BIOCHAR EFFECTS ON MAIZE PHYSIOLOGY AND WATER CAPACITY OF SANDY SUBSOIL

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Abstract:

Sandy soils facilitate maize growth in cold regions. However, Danish coarse sands have poor water and nutrient retention capacity which may constrain crop growth during dry spells. A greenhouse maize experiment was conducted in which straw biochar was applied to the subsoil at concentrations of 0, 1, 2, and 3%. All the plants were fully irrigated until flowering. Thereafter, half of the plants were subjected to drought until 76% of soil water content at field capacity was depleted in the control. Plant height and number of leaves were not significantly different at flowering although significantly lower for 3% biochar at stem elongation stage. Leaf water potential, stomatal conductance as well as photosynthesis and transpiration were maintained in biochar 2 and 3% during the drying cycle reflecting the increase in soil water holding capacity. In the drought treatments, plant biomass tended to be greater for biochar 2% but decreased for biochar 1 and 3%. Cob biomass was increased by biochar 3% but decreased by 1 and 2 %. Biochar however decreased plant biomass and cob biomass under irrigation.

KEYWORDS: DEFICITIRRIGATION, LEAF WATER POTENTIAL, STOMATAL CONDUCTANCE, COB BIOMASS, STRAW BIOCHAR, PHOTOSYNTHESIS

1. Introduction

The impacts of global climate change may result in drought in many areas with drought disaster affected areas projected to increase from 15.4% to 44% by 2100 (Li et al., 2009). Drought can result in significant grain yield reduction in maize especially during tasseling and or ear formation (Çakir, 2004). During drought, maize roots that detect drying of the soil communicate this change to the shoots (Blackman and Davies, 1985), as root derived abscisic acid eventually lower the stomatal conductance and thereby reduce transpiration and photosynthesis. This is particularly serious in sandy soils because of their low capacity for plant available water and their shallow rooting depth (Andersen and Aremu, 1991; Andersen et al., 1992).

Biochar is emerging as a solution to enhance crop growth, increase water and nutrient retention as well as increase soil carbon sequestration. It is a carbon-rich product formed when biomass such as wood, manure, or crop residues is heated in a closed container with little or no oxygen (Lehmann and Joseph, 2009). They are highly recalcitrant (Cheng et al., 2008) due to the condensed aromatic ring structure (McBeath et al., 2014) with an estimated mean residence time of between 90-1600 years (Singh et al., 2012). The key characteristic of biochar, depending on the pyrolysis treatment (Lee et al., 2010), is the cation exchange capacity due their large negatively charged surface area (Liang et al., 2006). In addition to the pyrolysis conditions biochar characteristics also depend on the type of feedstock. For instance, Jindo et al. (2014) have shown that biochar obtained from rice materials have unique chemical properties because of the incorporation of silica elements into its chemical structure. On the other hand, biochar produced from wood materials showed high carbon content and absorption character. This varied nature of biochar may be responsible for the differences and sometimes contrasting effects that have been reported in literature.

Danish coarse sandy soils restrict root growth and have poor water and nutrient retention (Bruun et al., 2014) with rooting depth limited to about 60 cm (Rasmussen, 1999). They pose a constraint on crop development because they have a high risk of drought in the dry seasons and of nitrate leaching from the root zone in the wet seasons (Bruun et al., 2014). In cold regions, sandy soil facilitates maize growth due to higher soil temperature than loamy soils (Odgaard et al., 2011). In Denmark cultivation of maize started increasing in the 1980´s (Olesen and Bindi, 2004). It is mostly for silage to feed cattle during winter, as this can be produced from maize vegetative parts and immature cobs (Andersen, 2000) as cited in Odgaard et al. (2011). Maize in Denmark is grown between May and October. There is some risk of some dry spells during summer which may be a major constraint for maize planted in sandy soils.

Several studies have shown that biochar application to soils increases soil water retention, root growth and yield (Abel et al., 2013; Abiven et al., 2015; Akhtar et al., 2014; Sun et al., 2014). A study by Bruun et al. (2014) showed that biochar increased water retention, and possibly improved root penetration and density in a Danish coarse sandy soil. Uzoma et al. (2011) observed significant increases in maize yield and nutrient uptake when they applied cow manure biochar to sandy soil. To the best of our knowledge, there is very limited literature on the effect of biochar amendment to coarse sandy soils under drought conditions and its implications for maize yield and crop physiological processes.

