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LAND DEGRADATION & DEVELOPMENT

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ENZYME ACTIVITY AS AN INDICATOR OF SOIL QUALITY CHANGES IN DEGRADED CULTIVATED *ACRISOLS* AT THE MEXICAN TRANS-VOLCANIC BELT

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ABSTRACT

Soils located at the Mexican Trans-volcanic Belt (MTB) have a worrying degree of degradation due to inappropriate management practices. Early indicators of soil changes are very useful to alert about negative impacts of wrong managements on these volcanic soils. The aim of this work was to evaluate the short-term effects (4 years) of different agricultural practices on soil organic matter (SOM) quality and to validate the potential of the selected biochemical properties as optimal early indicators of soil quality in Mexican cultivated *Acrisols*. During 2002–2005 four agronomic management systems: conventional (Tc); improved conventional (Ti); organic (To) and fallow (Tf) were assayed in plots located at the MTB. An uncultivated soil under grass cover (Sg) was used as reference. Soil samples were collected at 0–10 cm depth and were analysed chemically (soil organic C, total N, water-soluble C and humic C), and biochemically (total and extra-cellular enzyme activity). After 4 years, soil organic C, total N, water-soluble C and dehydrogenase activity had higher values in To, followed by Ti treatment. A similar response pattern was observed in the extra-cellular enzyme activity. The highest total enzyme activity was found in Sg, followed by Ti and To treatments, and the lowest values appeared in Tc and Tf. To and Ti increased SOM contents of the degraded *Acrisols* studied, while Tc and Tf managements decreased the quality of these soils. The results showed that the assayed soil enzymes can be used as indicators of quality changes of these Mexican volcanic soils. Copyright © 2010 John Wiley & Sons, Ltd.

KEY WORDS: volcanic soils; agricultural managements; extra-cellular enzyme activity; humic C; soil metabolic potential

INTRODUCTION

Soil is a non-renewable natural resource in the life span of a human being generation, therefore needs to be preserved and, if it is possible, its productive capacity and quality improved. Karlen *et al*. (1997) defined soil quality as ‘the capacity to function within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation’. Understanding soil functions is necessary to provide strategies for rehabilitation of degraded soils. In natural conditions soils tend towards maintaining equilibrium between pedogenetic properties and the natural vegetation (Parr and Papendick, 1997). Soil equilibrium can easily be disturbed, especially by human intervention (e.g. unsuitable agricultural practices) or natural events.

Since historical times soils located at the Mexican Trans-volcanic Belt (MTB) have undergone strong human impacts and have been subjected to overuse, which resulted in erosion, further limiting the soil fertility and reducing their productivity (Gallardo *et al*., 2005). The rehabilitation and improvement of these volcanic soils are essential as preliminary steps for sustainability and a better quality of life in the area.

The knowledge of many soil properties is necessary to define soil quality. Once these properties have been determined and quantified, the most suitable strategies for soil management can be undertaken. Soil chemical and physical parameters such as pH, texture, bulk density, water retention capacity, soil organic matter (SOM) or nutrient availability have been used to measure soil quality (Doran and Parkin, 1996; Gil-Sotres *et al*., 2005). However, these parameters change very slowly and several years are usually required to observe significant changes. On the other hand, soil biological and biochemical properties (such as C and N microbial biomass, mineralizable N, soil respiration or enzyme activity) are responsive to small changes in soils, thereby providing immediate and accurate information on changes in soil quality (Nannipieri *et al*., 1990; Yakovchenko *et al*., 1996).

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Few studies about the effect of organic amendments on soil micro-organisms for soil rehabilitation purposes have been conducted (Villar *et al*., 2004; Mabuhay *et al*., 2006). In addition to this, little information exists on the biochemical and biological properties of degraded soils developed from old volcanic deposits, such as those found at the MTB (Álvarez *et al*., 2000). According to the weathering degree, the young volcanic-ash soils contain allophanic minerals and Al and Fe-oxides with positively charge surfaces and, consequently, can establish strong covalent bonds with humic substances (Oades, 1995). Therefore, the enzymes of these volcanic soils (which have high contents of humic compounds) should have an elevated stability and resistance to physical and microbial degradation (Ceccanti and García, 1994). Humus-enzyme complexes are considered important for soil quality, since they represent the 'crossing-point' between mineral and organic reactions in soil (Ceccanti and Masciandaro, 2003). Information on this subject referring to soils derived from old volcanic materials is not available.

