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Effect of biochar amendment on morphology, productivity and water relations of sunflower plants under non-irrigation conditions

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ABSTRACT

Three biochars (B1: pine wood, B2: paper-sludge, B3: sewage-sludge) produced under controlled pyrolysis conditions and one produced in kilns (B4: grapevine wood) were used as organic ameliorants in a Calcic Cambisol, which represents a typical agricultural soil of the Mediterranean region. This field study was performed with plants of sunflower (*Helianthus annuus* L.) at the experimental station "La Hampa", located in the Guadalquivir river valley (SW Spain). The soil was amended with doses equivalent to 1.5 and 15 t ha^{-1} of the four biochars in two independent plantations. In addition, un-amended plots were prepared in both experiments for comparison purposes. The major goal of this study was the assessment of the effect of biochar amendment on the physiology and development of sunflower plants at field conditions. During most of the growing period plants of un-amended and amended plots showed no stress symptoms either by their appearance or by stress-sensitive biochemical parameters such as the stability of the photosystem II (QY). Biochar addition had no effect on seed germination. Addition of 1.5 t ha^{-1} biochar did not significantly change the pH of the soil, its electrical conductivity (EC) or its water holding capacity (WHC). Concomitantly the plant development and plant biomass production remained unaltered. Amendments with 15 t ha^{-1} slightly increased the WHC of the soil but showed no lasting impact on the soil pH. It stimulated plant growth and led to a greater leaf area, larger plant stems and wider inflorescences of the sunflower plants than those cultivated on the un-amended soil. At the end of the experiment, biochar amendment of soil caused no significant increase of the total biomass production excepting B4, the biochar with the lowest capacity of water retention, which exhibited the highest vegetative growth and seed production. The lack of rain during the last weeks caused a water shortage in the culture that produced greater QY loss in non-amended plants. Interestingly, better growth of amended plants during the drought period correlated with higher reduction of stomatal conductance, indicating that the greater water use efficiency is at the origin of the better crop performance of biochar-amended plants. This finding points to the agronomic relevance of biochar amendment of Mediterranean rain fed crops.

1. Introduction

Biochar is produced through the pyrolysis (thermal degradation under oxygen limited conditions) of biomass. It has been suggested as a soil conditioner to enhance plant growth by supplying and, more importantly, retaining nutrients and by improving soil physical and biological properties (Downie et al., 2009). As a C-rich material with a low turnover time, its application to soil is expected to significantly increase soil organic matter (SOM) contents, especially the slow SOM pool, while improving the quality of degraded soils (Lehmann and Joseph, 2009, 2015 and Liu et al., 2014). In many regions of the world, SOM is in critical decline. This problem is of particular interest in the case of agricultural areas in Mediterranean countries due to factors such as, overgrazing, intense agriculture and fire frequency (Almendros and González-Vila, 2012; Romanya and Rovira, 2011). Thus, amendment of soils with biochar may be an option to fight against further desertification. Concomitantly it enhances soil productivity since this approach has been found to improve soil fertility, which decreases fertilizer requirements (Lehmann et al., 2006; Sohi et al., 2010; De la Rosa et al., 2014 and Zhao et al., 2014). However, the effectiveness of biochar for enhancing plant production depends not only on soil type, climate and type of crop (Blackwell et al., 2009 and Obia et al., 2016) but also on the properties of the biochar (Van Zwieten et al., 2009; Cayuela et al., 2014 and Jeffery et al., 2011). The inherent variability of biochars due to different feedstock and production conditions implies a high variability of their effect on soil properties and productivity (Novak and Busscher, 2013 and Zaho et al., 2013). As a result, the effects of biochar on crop production are rather variable (Borchard et al., 2014; Jeffery et al., 2011, 2015a and Schultz and Glaser, 2012).

There is a lack of information on the effects of biochar on soil physical properties under field conditions in conjunction with crop development and plant yields (Mukherjee et al., 2014) and most of the published data derive from experiments in tropical, subtropical and

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temperate climatic zones. The effects of biochar application on crop cultivation in the Mediterranean region with its dry and hot summers and its typical calcareous soils are not well understood yet. De la Rosa et al. (2014) investigated the relationship between the characteristics of biochars from different feedstock and their effect as ameliorant on a calcic Cambisol. However that study consisted of a short-term pot experiment under controlled greenhouse conditions and optimal irrigation. Genesio et al. (2015) reported the increase of vineyard productivity at biochar amended soils from Tuscany. In addition, Vaccari et al. (2015) showed that the application of 14 t ha^{-1} of biochar to a tomato plantation with drip irrigation stimulated plant growth. Nevertheless, the necessity of data from field experiments providing specific attention to the effects of biochar amendment on plant physiology is clear.

One of the most important non-irrigated crop in Southern Europe is sunflower (*Helianthus annuus* L.). In Spain, the average surface of arable soils devoted to its cultivation comprises around 800,000 ha yr^{-1} (10% in irrigated lands) which accounts for a total production of approximately 1,000,000 t yr^{-1} (Magrama, 2015). In spite of this, studies on the impact of biochar on this crop are scarce. Whereas Tatarková et al. (2013) reported no positive effect of biochar on the growth of sunflowers, Alburquerque et al. (2014) described an increased plant production for very high application rates of ash-rich biochars, both studies were carried out in irrigated pots under controlled greenhouse conditions.