In the present study we therefore investigated the effect of biochar amendment to subsoil on crop physiological processes and yield of maize comparing irrigated and drought conditions.

2. Materials and Methods

2.1. Study location and Soil & Biochar

The study was conducted at Foulum Research station of Aarhus University, Denmark with soil collected from the Jyndevad research station. Soil materials were collected from 0 – 25 cm and 25 – 100 cm depth to represent topsoil and subsoil respectively. Information on the textural composition is presented in Table 1.

Table 1. Physical properties of soil used

Depth (cm)	Clay (%)	Silt (%)	Sand (%)	Organic matter (%)	Bulk density (g/cm^3)	Plant available water (%)
0-30	5.8	2.1	90.7	1.9	1.41	10.2
30-70	5.9	0.5	92.7	0.7	1.46	6

Source: (Ahmadi et al., 2009)

Wheat straw biochar from a commercial company, Frich A/S, Denmark was used for the experiment. It was produced by slow pyrolysis at a temperature of 500°C. Biochar properties are shown in Table 2.

2.2. Experimental set up

The pots used for this experiment had a dimension of diameter: 36 cm; length 70 cm. The soil columns were prepared under a shed in at AU-Foulum.To prevent root growth along the sides, the inside of the pots were coated with subsoil mixed with water insoluble wallpaper glue (Bostik Hernia Vaadrumslim) as done by Bruun et al. (2014) before filling the soil. The soil was filled in three layers. The layer at the bottom from 50 – 70 cm depth was filled with subsoil only.

Table 2. Some of the properties of the biochar used

Total C: 79.3 %, Total N: 0.7%, pH: 8.4, PAH: <0.50 mg/kg							
Nutrients							
NH_4 (mg/L)	NO_3-N (mg/L)	P_2O_5 (%)	K_2O (%)	MgO (%)	Na (mg/kg)	CaO (%)	S (%)
1.39	<5.0	1.1	5.3	0.44	996	1.81	0.12
Heavy metals (mg/kg)							
Cu	Fe	Mn	Zn	Pb	Cd	Cr	Ni
14.3	1300	86.1	81.4	<3.0	<0.20	19.9	32.0

It was then followed by subsoil with or without added biochar at the 20 – 50 cm depth. The topmost layer was filled with only topsoil at a depth of 8 – 20 cm. Prior to filling the pots, the soil and biochar were weighed to get the desired dry weight and proportion, and mixed for 5 minutes in a mechanical mixer. The soil was packed at a bulk density of 1.2 kg/m^3 and 1.3 kg/m^3 in the topsoil and subsoil sections, respectively. Packing was done by pressing the soil down with fingers followed by surplus irrigation and letting the soil settle to a "natural" bulk density, which was determined after harvest of the plants. TDR probes of 60 cm were placed in each pot for determining soil water content for irrigation scheduling. After filling all the pots, they were placed on an area with moist and lose soil to which peat had been added and irrigated in excess. The pots were then allowed to drain for three days after which field capacity was determined with time-domain reflectometry (TDR).

2.3. Treatments

Biochar was mixed with the subsoil in the following concentrations 0, 1, 2 and 3% on dry weight basis. With regards to irrigation, all the pots were initially fully irrigated and irrigation was carried out each time the soil water deficit exceeded 25% of the available soil water content. Drying cycle 1 was initiated at BBCH stage 5 (tasseling) at day 60 after planting (DAP). In the drying cycle, half of the pots were fully irrigated while the others were exposed to drought until an average soil water deficit of 76% was reached in the pots without biochar. Drying cycle 2 was initiated at BBCH stage 7 (fruit development) on day 78 after planting. This resulted in 8 treatments with 8 replicates shown in Table 3.

