



Short-term effects of byproduct amendments and lime on physicochemical and microbiological properties of acidic soil from Jiaodong Peninsula of China

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ABSTRACT: *The influences of byproduct amendments, containing silicon, calcium, magnesium and potassium, on acidic soil quality in Jiaodong Peninsula of China had been studied and compared with that of lime through monitoring physicochemical properties and enzyme activities of acidic soil over a 120-day period. Byproduct amendments (1125, 2250, 4500 and 9000 kg ha⁻¹) and lime 2250 kg ha⁻¹ was applied in the acidic soil. Results showed that both byproduct amendments and lime significantly increased the pH, EC and enzyme activities of soil. The by-product amendments inhibited microbial biomass carbon and soil respiration. Nevertheless, the lime-treated soil had a much more higher level of CO₂ emission than the by-product amendments-treated soil. Compared to the by-product amendments-treated soil, the lime-treated soil had the higher pH, peroxidase activity, phenol oxidase activity and invertase activity. Therefore, lime might be a better choice over by-product amendments to improve chemical and biological properties of the acidic soil in Jiaodong Peninsula of China. For soils lacking available calcium and magnesium, the mixture of 4500 kg ha⁻¹ amendments and 2250 kg ha⁻¹ lime was recommend to treat the soil.*

Key words: soil amendments, soil pH, soil respiration, enzyme activity.

Efeitos de alterações de subprodutos e cal em solo ácido da Península de Jiaodong da China

RESUMO: *As influências do emprego de subprodutos que contêm silício, cálcio, magnésio e potássio, sobre a qualidade do solo ácido na Península de Jiaodong da China, foram estudadas e comparadas com a da cal através do monitoramento das propriedades físico-químicas e atividades enzimáticas do solo ácido ao longo de um 120- período do dia. Os resultados mostraram que as alterações dos subprodutos e a cal aumentaram significativamente as atividades de pH, CE e enzimas do solo. As alterações dos subprodutos obviamente inibiram o carbono da biomassa microbiana e a respiração do solo, mas o solo tratado com cal apresentou um maior nível de emissão de CO₂. Em comparação com as modificações do subproduto, o solo tratado tinha o maior pH, a atividade da peroxidase, a atividade da fenol oxidase e a atividade da invertase. Portanto, o cal pode ser uma escolha melhor em relação às alterações dos subprodutos para melhorar as propriedades químicas e biológicas do solo ácido na Península de Jiaodong da China.*

Palavras-chave: emprego de subproduto, cal, solo ácido, pH, atividade.

INTRODUCTION

Soil acidity could reduce crop yield, pollute water and degrade forest, and further impede global sustainable development (CREGAN & SCOTT, 1998). Jiaodong Peninsula in China, which is located in the so-called “fruit belt” of North Latitude 36 of the world, has a long tradition of intensive horticultural crop production. In recent decades, its soil acidification is severe according to a preliminary investigation in 2007-2009. The topsoil pH in 60.4% of the 268 investigated orchard

sites was less than 5.5 (LI et al., 2014). Because acid deposition and excessive application of nitrogen fertilizers decrease the soil pH, and fruit trees also take out a great deal of cations from soils in each growing season (TANG et al., 2000), after a continuous period of time, soils may become more and more acidic. Subsequently, soil acidification may increase the bioavailability of metals and worsen their contamination, thus to limit the growth of horticultural crops. Therefore, it is necessary to improve the acidity soil, including increasing soil pH and ameliorating soil physicochemical properties, of

orchard at Jiaodong Peninsula in China to optimize the fruit growth and quality.

