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Review

Hydrolytic soil enzymes and their response to fertilization: a short review

Angélica Bautista-Cruz*, Yolanda Donají Ortiz-Hernández

National Polytechnic Institute, Oaxaca, México
*Correponding author, e-mail: mbautistac@ipn.mx

Abstract

Enzymes are proteins that catalyze chemical reactions in living systems, transforming specific substrates into the products needed in biological cycles and for many edaphic processes. Soil enzymatic activities have been proposed as soil quality indicators, due to their relation with soil biology. Although the long-term effects of organic and mineral fertilization on physical and chemical soil properties have been previously studied, little is known about their effects on microbial community structure, microbial biomass carbon, microbial activity and enzymatic activity. Some studies report that organic and mineral fertilizers can affect, be it positively or negatively, microbial biomass size as well as soil microbial activity. This work examines the effect of fertilization on the enzymatic activity of soil hydrolases.

Keywords: hydrolases, mineral fertilizers, organic fertilizers, soil

Enzimas hidrolíticas do solo e sua resposta à adubação: uma breve revisão

Resumo

Enzimas são proteínas que catalisam reações químicas em sistemas vivos, transformando substratos específicos para os produtos necessários em ciclos biológicos e para muitos processos edáficos. Atividades enzimáticas do solo têm sido propostos como indicadores de qualidade do solo, devido à sua relação com a biologia do solo. Embora os efeitos a longo prazo da adubação orgânica e mineral sobre as propriedades físicas e químicas do solo, foram previamente estudados, pouco se sabe sobre os seus efeitos sobre a estrutura microbiana, o carbono da biomassa microbiana, atividade microbiana e atividade enzimática. Alguns estudos relatam que os fertilizantes orgânicos e minerais pode afetar, seja ela positiva ou negativamente, o tamanho da biomassa microbiana, bem como a atividade microbiana do solo. Este trabalho examina o efeito da adubação sobre a actividade hidrolítica enzimática de solo.

Palavras-chave: fertilizantes minerais, fertilizantes orgânicos, hidrolases, solo

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Introduction

Enzymes are proteins whose function is to catalyze chemical reactions in living systems (Karigar & Rao, 2011). They act on specific substrates, transforming them into products necessary for biological cycles, and participate in many edaphic processes such as organic residue decomposition, humic substance synthesis, nitrification, oxidation, xenobiotic degradation and nitrogen fixation, among others (Cerón-Rincón & Melgarejo-Muñoz, 2005; Kumar & Varma, 2011). Enzymes are also related to ecological functions such as biomass production, contaminated soil recovery and ecosystem conservation (Cerón-Rincón & Melgarejo-Muñoz, 2005).

Hydrolase, transferase, oxidoreductase and lyase enzyme activity, all of which participate directly in the C, N, P and S cycles, has been detected in soil (Cerón-Rincón & Melgarejo-Muñoz, 2005). Due to their function, oxidoreductases (in particular dehydrogenases, catalases and peroxidase) and hydrolases (phosphatases, proteases and ureases) are the soil enzymes most studied. Organisms and plants release enzymes into the soil by secretion and cellular lysis upon death; a low percentage of these proteins remain immobilized and stabilized in interaction with different components of the soil solid phase, such as clay, organic molecules and organo-mineral complexes (Joinville et al., 2004). Depending on the components of the soil solid phase, this interaction is caused by mechanisms that include microencapsulation, transversal bonds, copolymer formation, adsorption, capture, ion exchange and covalent bonds (Dick & Tabatabai, 1992). Only a low percentage of the enzymes leaving the cells are stabilized, as they are released into an inhospitable environment where they can be subjected to non-biological denaturalization, adsorption or protease degradation (Cerón-Rincón & Melgarejo-Muñoz, 2005). The highest production of extracellular enzymes is attributed to microorganisms, due to their high metabolic activity and short life cycle, in contrast with other organisms that also release enzymes, such as plants and animals (Aşkın & Kızılkaya, 2006). High correlations have been found between soil microbial activity and enzymatic activity (Alvear et al., 2005, Reyes et al., 2011). Enzymes can also originate from organic matter applied to soil. Nutrients from organic fertilizers are released by microbial metabolism, thus making them available to the plants. In contrast, nutrients in mineral fertilizers can be directly acquired by the plant. The application of organic and mineral fertilizers to soil increases the nutrient content, microbial activity, enzymatic activity, humic fraction, soil structure and the ion exchange system (Okur et al., 2009).