Table 3: Treatment combinations

Biochar Conc. (%)	Irrigation Level	Designation
0	Full	BC01
0	Drought	BC00
1	Full	BC11
1	Drought	BC10
2	Full	BC21
2	Drought	BC20
3	Full	BC31
3	Drought	BC30

2.4. Planting and growth conditions

Maize was sown on 7th May, 2015 under the shed. Three seeds were planted per pot at a depth of 5 cm and a distance of 2 cm between seeds. The pots were moved into the greenhouse on 11th May, 2015. The pots were arranged in four rows in the greenhouse in a completely randomized design. The plants were thinned after emergence to one plant per pot twelve days after planting. The growth condition of the greenhouse was a maximum temperature of 28 ºC during the day and 10 ºC at night. Plants were subjected to natural lightening with no artificial lamp. Recommended amount of Garta liquid NPK fertilizer with chelated micro minerals corresponding to 160 kg N/ha 26.6 kg P/ha and 127 kg K/ha was applied in two doses at leaf development stages 12 and 15. The plants were harvested 104 days after planting (19th August, 2015) at BBCH stage 85.

2.5. Plant physiological measurements

Growth stage of the plants was recorded weekly according to the BBCH scale for maize and plant height was measured with a ruler. Leaf area was scanned non-destructively using the Licor Li-3000 portable area meter (LI-COR Inc, NE, USA) for leaves numbers 4, 6, 8, 10 when they were fully developed.

Stomatal conductance (g_s) and photosynthetic rate (A_n) were measured weekly with a portable photosynthesis system (CIRAS-2, PP systems, MA, USA). Measurements were performed between 12.00 – 16.00 at 400 – 420 ppm CO_2 and with PAR set to 1500 μmol m^2 s^{-1} and the humidity close to the humidity of the air in the green-house. The measurements were conducted on the last fully developed leaf from leaf stage 3 to the stem elongation stage. From the flowering stage onwards, the measurements were carried out on the fourth leaf from the top.

Leaf water potential measurements were made during the drying cycle in connection with the gas-exchange measurements. A leaf was enclosed in a plastic bag and cut off using a razor. The leaf with the plastic bag was then inserted in the pressure chamber (Soil Moisture Corp. CA, USA), and the pressure increased slowly. The equilibrium pressure was read, as soon as water flow from the cut mid-vein of the leaf was observed through a binocular microscope.

After harvest, the fresh weight of the cobs and the other shoot parts was determined. The dry weight was determined after drying at 60 ^{o}C to a constant weight.

2.6. Sap flow

Transpiration was measured by the sap flow technique (Dynagage, Tx, USA) in treatments that were either: fully irrigated with and without biochar; drought stressed without biochar; and drought stressed with 2 % addition of biochar. Eight heat balance sensors were mounted so that they surrounded the maize stems and monitored and recorded 10 minute values of sap flow with a CR1000 data logger (Campbell Scientific, Logan, UT, USA).

2.7. Soil water measurement

Soil water content was measured three times per week with the Campbell TDR-100 system connected to a handheld computer.

2.8. Statistical analysis

The data analysis was done with SigmaPlot 11 (Systat Software, San Jose, CA). All data were tested for normality. In case of normal distribution and equal variance, one way Analysis of Variance (ANOVA) was used. Otherwise, the Kruskal-Walis test was used. P values of less or equal to 0.05 indicate statistical difference.

3. Results

3.1. Soil water content

Volumetric water content at field capacity is shown in Fig 1. There was significant increase (P=0.001) with the highest biochar concentration of 3%. There was also an increase with 2% but a decrease with 1% biochar, however the latter differences were not statistical significant. The trend of volumetric soil water content during the growing season under irrigation and drought is shown in Fig 2. The trend was the same for both irrigation schemes before day 60 when drying was initiated.

Under drought, the trend shows a steep fall when the water content was depleting and a rise when the plants were irrigated at the end of the drying cycles.

