POTENTIAL UNIVERSAL APPLICABILITY OF SOIL BIOINDICATORS: EVALUATION IN THREE TEMPERATE ECOSYSTEMS

MIRTA G GONZÁLEZ [1]; JUAN F GALLARDO[2]; ELENA GÓMEZ[3]; GRAZIA MASCIANDARO[4]; BRUNELLO CECCANTI[4] & SILVIA PAJARES[2]

[1]FAUBA. Cátedra de Edafología. Av. San Martín 4453. (1417), Buenos Aires Argentina. mggonzal@agro.uba.ar
[2]CSIC Instituto de Recursos Naturales de Merinas, Aptado257, Salamanca 37071, Spain.
[3]Facultad de Cs Agrarias UNR Cátedra de Microbiología Agrícola. Campo Experimental Villarino. CC14. Zavalla CP2125. Sta Fe.
[4]CNR. Instituto de la Chimica del terreno. Via Condini. Pisa 7856125. Italy.

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ABSTRACT
Three selected soils from three countries with temperate climates have been analyzed. Two of the soils are silty loams (Buenos Aires, Argentina, and Salamanca, Spain) and the third one is a sandy loam (Peccioli, Italy). Soil samples representing three agricultural managements were obtained from the top layer (0-10 cm), i.e. intensively cultivated, cultivated and undisturbed native soils. Soil organic carbon (SOC), total nitrogen (Nt), ATP, urease, protease, phosphatase, β-glucosidase, dehydrogenase (DHA), and arginine ammonification (ARA) were determined and compared. SOC and Nt were significantly higher ($P < 0.01$) in native than in cultivated and intensively cultivated soils. A good correlation ($P < 0.05$) was found between ATP, hydrolase activities, ARA, and SOC and Nt, indicating that all these parameters were related to the biological properties and biochemical activity. Then, these biological parameters can be used as bioindicators of agriculture-induced changes in soils. DHA did not correlate with the SOC and Nt contents, or protease activities, indicating that the soil ecosystems had low concentrations of exogenous substrates to metabolize and that micro-organisms were in a reduced state of activity.

Key words. Soil quality, Soil degradation, Soil enzymatic activity, Argentine, Spain, Italy.

APLICACIÓN POTENCIAL UNIVERSAL DE BIOINDICADORES DEL SUELO: SU EVALUACIÓN EN TRES ECOSISTEMAS TEMPLADOS

RESUMEN
Se compararon las actividades enzimáticas de distintos ecosistemas con diferentes características de uso de suelo para utilizarlas como bioindicadores.

Se analizaron suelos de tres países de climas templados. Dos de los suelos presentan textura franco limosa (Buenos Aires, Argentina y Salamanca, España) y el tercero franco arenosa (Peccioli, Italia). Se obtuvieron muestras de 10 cm de profundidad provenientes de tres manejos diferentes en cada uno de ellos: agricultura intensiva, rotación cultivo-pastura y suelo nativo.

En todos los sitios se determinaron y compararon el Carbono orgánico (SOC), Nitrógeno total (Nt), contenido de ATP, acividad enzimática de la ureasa, proteasa, fosfatasa, β-glucosidasa, deshidrogenasa (DHA) y arginina (ARA).

Se encontró una buena correlación ($p < 0,05$) entre ATP, DHA, ARA con el SOC y Nt, indicando que estos parámetros del suelo están relacionados con las propiedades biológicas y la actividad bioquímica. No se encontró correlación entre DHA con el SOC, con el Nt , ni con la actividad de la proteasa en suelos de agricultura intensiva, indicando que en ecosistemas de bajos contenidos de sustratos exógenos para metabolizar, los microorganismos están en un nivel bajo de actividad. La similitud de los resultados obtenidos de los suelos de tres diferentes países confirman la utilidad de las variables bioquímicas como indicadores potenciales de la degradación del suelo causadas por las prácticas culturales.

Palabras clave. Calidad del suelo, Degradación del suelo, Actividad enzimática, Argentina, España, Italia.

INTRODUCTION

The degradative effects of poor agricultural management on natural and environmental resources have been widely discussed at global level and have led to increasing concern about the need to develop strategies aimed at ensuring a sustainable agriculture Gregorich *et al*. (1993); Doran *et al*. (1994).

In recent decades, studies carried out on the productive capacity of soils have revealed severe degradation and more than 10% of cultivated soils worldwide was affected by erosion, environmental pollution, excessive tilling, overgrazing, tree-felling, salinization, and desertification [Sanders, (1992); Lal, (1994)]. Management practices such as tilling, rotation, pesticide and fertilizer use, the addition of organic matter and composts, among others, have direct bearing on the quality of water, air, and soils [Mosier *et al*., (1991); Doran & Jones, 1996; Chander *et al*., (1997)]. Relative long periods of time (5-10 years) are required in order to detect significant changes in soil organic carbon and nitrogen as a result of management practices [Nannipieri *et al*., (1990); García & Hernández, (1997)].