The role of soil enzyme activities as early and sensitive indicators of management-induced changes in soil quality has been widely suggested (García *et al*., 1994; Dick *et al*., 1996; Masciandaro and Ceccanti, 1999). The study of different hydrolase-enzyme activities is important since they indicate the soil potentiality to carry out specific biochemical reactions and also maintain soil fertility (Burns, 1982). As examples, urease (URa) and protease (PRa) activities have been widely used in the evaluation of soil quality changes due to soil management (Pascual *et al*., 1999; Saviozzi *et al*., 2001). They act in the hydrolysis and transformation of organic N to inorganic forms, the former using urea-type substrates and the latter simple peptidic substrates. The acid phospho-monoesterase (PHa) activity is a good index of SOM quality and quantity and has been frequently used for estimating soil quality changes due to management (Bergstrom *et al*., 2000). PHa catalyses the hydrolysis of P organic compounds to phosphate esters. With regard to the enzymes involved in the C cycle, β-glucosidase (GLa) has been the most widely used to evaluate soil quality in soils subjected to different management systems (Saviozzi *et al*., 2001). GLa catalyses the hydrolysis of chains of β-glucosides to form β-glucose and indicates the potential for SOM decomposition.

Water soluble C (WSC) is the most degradable fraction of SOM, acting as an immediate energy source to the micro-organisms (Cook and Allan, 1992). The study of this fraction is also interesting in agricultural soils as it determines the soil's potential microbial activity (Ceccanti and García, 1994).

The aims of this work were: (a) to evaluate the effects of different agricultural management practices on SOM and biochemical properties related to soil microbial activity; and (b) to assess the effectiveness of the chosen biochemical properties as optimal early indicators of soil quality in representative Mexican cultivated degraded soils derived from old volcanic materials.

MATERIALS AND METHODS

Site Characteristics and Soil Management Practises

The experimental site is located in the West of the MTB, at the Atécuaro catchment, close to the city of Morelia (State of Michoacán: 19° 35′N; 101° 12′W; at 2270 m a.s.l.). The climate is temperate and sub-humid. Annual rainfall is close to $800\,\mathrm{mm\,y^{-1}}$, falling about 85 per cent of this total during the summer season (from June to September). Mean annual temperature is close to 14°C. *Andosols* (mostly in the mountains) and *Acrisols* (in downslopes and valleys, where agricultural areas are found) are dominant at the Atécuaro catchment, comprising near 70 per cent of the total surface area (Gallardo *et al*., 2005).

The soil at the experimental site is a degraded *Acrisol*, deep, acid (pH 4·9), rich in clay (50–60 per cent) and sesquioxides, derived from weathered volcanic material (Table I). The *Ap* horizon was poor in soil organic C (SOC $<18\,\mathrm{mg\,C\,g^{-1}}$), total N (Nt $<1{\cdot}6\,\mathrm{mg\,N\,g^{-1}}$), and available P (Bray-$P < 6\,\mathrm{mg\,P\,kg^{-1}}$). The Fe extracted with the dithionite, citrate and bicarbonate solution ($\mathrm{Fe_{DCB}}$) was the dominant Fe fraction in the soil (5·6 per cent), showing that this soil has mainly crystalline Fe oxides and oxy-hydroxides. Hidalgo, (Personal communication, unpublished results) found that the soil fine fraction ($<2\,\mu$m) of this *Acrisol* contained Fe oxides and oxi-hydroxides: goethite, hematite, akaganeite, magnetite, kaolinite (a clay with low activity, usual in tropical soils) and quartz. The

Table I. Characteristics of the cultivated and natural soils (2002)

Parameters soils	Texture	pH (H_2O) 1:2	CEC ($\mathrm{cmol_c\,kg^{-1}}$)	BS (%)	SOC ($\mathrm{mg\,g^{-1}}$)	Nt ($\mathrm{mg\,g^{-1}}$)	C/N	Bray$^-$P ($\mathrm{mg\,kg^{-1}}$)	$\mathrm{Fe_{OXA}}$ (%)	$\mathrm{Fe_{DCB}}$ (%)	$\mathrm{Fe_{OXA}}$/ $\mathrm{Fe_{DCB}}$
Cultivated	Clay	4·9	17·5	38·0	17·9	1·50	11·9	5·1	0·77	5·6	0·14
Natural (Sg)	Clay	5·3	18·2	45·2	18·7	1·74	10·7	7·5	—	—	—