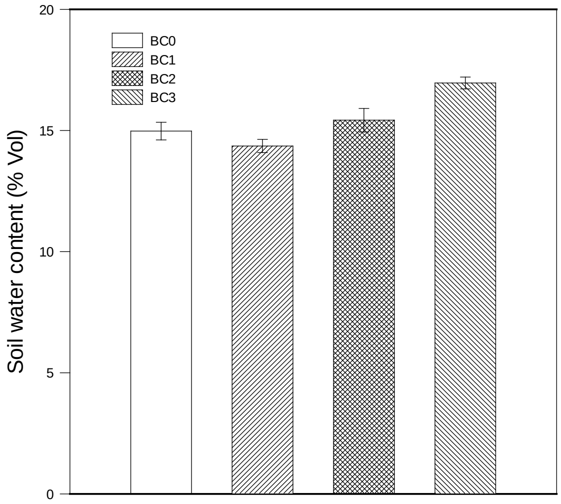

Fig. 1. *Soil water content with different biochar concentration at field capacity.*

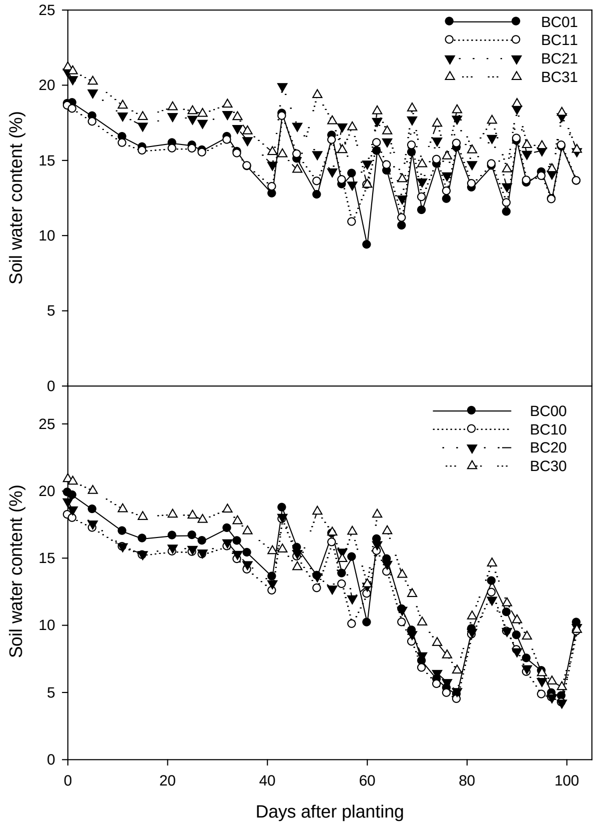

Fig. 2. *Volumetric water content during the growing season under full irrigation (upper) and drought (lower)*

3.2. Plant physiology measurements

The treatments showed similar trend for plant height and number of leaves shown in Fig. 3. The application of biochar had no significant effect on plant height until growth stage 3 (stem elongation) when the biochar 3% plant height was significantly lower than the remaining treatments (P=0.001). However, at growth stage 6 (flowering) there was no statistical difference between the treatments, (P = 0.094). Similar trend was observed for the number of leaves, showing a significant decrease for biochar 3% at stage 3 (P=0.013) and not significant at stage 6 (P=0.08).