Traditionally, lime and gypsum have been applied to improve surface acidity of acidic soils. However, their applications have been limited due to their high cost in Jiaodong Peninsula of China and very limited ability to increase the amount of K, P and other nutrient elements (SUN et al., 2000). Recently, scientists turn their attention to industrial byproducts for ameliorating acidic soil (LI et al., 2010), because, except of alkalis, industrial byproducts also contain a large amount of K, Ca, Mg and other nutrient elements (GARRIDO et al., 2003; TRAN et al., 2015). For example, alkaline slag and sugar foam had been used to raise soil pH, precipitate Al^{3+} and increase concentration of exchangeable Ca^{2+} and Mg^{2+} (GARRIDO et al., 2003; LI et al., 2010). In China, alkaline byproducts, a mixture of low-grade phosphate rocks, insoluble potassium-containing rock and coke powder, have been widely used as amendments to improve acidic soils for decades (ZHANG et al., 2015). However, their potential positive and negative impacts on the environment have not been studied.

The amendment was byproduct of nitro-compound fertilizer through calcination of potassium feldspar and limestone at a high temperature. The amendment has a high pH value with high-concentration of Ca, Mg, K, Si and P, and trace amount of heavy metals. Therefore, the alkaline byproduct amendment could potentially be used to ameliorate soil acidity and improve soil nutrient levels. Although, there are some reported studies on the effects of the amendments on the chemical and physical properties of acidic soils (ZHANG et al., 2015; CHEN et al., 2015; YU et al., 2015), little attention has been paid to their effects on soil microbial processes and soil enzyme activities. Therefore, in this study, experiments were designed and conducted to investigate the effects of byproduct amendments on the physicochemical properties and microbial activities of acidic soil, which was further compared with that of lime. Specific objectives were (1) to determine both positive and negative impacts of byproduct amendment and lime on the environment in short term, and (2) to determine proper amount of byproduct amendment and lime amendment in acidic soil amelioration. Therefore, two incubation experiments were conducted to investigate the impact of byproduct amendment and lime addition on changes in physicochemical properties of acidic soil and changes in inorganic N content, soil respiration, microbial biomass and enzyme activities.

MATERIALS AND METHODS

Lime was from Guoyao Shiji in China. The byproduct amendments, which was a mixture of low-grade phosphate rocks, insoluble potassium containing rocks and coke powder, was from Kingenta Ecological Engineering Group Co. It was crushed within a spinning hammer mill to particle size of <2 mm in diameter. pH value (8.80) and chemical compositions of byproduct amendments were listed in table 1.

Soil samples

Soil samples were randomly collected from different locations at depth of 0-20 cm of the orchards in Qixia, Northeast of Jiaodong Peninsula of China. These fresh soil samples were passed through a 2 mm Nylon sieve before use. Table 2 lists the basic chemical parameters of the studied soil samples. Recently, due to the insufficient input of organic fertilizer and the extensively application of nitrogen-based quick-acting fertilizer, organic matter in some orchards is seriously deficient. At the same time, exchangeable calcium and exchangeable magnesium in soil are also relatively insufficient.

Soil treatment with the byproduct amendments

One air-dried soil sample (150 g) with or without byproduct amendments was mixed with deionized water in a 200 mL plastic container to reach 40% of the soil holding capacity. The container was covered with plastic film with 10 holes to allow gas exchange to maintain moisture. The sample was incubated at 25 °C for a week to reduce variation in microbial activity. All 144 soil samples were assigned into six groups, including blank samples without byproduct amendments (CK), samples with 0.075 g (equivalent to approximately 1125 kg ha⁻¹ amendments distributed in 20 cm of topsoil at the field) of byproduct amendments

Table 1 - Chemical composition of the amendment.

----Macro-components----		-----Micro-components-----	
C	12.3 g kg ⁻¹	F	5 g kg ⁻¹
P ₂ O ₅	13.4 g kg ⁻¹	H	950 mg kg ⁻¹
K ₂ O	35.3 g kg ⁻¹	As	7.77 mg kg ⁻¹
MgO	34.5 g kg ⁻¹	Pb	18.60 mg kg ⁻¹
SiO ₂	223.8 g kg ⁻¹	Cr	56.8 mg kg ⁻¹
CaO	374.3 g kg ⁻¹	Cd	0.063 mg kg ⁻¹
Fe ₂ O ₃	23.1 g kg ⁻¹	Hg	0.01 mg kg ⁻¹

Table 2 - Chemical parameters of the studied Brown soil.

