



Influence of pig manure and its biochar on soil CO₂ emissions and soil enzymes



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ABSTRACT

Biochar production from manure wastes, including pig manure, could provide a valuable alternative to current waste management practices, while offering an opportunity to improve soil properties and to reduce the risk of contamination derived from the direct application of manure as a soil amendment. Two different biochar samples, produced from pig manure at 300 °C (BPC300) and 500 °C (BPC500) were used to evaluate the impact of biochar amendment on soil enzymatic activity and soil CO₂ emissions. An incubation experiment was designed as follows: selected soil (S) was amended with pig manure (PC) and two pig manure biochars prepared at 300 °C (BPC300) and 500 °C (BPC500) at a rate of 8 wt%. All samples were incubated during 219 days.

The results indicated that soil amendment with biochars decreased the carbon mineralization, in contrast to soil amended with the pig manure. Addition of pig manure increased dehydrogenase, phosphomonoesterase and phosphodiesterase activities, while B prepared at 300 °C resulted on a positive effect on dehydrogenase activity. In contrast, B prepared at 500 °C did not exhibit a positive effect on soil enzyme activity.

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1. Introduction

Previous studies have indicated that biochars can improve soil properties and also contribute to carbon sequestration when used as organic amendment. The amount of soil sequestered and the performance of biochar as a soil amendment depend on pyrolysis conditions and on the feedstock used for biochar production (Lehmann and Joseph, 2009; Paz-Ferreiro et al., 2012; Cely et al., 2015). However, biochars could also represent a risk due to their potential content of toxic compounds such as polycyclic aromatic hydrocarbons (PAHs) or heavy metals. There is an increasing body of literature providing evidence of biochar having a contrasting effect on different species of plants and microorganisms, depending on their sensitivity. Bastos et al. (2014) found the bioluminescent bacteria *V. fischeri* to be the most sensitive organism to a biochar produced from slow pyrolysis of mixed pine wood chips, however they did not observe any detrimental effect on the growth of the microalgae *P. subcapitata*. Besides, Cely et al. (2016) found

that some plant species, including lettuce and tomato, seemed to be more sensitive to the presence of phytotoxic compounds in biochars, while all biochars used in the same experiment acted as phytostimulant for lentils.

Enzymatic assays represent an important measurement in order to understand the metabolic activity in soils which underpins processes such as the mineralization and humification of organic matter. The latter in turn influences the biogeochemical cycles of elements including C, N, P and S (García and Hernández, 2003). Therefore, soil enzymes play an important role in organic matter decomposition and nutrient cycling (Walelign et al., 2014). Paz-Ferreiro et al. (2012) used the geometric mean of enzyme activities (GMea) as a soil quality index and reported a lower value of this index in soils amended with sewage sludge. On the contrary, sewage sludge biochar resulted in higher values of GMea than the control soil, indicating an improved soil quality.

Biochar, as a soil amendment, can increase soil organic matter stocks and stimulate soil microbial activity (Lehmann and Joseph, 2009). Microorganisms are largely responsible for the decomposition of the organic matter via a variety of enzymes and hence, application of biochar is a method to improve soil quality and

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soil biological status, which usually implies an increase in enzyme activity.

Soil organic matter content is a balance between inputs and decomposition rates and, as such, changes in agricultural practices can result in marked alterations in both the addition rates and decomposition rates of soil organic matter and therefore, nutrients (Waleign et al., 2014). The enhancement of C mineralization and microbial biomass, following biochar addition, indicates that the activity and mass of soil microorganisms have been promoted due to the presence of extra nutrients provided by the biochars (Zhao et al., 2015). Also, increasing application rates of biochar could enhance TOC and its labile fractions which improve the soil quality by increasing the microbial activities, aggregation and soil C sequestration. Cely et al. (2014) reported an increase in the amount of more humified or thermally stable organic matter after addition of different types of biochar. In addition, besides previous studies indicated that the CO₂ evolved after adding biochar to the soil could be related to the labile carbon content in biochars (Méndez et al., 2013; Cely et al., 2014).

The main purpose of this study was to evaluate the effects of pig manure and two biochars produced from pig manure at two different temperatures on soil carbon mineralization and soil microbial activities.

2. Methods

2.1. Soil samples selection and characterization

The soil sample was taken from the northeast of Toledo-Spain (40° 7' 6" N–4° 14' 29" W–595 m). The soil was classified as a Cambisols, with a pH of 7.66 and a low content in C_{Cr207} (%), N and P. Soil texture was sandy loam.

