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Biological response of a sandy soil treated with biochar derived from a halophyte (*Salicornia bigelovii*)

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ABSTRACT

Cultivation of halophytes for food and fuel is highly desirable because these plants can be irrigated with brackish or sea water, and they can be grown on saline non-arable soils. Of particular interest is the high oil seed content halophyte *Salicornia bigelovii*. The vegetative biomass remaining after harvesting of plant seeds could be used as feedstock for the production of biochar; a product potentially useful for saline agricultural systems. In this study biochar was produced from *S. bigelovii* biomass (350 °C, 6 h) and characterized; pH 8.6; BET surface area 1.72 $m^2 g^{-1}$; proximate analysis (29.8% fixed carbon, 27.4% volatiles and 41.1% ash), elemental O/C and H/C ratios of 0.2 and 0.9 respectively.

Treatment: of a sandy soil with 5% (wt.) biochar increased the organic carbon concentration from 10 to 26 g kg^{-1} in the soil, and did not affect plant available water. Subsequently, microbial biomass carbon, basal respiration and enzyme activities (β-glucosidase, acid phosphatase and alkaline phosphatase) were measured over a 30 day period. Higher respiration rates were observed over the course of the incubation study for biochar treated soil (~0.35 μg CO2-C g^{-1} soil h^{-1}) when compared to control soil (~0.05 μg CO2-C g^{-1}), but similar microbial biomass carbon measurements were found in both soils (~350 mg C Kg^{-1} soil). There was also a 1.5 fold increase in β-glucosidase and alkaline phosphatase activities in biochar treated soil when compared to control soil. The results collectively indicate that treatment of soil with *S.bigelovii* biochar stimulates biological activity and treatment does not appear to have an adverse effect on soil biological processes. Therefore, there is potential for the utility of halophyte derived biochars such as the one used in this study in saline agricultural systems.

1. Introduction

A growing practice for managing soil quality is land amendment with biochar (Beesley et al., 2011). This is a charcoal like material produced from biomass at temperatures between 300 and 550 °C, and at atmospheric pressure in the absence of oxygen (Beesley et al., 2011; Chan et al., 2007; Jeffery et al., 2011; Laird et al., 2010a; Novak et al., 2009). Numerous studies reported improved physical, chemical and biological properties of soil after treatment with biochar using application rates from 10 to 100 g Kg^{-1} dry soil (Akhtar et al., 2014; Dempster et al., 2012; Enders et al., 2012; Jeffery et al., 2011; Karhu et al., 2011; Laird et al., 2010b; Luo et al., 2011; Wu et al., 2014). For example, enhancement of water holding capacity in soil (Basso et al., 2013; Hardie et al., 2014; Karhu et al., 2011), suppression of soilborne diseases (Elad et al., 2012), improved soil fertility (Mukherjee and Zimmerman, 2013), increased biomass yields (Akhtar et al., 2014), and carbon sequestration in soil (Woolf et al., 2010). However, biochars vary considerably in their properties (pH, surface area, proximate analysis, elemental composition) depending on the type of biomass and heating conditions used (Enders et al., 2012; Kloss et al., 2012; Novak et al., 2009; Singh et al., 2010). For this reason a considerably large number of studies have been dedicated to biochar characterization and agricultural use.

Very few studies are available on biochar produced from biomass of halophytes (e.g. (Irfan et al., 2016)), which are plants that can survive and complete their life cycle in at least 200 mM salt (Flowers et al., 2010). There is a growing interest in growing halophytes for food and fuel because these plants can be irrigated directly with brackish/sea water (Ladeiro, 2012). Adoption of halophyte based agricultural systems (known as saline agriculture) would reduce the demand for freshwater and also make use of marginal land not suitable for the cultivation of conventional crops. The halophyte *Salicornia bigelovii* is an attractive candidate for saline agriculture because its biomass is suitable for human and animal consumption, and the high oil content of its seeds make it

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suitable for biofuel production (Glenn et al., 1992, 1991; Shahid et al., 2013). Furthermore, *S. bigelovii* can be irrigated directly with sweater (Grattan et al., 2008) and it is adaptable to arid/dry climates (Abdal, 2009). However, high plant yields and the implementation of suitable agronomic practices for salt tolerant species are required for saline agriculture to be cost effective and sustainable (Ladeiro, 2012). Incorporation of biochar produced from halophytic species maybe a cost effective strategy to improve soil quality in saline agricultural systems. For example, using a fraction of the harvested halophytic biomass for biochar conversion and application to soil can be a sustainable mechanism to increase organic carbon and also recycle back essential nutrients in soil.

Microbial biomass carbon, soil respiration and enzyme activities are linked to soil fertility and agricultural productivity (Anderson and Domsch 1993; Yan et al., 2003; Ghollarata and Raiesi 2007; Wang et al., 2009; Dempster et al., 2012; Prayogo et al., 2014). Their measurements in soil is a useful approach for detecting changes occurring early on in response to land management such as biochar application. In this study, *S. bigelovii* biomass is converted to biochar, characterized and subsequently applied to soil with the objective of identifying biological changes occurring immediately in response to treatment. Specifically, we measured microbial biomass carbon, microbial respiration and enzyme activities (β-glucosidase, alkaline phosphatase and acid phosphatase) of biochar treated soil compared to control soil over a 30 day laboratory incubation. To our knowledge, this is the first report of biochar production from *S. bigelovii* and its application to soil.

