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Biochar amendment increases maize root surface areas and branching: a shovelomics study in Zambia

Abiven, Samuel ; Hund, Andreas ; Martinsen, Vegard ; Cornelissen, Gerard

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Biochar amendment increases maize root surface areas and branching: A shovelomics study in Zambia

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Abstract

Background and Aims

Positive crop yield effects from biochar are likely explained by chemical, physical and/or biological factors. However, studies describing plant allometric changes are scarcer, but may be crucial to understand the biochar effect. The main aim of the present study is to investigate the effect of biochar on root architecture under field conditions in a tropical setting.

Methods

The presented work describes a shovelomics (i.e. description of root traits in the field) study on the effect of biochar on maize root architecture. Four field experiments we carried out at two different locations in Zambia, exhibiting non-fertile to relatively fertile soils. Roots of maize crop (*Zea mays L.*) were sampled from treatments with fertilizer (control) and with a combination of fertilizer and 4 $t.ha^{-1}$ maize biochar application incorporated in the soil.

Results

For the four sites, the average grain yield increase upon biochar addition was 45±14% relative to the fertilized control (from 2.1-6.0 to 3.1-9.1 ton ha^{-1}). The root biomass was approximately twice as large for biochar-amended plots. More extensive root systems (especially characterized by a larger root opening angle (+14±11%) and wider root systems (+20±15%)) were observed at all biochar-amended sites. Root systems exhibited significantly higher specific surface areas (+54±14%), branching and fine roots: +70±56%) in the presence of biochar.

Conclusions

Biochar amendment resulted in more developed root systems and larger yields. The more extensive root systems may have contributed to the observed yield increases, e.g. by improving immobile nutrients uptake in soils that are unfertile or in areas with prolonged dry spells.

Keywords: Biochar; Shovelomics; Root architecture; Plant allometric trends; Field experiment; Maize

Introduction

Biochar – defined as pyrogenic organic matter deliberately added to soil – has been proposed as a strategy for mitigating climate change as well as improving agricultural yields (Lehmann, 2007). A growing body of evidence shows that biochar does affect plant growth and yield (Crane-Droesch et al., 2013; Jeffery et al., 2011). However, the directions (an increase or decrease in growth and yield) and mechanisms behind these changes remain unclear. The majority of studies have presented increases in crop yield for soils amended with biochar as compared to control soils, however, examples exist where decreases have been observed (Rajkovich et al., 2012; Van Zwieten et al., 2009). According to a recent meta-analysis, soil properties are the main parameters explaining the potential of the biochar (Crane-Droesch et al., 2013).

With regard to the mechanisms operating, different processes have been proposed to explain the modifications of the soil properties after biochar inputs. These include: water retention increase (Novak et al., 2012), increased nutrient availability via the increase of the cation exchange capacity and the release of phosphate (Glaser et al., 2002), the promotion of mycorrhizae (Warnock et al., 2010) and a liming effect (Kimetu et al 2008). These mechanisms are all related to the properties of the soil, but the link to the plants themselves, i.e. through a systematic study of the nutrient content of plant tissues or via the observation of allometric changes occurring during plant growth, remains largely unexplained (Steiner et al 2007; Martinsen et al 2014).

An additional approach to elucidate this link would be to look at the effect of biochar from a more holistic point of view, and investigate how the plant may adapt to the new conditions created by the input of biochar. The plant's allometric trends and its architecture may provide information about the changes in the soil environment that occur during the growth of the plant (Körner, 2011; Poorter and Sack, 2012). In particular, the way in which the plant root system adapts to the prevailing soil conditions reflects the limitations of resources that the plant experienced. For example, maize plants root growth angles have been shown to become steeper under low nitrogen conditions (Trachsel et al 2013). Here "root system" refers to the overall root mass, whereas "root architecture" refers to its structure and quality.

Root system architecture is one major factor determining the biomass productivity, particularly under edaphic stress. For example, a deep rooting system may be beneficial during droughts (Benjamin and Nielsen, 2006), while a system exploring the topsoil may be useful to collect immobile nutrients, especially phosphorus (Ho et al 2005). Describing the root system architecture remains a technical challenge since its access is constrained by the soil. Several methods have been proposed in the laboratory including very artificial, but high-throughput setups such as hydroponic conditions, paper rolls or growth pouches, to more realistic set ups such as pot experiments (reviewed in Zhu et al (2011)). These designs reduce sampling demands and field heterogeneity, but are limited by many aspects including the volume of the soil or artificial climatic conditions. Field studies are much more time consuming and pose unique technical challenges associated to mature root system imaging under realistic conditions (Bucksch et al., 2014; Zhu et al., 2011). First attempts to capture root architecture features in the field were often restricted to visual scoring of few root system traits (Ekanayake et al 1985). Recently, a new approach, namely shovelomics, has been proposed to produce high throughput data from field studies (Trachsel et al., 2011). This technique consists of the excavation of a maize root system using a shovel, the cleaning of the root system followed by visual scoring and counting of root characteristics on a scoreboard. Up to now, shovelomics was mainly used to detect differences between genotypes of maize plants (Grift et al., 2011; Trachsel et al., 2011), primarily to support breeders allowing them to provide plant genotypes that are adapted to various conditions, whereby the soil (or substrate) and the amendments were the same for all treatments. Shovelomics has also previously been used to investigate the effect of nitrogen fertilisation on root architecture (Trachsel et al., 2013). A clear link between a deeper root system and a low fertilisation rate was identified, indicating that in instances of lower nitrogen (N) availability in the topsoil, some genotypes can explore the subsoil.

