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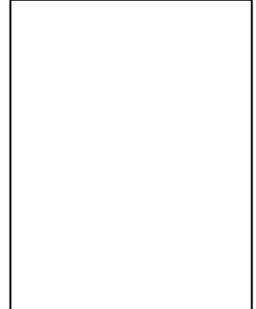
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Impact of a Biochar or a Compost-Biochar Mixture on Water relation, Nutrient uptake and Photosynthesis of *Phragmites karka*

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ABSTRACT

Soil water and nutrient status both are of major importance for the plant appearance and its growth performance. The objective of this research was therefore to study the effect of biochar (1.5% BC) and a biochar-compost mixture (1.5% BC + 1.5% Co) on the performance of *Phragmites karka* plants grown in a nutrient poor sandy clay (control, 50% sand 30% clay and 20% gravel) soil. The indicators of plant performance such as growth, lignocellulosic biomass, water relations, mineral nutrition status, leaf gas exchange, chlorophyll fluorescence parameters of *P. karka*, as well as soil respiration activity, were assessed. The sole amendment of BC led to higher growth rate and lignocellulosic biomass production in *P. karka* plants compared to the non-treated control. There was also significant increase in soil respiration with biochar treatments that stimulated microbial interaction. The increase in the water holding capacity after BC amendment caused a significant improvement in plant water status (water potential, osmotic potential, leaf turgidity) and plant ion content (K^+ , Mg^{++} and Ca^{++}) leading to an increase of net photosynthesis but also a higher energy use efficiency of the Photosystem II. Additionally, the BC plants managed to avoid oxidative stress, improved water use efficiency (WUE) and decreased dark respiration. However, the amendment of a biochar-compost mixture (BC + Co) led to even better improvement of physiological parameters such as growth, leaf turgor, photosynthesis and nutrient content and soil gas exchange of *P. karka*. Our results suggested that BC and Co promote plant growth with respect to nutrient uptake, water balance, and efficiency of the photosynthetic system. In summary both soil amendments might open an opportunity for *P. karka* to sequester CO_2 and to produce higher fodder, bio-active compounds and biomass for bio-energy on nutrient poor degraded soils.

Key Words: Biochar, chlorophyll fluorescence, eco-physiology, growth, photochemical efficiency, *Phragmites karka*.

INTRODUCTION

Availability of fresh water is one of the important factors that affects biological systems worldwide (Koyro *et al.*, 2011; Gallardo *et al.*, 2015). This is especially of importance in arid and

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semi-arid regions, where low rainfall imposes constraints on plant growth and development (Schlesinger *et al.*, 2015). Crop productivity in arid regions declined substantially due to the loss of top soil caused by severe grazing (McDowell *et al.*, 2015; Smith *et al.*, 2016). These conditions lead to typically low soil organic matter, carbon content, water holding capacity (WHC), higher evaporation and drainage rate causing shortage of water availability to roots (Mills *et al.*, 2014). Plants adapted to such an environment are characterized by morphological and physiological features, such as stimulated root growth, change in leaf development and dry matter partitioning to sustain tissue metabolic activity for its survival and reproduction (Albacete *et al.*, 2014; Knutzen *et al.*, 2015). These physiological characteristics require high energy leading to reduced plant productivity (Sumesh, *et al.*, 2008). Low or little productivity of crops due to water scarcity and reduced nutrient acquisition from the soil affects food security and results economic losses leading to further socio-economic problems (Koyro *et al.*, 2011; Golldack *et al.*, 2014). These problems must be addressed through sustainable agriculture practices (Dile *et al.*, 2013; Pittelkow *et al.*, 2015).

Soil amendments of biochar and compost along with soil mineralization are a promising approach to address desertification (Fischer and Glaser, 2012). The supplement of biochar to soil has recently been proposed as a way to improve soil water holding capacity (Karhu *et al.*, 2011; Busch *et al.*, 2012), water infiltration (Asai *et al.*, 2009; Ippolito *et al.*, 2012) nutrient and water retention capacity (Clough *et al.*, 2013; Ventura *et al.*, 2013), and soil aeration and respiration (Case *et al.*, 2012; Haider *et al.*, 2015). These beneficial effects jointly lead to a substantial increase of physiological and biochemical performance of plants, promote plant productivity, and food security. However, an improper use of biochar may also have negative impact on plant performance (Jeffery *et al.*, 2011). Freshly prepared biochar or its high amendments can reduce plant growth. This could be due to nutrient immobilization caused by the adsorption of mineral nitrogen and dissolved organic carbon (DOC) (Ding *et al.*, 2010; Graber and Elad, 2013). These negative effects of biochar can be minimized or even prevented by an appropriate addition but also by mixing it with organic or mineral nutrients, blending with compost, or co-composting (Bruun *et al.*, 2011; Albuquerque *et al.*, 2012; Fischer and Glaser, 2012; Joseph *et al.*, 2013;). Application of compost-biochar mixture to the soil increases the adsorptive surface for nutrients, stimulates microbial colonization (Pietikäinen *et al.*, 2003), degrades possible noxious pyrogenic substances (Tuomela *et al.*, 2000), and improves the biochar surface reactivity through accelerated oxidative ageing (Cheng and Lehmann, 2009; Zimmerman, 2010). Soil substrate besides other factors determines the quality and composition of biochar or compost. Plant biomass production on low quality soils primarily reflects suitability of individual physiological strategies to mitigate deleterious effects of soils when amended with different substances (Haider *et al.*, 2015).