pH	6.05	Exchangeable Ca	9.53 cmolc kg ⁻¹
Organic matter	1.36 %	Exchangeable Mg	2.53 cmolc kg ⁻¹
NH ₄ ⁺ -N	48.78 mg kg ⁻¹	Exchangeable Al	0.14 cmolc kg ⁻¹
NO ₃ ⁻ -N	84.20 mg kg ⁻¹	Base saturation	68 %

(75T), samples with 0.15 g (i.e. 2250 kg ha⁻¹) of byproduct amendments (150T), samples with 0.3 g (i.e. 4500 kg ha⁻¹) of byproduct amendments (300T), samples with 0.6 g (i.e. 9000 kg ha⁻¹) of byproduct amendments (600T) and samples with 0.15 g (i.e. 2250 kg ha⁻¹) of lime (150CaO).

Soil incubation experiments

Soil moisture content was maintained at 60% of the soil holding capacity during the experiment through injecting a certain amount of water every four days. Two sets of incubation studies were conducted. One set was used to study changes in physicochemical properties and the other set was used to study soil respiration. Generally, during soil samples were incubated at 25 °C, their enzyme activities, mineral nitrogen, microbial biomass, pH and electronic conductivity (EC) were measured on Day 0, 1, 3, 7, 15, 30, 60 and 120 with 4 replicates at each time point. A total of 24 soil samples in the four groups were incubated at 25 °C and CO₂ emission from each sample was measured at day 0, 1, 2, 5, 10, 30, 60 and 120 of incubation with 4 replicates at each time point.

Analysis methods

Characterization of soil physicochemical properties

After the fresh incubated soil sample was mixed with water at 1:2.5 ratio (w/v, soil to water) and shaken for an hour, its pH and EC were determined on a pH meter (METTLER TOLEDO S210-K pH meter, USA) and an electronic conductivity meter (DDSJ-308A, Shanghai Leici, China), respectively.

Analysis on soil enzyme activities

Enzyme activities of soil sample were determined with a 144-well microplate approach as reported (ŠNAJDR et al., 2008). Activities of phenol oxidase were measured with pyrogalllic acid as the reductive substrate (PERUCCI et al., 2000; VEPSALAINEN et al., 2001). Optical density was measured at 525 nm.

Peroxidase activities were measured with 3,3',5,5'-tetra methyl benzidine (TMB) as the reductive substrate (JOHNSEN & JACOBSEN, 2008). Optical density was measured at 450 nm.

Soil enzyme activity is expressed as μmol of product per hour per g of soil. After the soil was incubated with 15 mL of 8% sucrose solution and 5 mL of phosphate buffer (pH 5.5) at 37 °C for 24 h, invertase activity (mg glucose g⁻¹ 24 h⁻¹) was measured with glucose as the reducing sugar through colorimetry at 510 nm with 3,5-dinitrosalicylic acid (GUAN et al., 1986).

Analysis on microbial biomass

Microbial biomass was determined with chloroform fumigation extraction method (VANCE et al., 1987). Typically, after 24 hours of fumigation with chloroform, soil samples with and without fumigation were extracted with 0.5 mol L⁻¹ K₂SO₄ solution at 1:2 soil: solution ratio. The microbial biomass C (MBC) and microbial biomass N (MBN) were determined on an organic carbon analyzer (Vario TOC, ELEMENTAR, Germany) through ninhydrin reaction as reported (JOERGENSEN & BROOKES, 1990).

Analysis on soil respiration

Soil respiration was determined with gas chromatography (Agilent 7890A, USA). Before and after CO₂ was trapped in the tightly rubber-plugged container for 24 hours at 25 °C, 40 mL of gas sample was collected with a syringe and injected into the gas analyzer. Difference between the final CO₂ content and the initial CO₂ content was calculated as the CO₂ emission.

Statistical analysis

Duncan test was applied to compare the difference among different treatments. A *p* value less than 0.05 was considered as a statistical significance. Kinetic modeling of the experiment data was performed with Origin Pro 8.0.