Although the long-term effects of organic and mineral fertilization on physical and chemical soil properties has been previously studied, little is known about the effects on the microbial community structure, microbial biomass carbon, microbial activity and enzymatic activity (Böhme et al., 2005). Some studies have reported that organic and mineral fertilizers can have an effect, be it positive or negative, on the size of microbial biomass and soil microbial activity (Treseder, 2008; Okur et al., 2009; Beauregard et al., 2010). This work examines the effect of fertilization on the enzymatic activity of soil hydrolases.

Factors that affect soil enzymatic activity

Changes in the physical, chemical and biological composition of soil can affect enzymatic activity (Gianfreda & Ruggiero, 2006). Other factors that can influence soil enzymatic activity are the type of crop and anthropogenic activities such as management practices, contaminating events and the application of agro-chemicals such as fertilizers, pesticides, herbicides, etc. (Alvear et al., 2006; Balezentiene & Klimas, 2009). The expression and conservation of soil enzymatic activity can also be modified naturally by environmental factors such as seasonal changes, geographical location and thermal and hydric soil regimes (Gianfreda & Ruggiero, 2006).

Another important aspect of the complexity of soil enzymatic activity is its *in situ* distribution; for example, its spatial variability in a soil profile and its location in soil structural fractions. In general, enzymatic activity decreases with soil depth, due to the decline of microbial activity and certain macronutrients such as organic C and organic N (Gianfreda & Ruggiero, 2006).

Complexity in the quantification of soil enzymatic activity

The methodologies adopted for soil enzyme measurement are not universal and this often creates difficulties when making comparisons (Burns et al., 2013). Most investigations regarding quantification of soil enzyme activity are conducted directly by measuring its activity (Dick, 1997). An enzymatic assay consists of adding a known quantity of soil to a solution containing a standard concentration of a substrate and measuring the rate at which the substrate converts to the product (Bünemann et al., 2006). Enzymatic assays are based in the quantitative evaluation of products released or substrates consumed and do not therefore differentiate between the contributions of intracellular or extracellular enzymes and to the total enzymatic activity (Taylor et al., 2002). For an enzymatic assay to be reliable and applicable, it must be tested and validated with soils that have different properties. Indeed, the main problems can be due to both the adsorption of substrates or products on the soil particles and the possible interference of elements or compounds present in the soil. Another problem in the quantification of enzymatic activity is the lack of standardization due to the use of diverse procedures (Burns, 1978), factors such as incubation time and the soil/solution ratio vary notably, according to the method employed.

Normally, the determination of diverse soil enzymatic activities is carried out in conditions that guarantee optimal catalysis velocity, that is, optimal pH and temperature, at a substrate concentration in excess and testing conditions that allow the free diffusion of the substrate and facilitate interaction with the enzyme (Taylor et al., 2002).

Response of soil hydrolases to mineral fertilization

Changes in the composition and function of the soil microbial community due to agricultural practices can greatly impact the health and productivity of this important natural resource (Chaparro et al., 2012; Franco-Otero et al., 2012).

Some studies reveal that urease activity, an enzyme that catalyzes the hydrolysis of urea to CO_2 and ammonia, decreased in soils with long-term nitrogen fertilization, in comparison with unfertilized soils (Meysner et al., 2006; Mohammadi, 2011). The reduction in the activity of this enzyme was attributed to the absorption of mineral N by soil microorganisms (Marcote et al., 2001; Meysner et al., 2006), which confirms the hypothesis of Konig et al. (1996) that high quantities of ammonia reduce urease activity. The application of $N_{120}P_{90}K_{90}$ also diminished urease activity in soils with a different crop rotation (Balezentiene & Klimas, 2009).

Invertase, the enzyme that catalyzes the hydrolysis of sucrose to D-glucose and D-fructose, sugars that are important sources of energy for microorganisms (Jin et al., 2009); xylanase, the enzyme that hydrolyzes xylan to release xylobiose and xylose; and urease activity all diminished in soils fertilized with NP_2O_5K and copper sulfate (Cu_2SO_4) (Butt et al., 2008).