Plants can adjust their physiological and biochemical responses to establish a new homeostasis in presence of biochar and other soil amendments (Lehmann *et al.*, 2011). Maximizing growth rate in a changed environment leads to a new pattern of species in the modified ecosystem (Jeffery *et al.*, 2015). To observe responses of these organisms in arid habitats, at least four eco-physiological aspects need to be studied: (a) growth rate and plant morphology, (b) water relation, (c) regulation of CO₂/H₂O-exchange and photochemical efficiency of PSII and (d) nutrient balance. Plant-available nutrients and water accessibility are of major importance in arid areas and usually limit plant growth in sandy soils. In addition, the supplement of biochar could provide a higher availability of water, prevent desiccation with improved turgidity, enhance nutrient uptake, provide better conditions for synthesis of organic solutes, and reduce oxidative stress through high water use efficiency (H₂O loss per net CO₂ uptake) (Panaque *et al.*, 2016). Altogether such improvement of soil quality could contribute to a significant improvement of the physiological status of plants and thus their biomass production (Lehmann *et al.*, 2011). Low stomatal conductance results in an inhibited assimilation rate. This goes along with a reduced demand for energy (ATP) and reduction equivalents (NADPH). The consequence is an over-reduction of the photosynthetic electron transport chain leading to

increased formation of reactive oxidative species (ROS) (Foyer and Noctor, 2009). Therefore, photosynthetic organisms need strategies to maintain a balance between efficient light harvesting, photochemistry and photo protection. Such a strategy is needed for survival but also is of economic importance because it has an impact on the productivity and suitability for industrial purpose (Wijffels *et al.*, 2010).

Increasing competition between food and biofuel production will be intensified due to the predicted climate changes (Damerou *et al.*, 2016). The decreasing fresh water supplies along with problems caused by an ever growing desertification have risen new interest in plant species which are non-edible but capable of producing economically profitable biomass (Koyro *et al.*, 2014). This means that suitability of such plants depends on the productivity of a high quality biomass. Therefore, high productive plants not used in agriculture to date need to be domesticated. In order to achieve this goal, extensive studies have to be initiated to ensure sustainable growth conditions and optimal biomass production. Therefore, plans should be devised to use local diversity to identify such high yielding plants, based on basic eco-physiological research, and to subsequently select candidates as non-conventional crops. Latter research can uncover adaptation mechanisms as a basis to improve productivity for wide industrial application and stabilize social harmony. Besides economic feasibility the environmental protection can be seen as a fundamental part of sustainability. A central aspect of this type of research is the selection of suited species (Abideen *et al.*, 2011). One potential candidate is *Phragmites karka*, a high biomass producing perennial grass and distributed as pure population on saline and dry arid areas around Karachi (Zehra and Khan, 2007). This species has the potential to become a potential biofuel crop because of its suitable ligno-cellulosic content in its biomass (Abideen *et al.*, 2011). The aim of this study was to demonstrate the potential improvement of local soil quality by addition of biochar or a biochar-compost mixture. Therefore, we have monitored the physiological response of *Phragmites karka* on such soil mixtures, and have evaluated a potential improvement of productivity of the prospective bio-energy crop.

MATERIALS AND METHODS

Biochar and compost production and their characterization

For biochar production wood chips (80% hardwood plus 20% coniferous wood) were subjected to pyrolysis for a period of 36 hours at 750°C in a Schottdorf type reactor (Carbon Terra GmbH., Augsburg, Germany). Compost was produced following a standard protocol for professional aerobic quality composting (Amlinger *et al.*, 2008; Bernal *et al.*, 2009). Chemical and physical properties of biochar and compost are given in Table 1.

Table 01

Initial selected chemical and nutritional properties of the biochar and compost used for the

Parameters	Biochar	Compost
Salt content (g kg ⁻¹ DM)	3.28	13.4
pH (1:10 CaCl ₂)	9.50	7.20
Organic carbon (mass % DW)	75.3	27.8
Electric conductivity (μS cm ⁻¹)	578	-
Nitrogen (%)	0.43	1.28
Density (kg m ⁻³)	269	770
Magnesium (mg kg ⁻¹)	2580	7400
Calcium (mg kg ⁻¹)	20500	69100
Potassium (mg kg ⁻¹)	9030	11000
Phosphorus (mg kg ⁻¹)	810	8500

experiment.

Soil properties

Sand was mixed with clay and gravel at a ratio of 30:50:20 (% w/w/w) to simulate the natural and nutrient poor soil conditions of *Phragmites karka* at its natural habitat near Karachi (Sindh province in Pakistan). Substrates were provided by the Quarzsandwerk Mittelhessen GmbH Gießen and McMineral, Heuchelheim (both Germany). The materials were previously not agriculturally used, and had not received fertilizer. Basic physical soil parameters such as water content, electric conductivity, pH, and water holding capacity (WHC) of this mixture are presented in Table 2. The water holding capacity was individually determined for all pots used for plant growth (Veihmeyer and Hendrickson, 1931). Eight pots of each treatment were put in a large vessel and slowly flooded with water until the water level was above the soil surface, but 1 cm below the pot brim. The pots were covered with foil and wetted for 24 hours. The pots were covered with aluminum foil to prevent evaporation. Pots were weighed after 24 and 48 hours of drainage and then finally oven dried on 80 °C.

Table 02

Water holding capacity (WHC), pH, volumetric water content (WC) and electric conductivity (EC) of the three soil mixtures in use: control, biochar (1.5 %) and biochar-compost mixture (1.5% +1.5

Parameters	Control	BC	BC + Co
Water holding capacity (%)	22.70 ± 0.1a	26.18 ± 0.3b	29.02 ± 0.7c
pH	7.58 ± 0.1a	7.71 ± 0.1a	7.75 ± 0.1a
Water content (Vol/Vol)	6.96 ± 0.2a	9.83 ± 0.1b	11.56 ± 0.7c
Electric Conductivity ($\mu\text{S cm}^{-1}$)	103 ± 6.2a	114 ± 5.3a	120.3 ± 4.9b

%). Bonferroni letters on means ± SE, represent significant differences ($P < 0.05$) among treatments.

Experimental setup

Seeds of *Phragmites karka* (Retz.) Trin. ex Steud were collected from Karachi, Pakistan (24° 55'3" N and 67° 6' 19" E). Plants were grown in green-house condition (Temperature: 25 ± 2 °C; Relative humidity: 50%; Photoperiod 16-8 h day-night with 200-250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity) at Justus-Liebig-University, Germany. Seeds were germinated in plastic trays (10 cm x 25 cm) filled with wet clay along with Wuxal Super (Aglukon Spezialdünger GmbH & Co. KG., Düsseldorf, Germany) as a nutrient source for seedling establishment. After 35 days seedlings were transplanted into plastic tubes (35 cm length, 11 cm diameter; two plants per tube) filled with sandy clay soil. Six tubes each were used for three different treatments: (1) 0% BC (Biochar) (control), (2) 1.5% BC, and (3) mixture of 1.5% of BC (biochar) + 1.5% Co (Compost). The plants were irrigated twice a day (after 12 h interval) with ½ strength Hoagland solution-modified after Epstein (1972) in a quick check system (Koyro, 2003).