RESULTS AND DISCUSSION

Effect of amendments on soil physicochemical properties

The pH of the soil solution is very important to determine the chemical environment of plants (BOLAN et al., 2003) because any component of the soil or of its biological inhabitants is very sensitive to soil pH. The pH of soil without amendments is 6.05. As shown in Figure 1a, compared to pH of

soil treated with CK, the pH of soil was increased with the addition of byproduct amendments or the increase of incubation time, and the maximum value was observed in soil sample treated with 0.15 g of lime (150CaO). However, in the late stage of incubation period, the pH of soils with all amendments treatments was reduced significantly. The pH of soil with maximum of byproduct amendments (600T) increased from 6.05 to 6.69, but decreased to 6.32 after 120 days incubation. The pH of soil sample 150CaO increased sharply to 7.82 and significantly dropped to 6.53. Results indicated that both amendments could increase soil pH and the effect of lime was much larger. Biochemical processes associated with soil pH change are mainly as follows: the release of alkalinity, ammonification of organic nitrogen, nitrification of mineralized nitrogen, and cation-exchange reactions between cations and exchangeable acidity (MOKOLOBATE & HAYNES, 2002; WANG et al., 2009; ZHAO & XING, 2009). The alkali neutralization capacity could be the predominant factor for soil pH change, especially the soil with lime. The byproduct amendments contained large amount of cations, such as Ca^{2+} , Mg^{2+} and K^+ , with anions, such as SO_4^{2-} , F^- and SiO_4^{2-} . The cations can induce the release of H^+ and Al^{3+} and these anions can release OH^- due to the ligand exchange. Therefore, the soil pH with byproduct amendment was increased slightly until day 7 and decreased at day 15.

As shown in figure 1b, compared to CK treatment, byproduct amendments increased both pH and EC of soil, which might have negative effects on soil structural stability, bulk density and

permeability (TEJADA & GONZALEZ, 2005). Application of amendments increased the pH of soils and the variation tendency of soil EC was consistent with pH. Soil EC was significantly influenced by applied soil amendments. The maximum values were observed in the soil sample 150CaO at the beginning of the experiment. However, soil EC values of 150CaO, 75T and 150T dropped quickly after 60 days of incubation.

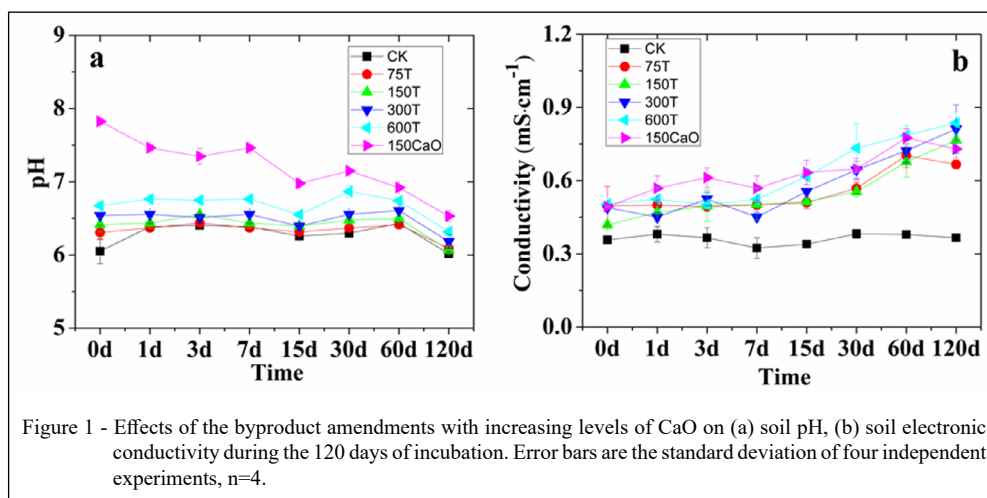
Effect of amendments on microbial biomass