CEC; cationic exchange capacity, BS; base saturation, SOC; soil organic C, Nt; total N, Bray-P; available P, $\mathrm{Fe_{OXA}}$; Fe extracted with acid oxalate, $\mathrm{Fe_{DCB}}$; Fe extracted with dithionite, citrate and bicarbonate solution.

amorphous Fe (Fe_{OXA}) content is lower than 1·0 per cent; then, the Fe_{OXA}/Fe_{DCB} ratio, an index of crystallinity degree of Fe minerals ('age of iron oxides'), is close to 0·1, a value reported for Andriesse (1978) for old tropical soils. The high clay content (>50 per cent) of this soil favours the formation of organic-Al complexes, highly resistant to the biological attack and the physical degradation (Pajares and Gallardo, 2007).

During 2002–2005 four terraces of 1000 m^2 each, with the same slope and bedrock, were cultivated for research purposes. The terraces were divided into two plots of 500 m^2 each. Due to particular field conditions (imposed by the geographic characteristics of the place, and the way of the farmer to cultivate the land) it was not possible to install a truly complete randomized treatment design. Thus, two replicates of each treatment were installed in each terrace (two plots per treatment). In 2002, at the beginning of the present research, preliminary soil analyses were performed in all the plots to ensure that the starting conditions were similar in all the cases (Table I).

Each terrace was subjected to different agronomic management system (Table II): (a) Conventional (Tc), ploughed every year using crop rotation with low fertilizer input, and full exportation of crop residues at the end of the agricultural cycle; (b) Organic (To), using organic sub-product inputs as nutrient sources every year (cow and poultry manure, and compost) and crop rotation; (c) Improved conventional (Ti), mulching with crop residues to protect the soil in 2004 and 2005, moderate inputs of inorganic fertilizers according to the crop demand, and crop rotation and (d) Fallow (Tf), 1 year in fallow and the next with a crop and so on, fertilization was low during the crop year and cattle grazed the native pasture during the fallow year. Prior to this experiment the landowner had cultivated the land employing Tf, the dominant system in this Mexican district.

Tillage of the experimental plots was done according to the traditional mechanical technology of local farmers (mules and sometimes tractor). Nitrogen fertilizer was applied as urea, and phosphorus fertilizer as triple superphosphate (2002 and 2003) and diamonic phosphate (2004 and 2005) in Tc, Ti and Tf. Fertilizers were added at sowing time every year. The crops of each particular year were determined by the farmer, depending on his needs. A soil under native spontaneous grass cover (Sg) and close to the experimental plots was used as reference.

Soil Physico–chemical and Biochemical Analyses

In August 2005, at the end of the experiment, three composite soil samples (with a minimum of 10 simple subsamples each) were taken from the top layer (0–10 cm, using a cylindrical probe) from each plot and from the reference soil. Soil samples were mixed, homogenized, air dried and sieved to a 2-mm mesh before chemical and biological analysis.

Soil pH was analysed in water (1:2 ratio). Soil organic C (SOC) was determined by dry combustion (TOCA, Shimadzu) and Nt was measured using the micro-Kjeldahl

Table II. Characteristics of the agronomic treatments under study

	Year	Tc (plots 1 & 2)	To (plots 3 & 4)	Ti (plots 5 & 6)	Tf (plots 7 & 8)
Crops	2002	Broad bean^{a}-vicia^{b}	Broad bean-vicia^{b}	Broad bean-vicia^{b}	Fallow
	2003	Oat^{c}-vicia^{b}	Oat^{c}-vicia^{b}	Oat^{c}-vicia^{b}	Oat^{c}-vicia^{b}
	2004	Maize^{d}-bean^{e}	Maize^{d}-bean^{e}	Maize^{d}-bean^{e}	Fallow
	2005	Maize^{d}-bean^{e}	Maize^{d}-bean^{e}	Maize^{d}-bean^{e}	Maize^{d}-bean^{e}
Total fertilization N-P-K (kg ha^{-1})	2002	40-30-14	None	60-96-40	None
	2003	60-40-0	None	80-40-0	60-40-0
	2004	140-100-0	None	140-100-0	None
	2005	140-100-0	None	140-100-0	140-100-0
Organic additions (mg ha^{-1})	2002	None	15 Mg WM ha^{-1} EB^{f}	None	None
	2003	None	15 Mg ha^{-1} compost	None	None
	2004	None	10 Mg ha^{-1} compost+2 Mg DM ha^{-1} poultry manure	Cover of 4 Mg DM ha^{-1} of wheat residues	None
	2005	None	6 Mg DM ha^{-1} poultry manure	Cover of 4 Mg DM ha^{-1} of wheat residues	None