Plant analysis

Non-destructive growth parameters (plant height, number of leaves and tillers) were recorded prior to the final harvest after 72 days after sowing in treated soils. Six pots (n=6) were harvested from each treatment and separated into leaf, stem and root tissue. The fresh weight (FW) of plant tissues was recorded immediately after harvest and incubated at 60 °C to determine dry weights (DW). Leaf area was recorded using a LICOR-3000C Portable Area Meter to subsequently calculate a leaf mass to area ratio (leaf area/plant mass, LAR). After the plant harvest root length (Himmelbauer *et al.*, 2004, Song *et al.*, 2017) was measured with a ruler and a root length per plant mass ratio (RLR) was calculated. The above ground plant material was air dried ground and stored in airtight plastic bags for lignocellulosic biomass analysis that linked with plant fiber estimation (AOAC, 2005). The method involves estimation of Neutral Detergent Fiber (NDF) which accounts for the cellulose, hemicellulose and lignin contents. Acid Detergent Fiber (ADF) was determined by subsequently using the residue left from NDF determination. The hemicellulose content was calculated by subtracting ADF from NDF (Jung and Vogel, 1992). The NDF and ADF treated plant material was then hydrolyzed with 72% H₂SO₄ to determine the cellulose content. Finally, the lignin content was calculated by converting the remaining residue from hydrolysis to ash.

The leaf water potential (WP) was measured at pre-dawn (when plants had been able to equilibrate their water potential) by pressure chamber as described in Scholander *et al.* (1965).

Osmotic potential (OP) was determined in leaf sap, extracted from frozen samples by using an ice cooled mortar. Prior to the measurement debris were removed by centrifugation for 5 min. at 4 °C and 3000×g. The extracted sap was heated at 50 °C for 2-3 min to denature enzymes and OP was determined in a freeze point depression cryo-osmometer (Osmomat 030, Gonotec). Osmotic potential (OP) was calculated using van't-Hoff equation as described by Guerrier (1996): $OP = -n \cdot R \cdot T$; (where n is the number of moles of solute, $R = 0.008314 \text{ J mol}^{-1} \text{ K}^{-1}$ (gas constant), and $T = 298.8 \text{ K}$ (absolute temperature). Turgor potential (TP) was calculated by subtracting OP from WP (Boyer and Potter, 1973). Relative water content (RWC) and moisture content in above and below ground parts were also determined in all treatments. Oven dried plant samples (20 mg of leaf, stem and root separately) were digested in 10 ml of 0.5% HNO_3 than placed in water bath (80 °C) for 12 h to extract inorganic ions. Cations (K^+ , Mg^{++} and Ca^{++}) were determined in the appropriate dilution of extracted material by atomic absorption spectrophotometer (AAS PE2100, Perkin Elmer). Data were expressed as total ion content (mmol) per respective plant organ.

Leaf gas exchange was measured using a portable infrared $\text{CO}_2/\text{H}_2\text{O}$ gas exchange cuvette system LI-COR 6400 (LI-COR, Lincoln, NE, USA) at $400 \mu\text{mol m}^{-2} \text{ s}^{-1} \text{ CO}_2$ and $300 \mu\text{mol m}^{-2} \text{ s}^{-1}$ flow rate. Net photosynthetic rate (P_N), respiration rate (R), stomatal conductance (Gs), intercellular carbon dioxide concentration (C_i), transpiration (E), C_i/C_a and water use efficiency ($\text{WUE} = P_N/G_s \cdot 1000$ in $\mu\text{mol}/\text{mmol}$) were measured on the youngest fully emerged leaf blades. Light intensity varied from 800 to $1250 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ depending on the saturating irradiation for each treatment. Saturation irradiance was calculated applying a regression analysis of a light response curve ($R^2 > 0.99$) at the range of 0 to $1500 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ photosynthetic photon flux density (PPFD). Relative chlorophyll content was analyzed by using SPAD 502 (Konica Minolta, Japan).

Chlorophyll fluorescence was determined using a pulse modulated chlorophyll fluorescence meter (Junior PAM, Walz, Germany) on the same leaves selected for gas exchange measurements. Minimal (F_o) and maximal fluorescence (F_m) values were measured subsequent to prior leaf exposure for 25 min. in the dark. These parameters were used to calculate the maximum photochemical quantum yield of PSII ($F_v/F_m = F_m - F_o / F_m$) by the method of Kitajima and Butler (1975). Whereas, steady-state (F_s), maximal (F_m') and minimal fluorescence (F_o') were measured on light-adapted leaves. Effective photochemical quantum yield of PSII ($Y(II)$) was calculated as $F_m' - F_s / F_m'$ (Genty *et al.*, 1989). Non-photochemical fluorescence quenching parameters (NPQ) were calculated as $\text{NPQ} = F_m' / F_m - 1$ while: $Y(NO) = F / F_m$ and $Y(\text{NPQ}) = F / F_m' - F / F_m$. (Bilger and Bjorkman, 1990). Apparent electron transport rate (ETR) was calculated based on the $Y(II)$ value as described by Krall and Edwards (1992): $\text{ETR} = \text{PSII} \times \text{PPFD} \times 0.5 \times 0.84$ (where PPFD: Photosynthetic photon flux density on the leaf; 0.5: factor that assumes equal distribution of energy between the two photosystems (PSII and PSI). A factor of 0.84 was assumed as leaf absorbance.

Soil respiration

Soil respiration was measured using a LI-8100 soil efflux chamber system (LICOR, Nebraska, USA) in combination with a dark survey chamber (10-cm diameter) within 30 min after removing the plant top from pots. The survey chamber fitted exactly to the brim of the pots that were used in the experiment for plant growth (Kammann *et al.*, 2011). The offset (height between soil surface and pot brim) of each pot was entered into the LI-8100 software for calculation of the correct system volume and thus of the soil CO_2 efflux. Measurement time and observation delay were set to 60 and 20 s, respectively, to provide a sufficient period of time for chamber volume mixing and monitoring CO_2 release. The increase in CO_2 -concentration always showed a linear slope with $R^2 > 0.99$. This justified to automatically calculate the CO_2 flux by the LI-8100 software that used the ideal gas law and linear regression. The respiration rate is given as CO_2 flux in $\mu\text{mol m}^{-2} \text{ s}^{-1}$.