Soil pH and electrical conductivity (EC) were determined with a soil: water ratio of 1:2.5 (g mL⁻¹) using a Crison micro-pH 2000 (Thomas, 1996) and a Crison 222 conductivimeter (Rhoades, 1996) respectively. CEC was determined by NH₄OAc/HOAc at pH 7.0 (Sumner and Miller, 1996). Organic carbon oxidized with dichromate was determined by the Walkley-Black method (Nelson and Sommers, 1996). N was determined by Kjeldahl digestion (Bremner and Mulvaney, 1982). P was determined according to Watanabe and Olsen (Watanabe and Olsen, 1965). Total metal content was determined using a Perkin Elmer 2280 atomic absorption spectrophotometer after sample extraction by digestion with concentrated HCl/HNO₃ following method 3051a (USEPA, 1997). Soil texture was determined following the methodology of Bouyoucos (1962). These analyses were performed in triplicate.

2.2. Pyrolysis of pig manure

Biochars were prepared as by Gascó et al. (2012) as follows: 100 g of pig manure were placed inside a covered steel cup and introduced in an electric furnace where the temperature was increased to 300 °C or 500 °C at a rate of 10 °C min⁻¹ and the final temperature was maintained for 1 h. As a result two biochar samples were obtained: pig manure biochar prepared at a temperature of 300 °C (BPC300) and pig manure biochar prepared at a temperature of 500 °C (BPC500).

2.3. Biochar characterization

Biochar samples were produced from a pig manure (PC) taken from a farm in Avila (Spain). All samples were air-dried, crushed and sieved through a 2 mm mesh prior to analysis.

The pH, CE, CIC, N Kjeldahl, total metal content and organic carbon oxidized were done as in section 2.1. The iodine number (mg I₂ g⁻¹), defined as the quantity of iodine adsorbed per gram of

activated carbon at an equilibrium concentration of 0.02 N was calculated according to D-4607 standard test method (ASTM, 1995). Proximate analysis was determined by thermogravimetry using a Labsys Setaram equipment. The sample was heated to a temperature of 600 °C under N₂ atmosphere and 30 °C min⁻¹ heating rate. Humidity was calculated as the weight loss from the initial temperature to 150 °C. Volatile matter (VM) was determined as the weight loss from 150 °C to 600 °C under N₂ atmosphere. At this temperature, air was introduced and fixed carbon (FC) was calculated as the weight produced when the final sample was burnt. The ashes were determined as the final weight of the samples (Cely et al., 2015; Gascó et al., 2012).

2.4. Treatments and soil respiration

The selected soil (S) was amended with the two biochar samples and with the pig manure (PC) at 8 wt% and mixtures were incubated at constant temperature (28 ± 2 °C) and humidity (60% water holding capacity) for 219 days. Treatments were named SPC (soil + pig slurry), SPC300 (soil + BPC300) and SPC500 (soil + BPC500).

Soil basal respiration (mg C kg⁻¹) was determined by static incubation as described previously (Paz-Ferreiro et al., 2012; Cely et al., 2014): each sample (100 g) was introduced into a 1 L airtight jar and the CO₂ produced during incubation was collected in 50 mL of a 0.3 NaOH solution, which was then titrated using 0.3 NHCl after precipitation of carbonates following BaCl₂ addition.

As a further experiment, it was studied if the application of the different amendments had an additive or synergistic effect in the soil (priming effect). In this way each sample (PC, BPC300 and BPC500) was incubated individually using the same experimental conditions. All treatments were performed in triplicate.

2.5. Determination of soil enzymes

Dehydrogenase, phosphomonoesterase, phosphodiesterase and β-glucosidase activities were determined following modifications of the original methods. While the amounts and concentrations of the substrates, buffers and other reagents were the same as in the original methods, different calibration curves were used for the control soil, soil amended with pig manure and soils amended with biochar. This correction has been described before (Paz-Ferreiro et al., 2009, 2012).

Dehydrogenase activity was determined by a modification of the method as reported by Camiña et al. (1998). The results were expressed as μmol idonitrotetrazolium formazan (INTF) g⁻¹ h⁻¹.

Phosphomonoesterase, phosphodiesterase and β-glucosidase were determined after incubating soils with a substrate containing a *p*-nitrophenyl moiety and then measuring the amount of *p*-nitrophenol released during enzymatic hydrolysis, using a spectrophotometer at a wavelength of 400 nm (Paz-Ferreiro et al., 2012). The activity of each of these three enzymes was expressed as μmol *p*-nitrophenol g⁻¹ h⁻¹.

Adsorption of the enzyme reaction product or substrate can occur in the surface of biochar, resulting in an inaccurate measurement of soil enzyme activity. Both products of the enzyme reactions (INT and *p*-nitrophenol) differ in their ability to be adsorbed in soil and in soil amended with biochar as reported in previous studies (Paz-Ferreiro et al., 2012). Thus, a different calibration curve was used for each treatment in order to obtain an accurate measure of enzyme activity. We also ensured that the reaction was not substrate limited.

Finally, the geometric mean (a general index to integrate information from variables that possess different units and range of

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