The shovelomics method is increasingly combined with image-based phenotyping techniques to enable reproducible results, which are independent of the person skills to evaluate the root stocks without systematic bias (Grift et al 2011). These image-based shovelomics methods were recently further optimized with regards to the sampling strategy and software solution (Colombi et al., 2015) where instead of sampling the whole root system, only half of the root system is sampled. This method enhanced on the one hand the transportation and cleaning process and enabled on the other hand a better insight into the root system without excessive overlapping of the roots on the image. A new software, "Root Estimator for Shovelomics Traits" (REST), was specifically developed to analyse pictures of root stocks. It automatically detects more than 10 parameters per root image including root angles, root system size, and root architecture.

Little is known about the influence of biochar on root architecture. Under laboratory conditions (soil columns), one study observed significantly larger barley root biomass in sandy soils after the amendment of biochar, by grid net counting after trimming by brushing (Bruun et al., 2014). To our knowledge, no study presents up to now observations from the field.

In this study, we applied the image-based shovelomics approach to samples from four field sites of two locations in Zambia, where biochar-treated, fertilized maize plots were compared to maize plots with only fertilizer input as well as nonfertilized controls. We hypothesised a modification of the root architecture upon biochar addition with greater effects of biochar in the sandy soils of Kaoma (based on observations of greater yield effects were observed in earlier studies (Cornelissen et al., 2013; Martinsen et al., 2014)) as compared to the loamy soils at Mkushi. This work will help in understanding why biochar can have positive effects on crop yield, and thus at which sites these beneficial effects can be expected. This is important since the main incentive for small-scale tropical farmers to implement biochar would be increased yields, where a global bonus would consist of the accompanying carbon sequestration.

Material and methods

Experimental sites

Farmer-led field experiments were carried out in 2013 and 2014 at four farms in Zambia. Two sites were located close to the town of Kaoma (referred to below as K3 and K4; S 14°50', E 24°58'; annual rainfall ~ 930 mm; altitude 1080 m; growth season temperatures 25-28 °C) in the west part of the country. The two other sites were located near the town of Mkushi (MK4 and MK7; S 13°48', E 29°03'; annual rainfall ~ 1220 mm; altitude 1320 m; growth season temperatures 23-26 °C) in the centre of the country. Both sites can experience weeklong dry spells even during the wet season in November-March, where practically all rainfall occurs. According to the Köppen-Geiger classification, the sites can be found in the “Cwa” climate zone (Kottek et al., 2006).

In this study, all experiments were conducted at farms practicing conservation farming (minimum tillage plus the retention of crop residues and the incorporation of legumes in the crop rotation (Hobbs et al., 2008)) with dry season preparation of planting basins (~16000 basins ha^{-1}) and addition of fertilizers to basins only. Table 1 presents the characteristics of the sites along with the analytical determination methods that were used and can be found in Martinsen et al (2014) and Cornelissen et al (2013). The four locations diverged mainly by their soil types characterised by an aeolian acidic sandy soil in Kaoma and an oxisol (sandy loam) in Mkushi.

Experimental design and field management

The same biochar was added to the four sites prior to seeding. The biochar feedstock was maize cobs, and the biochar was produced using a brick kiln. The charring temperature was around 350 °C, as measured by a digital thermocouple, and the pyrolysis time was seven days. The charred maize cobs were manually crushed to a coarse 1-5 mm powder before application in the field. The exact design of the field experiments as well as an extensive characterisation of the biochar can be found in Martinsen et al (2014). Amounts of fertilizer were 156 kg N ha^{-1} yr^{-1}, 56 kg P ha^{-1} yr^{-1} and 28 kg K ha^{-1} yr^{-1}, which corresponds to local standard recommendations. No lime was applied to the fields. The total size of each experiment was around 300 m^2 per farm. Each plot consisted of an area of around 50 m^2, three rows of 15 basins separated by one control row of 15 basins (Martinsen et al., 2014).

The amount of added biochar (4 tons ha^{-1} = 250 g $basin^{-1}$) corresponded to approximately 1.7 % biochar in the basins with a volume of ~10 l (corresponding to 15 kg soil $basin^{-1}$ with depth 20 cm, length 30 cm, width 16.7 cm and bulk density of 1.5 g cm^3). This amount corresponds to quantities potentially available on site based on the biomass resource locally accessible for biochar production. Fertilizer and biochar were added by mixing them into the soil of a planting basin.

The maize (*Zea mays* L.) was planted on November 29, 2013 (three seeds per basin). The same genotype (Maize Research Institute variety 634, Lusaka, Zambia) was used for the four sites.