Statistical analysis

All data were measured in six replicates ($n = 6$). Statistical analysis was carried out using SPSS Ver. 11.0 for Windows (SPSS Inc., Chicago, IL, USA) (SPSS 2012). Analysis of variance (ANOVA) was performed at $P < 0.05$ to identify significant difference among treatments while the Bonferroni test was used to compare individual means if the effects were significant. Data in the form of means and standard errors were used to construct graphs by SigmaPlot for Windows ver. 10.0 (Systat Software, San Jose, CA, USA).

RESULTS

Soil and growth parameters

Water holding capacity (WHC), water content (WC) and electric conductivity (Ec) of soil were higher after the amendment (BC or BC + Co mixture) than in untreated soil (0% biochar, Table 2). The application of BC and BC + Co increased shoot and root biomass but highest increase of growth was found in biochar-compost mixture (Fig. 1 and 2). Plant treated with BC or BC + Co showed about 50% more tillers than those grown under control condition (Fig. 2). Both treatments (BC and BC + Co) resulted in a substantially increase of leaf area but decreased the ratios of leaf area ratio and root length to plant biomass in comparison to untreated control (Fig. 3).



Fig. 01. *Phragmites karka* grown under control conditions, in soil amended with 1.5 % biochar and with (1.5% + 1.5%) biochar-compost mixture.

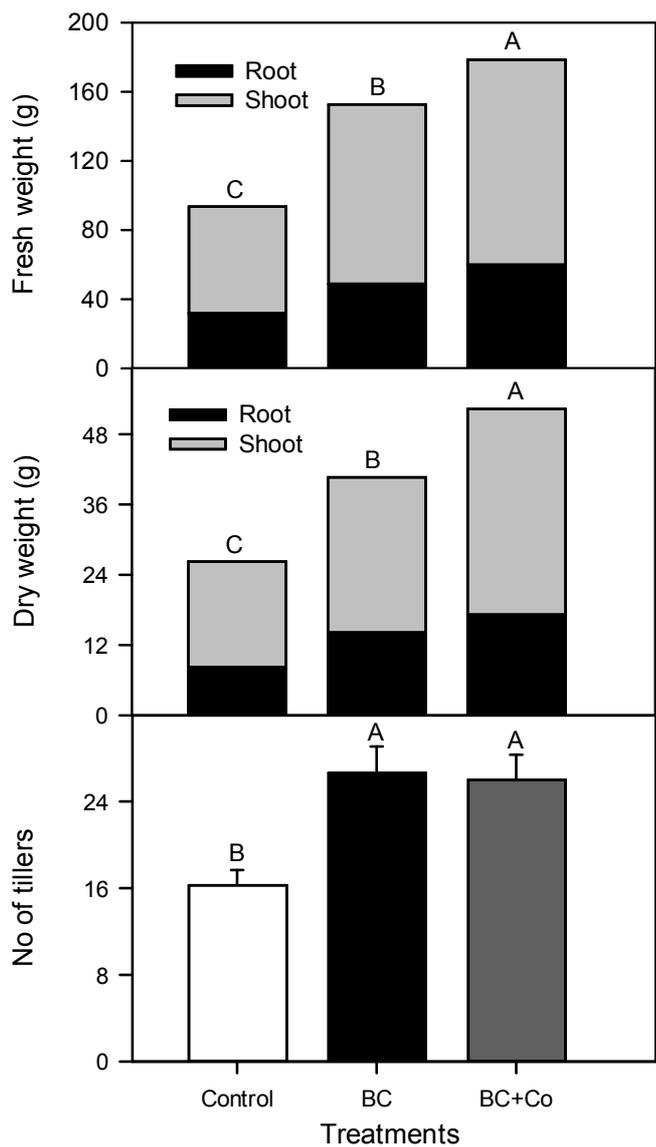


Fig. 02. Plant fresh weight, dry weight and no of tillers of *Phragmites karka* grown under control conditions, in soil amended with 1.5% biochar and with (1.5% + 1.5%) biochar-compost mixture, respectively. Bonferroni letters on means \pm SE, represent significant differences ($P < 0.05$) among treatments.

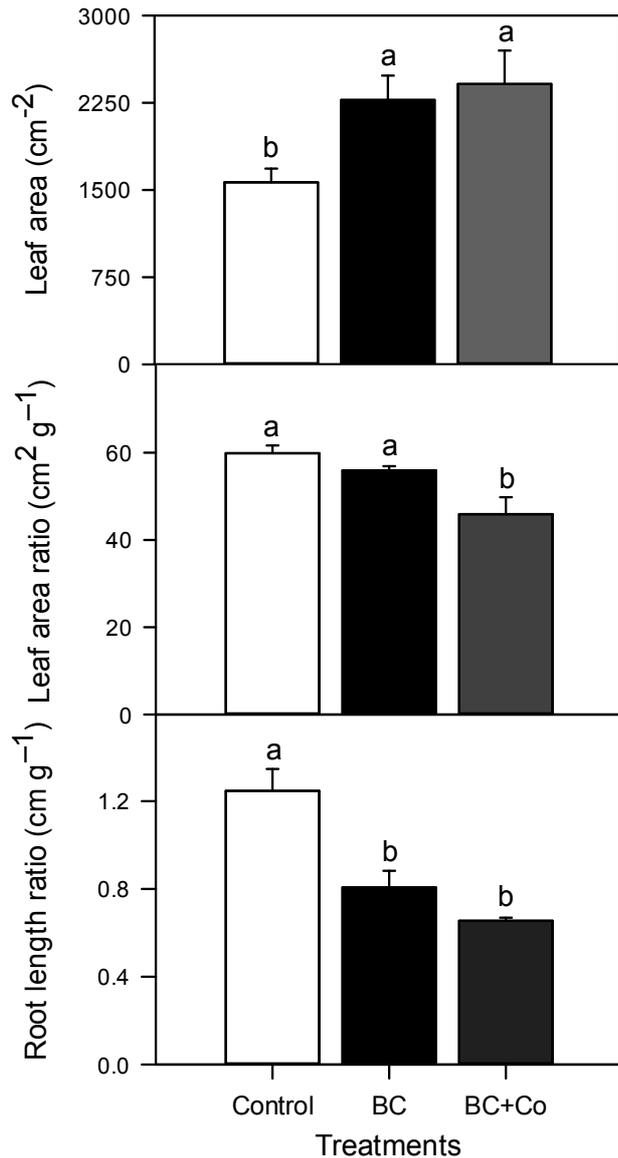


Fig. 03. Leaf area, leaf area ratio and root length ratio of *Phragmites karka* grown under control conditions, in soil amended with 1.5 % biochar and with (1.5% + 1.5%) biochar-compost mixture, respectively. Bonferroni letters on means \pm SE, represent significant differences ($P < 0.05$) among treatments.