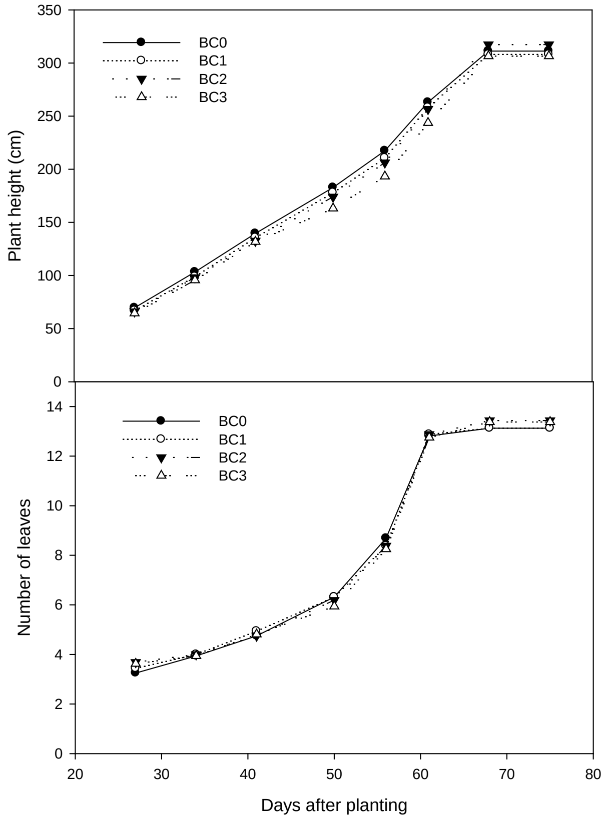

Fig. 3. *Weekly plant height (upper) and number of leaves (lower)*

Biochar tended to decrease the leaf area (Fig. 4) of leaves numbers 4, 6 and 8 with a less clear trend in leaf 10. The decrease was observed in the order of BC1, BC2 and BC3. The differences observed were however not statistically significant.

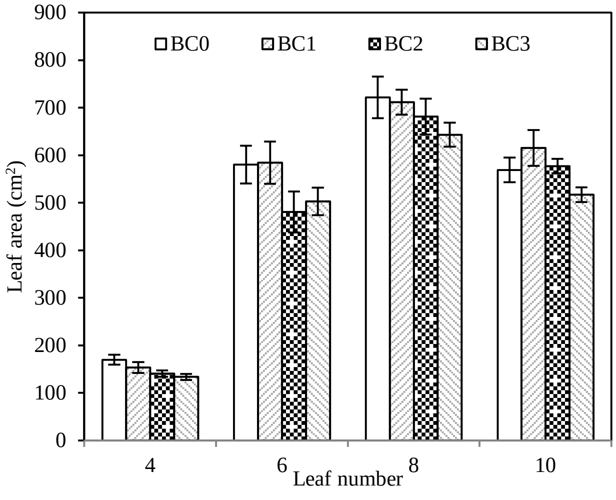

Fig. 4. *Leaf area for leaves numbers 4, 6, 8 and 10 when fully developed*

Net photosynthetic rate (A_n) (Fig. 5) and stomatal conductance (g_s) (graph not shown) decreased with an increasing rate of biochar prior to flowering (DAP 60). There was a strong correlation between A_n and g_s as shown in Fig 6. After flowering, biochar generally increased the A_n and g_s under both full irrigation and drought conditions. Under full irrigation, the trend continued until yield formation (DAP 92) when lower A_n and g_s were observed for biochar 2% than the control.

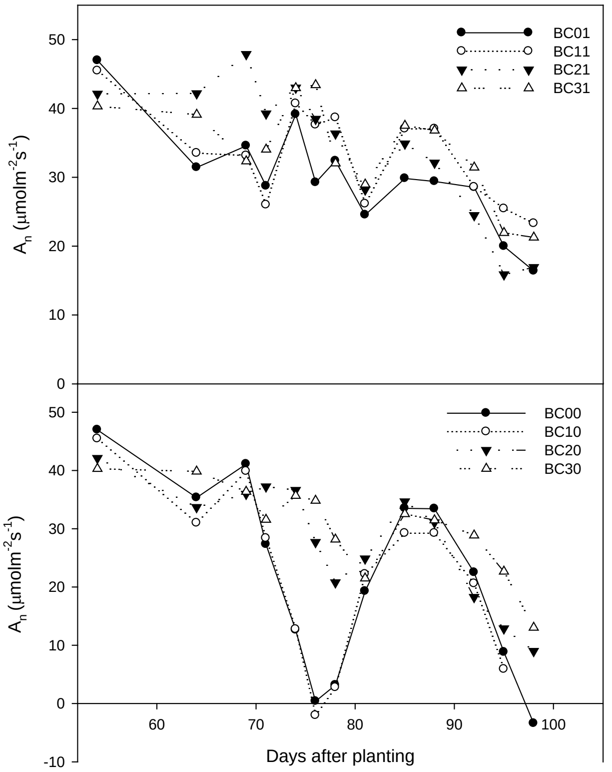

Fig.5. Photosynthetic rate under full irrigation (upper) and drought (lower)

At DAP 98, the lowest and similar values were observed for both control and 2% indicating ripening and approaching senescence. Under drought conditions, biochar generally increased the A_n and g_s after the initiation of dry cycle 1 (DAP 60). A steep downward decrease of A_n and g_s is observed at day 9 in drying cycle 1 (DAP 69) for the control and biochar 1%. Lowest A_n values of 0.04 and -2.03 µmol $m^{-2}s^{-1}$ were recorded for control and biochar 1% respectively indicating that leaves of the latter treatment had higher respiration than gross photosynthesis. The control and biochar 1% responded to irrigation at the end of dry cycle 1. Similar trends were observed for A_n and g_s in drying cycle 2.