Soil and biochar chemical analysis

pH was determined electrochemically (Orion, model 720, Orion Research Inc., Cambridge, MA, USA) in suspension with 0.01 M $CaCl_2$ (volume soil:volume solution ratio of 0.4). Samples were extracted with 1 M NH_4NO_3 and base cation concentrations were determined in the extracts. Extractable acidity was determined by titration with 0.05 M NaOH to pH 7. The sum of exchangeable base cations and exchangeable acidity was assumed to equal the cation exchange capacity (CEC). Organic carbon and nitrogen were determined by dry combustion after acidification, using a CHN analyzer (Leco CHN-1000; Leco Corporation, Sollentuna, Sweden).

Shovelomics

The roots were sampled with a sharp, flat shovel at the harvest of the maize on March 20 (MK4 and MK7) and March 30 (K3 and K4), 2014. They were excavated by removing a soil cylinder of approximately 40 cm diameter and 25 cm depth, with the plant stem in the middle of the cylinder. Root excavation, washing and photography were carried out by one and the same researcher to avoid bias from slight variations in sampling strategy. Sixteen plants were sampled per site; eight from the plots without biochar and eight from the plots with biochar (n=8 per site and treatment; total 64 samples). To this end, eight plants were sampled from eight out of fifteen basins from the middle row (of three rows) of each treatment, similar to Martinsen et al (2014) and Cornelissen et al (2013). The highest plant in each basin was selected for analysis. For four out of 64 selected planting basins, no maize plant had emerged and in these cases basin number 11 was therefore also sampled.

The root crowns were cut lengthwise through the middle, and carefully cleaned with water by soaking for 3 h followed by rinsing under a mild water flow for 15 to 30 min. A photograph (resolution 18 megapixel) of the root biomass was taken at constant light conditions on a black background with a HD camera (Canon EOS 60D).

The images were analysed using the software REST (Root Estimator for Shovelomics Traits – Colombi et al., 2015). This software was developed to provide an automated, high-throughput analysis of root architecture traits from images.

The root traits analysed with REST (Table 3) were divided in two categories, i) traits related to the size and expansion of the root stock and ii) traits related to root architecture within a given size. To provide a robust measure of the root stock dimensions, REST takes only the 95% interquartile width and the 95% interquartile rooting depth. This reduces the impact of single roots sticking out of the root system on these dimension parameters to a minimum. Within these dimensions a polygon is placed defining a convex hull embracing around 90% of the root system. Here, the area of the convex hull is used as a proxy measure for the root system size; it is defined as the area of the convex hull enclosing 90% of root-derived pixels in the image.

Certain traits (i.e. distinct variants of root characteristic phenotypes) related to the inner root architecture are calculated in a way that they can be considered as independent of root system size (see table 3 for the traits description). Such architecture traits are the fill factor (i.e. the proportion of root-derived pixel within the convex hull), the median gap size (i.e. the size of the holes with visible background within the root system) and the median thickness of measured root system. These traits are more related to branching density and root numbers, leading to a more developped root system. On the contrary, the trait “number of hole” is dependent of the root stem size (table 3).

The images were scaled based on markers present on the picture and the soil surface was set manually on the picture. The software can detect more than 10 different traits automatically. Figure 1 presents the picture and the post-treatment image of two plants, with and without biochar. Note that other software for root analysis is also available and have been applied before the REST software for different levels of complexity and data integration (Bucksch et al., 2014). However, numbers are compared between biochar-amended and non-amended plots. It is not expected that the type of software used will influence relative numbers and thus the conclusions on the effect that biochar has on root architecture. Furthermore, REST does not aim to describe individual roots but rather some basic characteristics of the root system.

Other parameters such as the projected area of root-derived pixel or the number of holes within the convex hull are affected by both size and inner architecture.

Statistical analysis

Statistical analysis was performed using the R software (R Development Core Team 2014). The experimental set up was a randomized block design. Data normality was confirmed by the Shapiro-Wilk-test ($p<0.05$). The significance of the differences between treatments was tested by a two-way Analysis of Variance (ANOVA) at a 95% confidence level using the biochar presence and the sites as factors. T-tests (95%) were used to compare the effect of biochar for a specific site.

Results

Yield, root biomass and root-to-shoot ratio

Maize grain yields from fertilized plots were 2 to 2.6 t ha^{-1} on the sandy soils of Kaoma (K3 and K4), and 5 to 6 t ha^{-1} on the more fertile loamy sands of Mkushi (MK4 and MK7). There was no significant site specific effect (i.e. no interaction) in grain yields upon biochar addition in Mkushi and Kaoma, but grain yields were significantly ($p<0.001$) greater in Mkushi as compared to Kaoma, and yields as well as total biomass were significantly (+20-30%; $p<0.05$; Table 2) smaller at fertilized, non-amended plots than at fertilized, biochar-amended ones. This was in accordance to observations reported for three previous seasons (2010-2013) at these plots (Cornelissen et al 2013; Martinsen et al 2014).

