar addition. This also implies the absence of nitrate bound to biochar particles and thereby limited nitrification. Improved soil aeration through biochar amendment (Kolb 2007) is also discussed to be potentially beneficial for microbial activity in soils and thereby for nitrification, but certainly a change in soil water conditions as discussed in Glaser et al. (2002) induced by biochar addition could counteract here. While increasing moisture may possibly even increase denitrification.

#### 3.7 Soil reaction (pH)

The pH values measured in water were slightly higher compared to pH values measured in CaCl<sub>2</sub>, both soil substrates





showing the same tendencies (Table 2). Therefore, only pH values in  $CaCl_2$  will be reported and discussed in the following. Initial pH<sub>CaCl2</sub> was  $6.30\pm0.10$  in sandy and  $6.37\pm0.03$  in loamy soil substrate. Composted biochar addition had no significant influence on pH in sandy soil substrate while pH significantly increased in loamy soil substrate (Table 2). Surprisingly, multiple linear regression analysis revealed that compost rather than biochar is responsible for this pH increase (Table 2). Conducting a SMR showed that pH value changes upon biochar and compost additions had only beneficial effects on plant heights in loamy substrate, being most significant predictor ( $R^2$ =0.345\*\*\*).

The fact that our amendments had no significant effects on pH in sandy substrate might be explained by the fact that the composted biochar showed pH value which was similar to the pH value of the used soil substrate. In addition, the pH values of the composted biochar varied only slightly between 7.2 and 7.6 (Table 1). Surprisingly, in the loamy soil substrate, pH correlated significantly with amount of applied compost (Table 2) although pH of both soil substrates was comparable and we added the same composted biochar.

Luo et al. (2011) used a range of biochars with pH value of up to three times as high as the pH of the soil. But even their biochar that showed a pH similar to that of the soil it was applied to had a stronger effect on the pH than our composted biochar with the biggest biochar fraction. Also the structure of our biochar should not have been limiting its ability to increase pH, although we used rather coarse charcoal. Already Tryon (1948) stated that finer charcoal works better for elevating the pH of soils.

#### 3.8 Electrical conductivity (EC)

Initial EC was  $13.5\pm2.1$  µs/cm in sandy soil and  $81.3\pm$ 1.3 µs/cm in loamy soil substrate (Fig. 5). Composted biochar addition significantly increased EC on both soil substrates, both biochar and compost contributing about equally to the EC increase (Table 2). Correlation between EC and plant growth was recorded for plant weights and heights on sandy substrate in soil samples from before and after vegetation period (t0 and t1). However, the picture was different on loamy substrate where EC at t0 significantly correlated with plant growth but not at t1 (Fig. 5), which could possibly be attributed to less drastically changed surface areas in loamy substrate that caused higher leaching rates compared to that in sandy substrate. In both soil substrates, biochar and compost effects contribution to EC decreased by a factor of about four during one vegetation period (Table 2) indicating either plant uptake or leaching of nutrients. As plant growth significantly correlated with EC, it is conservative to assume that plant uptake is higher than leaching of nutrients in composted biochar-amended soils.

Amounts of added composted biochar significantly correlated with EC in both soil substrates (Table 2). After one vegetation period, this effect declined and was only detectable in the loamy soil substrate while in the sandy soil substrate only compost contributed to significantly higher EC (Table 2). From these results, it is clear that both biochar and compost contained ionic elements being potentially plant-available. After one vegetation period, only compost provided further plant-available nutrients most probably via mineralization; though mineralization was lower the higher the biochar concentration was (Fig. 5) indicating enhanced stabilization of labile (compost) C with increasing biochar addition. This can be attributed to a negative priming effect also observed in other biochar compost experiments (Fischer and Glaser 2012).

Jones et al. (2011) showed that in their field study applying biochar and N fertilizer, biochar did not alter the EC significantly, the extracted biochar fractions themselves apparently showed a fourfold decrease in EC after the 3 years field trial. Hossain et al. (2011) showed a significant rise in EC after biochar additions, which might be largely due to the fact that their biochar was produced from sewage sludge which is very mineral-rich, contrasting to our biochar which was produced from hardwood. Steiner et al. (2007) reported that hardwood-derived biochars tend to contain considerable carbonate concentrations which might lead to an increased release of plant-available salts from the compost into the soil environment.

#### 4 Conclusions

Composted biochar addition to sandy and loamy soil substrates led to increased plant growth. This effect was the higher, the more composted biochar was added and the higher the biochar contribution to the applied mixture was. This could be explained by an increase in soil TOC content and by providing plant-available and mineralizable nutrients. Composting of biochar combined the high C sequestration of biochar (stable C-rich but N-poor material) and the fertilization potential of compost (labile organic matter rich in mineralizable nutrients). Our study clearly revealed that plant growth and soil fertility was higher the more composted biochar was added. In addition, this effect was higher, the higher the biochar amount was, indicating an optimum composition with respect to labile/stable organic matter and nutrient composition.

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