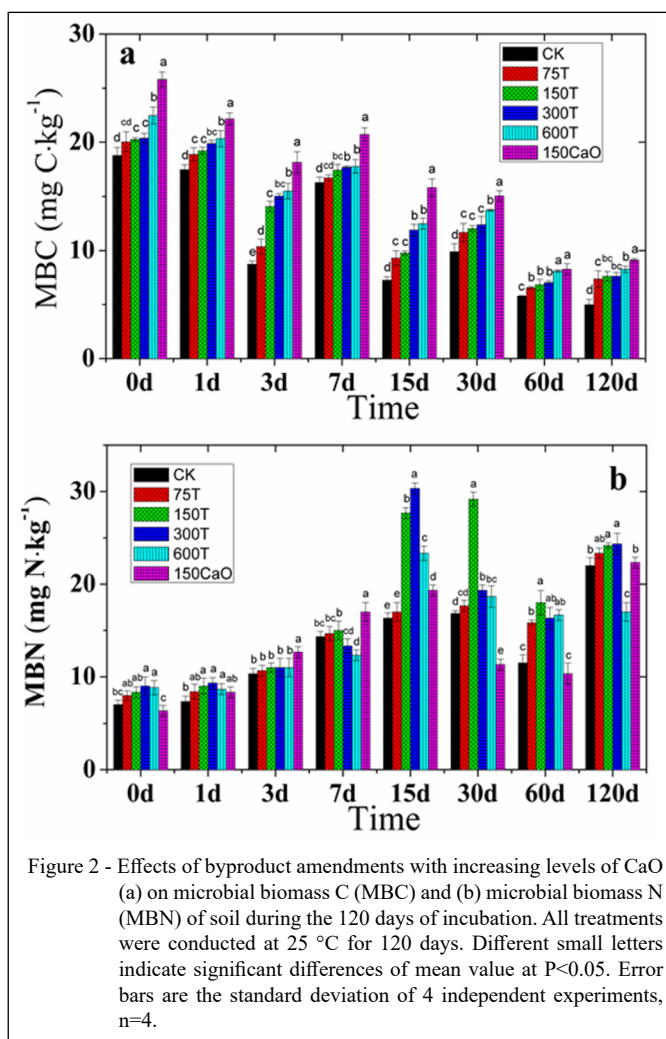
Microbial biomass carbon (MBC) of soils treated with byproduct amendments or lime was increased due to the increase of EC, compared to that of control soils without amendment. As shown in figure 2a, the 150CaO sample had the maximum content of MBC. And the MBC decreased along the time, indicating that the concentration of dissolved ions was increased during incubation (SARIG & STEINBERGER, 1994).

Similarly, both byproduct amendments and lime significantly influenced microbial biomass nitrogen (MBN) of soil samples; although, effects of byproduct amendments were larger than that of lime (Figure 2b). And soil samples 150T and 300T had the highest amount of MBN than others.

Soil respiration

Soil microbial respiration is a direct indicator of microbial activity and indirect indicator of the availability of organic materials (TEJADA et al., 2006). And the most common method to evaluate microbial activity of soil is based on the measurement of CO_2 evolved from soil as a consequence of





