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Restoration of carbon and microbial activity in salt-induced soil by application of peanut shell biochar during short-term incubation study

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HIGHLIGHTS

- Biochar from peanut shell alleviated soil C and enzymatic activities under saline environment.
- There is a scope to sequester soil carbon by using peanut shell biochar.
- Peanut shell biochar sustained the soil enzymatic activities under salinity, depending on rate and incubation interval.
- Phosphatases and urease activities emerged as most sensitive soil microbial parameters.

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ABSTRACT

For the present study, soil samples of four artificially-induced salinity gradients (S_0: control, S_1: 2.0, S_2: 4.0, S_3: 6.0 EC_{iw}) was incubated with fine-textured peanut shell biochar at various ratios (B_0: control, B_1: 2.5%, B_2: 5.0%, B_3: 10% w/w) for 30 days. At 1, 3, 7, 15, 30 days of incubation, samples were analyzed for soil carbon and selected enzyme activities. Results showed that biochar could increase soil organic carbon on application of highest rate of biochar addition (B_3), hence potentially restored the saline soils by less C mineralization, and more sequestration of soil C. However, soil enzyme activities were biochar rate(s), day(s) of incubation and enzyme dependent. The lowest rate of biochar addition (B_1) showed highest dehydrogenase (20.5 μg TPF g^{-1} soil h^{-1}), acid phosphatase (29.1 μg PNP g^{-1} soil h^{-1}) and alkaline phosphatase (16.1 μg PNP g^{-1} soil h^{-1}) whereas the higher rate (B_2) increased the urease (5.51 μg urea-N g^{-1} soil h^{-1}) and fluorescein diacetate hydrolyzing activities (3.95 μg fluorescein g^{-1} OD soil h^{-1}) in soil. All the positive changes persisted at higher levels of salinity (S_2, S_3) suggesting biochar-amended soil may be potential for better nutrient cycling. Soil enzymes were found to be correlated with soil carbon and with each other while principal component analysis (PCA) extracted the most sensitive parameters as the acid and alkaline phosphatases and urease activities in the present experimental condition. This is the first time report of examining soil microbial environment using peanut shell biochar under a degraded (saline) soil.

1. Introduction

The term 'biochar' refers to black carbon formed by the pyrolysis of biomass i.e. by heating biomass in an oxygen-free or low oxygen environment such that it does not (or only partially) combust. Biochar, produced by the pyrolysis of biomass under limited oxygen, is highly stable and resistant to microbial decay. Thus there is considerable interest in the concept of applying biochar to soil as a long-term sink for carbon (C) thereby mitigating climate change (Prayogo et al., 2014). Biochar application has received growing interest as a sustainable technology to improve highly weathered or degraded tropical soils (Beesley et al., 2011; Mukherjee et al., 2014). Protection of soil C could also be the result of greater aggregation, protecting both biochar and SOM from degradation, changes in microbial enzyme activity as a result of enzyme sorption to biochar (Prayogo et al., 2014). Before the application of biochar as a soil amendment, it is essential to characterise the biochar for

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efficient management since the physical and chemical properties of biochars are found to be governed by the feedstock properties and pyrolysis conditions such as highest treatment temperature and furnace residence time (Downie et al., 2009).

Biochar pores harbor and protect to almost all important soil microorganisms *viz*. bacteria (0.3–3.0 mm), fungi (2–80 mm), and protozoa (7–30 mm) and macropores (>200 nm) cater most ideal size to accommodate bacteria (Quilliam et al., 2013; Jaafar et al., 2014). Moreover biochar also contains micropores (<2 nm) and mesopores (2–50 nm) that efficiently store and supply moisture and dissolved substances required for microbial sustenance (Brewer and Brown, 2012). As extracellular enzymes are the immediate change-bringers of two most vital soil fertility phenomena: organic matter decomposition and nutrient cycling (Burns et al., 2013) hence the impact of biochar on activities of soil extracellular enzymes is immensely essential. Biochar controls the soil enzyme activity mostly based on (i) the interaction of substrate and enzyme with biochar (i.e. sorption and desorption phenomena at biochar CEC/AEC sites) (Bailey et al., 2011) and (ii) relation with the porosity and surface area of biochar (Lammirato et al., 2011). More porosity and surface area of biochar was found to reduce extracellular enzyme activity, since functional groups on biochar bind both substrates and extracellular enzymes, thus limiting the rate of substrate diffusion to the active site of enzyme catalysis (Bailey et al., 2011; Lammirato et al., 2011). This is supported by some recent studies (Ameloot et al., 2013; Chintala et al., 2014), who reported a reduction in dehydrogenase and fluorescein diacetate activity in proximity with biochar under short-term incubation experiments. However relatively long-term incubation studies revealed an increase of soil dehydrogenase, urease activities as well as microbial biomass C at lower rate of biochar with concomitant increase in organic C in soil (Demisie et al., 2014; Jiang et al., 2015). Although, there is every possibility that enzymes may behave differently under salt (or any other stress) affected soil in biochar-amended soil which eventually constitutes the projected hypothesis of the current paper.