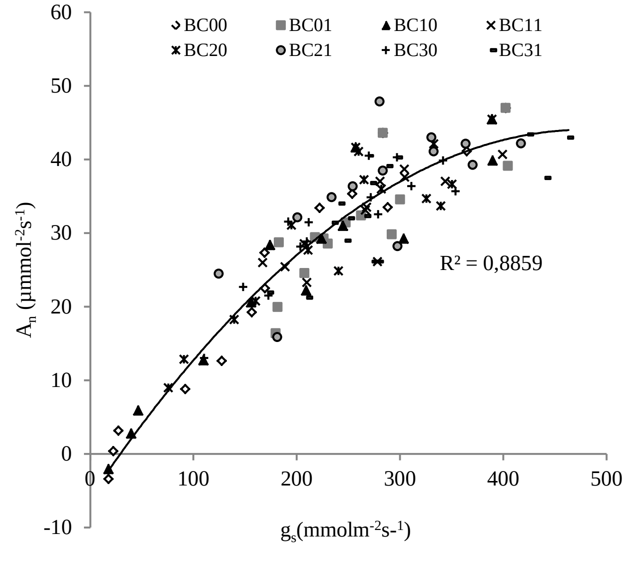

***Fig. 6.** Relationship between stomatal conductance (g_s) and photosynthetic rate (A_n)*

The trend for the leaf water potential during the drying cycles is presented in Fig. 7. Under drought, biochar increased the LWP as the drying cycle progressed. At the peak of the drying cycle the highest LWP (i.e. least negative) was recorded in the highest biochar level of 3% followed by 2%. Biochar level 1% on the contrary showed lower LWP than the control. Under full irrigation, biochar generally decreased the LWP except on DAP 69 and 81. This could be due the fact that the soil water deficit before irrigating on those days was much higher for the control than for the biochar treatment (Fig. 2 upper).

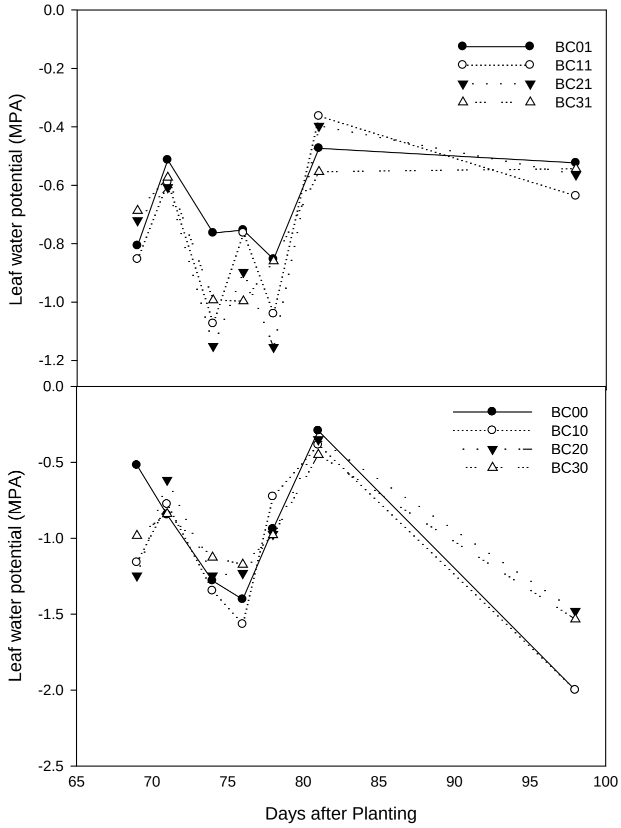

***Fig. 7.**Leaf water potential during drying cycle under full irrigation (upper) and drought (lower)*

3.3 Transpiration

The transpiration of each plant was normalized by expressing it as percent of the plants transpiration 94 DAP as shown in Figure 11. Drought treatments with biochar maintained transpiration during the drought periods, while the one without biochar did not.