Undetermined or guidance habit associated with maize, EB; Bovine manure/dung, DM; Dry material, WM; Wet material, Tc; conventional, To; organic, Ti; improved, Tf; fallow.

[a] Vicia faba.

[b] Vicia villosa.

[c] Oat strigosa.

[d] Zea mais.

[e] Phaseolus vulgaris.

method (Bremner, 1996). Extraction of WSC from the soil was carried out with distilled water (1:10 solid/liquid ratio) by shaking incubation at 50°C for 1 h, followed by centrifugation at 15 000 rpm for 15 min (García *et al.*, 1990). Humic acids were extracted from the soil by shaking the samples with $Na_2P_4O_7$ (0·1 M, pH 7·1) in a 1:10 solid/solution ratio. The extracts were centrifuged, filtered through a Millipore 0·45 μm membrane, and dialysed. After dialysis, extracts were concentrated (by ultrafiltration) and brought to the volume exhibited prior to dialysis (Masciandaro and Ceccanti, 1999); the extracts were used for the analysis of C content and enzyme activities. Pyrophosphate-extractable C (humic C, HmC) and WSC were determined by oxidation with hot $K_2Cr_2O_7$ (Nelson and Sommers, 1996).

Dehydrogenase activity (DHa) was determined by the reduction of 2-*p*-iodo-nitrophenyl-phenyltetrazolium chloride to iodonitrophenylformazan (INTF) using 1 g of soil at 60 per cent of field capacity for 20 h at 22°C in darkness. INTF was measured spectrophotometrically at 490 nm (Masciandaro *et al.*, 2000). The metabolic potential index (Masciandaro *et al.*, 1998; Caravaca *et al.*, 2002) was calculated as the ratio between the indicator of soil microbial activity (DHa) and the sources of energy for micro-organisms (WSC).

Regarding to hydrolytic enzymes, total enzyme activity was measured in soils samples and extra-cellular enzyme activity in dialysed pyrophosphate extracts (Masciandaro and Ceccanti, 1999; Benítez *et al.*, 2005). URa and PRa activities were analysed using urea and N-α-benzoyl-L-argininamide (BAA) as substrates, respectively. Both activities were determined as the NH_3 released in the hydrolysis reaction (Nannipieri *et al.*, 1980). PHa and GLa were determined using *p*-nitrophenyl phosphate disodium and *p*-nitrophenyl glucopyranoside as substrates, respectively. These assays are based on the release and detection of *p*-nitrophenol (PNP) in a spectrophotometer at 398 nm (Tabatabai, 1994).

Statistical Analyses

All the analytical assays were carried out in triplicate and results were analysed statistically. All response variables were normally distributed or could be transformed to normality with a square root transformation. Although the spatial design might initially be considered pseudo-replicated, we applied a *t*-student's test for individual treatment comparisons to corroborate that there were not significant differences between the two replicates of every treatment; then we applied another *t*-student's test comparing separately every treatment with the other treatments to determine overall differences among treatments. A Pearson correlation analysis was performed between the different parameters.

RESULTS

Because soil pH of these soils was acid and, therefore, could potentially be harmful to crops, 5·0 Mg ha^{-1} $CaCO_3$ was initially applied to the agricultural plots; then soil pH increased between 0·4 and 0·5 units in all the managements after 4 years of cultivation, while in Sg remained constant with time (Tables II and III).