On the other hand, biological components of the soil ecosystem can act as sensitive indicators since they participate in innumerable processes and functions occurring in the soil, such as the decomposition of organic residues, nutrient cycling, synthesis of humic substances, soil aggregation, and energy release, among others [Nannipieri *et al*., (1990); Smith *et al*., (1993); Turco *et al*., (1994)]. Therefore, the issue is the choice of the best biological properties as sensitive indicators able to detect early (less than 1 year) changes in soil degradation or in its recovery.

Among biological properties, soil enzyme assays are indicators of the soil potential to degrade or transform substrates. They can be an integrative index of past soil biological activity as influenced by soil management [Nannipieri, (1984); Dick *et al*., (1996)].

The term "biological activity" implies the contribution of all organisms inhabiting soil to the overall metabolic activity. Measurements conducted in the laboratory use homogenized soil samples in which remains of plants and meso-faune are eliminated. In this case, it is more correct to define the metabolic activity measured as soil microbiological activity (SMA).

Alef & Kleiner (1987) found that the arginine ammonification (ARA) is significantly correlated with the ATP content and soil respiration; as a consequence of this, ARA is considered to be a valid index of SMA, since the rate of ARA reflects the metabolic status of the microorganisms in the soil, whose numbers do not vary during the short incubation time (1 h). Presumably plants do not use arginine as a source of N and the fauna ammonifies arginine very slowly such that this method can reflect SMA under field conditions (Alef & Kleiner, 1987).

The aims of the present work are: 1) to assess through the use of different bio-markers the effect of cultivation practices on SMA under different managements: cultivated (crop rotation) intensively cultivated (monoculture), and the undisturbed soil; and 2) to compare the results obtained with such bio-markers in temperate ecosystems from different countries to assess their applicability to other similar cases.

MATERIALS AND METHODS

Soils from three countries representatives of temperate zones were studied: Peccioli (Italy), with a temperate and humid Mediterranean climate; Salamanca (Spain), with a semiarid Mediterranean climate, and Buenos Aires (Argentine), with a humid temperate climate (Table 1). In all the three areas, samples were collected from the surface layer (0-10 cm) from: i) native, un-

Table 1. Main characteristics of soils and climates in the three ecosystems studied.
Tabla 1. Características de los suelos y clima correspondientes a los tres ecosistemas estudiados.

	Peccioli (Italy)	**Salamanca (Spain)**	**Buenos Aires (Argentine)**
SOC (g C kg^{-1})	25	21	35
Nt (g N kg^{-1})	3.7	2.8	2.2
pH (H_2O)	6.0	6.2	6.5
Texture	Silty loam	Sandy loam	Silty loam
Tillage system	Conventional	Conventional	Conventional
Taxonomy (FAO, 1974)	*Dystric Regosol*	*Chromic Luvisol*	*Luvic Phaeozems*
Climate	Temperate, humid Mediterranean	Semiarid Mediterranean	Temperate, humid Continental

disturbed soils (NS); ii) cultivated soils, under crop rotation (maize, sunflower, and meadow; (CS) and iii) soils under continuous monoculture (maize, sunflower; (IS).

Soils samples were, mixed, homogenized, sieved (< 2 mm), and kept at 4 °C before the determination of microbiological and biochemical parameters, and air-dried for chemical determinations. All the analyses were made on three laboratory replicates.

Chemical analyses. The SOC and Nt were determined using the dichromate oxidation [Nelson & Sommers, (1982) and micro-Kjeldahl (du Preez *et al.,* (1987)] methods, respectively.

Biochemical determinations. Urease activity (URA) was measured according to the method of Zantua and Bremner (1977); protease activity (PTA) was determined using casein as substrate (Ladd & Butler, 1972). β-glucosidase (GCA) and phosphatase (PHA) activities were assessed following the method of Speir and Ross (1976). Dehydrogenase (DHA) activity was determined following the method proposed by Trevor (1984a) and was expressed as μg INTF g^{-1} h^{-1}. URA and PTA were expressed as μg N-NH_4 g^{-1} h^{-1}; PHA and GCA as μg PNP g^{-1} h^{-1}. ATP content was measured and expressed as μg ATP g^{-1}, except for Argentinean soils where the SMA was assessed by ARA and expressed as μg N-NH_4 g^{-1} h^{-1}.

Statistical analysis. Correlation analysis was performed to determine the association between bio-markers measuring SMA, SOC, and Nt as indicatives of soil fertility.

RESULTS

Results from the different enzymes measured related to Nt are exposed in Table 2.

The correlation matrix between URA, PTA, PHA, GCA, DHA, ATP, ARA, SOC, and Nt for soils from Peccioli, Salamanca, and Buenos Aires is presented in Table 3.

Figure 1 shows the variations in the activity of hydrolytic enzymes in the three soil ecosystems studied. The change of SOC, Nt, DHA, and ARA activities are shown in Figure 2.