Mineral fertilization (NPK) increased acid phosphatase activity (phosphatases catalyze the hydrolysis of esters and anhydrides of H_3PO_4 to release inorganic phosphorus, which is assimilable for plants) (Dick et al., 2000) but diminished alkaline phosphatase activity in soils cultivated with corn (Kalembasa & Symanowicz, 2012).

Nitrogen mineral fertilization stimulates phosphatase activity, most likely because phosphatase requires a substantial investment of N (Olander & Vitousek, 2000), and thus, adding N to the soil increases phosphatase activity. In contrast, P from mineral fertilization in the soil can inhibit phosphatase activity or slow down the synthesis of this enzyme (Saha et al., 2008; Emnova et al., 2012), which is consistent with the idea that phosphatase increases the available reserves of P when this nutrient is limited, and that the addition of P to the soil is an alternative for increasing the availability of this element.

Various studies have reported a negative effect of phosphorus fertilization on alkaline phosphatase (Kandeler et al., 1999; Sinsabaugh et al., 2008; Spohn & Kuzyakov, 2013) and acid phosphatase soil activity (Olander & Vitousek, 2000; Rosolem et al., 2014).

N×P mineral fertilization combined stimulates phosphatase activity. This occurs if nitrogen fertilization is a stronger control on phosphatase activity than phosphorus fertilization; nitrogen dictates phosphatase production as phosphatase cannot be produced without adequate N supplies (Marklein & Houlton, 2012).

N×P mineral fertilization combined also depresses phosphatase activity. This occurs if the effect of phosphorus fertilization is greater than the effect of nitrogen fertilization; it is not energetically favorable to obtain phosphorus through phosphatase production when inorganic phosphorus is abundant (Marklein & Houlton, 2012).

Response of soil hydrolases to organic fertilization

In general, recent incorporations of organic residues can stimulate enzymatic activity as a result of microbial proliferation or enzymatic induction in response to the added residues (Gianfreda & Ruggiero, 2006).

Various works have demonstrated that acid phosphatase activity increases as a consequence of organic fertilization (Chakrabarti et al., 2000, Chang et al., 2007) and decreases when mineral phosphorus fertilizers are employed (Clarholm, 1993; Olander & Vitousek, 2000). Organic fertilization can increase β-glucosidase, an enzyme that catalyzes the hydrolysis and biodegradation of various β-glucosidases found in the organic matter (Turner et al., 2002), and urease activity (Chakrabarti et al., 2000, Chang et al., 2007).

β-glucosidase, urease, phosphatase and protease (an enzyme involved in the progressive decomposition of the N contained in proteins) activity in soils treated with compost was greater than in soils that were not treated with compost (Crecchio et al., 2004). Likewise, other studies have revealed that the addition of organic fertilizers increases urease activity in soils with different tillage cultivated with wheat (Mohammadi, 2011).

Ros et al. (2006) evaluated the influence of compost from (i) urban organic waste; (ii) green compost; (iii) herd manure and; (iv) sewage sludge on hydrolase activity in a corn-wheat-barley crop rotation. Phosphatase activity was highest in soils treated with manure and sewage sludge and β-glucosidase activity was significantly higher in soils that received manure. Protease and urease activity were not significantly different between untreated and compost-treated soils. This is most likely due to an elevated NH_4NO_3 content in the composts that were added to the soil.

The addition of organic material to the soil in corn crops favored invertase, cellulase (the enzyme that decomposes cellulose, the most abundant polysaccharide in plant cellular walls), urease and phosphatase activity (Adriano et al., 2012). Kandeler et al. (1999) found similar results studying the dynamic of invertase and xylanase in soils amended with corn stubble. Martens et al. (1992) noted an increase in invertase activity in soils with straw residue. Yang et al. (2008) showed that the activity of this enzyme increased when horse manure was added to cucumber plants (*Cucumis sativus* L.).

Kautz et al. (2004) reported that cellulase activity in the soil increased significantly with the incorporation of straw and green compost. Debosz et al. (1999) found that β-glucosidase, cellobiohydrolase and endocellulase activity in soils fertilized with bio-solids, straw and green compost was higher in comparison to the activity of these enzymes in soils that only received nitrogen fertilization.

