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AMIDASE ACTIVITY OF SOILS UNDER POTATO CROPPING WITH CONVENTIONAL MANAGEMENT AND UNDER NATIVE GRASSLAND

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ABSTRACT

Amidase enzymatic activity was evaluated in soils under potato cropping with a conventional management with agrochemical application (PCA) and in soils under pasture without agrochemical application (PSA) in farms at Tausa, Villapinzón, and Zipaquirá located in the province of Cundinamarca (Colombia). Correlations between amidase enzymatic activity and other enzymatic activities related to the nitrogen cycle (e.g., urease and protease) and soil physicochemical parameters were also evaluated. The PCA samples showed null or low amidase activity compared to the PSA samples. The difference could be due to the application of agrochemicals and chemical supplements, which possibly affected soil microbiota and consequently the soil enzymatic activity. Similarly, the low amidase activity observed could be an indicator that carbofuran degradation via the amidase pathway did not occur at a significant level in the PCA soil. A direct relationship was observed between amidase, urease, and protease activities and the concentrations of soil microelements such as Mn, Fe, and Cu.

Key words. Edaphic enzyme, Urease, Protease, Nitrogen cycle.

ACTIVIDAD AMIDASA EN SUELOS BAJO CULTIVO DE PAPA CON MANEJO CONVENCIONAL Y BAJO PASTIZAL

RESUMEN

Se evaluó la actividad enzimática amidasa en suelos bajo cultivo de papa con manejo convencional de aplicación de agroinsumos (PCA) y en suelos bajo pastizal sin aplicación de agroinsumos (PSA), en fincas de las localidades de Tausa, Villapinzón y Zipaquirá en Cundinamarca (Colombia). Asimismo, se estableció su relación con la actividad de otras enzimas del ciclo del nitrógeno (ureasa, proteasa) y los parámetros fisico-químicos de dichos suelos. Se encontró que las muestras PCA presentaron nula o baja actividad amidasa con respecto a las PSA. Esto puede estar relacionado con la aplicación de plaguicidas e insumos químicos que afectan la actividad enzimática del suelo, o ser indicativo de que la degradación de carbofuran (plaguicida utilizado en el cultivo de papa) no se está llevando a cabo de manera significativa mediante la enzima amidasa. Se encontró una relación directa entre la actividad amidasa, ureasa, proteasa y microelementos del suelo (Mn, Fe y Cu).

Palabras clave. Enzimas edáficas, Ureasa, Proteasa, Ciclo del nitrógeno.

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INTRODUCTION

Soil quality is one of the factors that determines agricultural sustainability, environmental quality, and, consequently, plant, animal, and human well-being (Doran *et al.*, 1999). It depends greatly on long- term agricultural practices, which can affect soil biodiversity and fertility (Anderson, 2003; Nielsen & Winding, 2002; Masera *et al.*, 1999). Some parameters are useful as indicators of changes in physical, chemical, and biological soil properties (Nielsen & Winding, 2002), which include the evaluation of soil microbial populations and enzyme activities (Pankhurst & Doube, 1997; Cerón & Melgarejo, 2005), and are used to establish the soil quality conditions of a particular environment.

Enzymatic activities have been evaluated as soil quality indicators associated to the effects of xenobiotics used in industry and agriculture (Tabatabai & Warren, 1993) and have shown that the use of agro-chemicals influence enzymatic activity (Alvear *et al.*, 2006). Furthermore, enzymatic activity could be an indicator of the presence and effects (some harmful) of agrochemicals on soil quality (Burns, 1982; Klose & Ajwa, 2004; Avellaneda-Torres *et al.*, 2012). The amidase enzyme (important in the nitrogen cycle) occurs widely in nature and catalyses amide hydrolysis to produce ammonium and carboxylic acid by acting on C-N bonds (Frankenberger & Tabatabai, 1980a). Amidase activity has also been associated with the degradation of agro-chemicals (Kay-Shoemake *et al.*, 2000), including carbamates. It has been shown that the *mcd* gene (coding for the carbofuran hydrolase enzyme) is transferred in soil among microbial populations and contributes to the abundance and genetic diversity of N-methylcarbamate carbofuran-degrading bacteria (Desaint *et al.*, 2003; Plangklang & Reungsang, 2012). The N-methylcarbamate bond in carbofuran occurs as ester and amide bonds; therefore, carbamate bond hydrolysis occurs via amidase, esterase, or both, and the hydrolysis product can be identical for both pathways due to the instability of N-methylcarbamate acid (Chapalamadugu & Chaudhry, 1993).