Values of SOC and Nt ranged from 17·7 to 20·0 mg C g^{-1} and from 1·53 to 1·87 mg N g^{-1} in the soil samples, respectively (Table III); the highest values were found in To and Ti treatments, and the lowest values in Tf management. The highest C/N ratio was found under Tf (C/N:12·8) while To had the lowest value (C/N:10·7). HmC ranged from 1·8 mg C g^{-1} in Tf to 2·2 mg C g^{-1} in Ti and To; while WSC ranged from 399 to 517 μg C g^{-1}, with the highest value in To. Dehydrogenase activity ranged from 0·58 to

Table III. Physico–chemical and biochemical characteristics of cultivated and uncultivated soils

Parameters managements	Soil pH (H_2O)	SOC (mg C g^{-1})	Nt (mg N g^{-1})	C/N	HmC (mg C g^{-1})	WSC (μg C g^{-1})	DHa (μg INTF g^{-1} h^{-1})	DHa/WSC (μg INTF mg^{-1} C h^{-1})
Tc	5·3 a	17·8 c	1·60 b	11·1 ab	1·87 bc	399 c	0·58 b	1·45 b
Standard dev.	0·2	0·4	0·02	0·1	0·14	27	0·14	0·26
Ti	5·3 a	18·7 ab	1·70 ab	11·0 ab	2·21 a	485 b	1·25 a	2·57 a
Standard dev.	0·1	0·4	0·08	0·1	0·05	4	0·08	0·15
To	5·4 a	20·0 a	1·87 a	10·7 b	2·20 a	517 a	1·35 a	2·61 a
Standard dev.	0·2	0·8	0·11	0·2	0·12	11	0·16	0·25
Tf	5·4 a	18·3 bc	1·47 b	12·5 a	1·77 c	407 c	0·59 b	1·46 b
Standard dev.	0·1	0·6	0·15	1·4	0·15	15	0·01	0·04
Sg	5·3 a	18·5 bc	1·70 ab	10·9 ab	1·96 ab	423 c	0·67 b	1·59 b
Standard dev.	0·1	0·5	0·1	0·4	0·07	30	0·08	0·08

SOC; Soil organic C, Nt; total N, Hm-C; humic C, WSC; water soluble C, DHa; dehydrogenase activity, DHa/WSC; metabolic potential, INTF; iodonitrotetrazolium formazam, PNP; *p*-nitrophenol, Tc; conventional, To; organic, Ti; improved, Tf; fallow, Sg; grass cover.
Different letters for each variable indicate statistically different values (Student's *t*-test at $p < 0·05$).

1·35 μg INTF g^{-1} h^{-1} in these soils, with the highest values found under To and Ti. The metabolic potential index (DHa/WSC) ranged between 1·45 under Tc and 2·61 μg INTF mg C^{-1} h^{-1} under To. The Sg had intermediate values of all these parameters between the new implemented managements (To and Ti) and those conventionally applied (Tf and Tc).

With regards to total hydrolytic enzyme activity (Figure 1a), URa and PRa were lower in Tf management (16·4 and 42·2 μg NH$_3$ g^{-1} h^{-1}, respectively), having Sg the highest values (21·6 and 70·7 μg NH$_3$ g^{-1} h^{-1}, respectively). GLa showed the lowest values in Tc and Tf managements (78·3 and 86·6 μg PNP g^{-1} h^{-1}, respectively). The values of PHa were significantly higher in Ti and Sg (1833 and 1727 μg PNP g^{-1} h^{-1}, respectively) and lower in Tf (1302 μg PNP g^{-1} h^{-1}).

The highest extra-cellular activity of enzymes involved in C (GLa), N (URa and PRa) and P (PHa) cycles was found under To management and the lowest activity under Tc management (Figure 1b). GLa activity ranged from 15·9 to 34·4 μg PNP g^{-1} h^{-1}; PHa was higher under To and Ti (57·3 and 52·0 μg PNP g^{-1} h^{-1}, respectively) and lower under Tc (23·9 μg PNP g^{-1} h^{-1}); URa and PRa activities were also lower in Tc management (6·5 and 5·7 μg NH$_3$ g^{-1} h^{-1}, respectively).