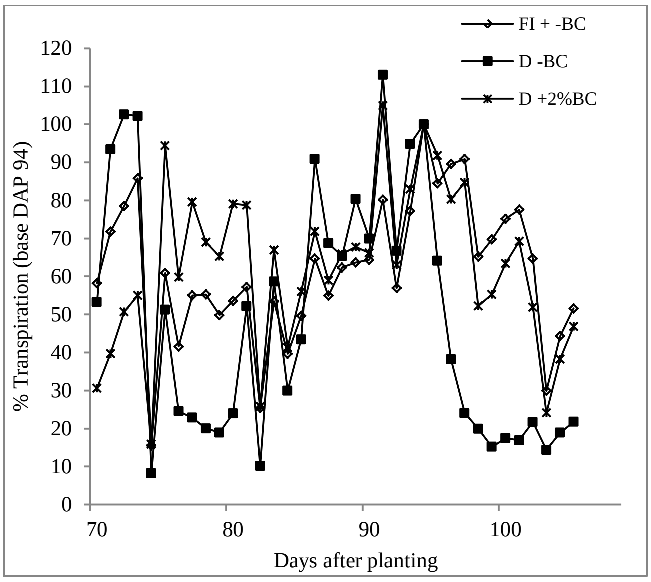

***Fig. 8.** Transpiration measured by sap flow technique*

3.4. Maize yield

The maize yields are presented in Fig. 9. The total above ground biomass was divided into the cob biomass and the remaining biomass is referred to as plant biomass. Biochar increased plant biomass in the 2% treatment. The application at the 1% and 3% levels however, resulted in a decrease. This trend was observed under both irrigation and drought conditions. The differences were not statistically significant with P values of P = 0.120 and 0.190, under irrigation and drought respectively. Cob biomass was decreased by the addition of biochar under full irrigation. The decrease was however not significant at biochar levels 2 and 3%. On the contrary, it was statistically significant at biochar 1% (P=0.048). Under drought the cob biomass was increased by biochar 3%. Cob biomass of 1 and 2% were lower than the control, however the differences were not statically significant (P=0.594).

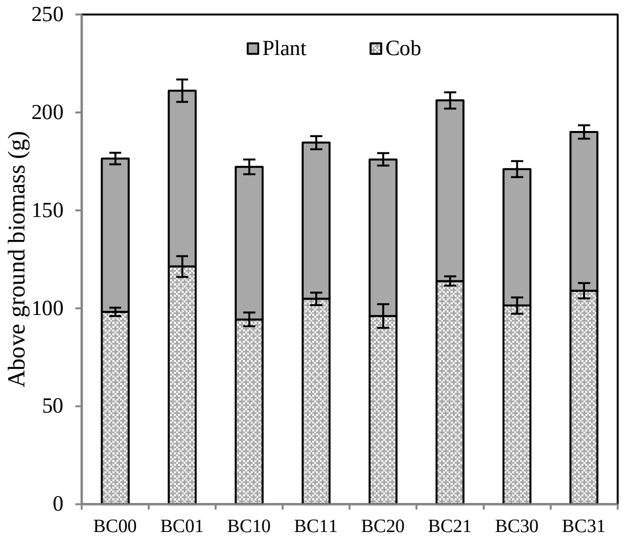

Fig. 9. *Aboveground biomass at harvest*

4. Discussion

4.1. Soil water content

The incorporation of straw biochar 2 and 3% increased the soil water content at field capacity. This result is consistent with findings from other studies on Danish coarse sandy soils (Bruun et al., 2014; Hansen et al., 2016). The increased water content can be attributed to the increased specific surface area (Glaser et al., 2002), the decrease in bulk density and increase in pore volume (Abel et al., 2013) of soil after biochar amendment. Our determination of bulk density after the experiment indicated a decrease of bulk density with increasing biochar concentration at the 30 cm and 50 cm. Biochar 1% however tended to decrease the soil water holding capacity.