2. Materials and methods

2.1. Biomass and biochar

Biomass of *Salicornia bigelovii* was provided by the Sustainable Bioenergy Research Center (SBRC) located in Abu Dhabi, UAE. The biomass was oven-dried at 110 °C overnight, milled into a fine powder and packed tightly into a sealed steel cylindrical container prior to carbonization in a muffle furnace at 350 °C for 6 h. The biochar conversion process in this study simulates traditional kiln production (Meyer et al., 2011), and the heating conditions were selected to induce slow thermal degradation of cellulose and lignin (Williams and Besler, 1996).

The pH of the materials were determined from suspensions of 1% (wt./v) biochar or biomass in MilliQ water after shaking at 150 rpm overnight at 25 °C. Surface area measurements were obtained from multipoint Brunauer–Emmet–Teller (BET) N_2 adsorption isotherms at 77 K using Nova2000e (Quantachrome Instruments,USA). Elemental composition (CHN) of materials were determined using a Flash2000 series (Perkin Elmers, USA). Proximate analysis of materials was determined as described earlier (Jouiad et al., 2015) using Thermal Gravimetric Analysis. Ash content was determined by measuring the difference in weight of material contained in porcelain crucibles before and after heating in a muffle furnace at 550 °C for 12 h and dividing by initial (before heating) sample weight times 100%. The oxygen content was estimated by taking the difference (O% = 100−ash%−C%−N%−H%). Biochar characterization analysis were completed in duplicate

2.2. Soil and experimental conditions

Soil (taxonomically classified as Entisol Psament) was collected from an area that is being prepared for the cultivation of the halophyte *Salicornia bigelovii* located at the SBRC facility in Abu Dhabi, United Arab Emirates (UAE). The soil used in this study is the most common in the emirate of Abu Dhabi and is the soil that will be used for commercial cultivation of the halophyte.

Biochar was added to moist soil using a 5% application rate (50 g per kg of oven-dried soil) and mixed manually to homogeneous consistency. Textural classification of non-treated (control) and biochar treated soils were determined using the hydrometer method. Elemental composition (CN) was determined using a Flash2000 series (Perkin Elmers, USA). Soil pH and EC measurements were made from a water saturated soil extract (1:2, soil: milliQ water). Total Organic Carbon was estimated using the loss-on-ignition method which involved calculating the difference between weights of the initial sample (dry soil) and final weights (soil heated in a muffle furnace at 440 °C over 24 h) divided by the initial sample weight times 100%. A pressure plate extractor unit (ICT international, Australia) was used to determine gravimetric water at field capacity (0.3 bar) and wilting point (15 bar). Plant available water was calculated by taking the difference in gravimetric water between field capacity and wilting point. Soil physicochemical characterization analysis were completed in duplicate.

Experiments were carried out in sealed rectangular plastic containers (length 19.5 cm; width 27 cm; height12 cm) in the dark at 37 °C in quadruplicates (n = 4) over 30 days. Soils were placed in the plastic containers after moistening to field capacity to a height of 7 cm using a packing density of approximately 1.4 g cm^{-3}. Composite aliquots of soil (50 g) were destructively sampled using a spatula from each of the control and treatment replicates on days 0, 6, 18 and 30 to carry out enzyme assays, and on days 0, 10, 20 and 30 to carry out microbial biomass carbon measurements. Soil moisture was monitored using a moisture probe (GS3stereo sensor, Decagon Devices Inc., USA) and maintained at field capacity throughout the duration of the experiment.

2.3. Soil enzyme assays

Enzyme assays were adapted from (Tabatabai and Bremner, 1969; Verchot and Borelli, 2005), and all activities were determined by colorimetric measurements (Absorbance 420 nm) of *p*-nitrophenol (pNP) linked substrates in buffer solution; pNP-β-D-glucopyranoside (β-glucosidase) or PNP-phosphate (acid and alkaline phosphatase). 1 g of soil was placed in a 50 mL falcon tube containing 10 mL of Modified Universal Buffer [2.44 g of TRIS, 2.32 g of maleic acid, 2.8 g of citric acid, and 1.26 g boric acid in 100 mL of 1 M NaOH titrated up to 1L of water pH 6.5 (β-glucosidase and acid phosphatase) or pH 11 (alkaline phosphatase) using 1 M HCl or NaOH]. The soil suspension was then placed on a rotary shaker for 30 min at room temperature (22 °C), centrifuged at 500 x *g* for 5 min and 1 mL of the supernatant was transferred into 15 mL falcon tube along with 0.5 mL of 50 mM pNP linked substrate and incubated at 37 °C for 1 h. The enzyme reaction was stopped by adding 1 mL of 1 M NaOH and 1 mL of 0.5 M $CaCl_2$, centrifuged at 500 x *g* for 5 min and 1 mL of the solution transferred to a cuvette for absorbance measurement. Controls (which also acted as blanks) in which pNP substrate was added after the 1 h incubation period were included to account for color development that might occur from chemical or soil humic interferences in the samples.

2.4. Microbial biomass carbon and basal respiration

Microbial biomass carbon was determined using 25 g of soil sampled from the incubation studies. The assay was carried out at 22 °C using the Substrate Induced Respiration (SIR) method (SIR) in airtight mason jars over 4 h with glucose as substrate and NaOH as the CO_2 trap (Höper, 2005). A digital titration device was used to neutralize the NaOH trap with HCl using phenolphthalein indicator. Glucose was added to soils at a rate of 6 g per Kg dry soil, which was determined to be the substrate saturating point for