Water relations and nutrient status

Leaf water potential (WP) and osmotic potential (OP) were significantly decreased in both treatments (BC and BC + Co) as compared to control condition (Table 3). Plants grown in biochar and biochar-compost treatment, showed about 1.5 times higher turgor potential than in control conditions (Table 3) while relative water content of plants was decreased in biochar and compost treatments. Moisture content was higher in both treatments (BC and BC + Co) as compared to control condition. BC treated plant showed a higher K^+ only in shoot tissue. However, the addition of BC + Co in medium enhanced the K^+ content in plant irrespective of tissue type (Fig. 4). The leaf Ca^{++} content was higher in plants exposed to BC or BC + Co while no difference was found in stem and root. The use of BC and BC + Co improved Mg^{++} content in leaf and root tissues but this effect was prominent only in the presence of BC + Co (Fig. 5).

Table 03

Leaf water potential (WP), osmotic potential (OP) and turgor potential (TP) of *Phragmites karka* plants treated without amendment (control), biochar (1.5 %) and biochar-compost mixture (1.5% + 1.5%). Bonferroni letters on means \pm SE, represent significant differences ($P < 0.05$) among treatments.

Parameters	Control	Biochar	Biochar-compost
WP(MPa)	$-0.23 \pm 0.09b$	$-0.38 \pm 0.15a$	$-0.36 \pm 0.20a$
OP(MPa)	$-1.25 \pm 0.01b$	$-1.32 \pm 0.01ab$	$-1.53 \pm 0.01a$
TP(MPa)	$0.98 \pm 0.10b$	$2.85 \pm 0.15a$	$2.50 \pm 0.20a$
RWC	$94.70 \pm 0.20b$	$93.70 \pm 0.37a$	$84.27 \pm 2.07a$

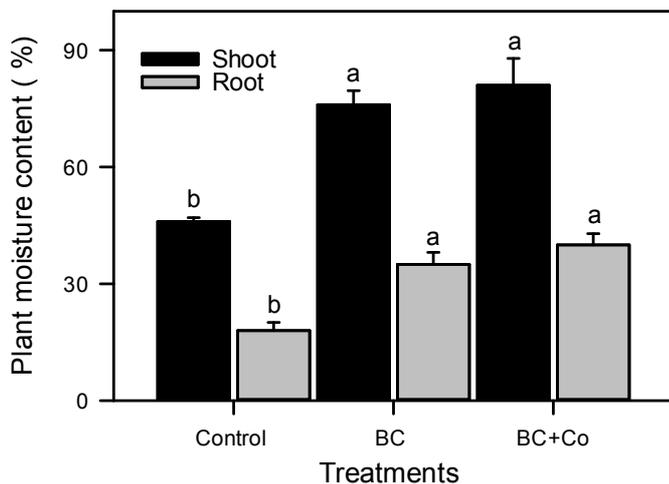


Fig. 04. Moisture content of *Phragmites karka* grown under control conditions, in soil amended with 1.5 % biochar and with (1.5% + 1.5%) biochar-compost mixture, respectively. Bonferroni letters on means \pm SE, represent significant differences ($P < 0.05$) among treatments.

Leaf gas exchange, SPAD content and chlorophyll fluorescence

Net photosynthesis rate was higher than control in both BC treatments particularly in BC + Co (Table 4). Stomatal conductance, internal CO₂ concentration and respiration rate were decreased substantially only in BC treatment. The application of BC and BC + CO increased WUE of plants but had no effect on transpiration rate and chlorophyll content (Table 4). Plants improved carboxylation efficiency in biochar treatment but this increase was more pronounced in plants treated with compost-biochar mixture. Potential quantum yield of photosystem (F_v/F_m) was unchanged during experiments but Y(II), ETR were increased by both BC and BC + Co treatments (Table 5). Non-photochemical quenching (NPQ) and Y(NPQ) were substantially lower in plants exposed to BC and BC + Co treatments as compared to control treatment while no difference was found in Y (NO) during experiments (Table 5).

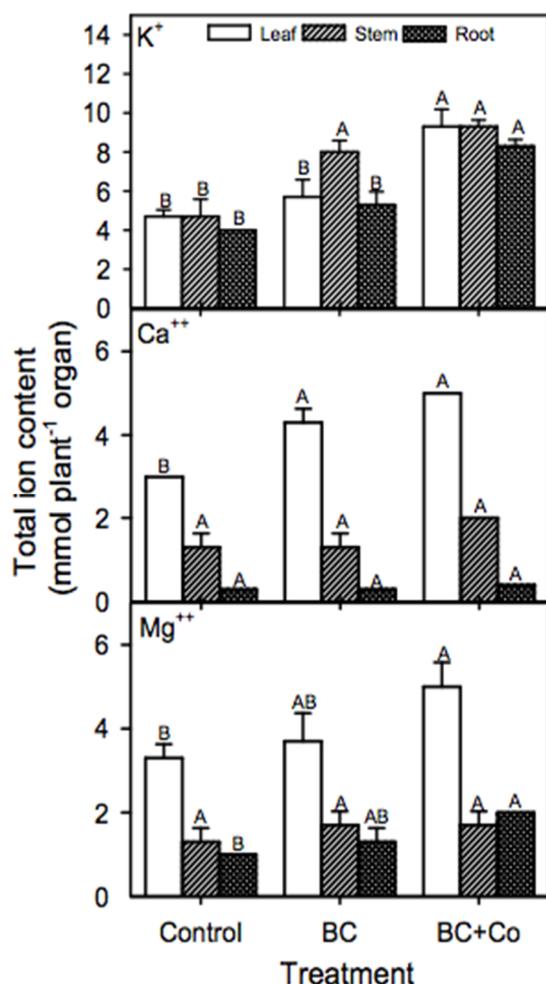


Fig. 05. Mean plant K^+ , Ca^{++} and Mg^{++} content of *Phragmites karka* grown under control conditions, in soil amended with 1.5 % biochar and with (1.5 % + 1.5 %) biochar-compost mixture, respectively. Bonferroni letters on means \pm SE, represent significant differences ($P < 0.05$) among treatments.