A number of studies have suggested that the effect of biochar is more pronounced in highly weathered, degraded and nutrient-poor soils than in well-structured, nutrient rich and high quality soils (Kookana et al., 2011; Jien and Wang, 2013). Salinity is one of the major threats to global food security. According to a recent estimate, 1128 Mha (million hectares) lands on global scale are affected by salinity and sodicity (Wicke et al., 2011). Salinity stresses plant by osmotic and ionic effects (Munns and Tester, 2008). Moreover, salinity also causes nutritional disorders (Grattan and Grieve, 1998) and limits the uptake of essential plant nutrients (K, Ca, Mg, P etc.) and ultimately results in crop yield losses. The influence of salt as a major stress to soil microorganisms has been the subject of several studies (Sarig and Steinberger, 1994; Pankhurst et al., 2001; Sardinha et al., 2003; Mamilov et al., 2004). A decrease in carbon dioxide (CO_2) production, enzyme activities, or microbial biomass in soil has often been observed in the field (Pathak and Rao, 1998) and under laboratory incubations (Ghollarata and Raiesi, 2007; Rietz and Haynes, 2003). Soil enzyme activities were found to decrease with increasing salinity but the degree of inhibition varied among the enzymes assayed and the amount of salt added (Frankenberger and Bingham, 1982).

While the effects of salinity on soil chemical and physical properties and plant growth are well known, their effects on soil biological characteristics remain relatively less prioritized, and there is limited information and poor consistency in studies of the salt effects on soil microbial activity. Since biochar is a stable source of carbon; it may contribute towards change in biological environment in soil apart from adding sources of carbon to soil. The objectives of this study were to: (i) assess the changes in soil C and enzyme activities under different rates of biochar application to soil over a short-term incubation span (ii) evaluate the potentiality of peanut shell biochar to ameliorate saline soil in terms of soil C-fractions and selected enzyme activities.

2. Materials and methods

2.1. Preparation of peanut shell biochar

The used peanut shell was collected from the research farm of the ICAR-Directorate of Groundnut Research, Junagadh (India) after harvesting and shelling of the pods. The shell was washed thoroughly and repeatedly with double distilled water to remove adhered soil and dust, and sun-dried for at least 8 h. The dried shell was then ground to a fine powder using an electrical grinder and sieved to 60-mesh size (of average particle size $\leq 250\ \mu$). The sieved material was again washed with double distilled water to remove the fine particles and dried in an oven at 80 ± 5 °C for 24 h. Methanol (125 mL) was added in the dried shell (50 g) and placed in a mechanical shaker for 5 h to increase the extraction recovery of organic impurities from the surface of the adsorbent and then again dried. Acid activation of peanut shell was followed by soaking it in 4N H_2SO_4 (200 mL acid solution/100 g of shell) for about 24 h under room temperature. This acid-treated material then washed to remove the excess acid with double distilled water and allowed to dry completely in an oven at the temperature 80 ± 5 °C for 4 h. The acid activated peanut shell was finally thermally activated at 300 ± 5 °C in a closed muffle furnace for 2 h to increase the surface area under limited oxygen environment. The chemically and thermally activated peanut shell was stored in a desiccator and used as a biochar material for the further study. The pH, electrical conductivity, total dissolved solids (Jackson, 1973; 1:2.5 biochar: water solution using Hanna make combined pH-EC-TDS Meter, model HI 991301) and cation exchange capacity (Sumner and Miller, 1996) of the prepared biochar were measured as 5.5, 3.76 dS m^{-1}, 1.88 ppt and 6.9 $cmol^{(+)}\ kg^{-1}$, respectively. To measure the ash content 2.0 g of each of the oven-dried samples of peanut biochar in powder form were accurately weighed and placed in crucible of known weight. These were ignited in a muffle furnace under air and ashed for 8 h at 550 °C. The crucible containing the ash was then removed, cooled in a desiccator and weighed and the ash content expressed in term of the oven-dried weigh of the sample. The prepared biochar was found to have a composition of 3.31% ash, 56.1% C (TOC-L, model SSM-5000A, Shimadzu, Japan) and 0.89% N (Bremner and Mulvaney, 1982).

The functional group variability of peanut biochar was investigated by analysing Fourier transform infrared spectroscopy (Shimadzu FT-IR-8400) using KBr pellet method in the scanned range of 4000–400 cm^{-1}. To determine the surface morphology of biochar, scanning electron microscopy (SEM) (Carl Zeiss, Oberkochen, Germany) analysis was carried out where the powder samples were mounted onto double-sided carbon tape covered copper stab with industrial glue and, coated by 20 nm thick palladium layers in a vacuum of 1.7 $e^{-0.005}$ m bar prior to analysis.

2.2. Imposition of treatments

The fresh soil sample (150 g) collected from the permanent salinity plots (maintained for last 12 years) of four different salinity gradients (S_0: control, S_1: 2.0 EC_{iw}, S_2: 4.0 EC_{iw} and S_3: 6.0 EC_{iw}) was kept into glass beakers and mixed with fine-textured peanut shell biochar in the month of August, 2014. The biochar were added as B0: no biochar (control), B_1: 2.5% biochar, B_2: 5.0% biochar and B_3: 10% biochar, based on the fresh weights of the soil samples taken and mixed with the soil thoroughly. Altogether sixteen treatment