The potato crop (*Solanum tuberosum*) is of worldwide importance, along with wheat, rice, and corn. Because of its high nutritional value and adaptability to diverse climates and cultivation systems, potato is one of the 10 most commonly cultivated crops in developing countries (Devaux, 2010), given that it offers a possible solution to worldwide problems of food security, malnutrition, and poverty (Bonilla *et al.*, 2008).

The present study evaluated the amidase enzymatic activity in soil used for potato cropping with conventional management and agrochemical application (PCA) and in grassland soil without agrochemical application (PSA) in farms at Tausa, Villapinzón, and Zipaquirá located in the province of Cundinamarca (Colombia). In addition, the relationship between the amidase activity and other enzyme activities of the nitrogen cycle (*e.g.,* urease and protease) and soil physicochemical parameters was evaluated using a principal component analysis.

MATERIALS AND METHODS

Experimental scheme and soil sampling

Three farms located in the province of Cundinamarca (Colombia) were evaluated in the following counties: Tausa (township of Páramo Bajo, farm: Páramo Bajo N 03°40'14,9''; W 073°39'51,1'' and N 03°35'50,4''; W 073°28'56,0''), Zipaquirá Z (township of Páramo Guerrero Oriental, farm: Puente de Tierra N 04°05'59,7''; W 072°54'34,6'' and N 03°37'32,9''; W 073°37'39,8''), and Villapinzón V (township of Salitre Alto, farm: Santa Ana N 03°43'43,5''; W 073°42'18,6'' and N 03°43'43,5''; W 073°42'18,6''). Two sample types were collected according to the method described by Avellaneda *et al.*, (2012): 1) Potato (*Solanum tuberosum*) mono-cropped soils, in the post-harvest phase, Parda Pastusa variety, with less than 10 years of agricultural use under a conventional agro-chemical application (PCA) management scheme; these samples were designated as TCA, ZCA, and VCA. The management including agrochemicals (Agricon®, Carbotox®, Nudrin®, Roxion®, Larvin®, Lorsban®, Lannate®, Eltra®, Methox®, Carbofed®, Furalimor SC, Alodrin®, Fursem®, Furadan®, among others), fungicides (Magricen 80%, copper oxychloride, Mancozeb, Acrobat®, Forum®, Antracol®, Fitoraz®, Previcur®, Rhodax®, Curzate®, Manzate®, Kasumin®, among others), herbicides (glyphosate, Sencor®, Gramafin®, Afalon®, Fusilade®, Gramoxone®, Reglone®), and N:P:K (10:30:10) chemical fertilisers applied at 200 kg ha^{-1} with further fertilisation using urea at 40 days (200 kg ha^{-1}); and 2) Soils under grassland (*Calamagrostis* sp.) with no agrochemical application (PSA), designated as TSA, ZSA, and VSA. Sampling was performed using a zigzag route in a one-hectare plot at each site. Three soil samples were collected from each site, each composed of ten subsamples (collected every 15 steps). Each subsample was collected from a soil depth of 20 cm and weighed 50 g. Finally, the samples were deposited in sterile plastic bags and transported at 4 °C to the laboratory for subsequent analyses (*i.e.,* determination of the enzymatic activities in moist soil, with values expressed in terms of dry soil).

Physicochemical analyses of soils

The following physicochemical parameters were evaluated by the Certified Service Unit of the Instituto Geográfico Agustín Codazzi (González & Malagón, 1990): moisture content, pH, organic carbon (OC) by the Walkley-Black method, total nitrogen (TN) by the Kjeldahl method, cation-exchange capacity (CEC) by the ammonium acetate method, the microelements manganese (Mn), iron (Fe), zinc (Zn), and copper (Cu) by *Dietilen-Triamin-Penta-Acetic acid* extraction, available boron (B) by hot water application, and available phosphorus (P) by the Bray II method.