Root biomass was twice as high for biochar-amended, fertilized plots than for fertilized plots without biochar (significant difference for pooled values for all plots; $p<0.01$; Figure 2). The increase in root biomass upon biochar addition was only significant for site K3 (Figure 2). Root-to-shoot weight ratios were 0.05 to 0.16 in the sandy soils of Kaoma, and smaller (0.02 to 0.05) in the more fertile loamy sands of Mkushi (Table 2). These values were similar to reported values of 0.06 to 0.12 for watered and non-watered maize plants (Sharp and Davies 1979) as well as to many reviewed values for root-to-shoot ratios under various CO_2 regimes (Rogers et al 1995). At all sites, ratios increased with biochar addition, but due to great variability between plants, these differences were not significant ($p>0.05$).

Root traits

The traits that were mainly affected by site ($p<0.05$) and only weakly affected by the biochar treatment ($p>0.05$) were: stem diameter, the median thickness, the median gap size and width of the root system and the root system depth and the convex hull area. The width of the root system (95% of the root system-derived pixel detected in the picture) of the controls ranged from 12.2 cm (K3) to 23.4 cm (MK4). The site was the main determinant for the stem diameter: 2.10 cm for the control plot at site K3 compared to 2.73 cm for the control plot at site MK7. In addition, site determined variations in the root depth of the controls (95% of root system-derive pixels detected on the picture), where 23.3 cm for site K3 was observed as compared to 29.8 cm for site K4. The observation that the site was the main factor in determining the root system dimension was also expressed in the convex hull area trait (the area of the convex hull enclosing about 90% of root-derived pixel in the image). The differences in this trait were highly significant between sites, where there were mainly significant differences between the control plots and the biochar-treated plots for site K3 ($p<0.01$) and for MK7 ($p<0.05$), although to a lower extent.

Traits that were mainly affected by biochar treatment were related to both the size and architecture of the inner root system. Seven out of 11 traits were significantly affected by biochar addition (stem diameter, 95% width, median thickness and median gap size were not significantly affected). The projected root area showed the most significant ($p<0.001$) change upon biochar addition (Table 3), where differences between the control and the treatments were significant ($p<0.05$) for three of the four individual sites (all except MK4; $p>0.05$). The greatest increase in the projected area in the presence of biochar was observed at site MK7 (increase from 206.2 to 328.6 cm^2). There was also a significant ($p<0.001$) effect of site for this trait with greater projected areas both for controls and for biochar amended plots in Mkushi as compared to Kaoma. Biochar affected the number of holes within the convex hull ($p<0.01$ for site K4 and all sites combined; $p<0.05$ for site MK7) and biochar treatment exerted significant effects on the root angle opening ($p<0.01$ for all sites combined). The root angle opening increased by around 20° for two of the sites (K3 and MK4), indicating a wider arc of the maize roots and a more extended root system in the presence of biochar. Biochar also influenced root depth, particularly for site K4 (29.8 cm for the control and 35.9 cm for the biochar treatment; $p<0.05$; Table 3). Of importance were the effects of the biochar treatment on parameters describing the inner architecture, such as the fractal dimensions ($p<0.05$ for site K3 and for all sites combined $p<0.01$) and the fill factor ($p< 0.01$ for all sites combined). The number of holes increased upon biochar addition at all sites and was doubled upon biochar addition for the sites K4 (from 1563 to 3785, $p<0.01$) and MK7 (from 23060 to 3904, $p<0.05$; Table 3)

Discussion

The effect of the soil and the effect of the biochar led to very different trait changes: a larger root system size (especially characterized by a significantly larger root opening angle ($p<0.005$) and a wider root system ($p<0.005$); Table 3) in the sandy loam soils of Mkushi compared to the aeolian sand of Kaoma, and root systems with significantly more intensive branching (more holes on the image; $p<0.01$)) and with a significantly larger surface area in the presence of biochar ($p<0.005$). The larger root systems in the Mkushi sandy loams compared to the Kaoma sands corresponded with a significant difference in crop yield – both, biomass and grain yield in Mkushi were double to triple the yields observed in Kaoma ($p<0.01$ both for nonfertilized, fertilized and biochar-amended plots; $n=11$). However, it is not necessarily the case that the larger root systems were causing the larger yields in Mkushi compared to Kaoma.

Biochar addition resulted in yield increases that were significant for all plots combined (+45±17%; $n=4$; $p<0.1$). This observation was corroborated by the root system size increases that were significant for some of the sites, such as those in the root area (+54±14%; $n=32$;

$p<0.005$) and related parameters such as root depth (+10±7%; n=32; $p<0.05$), root angle opening (+14±11%; n=32; $p<0.01$) and fine root development expressed by the number of holes in the images (+70±56%; n=32; $p<0.01$). Again, both the changes in root architecture and grain yield are caused by BC, but the larger yields are not necessarily caused by the root system changes – both are expressions of the fact that biochar causes significant changes in soil biology, chemistry and physics (Martinsen et al., 2014; Warnock et al., 2010; Yamato et al., 2006).