DISCUSSION

Values of SOC and Nt found in these experimental plots were lower than those found by Nishiyama *et al*. (2001) and Zagal and Córdova (2005) in cultivated volcanic soils. Traditional cultivation practices generally aggravate land degradation, decreasing SOC content due to disruption of the equilibrium between the competing processes of humus formation and mineralization (Masciandaro *et al*., 1998; Saviozzi *et al*., 2001). The regular use of organic manure tends to counterbalance these processes maintaining high levels of SOM (Wu *et al*., 2004). In the present case the highest values of SOC and Nt found in the soil under To management were due to organic manures and composts incorporated to the soil for 4 years (Table III). According to Chander *et al*. (1999), the continuous incorporation of organic residues favours the significant increase of SOC and maintains the agricultural productivity. The C/N ratio (an indicator of the intensity of SOM mineralization) was higher under Tf treatment, which could be due to the organic residues input (mainly roots) poor in N after the grazing period (Masciandaro *et al*., 2004; Covaleda *et al*., 2006[Q3]). Q3 The low value of C/N ratio under To was due to the poultry manures applied with this management. Poultry manure is known to be a rich source of N for plants (Moore *et al*., 1995)

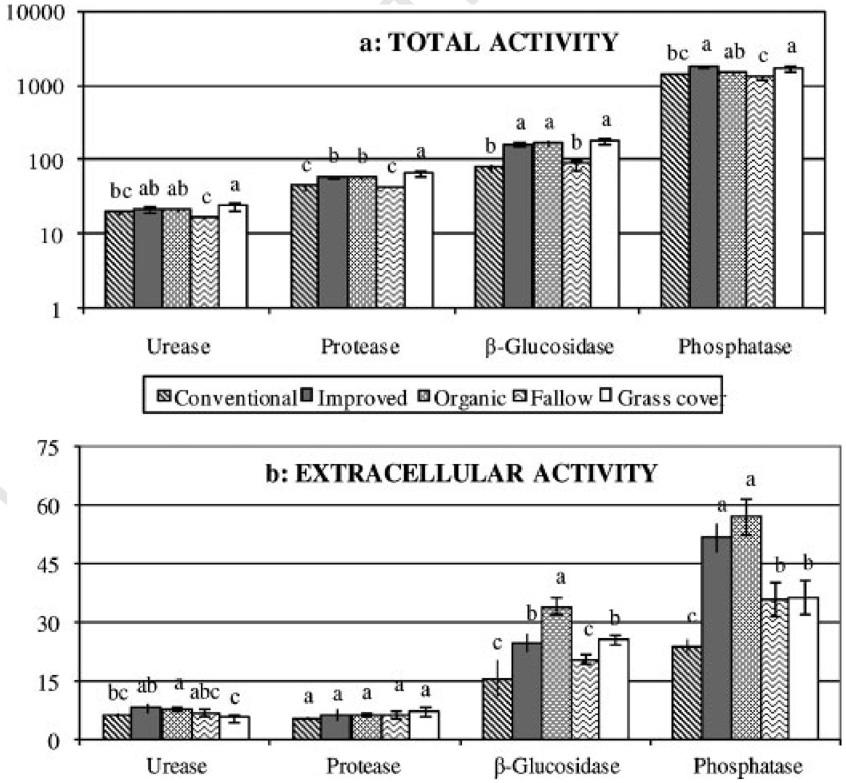

Figure 1. Total and extra-cellular enzyme activity: urease (μg NH$_3$ g^{-1} h^{-1}), protease-BAA (μg NH$_3$ g^{-1} h^{-1}), *β*-glucosidase (μg PNP g^{-1} h^{-1}) and phosphatase (μg PNP g^{-1} h^{-1}). Values for each parameter with the same letter are not significant at $p < 0.05$ according to *t*-Student's test.

and the C/N ratio in this treatment indicates an evolution of the organic residues towards more humified forms.

The lowest values of HmC in Tf and Tc managements were related to the low SOC contents and suggests a slow humification rate and/or a mineralization increase with respect to Sg and the other managements (To and Ti), where the applied organic manures and the mulching stimulated the humification process.

Water soluble C had the highest value under To. This finding is congruent with the yearly application of organic material that contains labile C to this treatment, which resulted in the highest SOC content. The highest value of DHa activity, an index of soil microbial metabolism (García *et al*., 1997), was also found in To and it was related to its high labile C content (WSC). Organic amendments improve the stability of soil structure and increase water retention, which stimulate soil enzyme activity (Marcote *et al*., 2001). The lowest DHa activity under Tc and Tf can be directly related to the low SOM contents in the *Acrisols* cultivated with these treatments, what could cause a decrease of the number of associate microorganisms (García *et al*., 1997). The values of the DHa/WSC ratio were higher in To and Ti, indicating higher soil microbial activities (likely due to the addition of easily decomposable organic compounds under these two managements). Similarly to other parameters, the DHa/WSC ratio was lower in Tc and Tf, suggesting a decrease of soil metabolic activity when these managements were applied.