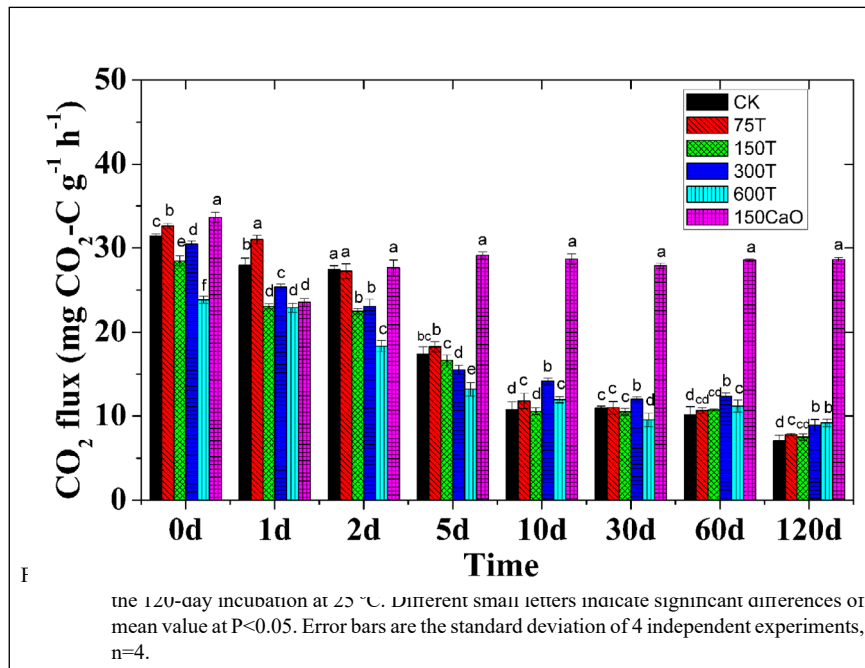
microbial respiration (SÁNCHEZ-MONEDERO et al., 2008). The CO_2 emission of all soil samples decreased gradually during the whole incubation period (Figure 3). Because CO_2 emission of soils was relatively low in alkaline amendments with high EC and high pH (KOWALENKO et al., 1978; RAO & PATHAK, 1996), compared soil sample treated with CK, soil sample treated with more byproduct amendments had less CO_2 emission. However, the soil 150CaO always had the largest amount of MBC and the maximum CO_2 emission during the 120-day experiment, probably because CaO could react with water and dry the soil, which could accelerate the release of CO_2 .

Soil enzyme activities

Enzyme activities are sensitive indicators of ecological stress. For example, peroxidase (POD),

an oxidoreductase, can reduce oxygen to H_2O_2 while oxidizing hydroxyls to carbonyls. In the soil, the generated soil peroxidase accelerates the degradation of humic materials and thus leads to more CO_2 release (DEC et al., 2003). And phenol oxidase (SPPO) can catalyze the oxidation of aromatic materials such as humic acids (FLOCH et al., 2007). Although, activities of these soil oxidative enzymes are independent of soil carbon content, they are sensitive to soil pH and other factors (SINSABAUGH et al., 2008; FLOCH et al., 2009).

As shown in figure 4a, compared soil sample treated with CK, soil sample 150CaO always had the highest peroxidase activity and CO_2 emission over the 30-day incubation period compared to other sample treated with byproduct amendments. And there was no significant difference among effects of byproduct amendments amount on the peroxidase activity.



As shown in figure 4b, the phenol oxidase (SPPO) activities of all samples were increased within 30 days of incubation and were then decreased, and sample 150CaO also always had the highest SPPO activity over the 30-day incubation period at 25 °C (Figure 4b), which was in accordance with the tendency of soil pH. These results further proved that phenol oxidase activity and stability of soil were correlated positively with its pH (SINSABAUGH, 2010).

Since the main energy of soil microorganisms is from sucrose and free simple sugars that should be hydrolyzed and broken down with the enzyme invertase (S-SC) (FRANKENBERGER & JOHANSON, 1983), invertase is more efficient than other enzymes to reflect soil fertility and biological activity. As shown in figure 4c, the soil S-SC activity was significantly increased with all amendments during the incubation period, and sample 150CaO always showed the highest S-SC activity compared to other samples over the 30-day incubation period at 25 °C. Additionally, the S-SC activity was increased with the increase of byproduct amendments. These results were in accordance with the tendency of soil pH in all soil samples, which further proved the S-SC activity and stability of soil were influenced significantly by its pH and ionic strength (GUAN, 1986; GIANFREDA & BOLLAG, 1996;

ACOSTA-MARTINEZ et al., 2000; EKENLER & TABATABAI, 2003).