4.2. Plant physiology

The number of leaves and plant height followed a similar trend. The similarity in the trend at the different growth stages was also reported by Uzoma et al. (2011). Contrary, to their results which showed a significant increase in maize height and number of leaves with the addition of biochar, our results indicate that there was no significant effect of biochar on height and leaves except at the growth stage 3 (stem elongation) when biochar 3% resulted in significantly lower height and number of leaves. Simultaneously at this stage, it was observed that some of the roots of this treatment lost their geotropism and grew upwards after entering the top soil (Fig. 10).We suspect ethylene gas might have been the cause of this effect. Ethylene is a plant hormone that regulates many aspects of plant growth and development (Sisler and Yang, 1984) and in high concentration it can negatively impact plant growth. Several studies have shown that ethylene in soil can accumulate under anaerobic conditions (e.g. Rigler and Zechmeister-Boltenstern, 1999). Neljubow (1901) as cited in (Fulton et al., 2013) was the first to demonstrate that ethylene affects tropism. Recent studies by (Fulton et al., 2013) have reported that fresh biochar can emit significant concentrations of ethylene. They recommend that biochar should be stored for three months in the open to allow ethylene stored in fresh biochar to degas before using. In our experiment the bags with biochar were only opened when it was used to mix with soil and the soil-biochar mix was planted one week after filling the pot. The loss of geotropism in the 3% biochar treatment and general decrease of leaf area with increasing biochar concentration could be due to ethylene emitted directly by the biochar we used which was further compounded by anaerobic conditions most likely created in the biochar 3% treatment. Biochar consists of a labile fraction and the recalcitrant fraction. Decomposition of the carbon takes up oxygen and in the case of biochar 3% the very high labile fraction means that the oxygen could be depleted, leaving the soil in an anaerobic condition. Since there was no significant difference between the leaf number and plant height at flowering, we assume the ethylene to have dissipated at that time.

Drought stress has been shown to decrease photosynthesis, stomatal conductance, leaf water potential and transpiration. The reduction of LWP and A_n that occurred in the late drying period is consistent with results by Bahrun et al. (2002).Our results show a decrease in A_n. g_s, LWP and T during the drying cyles for the control and biochar 1%. To the best of our knowledge there is no study of the effect of biochar on physiological processes on sandy soil with maize so it was not possible to compare these results. However, when compared for grapes on sandy-clay-loam (Baronti et al., 2014) the improved soil water status was associated with increased photosynthesis, stomatal conductance and leaf water potential in biochar treatments, results which are consistent with our findings. Similar results were also reported by (Hansen et al., 2016) who showed a decrease in stomatal conductance of barley on coarse sandy soil under drought conditions.

Fig. 10. *Roots of 3% biochar treatment growing out of topsoil*

Our results showed that drought reduced the yields of maize in all treatments irrespective of the biochar concentration compared to their fully irrigated counterparts. Under full irrigation biochar reduced both cob and plant biomass. Reported effects of biochar on yield have been rather mixed. Whiles some studies e.g. Uzoma et al. (2011) have reported an increase in yield of up to 150% when they used cow manure biochar, other studies such as Major et al. (2010) observed no significant increase in maize yields in the first year of application of wood biochar but significant increases over the next three years. In temperate soils, the use of pure biochar has often shown moderately-negative to -positive yield effects (Kammann et al., 2015). Even though we observed that biochar maintained photosynthesis in our drought treatments, there was no significant increase of yield. This could be due to the concomitant reduction of leaf area of the plants, which was generally reduced by biochar.

Thus the photosynthesis per plant integrated over time and leaf area may not have been enhanced by biochar, which may be the reason for no yield increase even under drought.

5. Conclusion

Biochar increased soil water content in Danish coarse sandy soil. Under drought, photosynthesis, leaf water potential and transpiration were maintained by 2 and 3% biochar addition to the subsoil. Thus, the use of biochar has the potential to enhance agricultural productivity in drought prone areas. However, some negative effects and the lack of corresponding increase in yield requires further studies of long term effects after the suspected short term toxicity of the biochar has subsided.

6. Literature

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