Determination of amidase activity in soil

Amidase (EC 3.5.1.4) activity measurements were performed according to Frankenberger & Tabatabai (1980a) using formamide as a substrate. The final detection of N-NH_4 (ammonium nitrogen) produced by the enzyme's activity was performed by distillation with 25% NaOH and titration with H_2SO_4 (0.005M). Two grams were used from each soil sample with a particle size of <2 mm, weighed in triplicate, and the reagent ratio was maintained according to the amount of sample.

Statistical analysis and relationship to other enzymes of the nitrogen cycle

The variance homogeneity and normality assumptions were analysed using Bartlett and Shapiro-Wilk tests, respectively, and by performing an analysis of variance with a factorial design (DCA) by means of Statistix 9.0. A principal component analysis was performed between amidase (EC 3.5.1.4), urease (EC 3.5.1.5), protease (EC 3.4.2.21.24) activity and physicochemical parameters. The results of urease and protease activity for the studied soils were reported by Avellaneda (2008) and Anacona (2008), respectively. The principal component analysis (PCA) was performed using Primer 6 V 6.1.14 and Permanova+ Version 1.0.4.

RESULTS AND DISCUSSION

Physicochemical analyses of soils

Table 1 shows that the soils were acidic (pH less than 5.5) with a high CEC (>20 meq/100 g) and high levels of TN (>0.5%, except ZSA). The total OC content was high for all soil samples (>5%). There were Zn (<3 ppm), Cu (<1.5 ppm), and Mn (<15 ppm) deficiencies but high Fe (>30 ppm) and B (0.6-1.5 ppm) concentrations.

Furthermore, the PCA samples had higher phosphorus contents than did the PSA sample (statistically significant), which is consistent with the application of synthetic phosphorus through the addition of N:P:K fertilisers. Likewise, the OC content was greater in the PCA samples than in the PSA samples (statistically significant); this difference can be attributed to movements of the soil during potato cropping in the PCA samples, which cause a shift in the soil layers and a possible increase in the clay contents on the surface, as well as a decrease in organic matter; meanwhile, in the PSA samples, more organic matter can be accumulated due to the absence ofthe mechanic activities on the soil. . The soil moisture content was higher in the PSA samples, possibly due to the sunlight protection provided by the grassland, in contrast to PCA samples that are subject to a constant turnover by farmers and can lose water through evaporation due to direct solar radiation.

Amidase activity and its relationship to urease and protease activities

The PSA soil samples showed a greater amidase activity than did the PCA samples. These differences are statistically significant between treatments with and without a record of potato cropping and agrochemical application, and also

Table 1. Physicochemical parameters of soils evaluated in this study. TSA, VSA, and ZSA: Tausa, Zipaquirá, and Villapinzón without the application of agrochemicals. TCA, VCA, and ZCA: Tausa, Zipaquira and Villapinzón with the application of agrochemicals. H: moisture content, CEC: cation-exchange capacity, OC: organic carbon, TN: total nitrogen.

Tabla 1. Parámetros fisicoquímicos de los suelos evaluados en este estudio. TSA, VSA, ZSA: Tausa, Zipaquirá y Villapinzón sin aplicación de agroquímicos. TCA, VCA y ZCA: Tausa, Zipaquirá y Villapinzón con aplicación de agroquímicos. H: humedad, CEC: Capacidad de intercambio catiónico, OC: carbono orgánico, TN: nitrógeno total.

