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Application of holm oak biochar alters dynamics of enzymatic and microbial activity in two contrasting Mediterranean soils

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ABSTRACT

The application of biochar in Mediterranean region has been considered a promising mean to enhance the soil quality and health. However, the results of previous studies are inconsistent and the effects of biochar on biological activity seem to depend on the initial soil properties. To elucidate this relation, the evolution of microbial and enzymatic activity in two contrasting soils was evaluated in the course of 12 weeks of greenhouse incubation after biochar application and fertilization with nitrogen, phosphorus and potassium. Biochar enhanced the activity of soil biota in an acid Acrisol as a result of soil pH neutralization which was observed as an increase of microbial biomass carbon (MBC) and soil basal respiration (SBR) during the initial six weeks of the incubation. On the other hand, in an alkaline Calcisol, the effect of biochar application on SBR and MBC was short-term (three weeks) and seemed to be related to the nitrogen availability. Dehydrogenase and urease activity in Acrisol were enhanced by biochar application while the activity of all the other enzymes decreased or remained unaffected by biochar application. Biochar application enhanced the aggregates stability in both soils. In summary, general decrease of the enzymatic activities and the inconsistency of the soil biological properties, specifically MBC and SBR, highlight the need of long-term investigation and periodic sampling to target the dynamic changes induced by biochar application. Nevertheless, the improved aggregation of both soils could indicate biochar as a useful mean to combat soil degradation in Mediterranean areas.

1. Introduction

Biochar is a carbonaceous material obtained by pyrolysis of organic matter, differing considerably from its original feedstock. The pyrolysis process transforms the major part of organic carbon (C) into stable form which is generally recalcitrant towards biotic and abiotic oxidation and which is believed to persist in soil for centuries or even up to thousands of years [1]. Although only minor part of original C content remains labile and available for the use by soil biota [2], addition of biochar to soil may alter soil biogeochemical processes as a result of organic C addition and the unique properties of biochar. Pyrolysis induces the formation of large amount of surface functional groups which are directly linked to biochar properties such as electric conductivity (EC) and pH [3] or sorption capacity [4] and together with ash content lead to often observed changes in soil pH. These biochar properties are affected by both feedstock material [5] and pyrolysis temperature with more alkaline biochars with higher sorption capacity formed at higher temperatures [6]. These characteristics have high importance in degraded soils where acidity can limit microbial activity and plant growth, such as many Mediterranean soil where inappropriate agricultural practices caused drastic reduction of soil C stocks and soil acidification. It has been suggested that changes in soil pH and salinity could be the key drivers of microbial changes in biochar-amended soils [7,8].

Abbreviations: C_{ox}, dichromate oxidizable carbon; EOC, extractable organic carbon; MBC, microbial biomass carbon; SBR, soil basal respiration; SIR, substrate induced respiration; SOM, soil organic matter; TC, total carbon; TN, total nitrogen; WSA, water-stable aggregates; WSC, water-soluble carbon; WSN, water-soluble nitrogen

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Biochar can sequester C in soil, while simultaneously making use of organic wastes [5] and potentially increasing soil quality. Once in soil, biochar has been observed to induce changes in nutrient cycling which could be attributed to the addition of C substrate [9] or nutrients, or to transformation of chemical or physical properties. Aside from these variable interactions in soils, the duration of change [10] also depends on the rate of decomposition and the initial soil properties [11] and biochar feedstock [12]. The inherit variability within soils and biochar amendments calls for long-term studies with multiple sampling points to capture complex dynamics.

The interaction of biochar a soil will also impact microbial abundance and activity of intra- and extracellular enzymes [13]. These are critical for key steps of soil organic matter (SOM) decomposition, facilitating the liberation of nutrients for plant or microbial uptake. Given that enzyme activity is considered a sensitive indicator of soil health, the effect of biochar on soil enzymes is a key to understanding changes to short and long-term impacts on microbial nutrient cycling [7,14]. Incorporation of biochar into the soil may have several direct or indirect impacts on soil biota, including alteration of abiotic factors including soil pH and increased availability or altered quality of substrate as a source of energy [15].

Despite their key role in soil nutrient cycling and increasing body of literature, the effect of biochar on the activity of soil enzymes remains largely unclear. For example, dehydrogenase is an intracellular enzyme and its activity in soil is linked to respiratory processes, often tightly correlated with organic C availability. Nevertheless, strong enhancing effect of soil pH have also been detected in degraded acid soils [16–18], suggesting that biochar could affect dehydrogenase activity either by labile C input or through soil pH neutralization. Similarly, the activity of β-glucosidase, the enzyme catalyzing the final step of cellulose degradation releasing glucose, has been observed to be increased, unaffected or decreased by biochar application [19–21] as well as the activity of other hydrolases involved in SOM transformation and nutrient cycling, such β-glucosaminidase [21], phosphatase [20,21] or urease [18]. Soil organic C content is directly linked to the formation and stabilization of soil aggregates which are the key element in soil structure rebuilding and erosion prevention. The increase of soil C leads to stimulation of biological activity in soil including the activity of soil enzymes, which in turn result in enhanced formation of soil macroaggregates and their stabilization [22].

