



# Manure biochar influence upon soil properties, phosphorus distribution and phosphatase activities: A microcosm incubation study



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## HIGHLIGHTS

- Manure biochar can be utilized as a soil amendment for P supply.
- Swine manure biochar enriched inorganic P such as orthophosphate and pyrophosphate.
- A novel solution P-31 NMR method was adopted to analyze P forms quantitatively.
- Manure biochar can improve soil properties and phosphomonoesterase activities.

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## ABSTRACT

Using manure-derived-biochar as an alternative phosphorus (P) source has bright future prospects to improve soil P status. A 98-day microcosm incubation experiment was set up for two soils which were amended with manure biochar at proportions of 0, 0.5% and 1.5%. Swine manure samples were air-dried and manure biochar was prepared by pyrolysis at 400 °C for 4 h. As determined by P-31 nuclear magnetic resonance (<sup>31</sup>P NMR) spectroscopy, manure biochar mainly increased the contents and fractions of orthophosphate and pyrophosphate in two soils, while decreased those of monoesters ( $P < 0.05$ ). At the end of incubation, 1.5% of manure biochar raised soil pH by 0.5 and 0.6 units, cation exchange capacity by 16.9% and 32.2%, and soil total P by 82.1% and 81.1% for silt loam and clay loam soils, respectively, as compared with those soils without biochar. Simultaneously, 1.5% of manure biochar decreased acid phosphomonoesterase activities by 18.6% and 34.0% for clay loam and silt loam, respectively; while it increased alkaline phosphomonoesterase activities by 28.5% and 95.1% for clay loam and silt loam, respectively. The enhancement of soil P availability after manure biochar addition was firstly due to the orthophosphate and pyrophosphate as the major P species in manure biochar which directly increased contents of soil inorganic P, and also attributed to the decomposition of some organic P like monoesters by enhanced alkaline phosphomonoesterase activities from manure biochar addition.

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## 1. Introduction

Biochar is a carbon-rich material derived from pyrolysis of biomass under limited or no oxygen atmosphere. Due to biochar's porous and recalcitrant characteristics, there is a growing interest in recent years in applying biochar as soil amendment to supply nutrients (Chan et al., 2008; Goloran et al., 2014). Biochar has positive effects on the soil ecosystem, which has been proposed to derive either directly from nutrients within biochar itself, or

indirectly from its ability to sorb and retain nutrients (Mukherjee and Zimmerman, 2013). Application of biochar derived from various biomass into soil has many advantages in terms of supplying soil nutrients, reducing soil remediation and mitigating greenhouse gas emissions. For example, compared with direct-amendment of sewage sludge into soil, addition of sewage-sludge-derived biochar can significantly reduce the bioaccumulation of polycyclic aromatic hydrocarbons and potentially toxic elements and potentially increase plant biomass (Khan et al., 2013; Waqas et al., 2014b). Combined application of hardwood-derived biochar with endophytes can improve soybean growth and enhance crop production under heavy metal stress

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(Waqas et al., 2014a). Most of these studies focused on the biochars made from common materials like wood residues or other agro-waste raw materials rather than manure. Manure biochar may contain more mineral nutrients than materials derived from wood (Gunes et al., 2014).

Phosphorus (P) is a macronutrient in soil and is essential to plant development. In the perspective of a progressive depletion of world reserves of P fertilizers, manure biochar, being more easily storable in time and space than manure, appears to be a valuable source of available P for crops (Marchetti and Castelli, 2013). Furthermore, manure biochar has a porous texture and abundant nutrients, and these properties make it pertinent to soil contaminant immobilization and soil fertility improvement (Tsai et al., 2012). Thus, biochar made from animal waste as an alternative P source has been suggested as a sustainable solution to the global problem of P scarcity (Vassilev et al., 2013). Technically, manure biochar addition enhances soil P availability mainly due to factors similar to other biochars: (1) it may increase soil pH and change the activity or availability of cations (i.e.,  $\text{Al}^{3+}$ ,  $\text{Fe}^{3+}$ , and  $\text{Ca}^{2+}$ ) that decrease P sorption or increase P desorption in soil (Xu et al., 2014); (2) it contains highly soluble P in itself that will directly increase the extractable P within the soil solution (Vassilev et al., 2013); (3) it can improve the soil micro-environment for phosphatase activities which play an important role in transforming organic P into inorganic P in soils (Neble et al., 2007).