SAMPLE	pH	Pppm	H%	OC%	TN%	CECmeq/100 mg	Mnppm	Feppm	Znppm	Cuppm	Bppm
TSA	5.1a	18.8a	50.4a	9.5a	1.10a	65.1a	0.64a	116a	3.20a	0.08a	0.52a
TCA	4.7a	67.3b	47.1a	18.6b	1.20a	76.4a	0.88a	81.2b	1.30a	0.18a	1.50b
VSA	3.9b	70.1b	32.6b	8.1c	0.57b	42.1b	3.40b	368a	2.00a	0.16a	0.86a
VCA	3.9b	198c	33.7b	9.3a	0.56b	46.4b	1.80c	233a	0.52b	0.22a	0.66a
ZSA	4.1b	18.8a	33.6b	5.6d	0.42b	30.4c	1.70c	314a	1.10a	0.40a	0.51a
ZCA	4.0b	76.4b	30.1b	7.4c	0.50b	41.0b	2.70c	305a	0.48b	0.26a	0.82a

between sites (Fig. 1). In the case of the TCA and VCA samples, no amidase activity was detected, which can be associated with hydrolysis products of the chemical supplements applied (i.e., those that modify soil pH), the type and concentration of chemical substances present in the soil, and a greater biological activity related to a greater organic matter content in the PSA samples, as mentioned in the previous section. According to Ochoa *et al.* (2007), both microbial activity as well as microbial enzyme production can be adversely affected in the presence of inorganic fertilisers used in conventional agricultural systems. Moreover, the obtained results indicate a possible decrease in the biological activity of soils under cultivation and agrochemical application. Klose and Ajwa (2004) reported that the presence of agrochemicals can affect soil quality. In addition, Avellaneda *et al.* (2012) reported different extracellular enzymatic activities (urease, protease, acid phosphatise and alkaline phosphodiesterase, β-glucosidase, arylsulfatase) in the bacterial consortia (*Bacillus subtilis, Brevundimonas diminuta, Flavimonas oryzihabitants*) from the same soils used in this study (PCA and PSA) from Tausa, Villapinzón, and Zipaquirá counties . The type of land use and the location affected the enzymatic activities, observing higher protease, alkaline phosphatase, and arylsulphatase activities in the bacterial consortia for the PSA samples compared to the PCA samples (50, 83, and 58% increases, respectively) in Villapinzón. The same behaviour was reported at Zipaquirá for protease, alkaline phosphatase, and arylsulphatase activities (63, 67, and 21% increase, respectively), and at Tausa for urease and arylsulphatase (70 and 81% increase, respectively), indicating a possible inhibitory effect due to fertilisation, agrochemical use, and conventional tillage practices.

The pesticide carbofuran is widely used as an insecticide, acaricide, and nematicide throughout the world (Chelinho *et al.*, 2011) and in Colombia (Fedepapa, 2005). There have been reports of carbamate hydrolase enzyme participation in methylcarbamate degradation (Tomasek & Karns, 1989) in bacterial-mediated processes (Parekh *et al.,* 1994) as well as in soils used for the cultivation of a unique potato variety in the municipality of Silos (department of Norte de Santander, Colombia) with different temporal history of plaguicide application than in the sites studied here Castellanos *et al.,* 2013). Carbofuran can be hydrolysed by amidases or esterases (Chapalamadugu & Chaudhry, 1993).

The results obtained for the PCA soils with no amidase activity (TCA and VCA) and low amidase activity (ZCA) suggest that the application of various agrochemicals is preventing the establishment of an adequate enzymatic stability and activity, and therefore, agrochemical degradation via amidase is not a significant process in the potato crop. Currently, there is quantitative evidence of carbofuran bacterial degradation in the studied soils