The application of vermicompost can also cause phosphatase and β-glucosidase activity to increase significantly (Bastida et al., 2008; Saha et al., 2008).

Martens et al. (1992) found that the long-term addition of organic material maintains high levels of phosphatase activity in the soil. Álvarez-Solís et al. (2010) demonstrated a positive effect on acid and alkaline phosphatase activity with the application of organic fertilizers to soils cultivated with corn under seasonal conditions.

The literature indicates that the increase of enzymatic activity in soils treated with organic residues has been attributed to a combined effect of (i) a higher degree of enzyme stabilization with the formation of organo-mineral complexes as a result of the increase in humus content (Chang et al., 2007) and (ii) an increase in the microbial biomass due to the increase of the organic carbon content.

Response of soil hydrolases to simultaneous organic and mineral fertilization

Urease activity and acid and alkaline phosphatase activity increased when nitrogen and organic fertilizers were added simultaneously to the soil (Eivazi et al., 2003). Mohammadi (2011) also reported that urease and alkaline phosphatase activity increased significantly with the addition of compost and mineral fertilizers to the soil. Likewise, Datt et al. (2013) noted that the simultaneous application of organic and mineral fertilizers promoted urease and phosphatase activity in soils cultivated with beans. Acid and alkaline phosphatase activity increased in soils cultivated with taro (*Colocasia esculenta)* with the simultaneous application of green composts, ½ NPK and lime (Hota et al., 2014).

Mijangos et al. (2006) studied the effect of mineral (NH_4NO_3, P_2O_5 and KCl) and organic (cow manure) fertilizers on β-glucosidase and acid phosphatase activity in soil cultivated with corn and found that the plots of land treated with organic fertilizers had a higher enzymatic activity than those treated with mineral fertilizers.

Mandal et al. (2007) studied the effect of 34 years of fertilization with N, NP, NPK, NPK+S and NPK+compost on the enzymatic activity of Typic Haplustepts with a corn-wheat rotation. The results revealed that the NPK+compost treatment had higher acid and alkaline phosphatase activity.

The combined application of slow-release mineral fertilizers and compost increases phosphate activity in the rhizosphere of the mezcal maguey (*Agave angustifolia* Haw.) (Martínez-Gallegos et al., 2012).

Saha et al. (2008) reported the highest value of invertase activity with simultaneous organic and mineral fertilization in a wheat-soya rotation under seasonal conditions.

β-glucosidase was higher in soils treated with long-term organic fertilization (with or without NPK) in a beet-barley-potato-wheat crop rotation in a Typic Mollisol (Böhme & Böhme, 2006). β-glucosidase and alkaline phosphate activity were also higher with the combined application of vermicompost and mineral fertilizer (NPK-100:80:80), in comparison with the activity of these enzymes when the fertilizers were applied individually to soil cultivated with onion (Srivastava et al., 2012).

The combined application of organic and mineral fertilizers can increase enzymatic soil activity due to the easily-available, enriched nutrients from mineral fertilization and the high levels of organic material and biological activity promoted by the addition of organic fertilizers.

Among the hydrolases, phosphatase activity has been the parameter most often used to estimate changes in soil quality that are due to either management or to the presence of contaminants (Gil-Sotres et al., 2005). With respect to the enzymes involved in the carbon cycle, β-glucosidase has been the most used to evaluate the quality of soil that has been subjected to different types of management. Urease has also been widely used in the evaluation of management-related changes in soil quality.

Conclusions

Hydrolytic enzyme activity responded to different forms of fertilizers. Organic restitutions stimulate hydrolytic enzymatic soil activity. In contrast, mineral fertilization can inhibit or slow down the synthesis of these enzymes. The combined use of mineral and organic fertilizers generates a greater positive effect on enzymatic activity, due to the easily-available, enriched nutrients from mineral fertilization and the high levels of organic matter and biological activity promoted by the addition of organic fertilizers. The works consulted show that phosphatase and urease activity were the biochemical parameters analyzed with the highest frequency to evaluate the effect of fertilization on soil enzymatic activity.

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