Total hydrolytic-enzyme levels were significantly lower in Tf and Tc than in Sg, which has not suffered from human intervention (Figure 1a). The existence of plant cover and its progressive mineralization was the cause of the highest enzyme activities in Sg. In addition, the highest values of GLa in To, Ti and Sg suggest an enrichment of fresh-plant materials of cellulolytic nature acting as substrate for the β-glucosidase enzyme (Caravaca *et al*., 2002). Although different studies have shown that PHa activity increases as a consequence of organic additions (Pascual *et al*., 1999; Chakrabarti *et al*., 2000) and decreases when P fertilizers are used (Olander and Vitousek, 2000; Böhme and Böhme, 2006), the PHa activity was significantly higher in Sg and Ti (where P fertilizers were applied) than in the other managements. According to Saviozzi *et al*. (2001), URa and PRa activities decrease as a result of ploughing (Tc management in this study).

Enzyme activity of fine texture soils (like in this case) depends in part on the activity of extra-cellular enzymes immobilized by mineral colloids, which may not be as sensitive to environmental factors as microbial activity (Nannipieri *et al*., 1996). The enzymes linked to humic colloids retain their enzymatic activity in soil extracts due to the protection from physical–chemical denaturation and microbial attack (García *et al*., 1994; Nannipieri *et al*., 1996). In general, the extra-cellular enzyme activity was lower under Tc management and higher under To treatment, where available organic substrates (WSC) were higher (Figure 1b); suggesting an interaction between the available energy-rich compounds and the biochemical energy accumulated and preserved in humus-enzyme complexes (Masciandaro and Ceccanti, 1999). The GLa and PHa extra-cellular activities were the most significantly affected by the applied managements, whereas the URa and PRa extra-cellular activities showed less variability among the different applied treatments; indicating that the latest two enzymes[Q4] had higher resistance to the physical–chemical stress and microbial attack (and therefore less influenced by the agricultural managements) than the former two enzymes.

Q4

Specific enzyme activities have been used to compare values of enzymatic activities in soils with different organic matter contents (Masciandaro and Ceccanti, 1999; Benítez *et al*., 2005) and could be considered as simple indexes of soil quality. In this study specific extra-cellular activities for each hydrolytic enzyme were calculated per unit of HmC (pyrophosphate-extracted C) to evaluate the intensity of the biochemical activity of the humic fraction of the SOM according to each soil management (Table IV). Specific extra-cellular activities showed a similar tendency in relation to the absolute extra-cellular activities (excepting specific PRa, which did not show significant differences); in general the lowest values were found in Tc treatment and the highest values in To treatment. Therefore, the application of organic residues to the soil increased the enzymatic activity due to the direct addition of microorganisms and enzymes to the soil or indirectly by the addition of easily available substrates for microorganisms that increase their activity (García *et al*., 1994; Benítez *et al*., 2005).

According to these results, enzyme activities are related to SOM content (Table V); then, the different managements applied in these soils derived from old volcanic materials modified the SOM content, affecting the humus-enzyme complexes activity and the soil metabolism.

Table V shows the correlation matrix for all the studied parameters. There was a positive correlation between SOC, Nt, HmC, WSC, DHa and GLa, which means that these enzyme activities were influenced by the energy availability (SOC and WSC contents). A very significant correlation was also found between DHa and WSC ($r = 0{\cdot}974$, $p < 0{\cdot}01$), suggesting a relationship between the availability of labile and easily mineralizable SOC and the activity of microbial populations. The close correlation between DHa and HmC ($r = 0{\cdot}921$, $p < 0{\cdot}01$) indicated that SOM humification is a biological process controlled by micro-organisms activity (Ceccanti and García, 1994). A high positive correlation existed among the overall total enzyme activities (URa, PRa, GLa and PHa), suggesting an equilibrium between the principal nutrients cycles. In general, extra-cellular enzyme

Table IV. Specific enzyme activity (absolute extra-cellular enzyme activity/ humic C) of cultivated and uncultivated soils