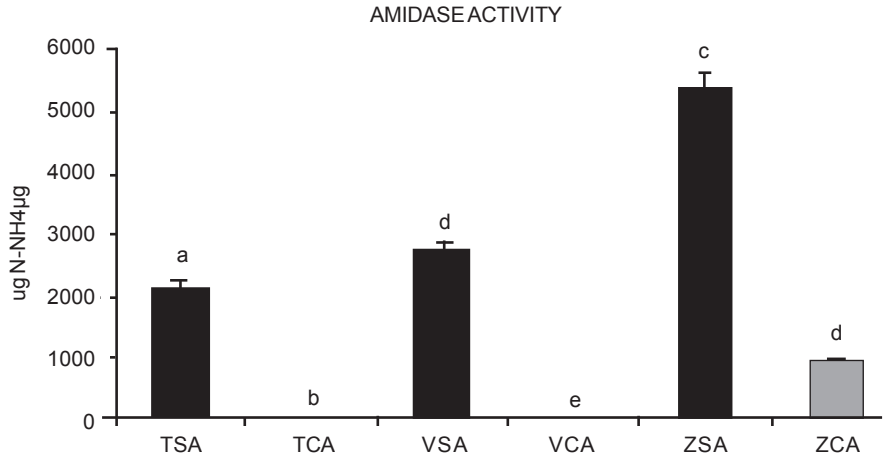

Figure 1. Amidase activity of soils evaluated in this study (ugN-NH_4/g dry soil). TSA, VSA, and ZSA: Tausa, Zipaquirá, and Villapinzón without agrochemical application. TCA, VCA, and ZCA: Tausa, Zipaquirá, and Villapinzón with agrochemical application.

Figura 1. Actividad amidasa de los suelos evaluados en este estudio (ugN-NH_4/g de suelo seco). TSA, VSA y ZSA: Tausa, Zipaquirá y Villapinzón sin aplicación de agroquímicos. TCA, VCA y ZCA: Tausa, Zipaquirá y Villapinzón con aplicación de agroquímicos.

(Sánchez, pers. com.; data not shown), indicating that this activity is being performed by another type of hydrolase (*e.g.,* an esterase).

Burns and Wallenstein (2010) reported that soil enzyme stability represents a potential reservoir because enzymes represent the first catalytic response towards changes in substrate availability in soil. Furthermore, this give rise to the production of signalling molecules for substrate sensing by microbial communities as well as population growth sensing, in which gene function is related to cellular density. As a consequence, enzymes are only synthesized when cell numbers are sufficiently high.

Avellaneda (2008) reported urease activity values (µg N g^{-1} dry soil 2 h^{-1}) for the studies soils without agrochemical application (TSA: 4.1; VSA: 4.8; ZSA: 22.7) and with agrochemical application - PCA - (TCA: 2.8; VCA: 2.1; ZCA: 20.7). In addition, Anacona (2008) reported protease activity values (µg Tir g^{-1} dry soil 2 h^{-1}) for the same soils without agrochemical application - PSA - (TSA: 133; VSA: 199; ZSA: 437) and with agrochemical application - PCA - (TCA: 258; VCA: 168; ZCA: 273). The urease and protease activities showed the same tendency as the amidase activity, with the exception of the protease enzyme in TCA. The PSA samples showed greater enzymatic activity than PCA samples. It is likely that the contrasting protease activity values obtained for the TSA and TCA soils could be associated with variations in specific physicochemical and environmental soil characteristics unique to each site and well as variations in the temperature, nutrient availability, and litter fall, as observed for dehydrogenase, urease, phosphatase, and arylsulphatase activities in a forest soil in Korea (Kang *et al.*, 2009). In addition, Ahmad *et al.* (2001) reported that microorganisms and plant roots are the greatest contributors to soil enzyme sources; however, enzyme production is unstable due to factors such as seasonal variations, low enzyme levels in plant tissues, the influence of the soil status and the fractions with which these enzymes can associate, the clay mineral content, organic matter levels, and the aqueous soil phase (Burns, 1982).

The principal components analysis (PCA) obtained for the various studied parameters is illustrated in Fig. 2. These results show an accumulated variance of 76.8% for axes 1 and 2, which explains a high percentage of the data