Biochar amendment resulted in a larger number of significant root trait changes at the Kaoma site (three at both Kaoma sites, none at MK4, three at MK7) than at the Mkushi site, and even though this was not expressed in greater increases in crop yield in Kaoma than in Mkushi during the particular cropping season reported here (2013-2014). However, earlier crop yield responses to biochar (in previous seasons) were significantly stronger at Kaoma than at Mkushi. For the 2010-2011 season, namely, maize grain yields were tripled ($p<0.05$) upon biochar addition at Kaoma, whereas yields were slightly (and none significantly) reduced at the Mkushi sites (Cornelissen et al 2013). This picture was the same in the 2011-2012 season, when there was an increase in relative yields of 178% and 289% ($p>0.05$) at 2 and 6 t biochar per ha, respectively in Kaoma and 109% and 110% at 2 and 6 t biochar per ha, respectively in Mkushi ($p>0.05$)(Martinsen et al 2014). This corroborates previous findings indicating that biochar has generally a more positive effect in soils with low fertility (Crane-Droesch et al., 2013).

Biochar effects on root architecture are at the moment poorly understood (Bruun et al, 2014). Actually, a more developed root architecture with a higher surface area could be the result of two contradicting biochar actions: a negative effect, i.e. a toxicity effect which would force the plant to develop more root to uptake the water and nutrients, or a positive effect, where the biochar would improve soil properties and promote root development. Our data rather suggest a positive effect of biochar. The proliferation of primary and secondary lateral roots is a well-observed answer of plants to higher availability of nutrients in a specific zone of the soil (Hodge, 2004). This specific development of roots is particularly observable for immobile nutrients like phosphorus (Lynch, 2011). Mobile nutrients higher availability rather result in a deeper root system (Hodge, 2004; Peng et al., 2010).

It is speculated that the here observed effects are consequences in physical and/or chemical changes in soil brought about by biochar. For example, biochar decreases soil density (Glaser et al, 2002), and may facilitate root proliferation by creating wider or additional pores (Bruun et al, 2014). This density effect has explicitly been shown for the Kaoma and Mkushi soils in a parallel soil physics study (Obia et al, submitted). Another physical effect of biochar amendment observed for the currently studied Zambian soils is an increase in plant-available water measured via pF curves (Cornelissen et al, 2013; Martinsen et al, 2014). Larger root proliferation may indicate more available water locally in the basins. However, like for mobile nutrients, water rather induces an elongation of the root system (Bengough et al., 2011), which we did not observe here.

With regard to chemical effects, biochar has been observed to result in higher K contents in both the soil solution (from around 150 to around 300 µg cm sampler^{-2} month^{-1} in plant root simulator ion exchange membranes) and in plant tissue (from around 5000 to 8000 mg kg^{-1}) at these two sites in Zambia (Martinsen et al, 2014). Also P availability can be expected to increase with the pH increase brought about by the biochar (Kaoma, pH from 4.6 to 6.3, Mkushi, pH from 5.3 to 5.9). Lastly, the concentrations of available Al^{3+} decreased from 0.14-0.18 to 0.01-0.06 cmolc kg^{-1}; even though 0.14-0.18 cmolc kg^{-1} is not an excessively high Al concentration, Al is very toxic to plant roots and this toxicity is alleviated by biochar amendement (Barceló and Poschenrieder, 2002).

Overall, it appeared that a better developed root architecture, likely in the form of lateral root branching, in the presence of biochar can contribute to larger yields and thus, a larger amount of roots, aid in achieving increases in plant growth. The presence of biochar would thus improve the ability of the plant to resist environmental stress factors such as drought (Malamy, 2005). Biochar has also been cited as a major asset in order to avoid nitrate leaching and a higher nitrate assimilation efficiency (Dunbabin et al., 2003) or phosphorus uptake (Lynch, 1995). This is extremely important in the easily leached, low-CEC soils such as the ones studied here.

Early work (Breazeale 1906; Nutman 1952, cited in Lehmann et al., 2011) reported an increase in biomass root growth in the presence of biochar type materials. (Lehmann et al., 2011) reviewed the changes of root biomass induced by the application of biochar as compared to a non-amended control and observed that in most cases, an increase in root biomass was related to an increase in shoot biomass. Our results are in line with these findings. The improvement of key properties such as inherent nutrient and water conditions results in a more developed root system. However, in most of the cases reported by Lehmann et al (2011), the root-to-shoot ratio also decreased, while in our study it was systematically increased (Table 2). The soils we considered here are of a lower quality than those reported, thus one possible explanation for such an effect could be that for low quality soils the root architecture improvement is even more sensitive than for that in more fertile soils.

Conclusions

Our results suggested that biochar application in the sandy and sandy loam soils did not only increase the root biomass, but also extensively modified its architecture, leading to a more developed root system. Such an improvement of root architecture could have major implications for the plant, in particular related to its ability to resist climatic events such as droughts.

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Figures

Figure 1: Original picture (left) and processed image (right) of the investigated root system for the site MK4 without (a) and with biochar input (b). On the processed image, the blue rectangle represents about 90% of root-derived pixels, the red horizontal line the soil surface and the other red lines the root angles to the soil surface.

Figure 2: Root biomass (g dry mass) for the four sites (K3, K4, MK4 and MK7), with (black bars) and without (white bars) biochar addition. The bars represent the standard error. The results of the ANOVA are presented above the figure and the statistical comparison between the biochar amended and the control plot at each site are presented above the bars corresponding to the sites.