Most of the studies on biochar effects on plant growth focus on the analysis of total biomass production. However, for a better understanding of how plant growth can be affected, it is necessary to explore other parameters that are indicative of the physiological status of the plant. For instance, Abiven et al. (2015) reported a significant increase in the maize branching due to biochar addition. Given that we are exploring in this study the effects of biochar on plants cultivated under water shortage conditions, physiological parameters including leaf transpiration and the efficiency of photosystem II (PSII) have been determined to reveal the impact of biochar on plant health and plant water status. Quantum yield (QY) is a well-known plant-stress marker that quantifies the Photosystem II (PSII) efficiency. Presently very few reports are available where these parameters have been tested (Kammann et al., 2011 and Alburquerque et al., 2013).

Therefore, the major goal of the present work was to assess the effect of the soil amendment with biochar produced from diverse feedstock (conifer chip-wood, pulp paper sludge, sewage sludge and grapevine wood) on germination, plant growth and productivity, as well as stress and water parameters, in sunflower plants grown in field conditions under non irrigation regime in a Calcic Cambisol (WRB, 2007), a typical Mediterranean soil from the Guadalquivir river valley in Andalusia, Southern Spain. This study also intends to show the usefulness of innovative physiological parameters as a tool to evaluate the effect of biochar amendment on the plant development and to understand how it is affected by biochar characteristics.

2. Materials and methods

2.1. Biochar samples

Three of the four biochars used in this experiment were provided by the COST action TD1107 "Biochar as option for sustainable resource management". A detailed description of the pyrolysis conditions and nature of the feedstock are provided in Bachmann et al. (2016). Briefly, they were produced by fast pyrolysis (pyrolysis temperature 500–620 °C; 20 min pyrolysis time) from pine wood (B1), paper-sludge (B2), sewage sludge (B3). The fourth biochar derived from grapevine wood (B4) and was produced by the company "*Bodegas Torres*" (Spain) applying the traditional kiln technique. A more detailed description of the production conditions, the feedstock, and the chemical and physical properties of the four biochars is given in Table 1. All samples were kept in sealed opaque plastic bags and maintained at 4 °C until they were used to avoid their alteration or microbial degradation.

2.2. Field experiments and soil characteristics

Two field experiments were carried out by seeding *Helianthus annuus* L. in a typical Mediterranean agriculturally managed soil classified as Calcic Cambisol (WRB, 2007). This sandy loam soil is located at the experimental station "La Hampa" of the "Instituto de Recursos Naturales y Agrobiología de Sevilla", in the Guadalquivir river valley (SW Spain; 37° 21.32′ N, 6° 4.07′ W), Coria del Río, Seville. Elemental (EA) analysis was carried out by dry combustion in a flash 2000 HT (C, H, N, S) elemental micro-analyzer (Thermo Scientific, Bremen, Germany) at a combustion temperature of 1020 °C. Total nitrogen (TN) and carbon (TC) were measured in triplicated and total organic carbon (TOC) of soils was determined after the removal of carbonates by treating the soils samples with 1 M HCl. Bulk Cambisol contains 21 g of TC kg^{-1} of which 10 g kg^{-1} corresponds to TOC, and 1 g kg^{-1} of TN. Soil pH (H_2O) is 8.5 and its WHC and ash content are 49% and 95%, respectively. Those parameters are typical values reported for Cambisols of cultivated lands around the area of Aljarafe which is located within the province of Seville (Mudarra-Gómez, 1988).

All biochar samples were homogenized and oven-dried at 40 °C for 72 h before being applied to the soil. In the case of B4, the material was previously homogenized by crushing and sieving (<1 cm).

On the 20th of February 2014, the first experiment was started by amending the soil with a biochar dose equivalent to 15 t ha^{-1}. For this purpose a plot of 150 m^2 of surface was divided into 5 equal areas. Each biochar was moistured (biochar:water, 1:1) and subsequently applied to one of the five section by mixing it with the first 5 cm of the topsoil. No biochar was applied to the fifth plot-section, since it was used as control. For each treatment, 24 certified seeds of *Helianthus annuus* L. were planted. On the 4th of March 2014 a second field experiment was initiated by using the same experimental approach as described above, but with a biochar-application rate of 1.5 t ha^{-1}, in order to test the efficiency of a low rate of biochar-application dose under Mediterranean climate conditions. Moisture of the topsoil (0–5 cm) at seeding time was 22% and 24% for the first and second experiment respectively. In both experiments, the number of germinated seeds and living plants were counted during the first 30 days of the trail (in order to assess the effect of the kind and amount of each biochar on the germination of the seeds and on the plant-survival). After 30 days, 12 plants from each plot were carefully removed. The length of the plant stems were recorded periodically for each plant until reaching the maximum height. In addition, at the end of the experiment (132 and 140 days after seeding (DAS) for 1.5 t ha^{-1} and 15 t ha^{-1} respectively) the number of leaves was counted and the total leaf area (LA) was calculated by non-destructive measurements as described in Rouphael et al. (2007). Data are expressed in cm^2.

The heads of the sunflowers were harvested, dried in a forced-air oven (72 h at 65 °C) and weighted. The seeds produced by each plant were manually separated from the heads, dried in an oven (65 °C; 72 h) and then weighted for assessing the sunflower-seed production. At the beginning and at the end of the experiment (t_0 and t_f respectively), WHC, pH (H_2O) (1:2.5) and EC of the soils were analyzed.

From the 20th of February until the 30th of April 2014 the total precipitation of rain accumulated to 150 L m^2, whereas from the 1st of May until the 15st of July 2014 it accounted only for 20 L m^2. The average temperature increased gradually during the experiment. Due to different climatic conditions during the initial growing phase of the plants in experiment 1 and 2, the plant productivity of the plots with different biochar amendment doses were not compared directly. Therefore, the comparison was performed by relating the effect induced by the biochar to the result of the plants growing on the respective control plot (un-