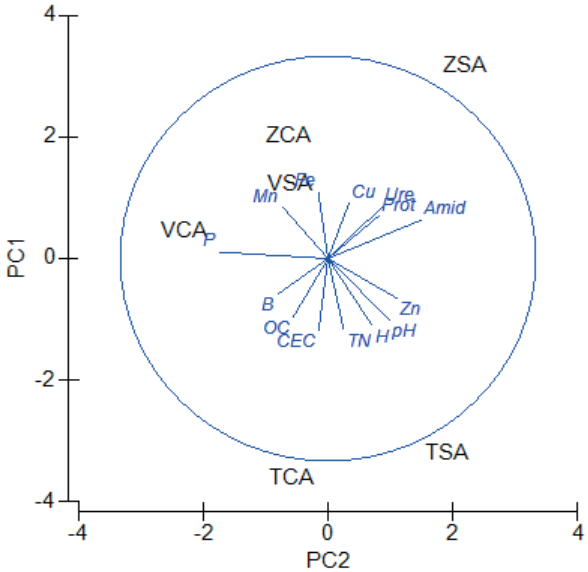

Figure 2. Principal component analysis of enzyme activities and physicochemical parameters. TSA, VSA, and ZSA: Tausa, Zipaquirá, and Villapinzón without agrochemical application. TCA, VCA, and ZCA: Tausa, Zipaquirá, and Villapinzón with agrochemical application. Amid: amidase, Ure: urease, Prot: protease, H: humidity, CEC: cation-exchange capacity, OC: organic carbon, TN: total nitrogen, Cu: copper, Fe: iron, Mn: manganese, P: phosphorus, B: boron, Zn: zinc.

Figura 2. Análisis de componentes principales de actividades enzimáticas y parámetros fisicoquímicos. TSA, VSA y ZSA: Tausa, Zipaquirá y Villapinzón sin aplicación de agroquímicos. TCA, VCA y ZCA: Tausa, Zipaquirá y Villapinzón con aplicación de agroquímicos. Amid: amidasa, Ure: ureasa, Prot: proteasa, H: humedad, CEC: capacidad de intercambio catiónico, OC: carbono orgánico, TN: nitrógeno total, Cu: cobre, Fe: hierro, Mn: manganeso, P: fósforo, B: boro, Zn: zinc.

variance. These demonstrate that the PSA samples (TSA, VSA, ZSA) had an overall greater activity of enzymes associated with the nitrogen cycle compared to the PCA samples. The activities of three enzymes showed a direct relationship, as indicated by the location of the vectors. This correlation suggests a synergistic relationship among these enzymes without antagonistic or competitive effects with respect to soil biochemistry. Therefore, this result is consistent with amidase catalysis of amide hydrolysis resulting in the production of ammonium and carboxylic acid as well as ammonium nitrogen and CO_2 resulting from urea-type compounds produced by urease; these findings are also consistent with soil protein hydrolysis and degradation by proteases. Likewise, the PCA results indicated a direct relationship between these enzymes and soil microelement levels (Mn, Fe, and Cu), which have been suggested as possible cofactors of these enzymes associated with the nitrogen cycle. In contrast, Frankerberger and Tabatabai (1981) reported an inhibitory effect of trace elements on amidase activity based on assays indicating a 3% inhibition due to the effects of Cu (I), Ba (II), Cu (II), Fe (II), Ni (II), Al (III), Fe (III), Ti (III), V (IV), As (V), Mo (VI), W (II). Likewise, Frankerberger and Tabatabai (1985) indicated that the soil components notably influence the reactions catalyzed by this enzyme, since, according to comparative studies of the characteristics a soil amidase and an amidase obtained from a soil bacterial isolate, they reported that the bacterial amidase had a 25% inhibitory percentage in the presence of different elements such as Ag (I), Cd (II), Cu (II), Hg (II), Ni (II), Pb (II), Zn (II), Al (III), Se (IV). In contrast, Frankerberger and Tabatabai (1980a) indicated that amidase activity requires Mg^{2+} and/or Ca^{2+} ions. Using dialysis and carbamate hydrolase enzyme inactivation assays, Alam (2009) indicated that amidase activity was recovered by the addition of Mn (II) and Co (II) as cofactors; furthermore, Karns and Tomasek (1991) reported the requirement of Mn (II) for adequate enzymatic activity of the carbofuran hydrolase of *Achromobacter* sp. strain WM II.

In summary, PCA soil samples (TCA, VCA, ZCA) showed a null or low amidase activity compared to PSA samples (TSA, VSA, ZSA). This difference indicates a possible inhibitory effect of agrochemicals on the amidase activity and suggests that the degradation of carbofuran (an agrochemical used in potato cropping) via amidase activity is not significant. In addition, there is a direct relationship among amidase, urease, and protease activities as well as the levels of soil microelements (Mn, Fe, and Cu).

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