Table 04

Gas-exchange parameters (P_n , net photosynthesis; G_s , stomatal conductance; C_i , intercellular concentration of CO_2 ; E , transpiration rate; R , respiration, WUE_i , intrinsic water use efficiency and SPAD values (represent chlorophyll content) of *Phragmites karka* leaf. Plants were grown in untreated soil (control), 1.5 % biochar and (1.5% + 1.5%) biochar-compost mixture. Bonferroni letters on means \pm SE, represent significant differences ($P < 0.05$) among treatments.

Parameters	Control	Biochar	Biochar-compost
P_n ($\mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$)	$18.04 \pm 1.43b$	$20.51 \pm 0.74ab$	$23.39 \pm 0.83a$
G_s ($\text{mol } H_2O \text{ m}^{-2} \text{ s}^{-1}$)	$0.28 \pm 0.01a$	$0.26 \pm 0.05b$	$0.27 \pm 0.01a$
C_i ($\mu\text{mol m}^{-2} \text{ s}^{-1}$)	$273.01 \pm 7.11a$	$242.34 \pm 5.95c$	$237.21 \pm 1.45b$

Ci/Ca	0.70 ± 0.01a	0.62 ± 0.01b	0.56 ± 0.05c
R ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	-4.17 ± 0.56a	-1.88 ± 0.32b	-0.41 ± 0.11c
E ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$)	5.36 ± 0.28a	4.96 ± 0.97a	5.67 ± 0.16a
WUE _i ($\mu\text{mol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$)	64.34 ± 2.09b	81.23 ± 0.90a	84.01 ± 0.03a
SPAD (Arbitrary values)	40.51 ± 0.57a	40.02 ± 0.47a	39.81 ± 0.32a

Table 05

Chlorophyll fluorescence parameters (Fv/Fm, maximum photochemical quantum yield of PSII; Y(II), Effective photo-chemical quantum yield of PSII; ETR, apparent Electron transport rate; NPQ, Non-photochemical quenching of fluorescence; Y(NPQ), yield for heat dissipation; Y(NO), yield of non-photochemical losses other than heat of *Phragmites karka* plants were grown in untreated soil (control), biochar (1.5 %) and biochar-compost mixture (1.5% + 1.5%). Bonferroni letters on means ± SE, represent significant differences ($P < 0.05$) among treatments.

Parameters	Control	Biochar	Biochar-compost
F _v /F _m	0.82 ± 0.01a	0.81 ± 0.01a	0.81 ± 0.00a
ETR	36.73 ± 1.07b	44.40 ± 1.91a	47.53 ± 1.48a
NPQ	1.09 ± 0.09a	0.55 ± 0.09b	0.43 ± 0.03b
Y(II)	0.46 ± 0.01b	0.56 ± 0.02a	0.60 ± 0.02a
Y(NO)	0.26 ± 0.01a	0.29 ± 0.00a	0.28 ± 0.01a
Y(NPQ)	0.28 ± 0.01a	0.16 ± 0.02b	0.12 ± 0.01b

Soil respiration and lignocellulosic biomass content

Soil respiration (CO₂ efflux) measured directly after cutting the plant tops (with the roots still in the soil) was maximal (three fold higher) in compost-biochar soil mixtures, while pure biochar treatment resulted in a twofold stimulation of the respiration rate of untreated soil (Fig. 6). Cellulose content was highest in plants grown in BC + Co treated soil, while some stimulation was found when plants were grown in BC treated soil. No difference between the treatments was found with respect to hemicellulose and lignin contents (Fig. 7).

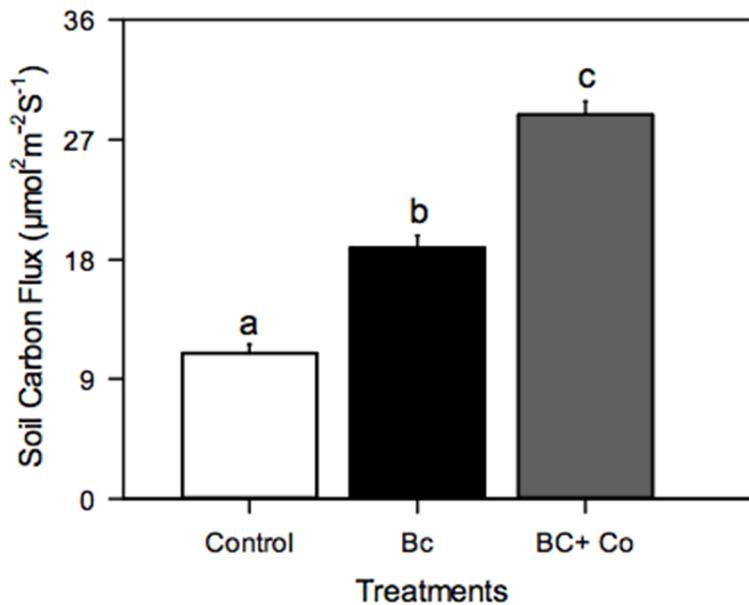


Fig. 06. Soil carbon flux CO_2 ($\mu\text{mol m}^{-2} \text{s}^{-1}$) of *Phragmites karka* grown under control conditions, in soil supplied with 1.5 % biochar and with (1.5% + 1.5%) biochar-compost mixture, respectively. Bonferroni letters on means \pm SE, represent significant differences ($P < 0.05$) among treatments.