Table 1: Mean (± sd) chemical and physical soil characteristics (0-20 cm) at individual farms. "-" indicates values below the detection limit. "nd" indicates values that were not determined. CEC, cation exchange capacity; OC, Organic Carbon; BD, bulk density.

Farm	**Location**	**pH**	**CEC**	**OC**	**Total N**	**BD**	**Sand**	**Silt**	**Clay**
		0.01 M $CaCl_2$	$Cmol_c.kg^{-1}$	*%*		$g.cm^{-3}$		*%*	
K3	Kaoma	5.18 ± 0.16	2.82 ± 1.83	0.61 ± 0.29	-	1.53 ± 0.01	81.7	15.3	3.0
K4	Kaoma	5.38 ± 0.19	3.89 ± 1.11	0.39 ± 0.08	-	1.53 ± 0.01	85.4 ± 0.8[a]	11.8 ± 0.5[a]	2.8 ± 0.5[a]
Biochar	Kaoma	7.1	32.5	70 ± 5	0.60 ± 0.02	0.098	n.d.	n.d.	n.d.
MK4	Mkushi	6.08 ± 0.14	2.72 ± 0.29	0.39 ± 0.02	-	1.46 ± 0.01	72.8	19.8	7.4
MK7	Mkushi	5.77 ± 0.38	3.65 ± 0.86	0.66 ± 0.10	0.01 ± 0.01	1.45 ± 0.09	79.1	9.4	11.5
Biochar	Mkushi	8.8	57.8	81 ± 5	0.70 ± 0.02	0.098	n.d.	n.d.	n.d.

[a] n=11, to test the heterogeneity for one of the sites

Table 2: Grain yield, total biomass and root-to-shoot ratios for the 2013 to 2014 season for the particular farm plot where shovelomics samples were taken. For comparison, grain yields (season 2013-2014) are also presented for the average of the farms studied at one location. Average grain yields and total biomass for all farms at one location for previous seasons (seasons 2011-2012 (lower fertilizer rates) and 2012-2013) can be found in Martinsen et al. (2014).

Farm	**Location**	**Maize Grain Yield**[a]			**Total Biomass**[a]			**Root-to-shoot ratio**[b]	
		Control[c]	**Control + NPK**[d]	**Biochar + NPK**[e]	**Control**[c]	**Control + NPK**[d]	**Biochar + NPK**[e]	**Control + NPK**[d]	**Biochar + NPK**[e]
		t ha^{-1}	*t ha^{-1}*	*t ha^{-1}*	*t ha^{-1}*	*t ha^{-1}*	*t ha^{-1}*	*g g^{-1}*	*g g^{-1}*
K3	Kaoma	0.6	2.1	3.3	1.2	3.6	5.0	0.047 ± 0.049	0.101 ± 0.080
K4	Kaoma	0.7	2.6	3.1	1.3	4.0	4.5	0.118 ± 0.107	0.158 ± 0.128
Average 6 farms	Kaoma	1.1 ± 0.7	3.4 ± 1.6	4.2 ± 1.9					
MK4	Mkushi	3.4	6.0	9.1	9.1	15.7	22.2	0.023 ± 0.010	0.037 ± 0.032
MK7	Mkushi	3.6	4.8	7.2	7.2	13.6	18.8	0.024 ± 0.025	0.045 ± 0.034
Average 5 farms	Mkushi	4.3 ± 1.1	7.9 ± 2.6	9.6 ± 2.1					

[a] Derived from harvesting ten planting basins in the middle of the plots (see text).

[b] Calculated from total biomass by assuming the emergence of two plants per planting basin, which may result in a systematic deviation but in similar relative numbers.

[c] Control without biochar or fertilizer.

[d] Only fertilizer added (156 kg N ha^{-1} yr^{-1}, 56 kg P ha^{-1} yr^{-1} and 28 kg K ha^{-1} yr^{-1}). No lime applied.

[e] Fertilizer and maize biochar (4 t ha^{-1}) added. No lime applied.

Table 3: Mean values of the root system traits for the four study sites. Values are the average of eight replicates. Standard errors are indicated between brackets. Stars indicate the level of significance between treatments. For individual sites, statistics were carried out to identify differences between control and biochar plots and results are shown next to the higher value if significant. The ANOVA results are presented in the last column. The interactions between treatment and site were significant only for one trait (stem diameter, $p<0.1$). ***: $p < 0.005$; **: $p < 0.01$; *: $p < 0.05$.

	Description of the trait	**K3**		**K4**		**MK4**		**MK7**		**Analysis of variance across sites**
		Control	Biochar	Control	Biochar	Control	Biochar	Control	Biochar	
Root angle opening (°)	Opening of the angle between left and right of the root system	75.3	97.4	63.5	66.7	105.6	123.2	119.6	127.2	Biochar:**
		(7.0)	(10.7)	(6.0)	(5.5)	(8.3)	(8.2)	(10.4)	(6.9)	Site:***
Fractal dimension (dimensionless)	Indication of the branching degree	1.72	1.77*	1.73	1.78	1.75	1.78	1.78	1.81	Biochar:**
		(0.02)	(0.01)	(0.02)	(0.03)	(0.02)	(0.02)	(0.01)	(0.02)	Site:*
Area (cm^2)	Surface covered by the roots	84.5	129.1*	126.2	212.5*	172.0	231.3	206.2	328.6*	Biochar: ***
		(11.4)	(12.4)	(14.2)	(27.8)	(26.6)	(27.6)	(22.6)	(48.3)	Site ***:

Convex hull area (cm^2)	Area of the convex hull enclosing about 90% of root-derived pixel in the image, based on the 95% width and 95% depth traits.	256.5 (19.6)	348.8** (24.3)	430.9 (49.3)	501.3 (57.8)	541.2 (95.3)	564.7 (58.3)	525.8 (48.5)	686.4* (71.9)	Biochar:* Site:***
Fill factor (Number of pixels in the convex hull)	Number root derived pixels divided by number of total pixels within convex hull.	0.33 (0.03)	0.37 (0.01)	0.31 (0.03)	0.44 (0.06)	0.34 (0.03)	0.41 (0.03)	0.40 (0.04)	0.46 (0.04)	Biochar:** Site: n.s.
Stem diameter (cm)		2.10 (0.08)	2.40 (0.09)	2.17 (0.11)	1.63 (0.18)	2.64 (0.28)	2.76 (0.13)	2.73 (0.08)	2.91 (0.18)	Biochar:n.s. Site: ***
95% depth (cm)	Height of 95 % of root-derived pixel, cutting of 5% of root pixels at the bottom to remove root that stick out of the root stock.	23.3 (0.65)	25.7 (1.01)	29.8 (2.00)	35.9* (1.30)	24.7 (1.83)	26.1 (1.65)	26.3 (1.37)	27.5 (1.14)	Biochar:* Site: ***
95 % width (cm)	Width of 95 % of root-derived pixel, cutting of 2.5 of root that stick out of the root stock	12.2 (0.8)	14.8 (1.0)	16.1 (1.2)	15.3 (1.5)	23.4 (3.0)	23.2 (1.3)	20.8 (1.1)	26.8 (2.8)	Biochar:n.s. Site: ***

	on either side									
Number of holes	Background patches which are enclosed by root-derived pixels. This trait is root system size dependent.	1298 (351)	1373 (210)	1563 (206)	3785** (973)	1205 (205)	1983 (499)	2306 (552)	3904* (714)	Biochar:** Site: **
Median thickness (cm)	Median thickness of the root system within the convex hull	0.10 (0.01)	0.10 (0.01)	0.10 (0.01)	0.13 (0.01)	0.16 (0.02)	0.16 (0.01)	0.15 (0.03)	0.13 (0.01)	Biochar:n.s. Site: **
Median gap size (cm^2)	Background patches which are enclosed by captured root-derived pixels. .	0.0013 (0.0001)	0.0015 (0.0003)	0.0018 (0.0001)	0.0016 (0.0001)	0.0012 (0.0002)	0.0016 (0.0002)	0.0018 (0.0001)	0.0018 (0.0002)	Biochar:n.s. Site: *