Extensive areas in Mediterranean regions are characterized by severely degraded soils resulting from an unsustainable soil management causing a reduction of SOM and often related soil acidification [23], such as for example large areas of SW Spain covered with degraded Acrisols (FAO). Climax vegetation these areas is a cork oak (*Quercus suber* L.) forest, which has been largely substituted by holm oak (*Quercus ilex* L.), olive groves, pastures or agricultural lands. Holm oak agroforestry systems (*Dehesa*) are of high economic and traditional importance due to Iberian pigs, which are largely or exclusively fed on acorns. However, the value of utilization of holm oak pruning residues declined drastically in the past decades and their conversion to biochar could simultaneously solve the residues problems as well as improve degraded soil properties and increase their productivity. Under Mediterranean climate, often severely C-poor Calcisols occupy the largest area of all World Reference Base (WRB) groups (85 million ha) [24]. These soils are often poor in nutrients and SOM and have high soil pH. There is limited amount of information about the effect of biochar application on Calcisols, despite their large extension areas under Mediterranean climate [10,25].

The aim of this study is the evaluation of enzymatic and microbial activity dynamics in two contrasting soils (an alkaline Calcisol with low organic C content and an acid degraded Acrisol) after biochar application and fertilization in a greenhouse experiment with a special emphasis on development of biochar-induced changes over twelve weeks since application. We hypothesized that the biochar will increase microbial biomass and microbial activity, related to labile C inputs in the Calcisol and related to pH neutralization in the Acrisol. Furthermore, biochar will enhance stabilization of soil aggregates in both soils as a result of stimulated microbial activity and that biochar induced changes will decrease over time.

Table 1
Selected soil and biochar properties.

Soil properties	Acrisol	Calcisol	Biochar properties	
pH	5.65	8.00	pH	10.2
Electric conductivity (μS cm^{-1})	49.7	570	Electric conductivity (μS cm^{-1})	940
CEC ($cmol_c$ kg^{-1})	2.73	8.84	CEC ($cmol_c$ kg^{-1})	35.1
TOC (g kg^{-1})	25.8	9.55	TC (%)	68.2
TN (g kg^{-1})	1.28	0.90	TN (%)	0.67
Carbonate content (%$CaCO_3$)	n.p.	21.9	Carbonates content (% $CaCO_3$)	11.9
WSC (mg kg^{-1})	78.3	29.1	WSC (mg kg^{-1})	149
WSN (mg kg^{-1})	19.0	49.2	WSN (mg kg^{-1})	93.4
Field moisture capacity (%)	16.9	18.3	C_{ox} (%)	4.70
Sand (%)	80.1	29.0	C:N ratio	102
Silt (%)	6.10	42.0	Ash content (%)	3.49
Clay (%)	13.8	29.0		

CEC, cation exchange capacity; TOC, total organic C; TN, total N; WSC, water-soluble C; WSN, water soluble N; TC, total C; n.p., not present.

2. Materials and methods

2.1. Soil and biochar characterization

Two contrasting soils (0–10 cm upper soil layer), originating from the Mediterranean climate were selected (Table 1). Acid sandy Acrisol (FAO), corresponding to Palexerult (USDA), characterized by low pH, low content of exchangeable bases, low available phosphorus (P) content and Al-dominated exchange complex was collected from Cañamero's raña formation in SW Spain (39°19′N 05°19′W) which is severely degraded due to long-term tillage. The second soil, a Haplic Calcisol (FAO), classified as Calcixerept by USDA, was used as a second selected soil and was collected from “La Chimenea” Field Station (40°03′N, 03°31′W) near Aranjuez (Madrid, Spain). This soil is characterized by high pH and carbonate content and loamy texture. Samples were transported to the laboratory, homogenized and sieved at field-moist state (< 5 mm) within two days. Part of the composite sample (composed of ten subsamples taken along a transect at least 10 m apart) was air-dried and sieved to 2 mm for laboratory analysis. Soil pH and EC were determined in soil:deionized water (1:2.5 w/v) after 1 h of shaking using pH meter and conductivity meter, respectively. Ammonium acetate (1 M, pH 7) method was used for cation exchange capacity (CEC) determination method [26]. Soil organic carbon content was measured by dichromate oxidation after carbonates reacted with HCl (Calcisol). The contents of water-soluble carbon (WSC) and water soluble nitrogen (WSN) were determined by extraction with deionized water (1:10 w/v), followed by analysis with automatic analyzer (multi N/C 3100, AnalytikJena, Jena, Germany) for C and N content, respectively. Field moisture capacity was quantified by pressure plate extractors at −0.33 kPa (Soil Moisture Equipment Corp., Santa Barbara, CA).

Biochar used in this study originated from holm oak pruning residues at temperature of 600 °C in oxygen-restricted environment in batch system and crushed to pass 2-mm sieve for chemical analysis (Table 1). Biochar pH and EC were measured by pH meter and electric conductivity meter, respectively, after 1 h shaking with deionized water (1:10 w/v). Total C (TC) and total N (TN) were analyzed by automatic analyzer LECO Instrument TruSpec CN (LECO Corporation, St. Joseph, MI, USA), dichromate oxidizable C (C_{ox}) by dichromate oxidation and carbonate content by calcimeter. The content of WSC and WSN were determined in the same way as in the soil samples. The modified