Parameters managements	URa ($\mu g\,NH_3\,mg^{-1}\,C\,h^{-1}$)	PRa	GLa ($\mu g\,PNP\,mg^{-1}\,C\,h^{-1}$)	PHa
Tc	3·47 bc	3·10 a	8·5 c	13·9 d
Standard dev.	0·06	0·49	1·5	0·1
Ti	3·75 ab	2·96 a	11·2 b	23·5 ab
Standard dev.	0·35	0·86	0·9	1·1
To	3·69 a	2·99 a	15·7 a	26·0 a
Standard dev.	0·02	0·43	1·9	0·6
Tf	3·64 ab	3·78 a	11·7 b	19·5 bc
Standard dev.	0·20	0·90	0·3	4·2
Sg	2·81 c	3·75 a	13·0 b	18·6 c
Standard dev.	0·39	0·76	0·1	1·5

URa; Urease, PRa; protease-BAA, GLa; β-glucosidade, PHa; phosphatase, PNP; *p*-nitrophenol, Tc; conventional, To; organic, Ti; improved, Tf; fallow, Sg; grass cover.
Different letters for each variable indicate statistically different values (Student's *t*-test at $p<0{\cdot}05$).

Table V. Correlation coefficients between different parameters

	SOC	Nt	HmC	WSC	DHa	URa-tot	PRa-tot	GLa-tot	PHa-tot	URa-ext	PRa-ext	GLa-ext	PHa-ext
SOC	1												
Nt	0·832^{b}	1											
HmC	0·755^{a}	0·780^{b}	1										
WSC	0·868^{b}	0·729^{a}	0·920^{b}	1									
DHa	0·802^{b}	0·638^{a}	0·921^{b}	0·974^{b}	1								
URa-tot	0·367	0·595	0·561	0·397	0·346	1							
PRa-tot	0·433	0·536	0·586	0·477	0·413	0·919^{b}	1						
GLa-tot	0·648^{a}	0·667^{a}	0·755^{a}	0·688^{a}	0·639^{a}	0·818^{b}	0·943^{b}	1					
PRa-tot	0·207	0·347	0·620	0·401	0·423	0·779^{b}	0·810^{b}	0·776^{b}	1				
URa-ext	0·664^{a}	0·507	0·739^{a}	0·780^{b}	0·810^{b}	0·012	−0·013	0·239	0·203	1			
PRa-ext	0·525	0·513	0·311	0·280	0·164	0·601	0·598	0·592	0·438	0·176	1		
GLa-ext	0·902^{b}	0·722^{a}	0·750^{a}	0·864^{b}	0·797^{b}	0·475	0·603	0·780^{b}	0·289	0·497	0·502	1	
PHa-ext	0·846^{b}	0·528	0·781^{b}	0·918^{b}	0·916^{b}	0·246	0·420	0·663^{a}	0·362	0·737^{a}	0·335	0·858^{b}	1

[a] Correlation coefficients are significant at $p<0{\cdot}05$ (two tailed).
[b] Correlation coefficients are very significant at $p<0{\cdot}01$ (two tailed).

activities also showed a good correlation with the different forms of C and N. The absence of correlation between extra-cellular and total enzyme activities confirmed the humic stabilized nature of these enzymes and that the extra-cellular enzyme activity is not so dependent of the micro-organisms action.

In summary, these correlations showed that the studied biochemical indices can provide good indications of SOM quality under different agronomic management systems in this degraded volcanic soil.

CONCLUSIONS

Retention of crop residues and addition of composts and farm manures (organic management), or mulching and fertilizer inputs adjusted to crop demand (improved management) resulted in an improvement of soil quality, mainly by increasing of SOC and N contents, and biochemical parameters related to microbial activities involved in C and bio-elements cycles in soils derived from old volcanic materials. Both treatments contributed to improve the organic reserves and the quality of the degraded *Acrisol* selected for this field experiment.

The activity of the assayed soil enzymes was strongly depressed under intensive agronomic use (conventional and fallow systems) and was enhanced under conservative managements (organic and improved managements). In the short-term (4 years) these enzyme activities, particularly those linked to stable humus complex (especially *β*-glucosidase and phosphatase), were more sensitive to agricultural practices than changes in SOC and HmC contents in this degraded *Acrisol*. Consequently, the assayed soil enzymes activity can be considered as good indicators of soil quality changes. In addition, water soluble C and the DHa/WSC ratio were also evidenced as good indicators of SOM quality of this *Acrisol*.

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