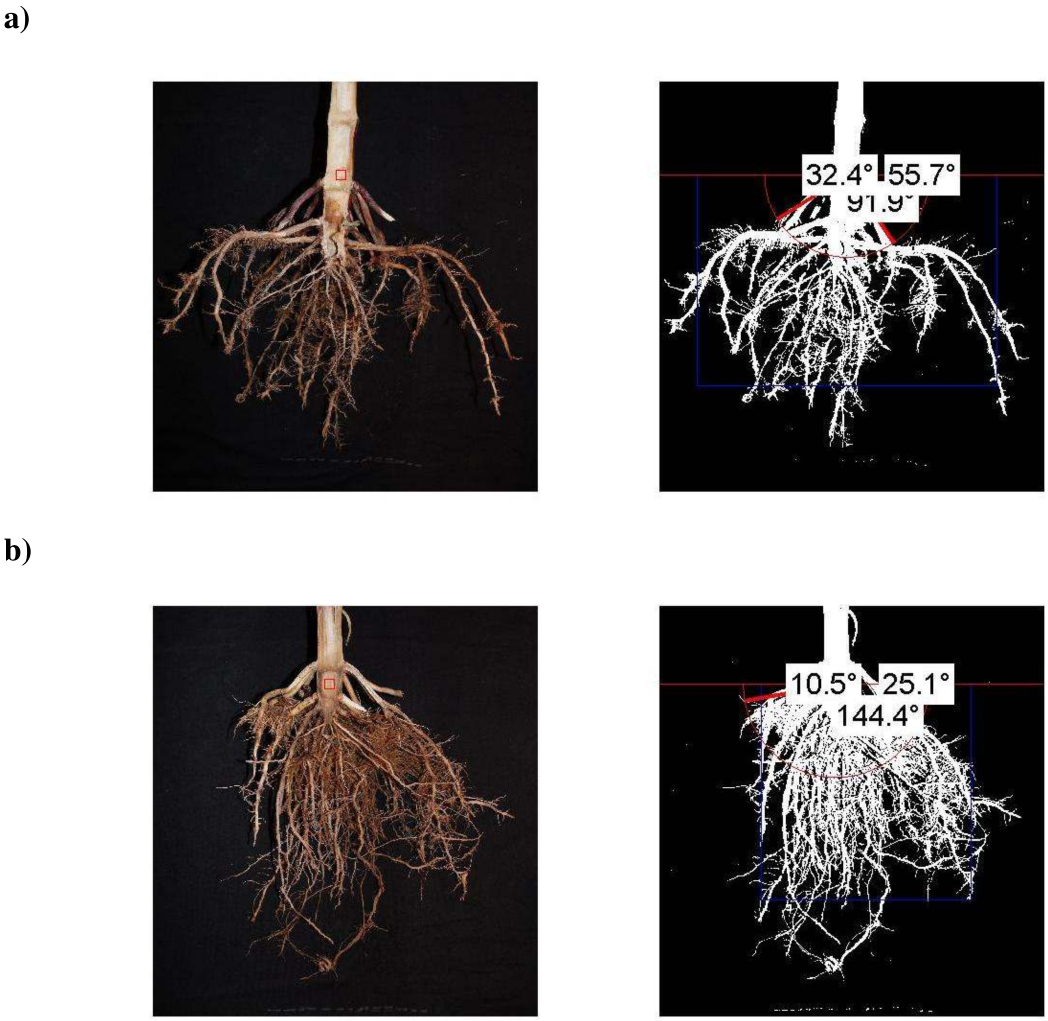
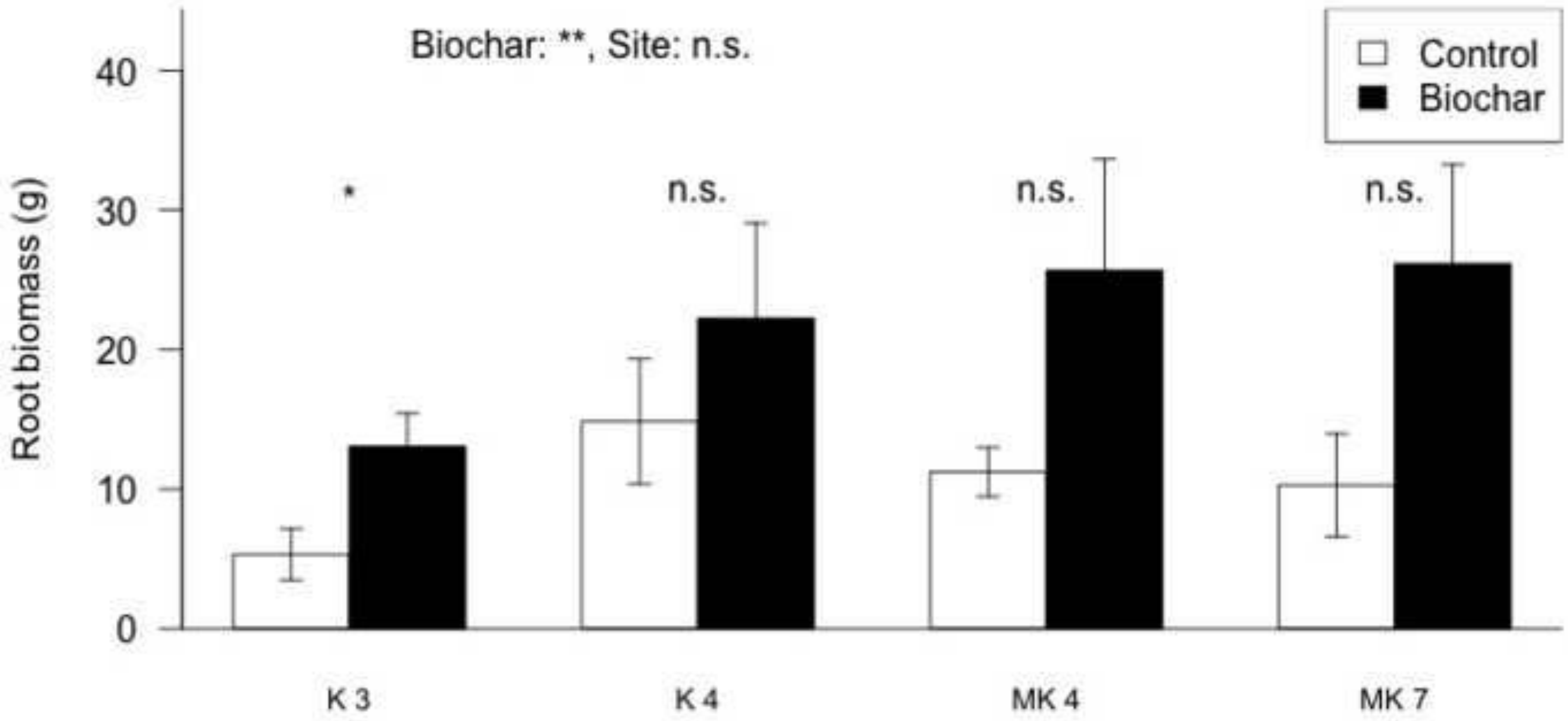


Figure 1


Biochar: **, Site: n.s.
Control
Biochar
Root biomass (g)
40
30
20
10
0
*
n.s.
n.s.
n.s.
K 3
K 4
MK 4
MK 7