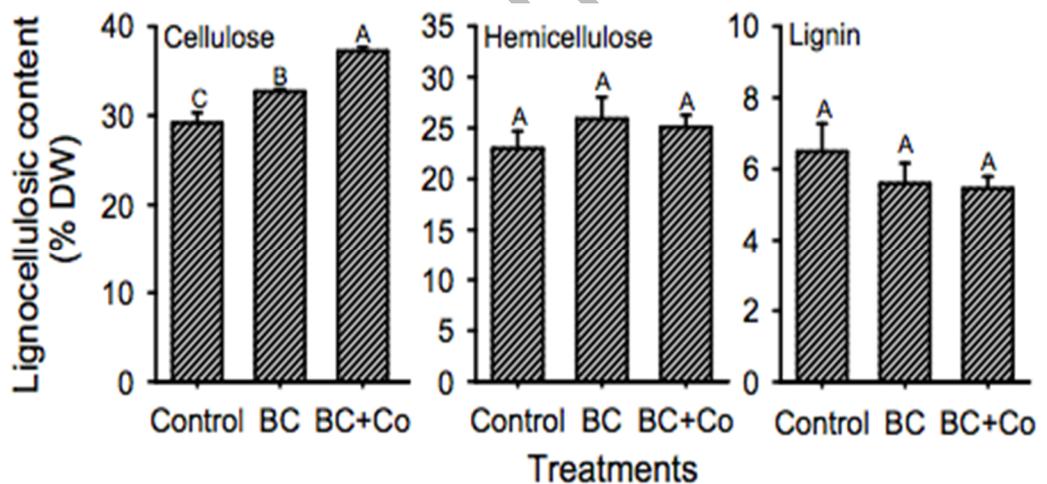


Fig. 07. Ligno-cellulosic biomass (cellulose, hemicellulose and lignin) of *Phragmites karka* grown under control conditions, in soil supplied with 1.5 % biochar and with (1.5% + 1.5%) biochar-compost mixture, respectively. Bonferroni letters on means \pm SE, represent significant differences ($P < 0.05$) among treatments.

DISCUSSION

Availability of water plays a key role in biomass production of plants in arid and semi-arid regions (Aguilera *et al.*, 2016). The knowledge about plant adaptation mechanisms is of high priority to maintain crop value and protect water resources in order to amend sustainable use of arid ecosystems. Low nutrient soil when treated with pure-biochar and compost-biochar mixture can improve water relation (Baronti *et al.*, 2014; Paneque *et al.*, 2016) photosynthesis and growth of *Phragmites karka* which resulted in higher yield of fodder (Koirala and Jha, 2013), bio-active compounds (Qasim *et al.*, 2014 and 2016) and biomass for bio-energy (Gul *et al.*, 2013).

Pure biochar and compost-biochar mixture translates into higher biomass production in *P. karka* by water and nutrient acquisition resulting in a beneficial effect on plant physiology (Eyles *et al.*, 2015; Haider *et al.*, 2015; Kamman *et al.*, 2015). Biomass increased by 60 - 80% (fresh weight) and by 40 - 50 % (dry weight) (Fig. 2) when plants were grown on soil improved by an added compost-biochar mixture as compared to results achieved on soil improved by biochar only and/or non-treated control. The observed increase in biomass of *P. karka* correlated with higher number of tillers and leaf canopy on biochar or compost-biochar treated soil (Fig. 3). Leaf area to plant biomass ratio (LAR) and root length to root mass ratio (RLR) decreased gradually by biochar and compost-biochar treatments (Fig. 3). Lower leaf area to biomass ratio indicated that leaves represented a lower fraction of total plant biomass, especially in compost-biochar treatment, in comparison with control plants grown on untreated soil. Additionally, *Phragmites karka* increased leaf area rather than leaf thickness and leaf number. This allowed an increase of photosynthetic active area at minimal investment of biomass (Hikosaka *et al.*, 2014; Enrique *et al.*, 2016). In addition, biochar and compost-biochar treatments caused a rise of nutrient retention in the soil, which enables plants to decrease root length to root biomass ratio (Schmidt *et al.*, 2014) and an increase shoot to root ratio. Latter one can be interpreted as a preferred investment of biomass in photosynthetic active tissues (Poorter *et al.*, 2012). Thus, *P. karka* was able to increase biomass production due to improved availability of nutrients in pure biochar and compost-biochar treatments, whereas without biochar, in nutrient poor habitats, a higher biomass fraction was allocated to root development, and thus a lower proportion of above ground biomass. It was investigated previously that *P. karka* can increase root length to biomass ratio in nutrient stress conditions such as salinity and drought (Abideen *et al.*, 2017 unpublished). Allocation of biomass to roots seems to be important at low water availability in soils to achieve higher root length rather than the changing root morphology (Poorter *et al.*, 2012). These results indicated the presence of high plasticity in root morphology of *P. karka* in different environmental conditions.

Plant water status plays an important role in growth and photosynthetic performance of plants (Baronti *et al.*, 2014; Paneque *et al.*, 2016). *Phragmites karka* lowers the water and osmotic potentials of leaves when exposed to biochar and compost-biochar treated soils (Table. 3). Individuals of *P. karka* increase leaf osmolality by accumulation of osmotically active organic and inorganic substances to attain osmotic adjustment (Abideen *et al.*, 2014). This results in 1.5-fold higher leaf turgidity. Similar observations were made in maize plants when treated with biochar (Haider *et al.*, 2015). Increased leaf turgidity and improved availability of water, is beneficial for higher biomass production on low quality sandy soil (Akhtar *et al.*, 2014). Some recent studies on biochar as soil amendment showed that biochar does enhance soil water permeability and leaf turgidity, but this would be more difficult to achieve in soils with high clay content (Asai *et al.*, 2009; Ahmad, 2015). The growth promotion in sandy, low nutrient soil can be of high ecological and economic significance to plan cultivation in poor quality lands to obtain profitable biomass for industrial uses.