CONCLUSION

Both byproduct amendments and lime significantly increased pH, EC and enzyme activities of the soil. The byproduct amendments inhibited microbial biomass carbon and soil respiration, but the lime-treated soil had a much more higher level of CO₂ emission than the byproduct amendments-treated soil. The soil treated with lime had the highest levels of pH, peroxidase activity, phenol oxidase activity and invertase activity among all soil samples. Therefore, lime might be a better short-term choice over byproduct amendments to improve the chemical and biological properties of the acidic soil in Jiaodong Peninsula of China. Byproduct amendments not only increased soil pH but also contained plentiful cations and anions, such as Ca, Mg, Si and P. Plentiful Ca and Mg in the byproduct can alleviate Al and Mn toxicity to plants and microorganisms by competitive adsorption. Consequently, the use of byproduct as amendments for acidic soil has some great potential as the traditional used lime in short time; although, its advantages are not dominant. Therefore, soil treated with 4500 kg ha⁻¹ byproduct amendments (300T) mixed with 2250 kg ha⁻¹ lime (150 CaO)

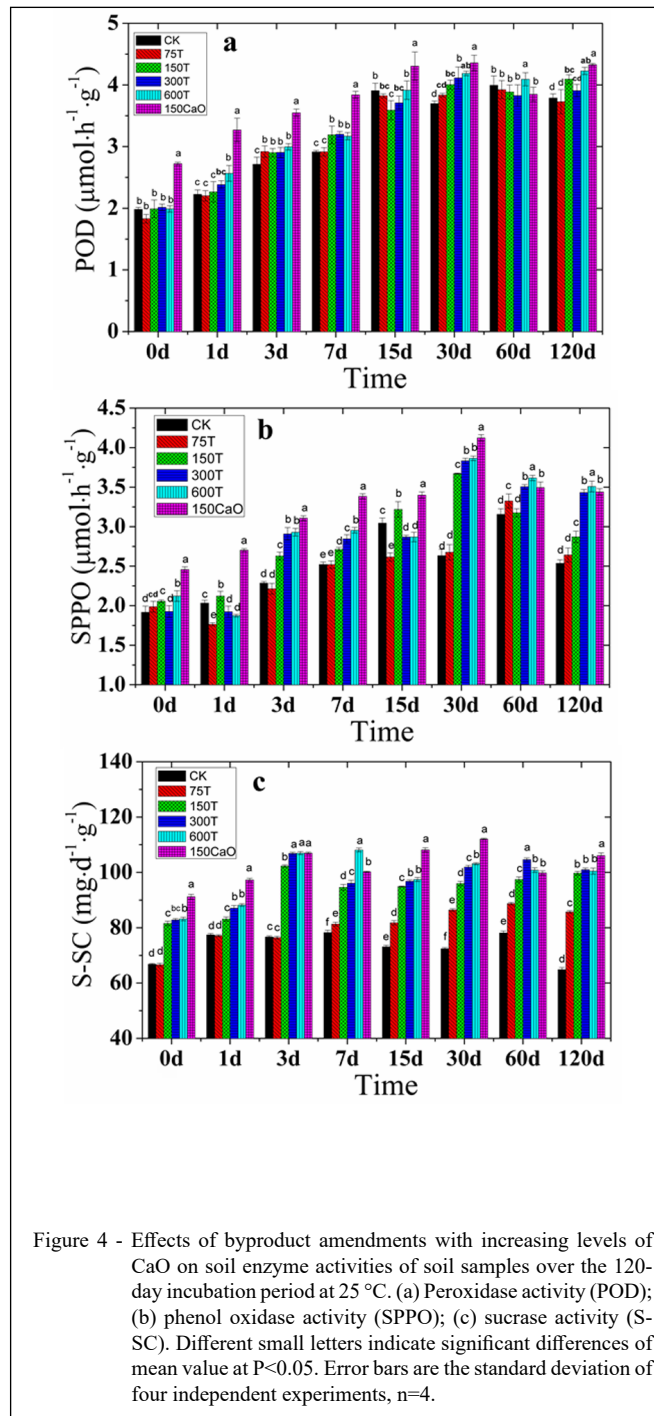


Figure 4 - Effects of byproduct amendments with increasing levels of CaO on soil enzyme activities of soil samples over the 120-day incubation period at 25 °C. (a) Peroxidase activity (POD); (b) phenol oxidase activity (SPPO); (c) sucrose activity (S-SC). Different small letters indicate significant differences of mean value at P<0.05. Error bars are the standard deviation of four independent experiments, n=4.

was recommend applied in acidic soil of Jiaodong Peninsula of China.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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