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Phosphomonoesterase Activities, Kinetics and Thermodynamics in a Paddy Soil After Receiving Swine Manure for Six Years

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ABSTRACT

Soil phosphomonoesterase plays a critical role in controlling phosphorus (P) cycling for crop nutrition, especially in P-deficient soils. A 6-year field experiment was conducted to evaluate soil phosphomonoesterase activities, kinetics and thermodynamics during rice growth stages after consistent swine manure application, to understand the impacts of swine manure amendment rates on soil chemical and enzymatic properties, and to investigate the correlations between soil enzymatic and chemical variables. The experiment was set out in a randomized complete block design with three replicates and five treatments including three swine manure rates (26, 39, and 52 kg P ha^{-1}, representing low, middle, and high application rates, respectively) and two controls (no-fertilizer and superphosphate at 26 kg P ha^{-1}). The results indicated that the grain yield and soil chemical properties were significantly improved with the application of P-based swine manure from 0 to 39 kg P ha^{-1}; however, the differences between the 39 (M_{39}) and 52 kg P ha^{-1} treatments (M_{52}) were not significant. The enzymatic property analysis indicated that acid phosphomonoesterase was the predominant phosphomonoesterase in the tested soil. The M_{39} and M_{52} treatments had relatively high initial velocity (V_0), maximal velocity (V_{max}), and activation grade (lgN_a) but low Michaelis constant (K_m), temperature coefficient (Q_{10}), activation energy (E_a), and activation enthalpy (ΔH), implying that the M_{39} and M_{52} treatments could stimulate the enzyme-catalyzed reactions more easily than all other treatments. The correlation analysis showed that the distribution of soil phosphomonoesterase activities mainly followed the distributions of total C and total N. Based on these results, 39 kg P ha^{-1} could be recommended as the most appropriate rate of swine manure amendment.

Key Words: activation energy, activation enthalpy, enzyme-catalyzed reaction, maximal velocity, Michaelis constant

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INTRODUCTION

Phosphorus (P) is a limiting nutrient to sustain crop yields in most agro-ecological zones over the world (Khan and Joergensen, 2009; Ramaekers *et al.*, 2010) since some of them are bound in the detritus as organic P and others are bound with Ca^{2+}, Fe^{3+}, and Al^{3+}, remaining inaccessible to plants (Miller and Fox, 2011; Huang *et al.*, 2012). Farmers usually add organic manures and mineral P fertilizers to remedy P deficiency (Ayaga *et al.*, 2006; Malik *et al.*, 2012). Mineral P fertilizer provides available P for plants growth but does not contribute to the improvement of soil physical conditions. Organic manure application can improve soil chemical and biological properties (Liu *et al.*, 2013). Swine manure is a relatively inexpensive form of organic manure and has been one of the most commonly applied fertilizers in organic rice production in developed agricultural regions such as the Taihu Lake region of southeastern China. Actually, the lower cost of production make organic paddies more profitable than conventional mineral fertilizer paddies (Liang *et al.*, 2013).

Swine manure presents management challenges due to its inconsistent nutrient content and rate of release (Guo *et al.*, 2004; Liang *et al.*, 2013). Generally, swine manure contains a relatively high total P content but the P availability is quite low in manure applied rice fields. Long-term high application rate of swine manure can lead to the increase of P precipitation in topsoil and P loss from farmland runoff to rivers and streams (Koopmans *et al.*, 2007; Xavier *et al.*, 2009).

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A large part of P has been found in organic P such as phosphoinositide, phosphorus esters and nucleotides in organic matter-amended soils (Nèble *et al.*, 2007). However, microorganisms and plants can only assimilate mineral orthophosphate P in the soil (Rao *et al.*, 1996). Most of organic P must be hydrolyzed into effective P forms *via* phosphatase which mostly originates from soil microorganisms and plant root exudates (Criquet and Braud, 2007; Saha *et al.*, 2008; Albrecht *et al.*, 2010). Soil phosphatase plays a pivotal role in the overall process of mineralizing organic P. Phosphomonoesterases are considered as the predominant phosphatases in most soils and are divided into acid phosphomonoesterase (ACP) (pH 4–6) and alkaline phosphomonoesterase (ALP) (pH 8–10) according to their pH optima in the enzymatic reactions (Tabatabai, 1994; Lu, 2000; Criquet *et al.*, 2004; Kunito *et al.*, 2012).