The complex chemical composition of the cell walls components cellulose, hemicellulose and lignin can be a limiting factor when extracting raw material from biomass of plants for biofuel production. Generally, plant biomass with high lignin content is not attractive for commercial application (Gul *et al.*, 2013; Wells *et al.*, 2015). *Phragmites karka* is characterized by a high cellulose + hemicellulose to lignin ratio of the cell walls although it can grow on low quality lands. This optimal lignocellulosic ratio is comparable to the one found in crops such as sugar beet and maize or trees, such as eucalyptus and poplar (Abideen *et al.*, 2011). The chemical composition of bioenergy feedstocks depends on multiple factors, including plant type, climate conditions, use of fertilizers, and physical and chemical composition of the soil. In this study lignocellulose biomass in *P. karka* was significantly stimulated by biochar plus compost treatment of the soil (Fig. 7). This makes *P. karka* a promising bio-energy plant, especially on dry and degraded lands which seemed to be useless before. The desire to reduce the dependence on edible crops and lands and to mitigate

greenhouse gas emissions can be achieved, if increased efforts are taken to develop technologies to acquire more energy from non-edible renewable resources.

Biomass production in plants is related to nutrient uptake from the growth medium and subsequent translocation to the shoot (Schmidt *et al.*, 2014). During soil drying cycles, changes in the level of soil water saturation can influence uptake of K^+ , Ca^{++} and Mg^{++} (Nguyen and Marschner, 2005). Increase in root potassium content during our experiment (Fig. 5) may result from the role of compost-biochar mixture as a nutrient carrier. Biochar in combination with compost is functioning as an ion exchange matrix and can bind high amounts of cations (Lehmann *et al.*, 2005; Yamato *et al.*, 2006; Chan *et al.*, 2007) and seems to be a sustainable source of nutrients retention (K^+ , Ca^{++} and Mg^{++}) in *P. karka*.

Photosynthetic rate was increased in plants grown in biochar treated soil and it improved further when biochar and compost was used together, while stomatal conductance and respiration rate remained the same in plants grown in all treatments (Table. 4). It may be concluded that both parameters, Pn and R, lead to the stimulation of biomass production observed on treated soil. Plants treated with biochar are known to minimize substantial water loss through stomatal closure and transpiration. This helps maintaining water balance and leaf turgidity when plants are cultivated in low quality soil. Thus, improvement of soil ultimately supports photosynthetic performance (Liu *et al.*, 2005; Akhtar *et al.*, 2014; Genesio *et al.*, 2015). Improved WUE of biochar treated plants was more likely due to decrease in transpiration and regulated stomatal conductance (Table. 4). The decrease of internal CO_2 concentration of *P. karka* leaves grown on treated soils indicates that photosynthesis is limited by stomatal conductance (Geissler *et al.*, 2015). Plants are keeping their water status on the expense of photosynthetic activity.

Photosynthetic activity of *Phragmites karka* is limited by stomatal conductance when plants are grown on treated soils (see above). This would result in an increased risk of over-reduction of the photosynthetic electron transport chain and concomitant ROS production (Geissler *et al.*, 2015). This, in turn, might cause damage of the photosynthetic system (Koyro *et al.*, 2006). However major damage could not be detected (Table. 4). The SPAD values shown in Table. 4 indicate no effect on the chlorophyll concentration. Under our experimental conditions soil treatments do not have any effect on the antenna size of photosystem PS (II) (Lu *et al.*, 2003; Redondo-Gomez *et al.*, 2006). This is confirmed by measurements of maximum quantum yield of chlorophyll fluorescence (Fv/Fm) which is unchanged among treatments. (Table. 5). The question is how plants in treated soils can be more productive and how they escape from ROS damage. The first indicator is the observation that apparent electron transport rate (ETR, Table. 5) is increasing while stomatal CO_2 exchange is decreasing with soil treatments (Table. 5). Obviously plants are able to generate an additional electron sink. Though there is no final proof, the location of this sink can be narrowed based on chlorophyll fluorescence data.

As expected, non-photochemical quenching (NPQ) decreased with increasing photosynthetic electron transport rate (ETR Tab. 5). The same correlation holds true for energy dissipation of absorbed light energy by heat (Y(NPQ)). Losses by other factors (Y(NO)) remain unchanged. In contrast to these correlations the efficient use of absorbed energy of light quanta Y(II) apparently is increasing with soil treatments alike apparent electron transport rate. This indicates that the electron sink occurred in *P. karka* when grown on treated soil, and was located on the donor side of the photosynthetic electron transport chain. Moreover, it shows that soil treatments are stimulating consumption of photosynthetic electrons, thus preventing electron transfer to oxygen (i.e. light mediated ROS production and subsequent damage). Nitrate is the main nitrogen source for plants. It has to be reduced to ammonia to be assimilated by synthesis of amino acids, nucleic acids, alkaloids, and other metabolites. Reduction occurs in plastids on expense of electrons from the photosynthetic electron transport chain. In non-woody plants up to one third of photosynthetic electrons may be consumed by nitrate reduction. Thus nitrate is significantly competing for electrons with CO_2 and nitrogen fertilization can affect the rate of CO_2 fixation (Bloom *et al.*, 2010). Based on these

arguments it may be discussed, whether the observed effects of soil treatment can be explained in terms of improved nitrate reduction when growing *P. karka* on soils of improved nutrient content. Alternative explanations of an additional electron sink would be the enhanced rates of photorespiration or the Mehler reaction (Vanlerberghe *et al.*, 2016). But these two reaction sequences would not concur with our observations: They rather would result in reduced growth rate, while improved growth rates have been measured in our experiments (Fig. 2).

CONCLUSIONS

The findings of the present research indicate that the amendments of biochar or a combination of compost and biochar can be recommended as soil ameliorant under the studied conditions. Both amendments are leading to an increase of the soil water holding capacity, soil carbon flux and electrical conductivity. This finally contributes to an improvement of the plant nutrient uptake (K^+ , Mg^{++} and Ca^{++} content) and plant water status (water potential, osmotic potential, turgor). It also supports the increase of net photosynthesis, a reduced respiration and a higher water use efficiency but also a higher efficiency of the photosystem II (Y(II) and ETR). There is a good reason to believe that these factors can contribute to an enhanced biomass production and water savings in cultures of the perennial crop *P. karka* on arid soils with low nutrient content. In addition, it might open the opportunity to sequester CO_2 and to produce higher yields of fodder, bio-active compounds and high quality biomass for bio-energy on degraded soils. Particularly the improvement of nutrient and water availability of biochar and its mixture with compost suggest the implementation of further studies with *P. karka* under conditions of low water availability or unfavorable ion supply (such as in saline soils) in order to gain insights into its potential to be grown on underutilized wasteland.

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