Basically, the impact of biochar addition of soil P availability depends on its effect on the abundance of inorganic orthophosphate and pyrophosphate in soils and is also reflected by dominant phosphatase activities (i.e., acid and alkaline phosphomonoesterases). There is a large amount of researches which have discussed the effects of biochar addition on soil quality and productivities related to P availability. For example, Qian et al. (2013) studied the effects of environmental conditions on the release of P species from raw biochars and found that inorganic orthophosphate and pyrophosphate accounted for more than 50% of the total P released from biochar. Chintala et al. (2014) added a pine wood residue biochar to soils with neutral to high pH to enhance the soil P availability. In low fertility soils, addition of biochar often had a positive effect on P availability due to its ability to bind nutrient cations, to increase pH, and to improve soil texture, structure, and particle size distribution, although the effect is generally inconsistent (Xu et al., 2014). However, the direct and indirect influence of manure biochar on the P cycle in soils has been rarely reported (Paz-Ferreiro et al., 2012). Knowledge of the composition of various P species in biochar-amended soil is essential to understand soil P availability. To date, the influences of manure biochar addition on P species and phosphatase activities in soils are poorly understood, and whether it is safe or not to use manure biochar as a soil amendment is still an open question and worthy of clarification. There are some studies on the analysis of P availability in biochar by chemical methods (Wang et al., 2012; Uchimiya and Hiradate, 2014). In this study, we used P-31 nuclear magnetic resonance ( $^{31}\text{P}$  NMR) spectroscopy to gain insight on the variations of the composition of P species in soils amended with swine-manure biochar. To complement this information, alkaline and acid phosphomonoesterase activities in response to the addition were also determined by the hydrolysis methods. Our hypothesis was that manure biochar might have different influences upon soil physicochemical properties, P forms and phosphatase activities according to the addition rates and soil types. The objectives of this study were to investigate the composition variations of P species in two soils (silt loam and clay loam) with swine-manure biochar addition and also determine phosphomonoesterase activities in response to applied biochar.

## 2. Materials and methods

### 2.1. Soil samples and biochar preparation

A silt loam soil sample was collected from a mulberry field on the Huajiachi campus of Zhejiang University and a clay loam soil was collected from a rice paddy field under organic fertilization at Jiaying agricultural research station. The two sites are located in Taihu lake basin, southeast China, and both are in a subtropical monsoon climate. More than 20 kg topsoil (0–15 cm) was taken from ten points at each site, air-dried, ground to pass through 1-mm sieve and stored at 4 °C before use.

Fresh swine manure samples were air-dried for 3 days, lyophilized for 24 h to reduce the bound moisture content, and then ground to pass through 1-mm sieve. Manure biochar was produced in a vacuum tube furnace (OTF-1200X, Hefei Kejing Ltd., China) by pyrolyzing lyophilized manure biomass at 400 °C for 4 h under an  $\text{N}_2$  atmosphere at a flux of 60 mL  $\text{min}^{-1}$  to ensure oxygen-limited conditions. The pyrolysis temperature was raised at a rate of 20 °C  $\text{min}^{-1}$ . After fully drying in a desiccator for 2 h, the manure biochar was ground in an agate mortar to pass through a 0.15-mm sieve. The yield and ash contents of manure biochar were determined according to the analysis method of American society for Testing and Materials (ASTM) D1762-84. The C and N contents of manure biochar were measured on an elemental analyzer (Vario MAX CNS, Elementar, Germany). The pH of manure biochar in water was measured at 1:20 (w/v) ratio after gently shaking for 30 min. The cation exchange capacity (CEC) was measured after extraction with 1 M ammonium acetate (pH = 7). The anion exchange capacity (AEC) of soil and biochar was determined using the point of zero net charge method (Mukherjee et al., 2011).

### 2.2. Soil microcosm incubation

The air-dried soils were pre-incubated at 25 °C and 40% of water-holding capacity for 10 days in an incubator in the dark, in order to revive soil microbial activity. The experiment was conducted in 250 mL amber bottles containing 50 g soil and manure biochar incorporated at rates of 0 (CK), 0.5% ( $M_1$  treatment) and 1.5% ( $M_2$  treatment) by mass. The containers were covered with tinfoil, and kept aerated in the incubator at 25 °C for 3, 7, 14, 28, 42, 63, 84, 98 days. During the incubation period, soil moisture was kept constant at 40% by adding deionized water every 7 days based on weight loss.

### 2.3. Soil physicochemical and phosphatase analysis

Soil pH was measured at a soil to water ratio of 1:5 (w/v) with a pH meter. Soil CEC was determined according to the ammonium acetate method, suitable for neutral and acidic soils. Total soil C (TC) and total soil N (TN) were determined using an elemental analyzer (Vario MAX CNS, Elementar, Germany). Soil organic C (SOC) was determined by a colorimetric method based on 105 °C digestion with  $\text{K}_2\text{Cr}_2\text{O}_7\text{--H}_2\text{SO}_4$ . Total soil P (TP) was determined by the molybdenum blue method after digestion with  $\text{H}_2\text{SO}_4\text{--HClO}_4$  at 300 °C for 2 h. Olsen-P was extracted by 0.5 M sodium bicarbonate and determined by the molybdenum blue method. Soil available K was digested with NaOH and measured using ICP-AES (6300, Thermo scientific).

The activities of acid and alkaline phosphatases were determined using a modification of the methods of (Acosta-Martinez and Tabatabai, 2000). Specifically, for phosphomonoesterase activities analysis, 1 g moist soil was mixed with 4 mL of universal buffer (pH 6.5 for acid phosphomonoesterase and pH 11 for

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