Soil phosphomonoesterase is involved in soil P cycling and catalyzes the conversion of soil P from unavailable to available forms. Therefore, it is imperative to discover the mechanism of swine manure effect on soil phosphomonoesterase activities and kinetics in the process of organic P transformation. A lot of studies were focused on soil phosphomonoesterase activity changes in a single sampling date and ignored the dynamics of phosphomonoesterase relative to other soil chemical factors over time. Previous researches have pointed out soil phosphomonoesterase activity was directly and indirectly affected by substrate concentration, reactive time and temperature (Koch *et al.*, 2007; Wallenstein *et al.*, 2009; Stone *et al.*, 2012). The paddy soils were sampled during rice growth stages to study the kinetic and thermodynamic properties of phosphomonoesterase by measuring *p*-nitrophenol released in reactions when the substrate concentration, reactive time and temperature changed, so as to figure out the kinetic and thermodynamic parameters. Soil phosphomonoesterase kinetic parameter $V_{\max}$ indicates the maximal velocity of enzyme-substrate complexes decomposing into enzyme and reaction products and parameter Michaelis constant K_{m} reflects the binding affinity between enzyme and substrate (German *et al.*, 2011). Thermodynamic parameter Q_{10} (temperature coefficient) is the factor by which a biological process changes in response to a 10 °C temperature increase. Activation energy (E_{a}) is the energy differential between reactants and transitional substances that subsequently decompose into products. The value of activation enthalpy (ΔH) is related to the events necessary to the formation of transition state. Activation grade (N_{a}) is used to describe the activation level of substance (per mole) transformed into the activated state in the enzymatic reaction.

Our primary objectives were i) to evaluate soil phosphomonoesterase activities, kinetics and thermodynamics during the rice growth stages after 6 years of consistent swine manure applications, ii) to understand the impacts of swine manure amendment rates on soil chemical and enzymatic properties, and iii) to investigate the correlations between soil enzymatic and chemical variables.

MATERIALS AND METHODS

Experiment

The study was carried out at the experimental farm of ShuangQiao, located in Jiaxing City (120°40′ E, 30°50′ N) in the Taihu watershed, Zhejiang Province of China. It has the typical characteristics of subtropical monsoon climate. The mean annual temperature and rainfall were 15.7 °C and 1 200 mm, respectively. The soil type is gleyed paddy soil (clay loam), which is the typical soil in the Taihu Lake region of Southeast China.

The experimental site was established in 2005. The cropping system was rice (*Oryza sativa* L.)-oilseed rape (*Brassica napus* L.) rotation pattern. Generally, the rice season was from June to November and the oilseed rape season from November to May of the following year. Fifteen plots in dimension 4 m × 5 m were laid out two parallel rows and barriers were constructed between them with concrete. Five fertilization treatments in a randomized complete block design with three replicates consisted of: no-fertilizer control (P_0), 26 kg P ha^{-1} used as the conventional fertilizer superphosphate (12% P_2O_5) (P_{26}), and 26 (low), 39 (middle), and 52 (high) kg P ha^{-1} used as different P-based swine manure (M_{26}, M_{39}, and M_{52}, respectively). The swine manure contained organic matter 150 g kg^{-1}, N 5.6 g kg^{-1}, P 4.3 g kg^{-1}, and K 4.0 g kg^{-1}. The superphosphate and swine manure were applied as basal fertilizer in June and the urea fertilizer as topdressing in July and August. Generally, a typical rice growth stage includes before plowing (BP), seedling stage (SS), tillering stage (TS), heading stage (HS) and maturity stage (MS).

Soil surface layer (0–20 cm) samples were collected from a 6-year field over rice growth stages. Fifteen soil cores were taken randomly from each plot and pooled together as a composite sample. After sampling, the visible leaves and roots were removed from the fresh soil samples and then the samples were stored in refrigerator at 4 °C for enzymatic analysis. Half of the