SOC and Nt contents were lower in cultivated soils (CS and IS); in the same way, URA, PTA, PHA, ß-GCA, and DH activities of cultivated soils decreased significantly (P <0.05) with respect to that found in the NS (Fig. 1). ARA was also higher under NS and reflects the high ammonification capacity of this native soil. Among cultivated soils, those undergoing intensive agricultural use (IS) had the lowest enzyme activity, in both the Argentinean and European samples. In biological systems, all reactions are practically catalyzed by enzymes.

Table 2. Nt (mg N g^{-1}), C/N, DHA/N, URA/N, PTA/N, PHA/N, and GCA/N ratios in the native soils (NS), cultivated soil under rotation (CS) and intensively cultivated soil (IS).

Tabla 2. Relación de Nt (mg N g^{-1}), C/N, DHA/N, URA/N, PTA/N, PHA/N y GCA/N en suelo nativo (NS), suelo cultivado bajo rotación (CS) y suelo bajo cultivo intensivo (IS).

Peccioli (Italy)

	NS	CS	IS
Nt	0.40	0.09	0.05
C/N	10.2	11.5	17.2
DHA/N	12	27	30
URA/N	182	330	340
PTA/N	23	37	40
PHA/N	6	19	20
GCA/N	7	19	20

Salamanca (Spain)

	NS	CS	IS
Nt	0.49	0.08	0.04
C/N	8.2	10.1	15
DHA/N	3	13	16
URA/N	245	671	850
PTA/N	22	23	27
PHA/N	22	36	45
GCA/N	11	23	27

Buenos Aires (Argentine)

	NS	CS	IS
Nt	0.40	0.23	0.11
C/N	10.0	8.3	13.6
DHA/N	13	16	18
URA/N	129	130	140
PTA/N	43	45	50
PHA/N	31	34	36
GCA/N	23	25	28

The difference in activity between the cultivated and (NS) soils reveals the perturbation caused to the ecosystem by cultivation practices. Therefore, intensive agricultural practices cause a loss of soil quality, probably due to a combination of factors, as low level of (SOC), nutrients, and (SMA).

Table 3. Correlation matrix between enzymatic activities, ATP, SOC and Nt in three temperate ecosystems: Peccioli (Italy) Salamanca (Spain) and Buenos Aires (Argentine).

Tabla 3. Matriz de correlación entre actividades enzimáticas, ATP, SOC y Nt en tres ecosistemas templados: Italia (Peccioli), España(Salamanca) y Buenos Aires (Argentina).

Peccioli (Italia)

	Urease	**Protease**	**Phosphatase**	**β-glucosidase**	**DHA**	**ATP**	**SOC**
Urease							
Protease	0.99**						
Phosphatase	0.99**	0.98**					
β-glucosidase	0.99**	0.99**	0.99**				
DHA	0.93*	ns	0.94**	0.93*			
ATP	0.99**	0.99**	0.98**	0.98**			
SOC	0.96*	0.99**	0.95*	0.96**	ns	0.99**	
Nt	0.96*	0.99**	0.95*	0.96**	ns	0.99**	0.99**

Salamanca (Spain)

	Urease	**Protease**	**Phosphatase**	**β-glucosidase**	**DHA**	**ATP**	**SOC**
Urease							
Protease	0.98**						
Phosphatase	0.99**	0.99**					
β-glucosidase	0.99**	0.98**	0.99**				
DHA	0.94*	ns	ns	0.94*			
ATP	0.99**	0.97**	0.99**	0.99**	0.96**		
SOC	0.99**	0.99**	0.99**	0.99**	ns	0.99**	
Nt	0.98**	0.99**	0.99**	0.98**	ns	0.97**	0.99**

Buenos Aires (Argentine)

	Urease	**Protease**	**Phosphatase**	**β-glucosidase**	**DHA**	**ARA**	**SOC**
Urease							
Protease	0.97**						
Phosphatase	0.98**	0.90*					
β-glucosidase	0.96**	0.91*	0.98**				
DHA	0.91*	ns	ns	0.90*			
ARA	0.99**	0.90*	0.95*	0.97**	ns		
SOC	0.97**	0.93*	0.98**	0.98**	ns	0.99**	
Nt	0.95*	0.92*	0.98**	0.97**	ns	0.95*	0.98**

*p<0.05; ** p< 0.01; ns: non significant

Figure 1. Variations in the activity of soil hydrolytic enzymes in the three ecosystems studied (urease and protease expressed as µg N-NH_4 g^{-1} h^{-1}; phosphatase and β-glucosidase, as µg PNP g^{-1} h^{-1}). NS: native soil, CS: cultivated soil under rotation; IS: intensively cultivated soil.

Figura 1 Variación de la actividad del suelo de las enzimas hidrolíticas en los tres ecosistemas estudiados (Ureasa y proteasa expresado como µg N-NH_4 g^{-1} h^{-1} fosfatasa y β-glucosidasa, como µg PNP g^{-1} h^{-1}). NS : suelo nativo, CS: cultivado y suelosbajo rotación; IS: suelo bajo cultivo intensivo.

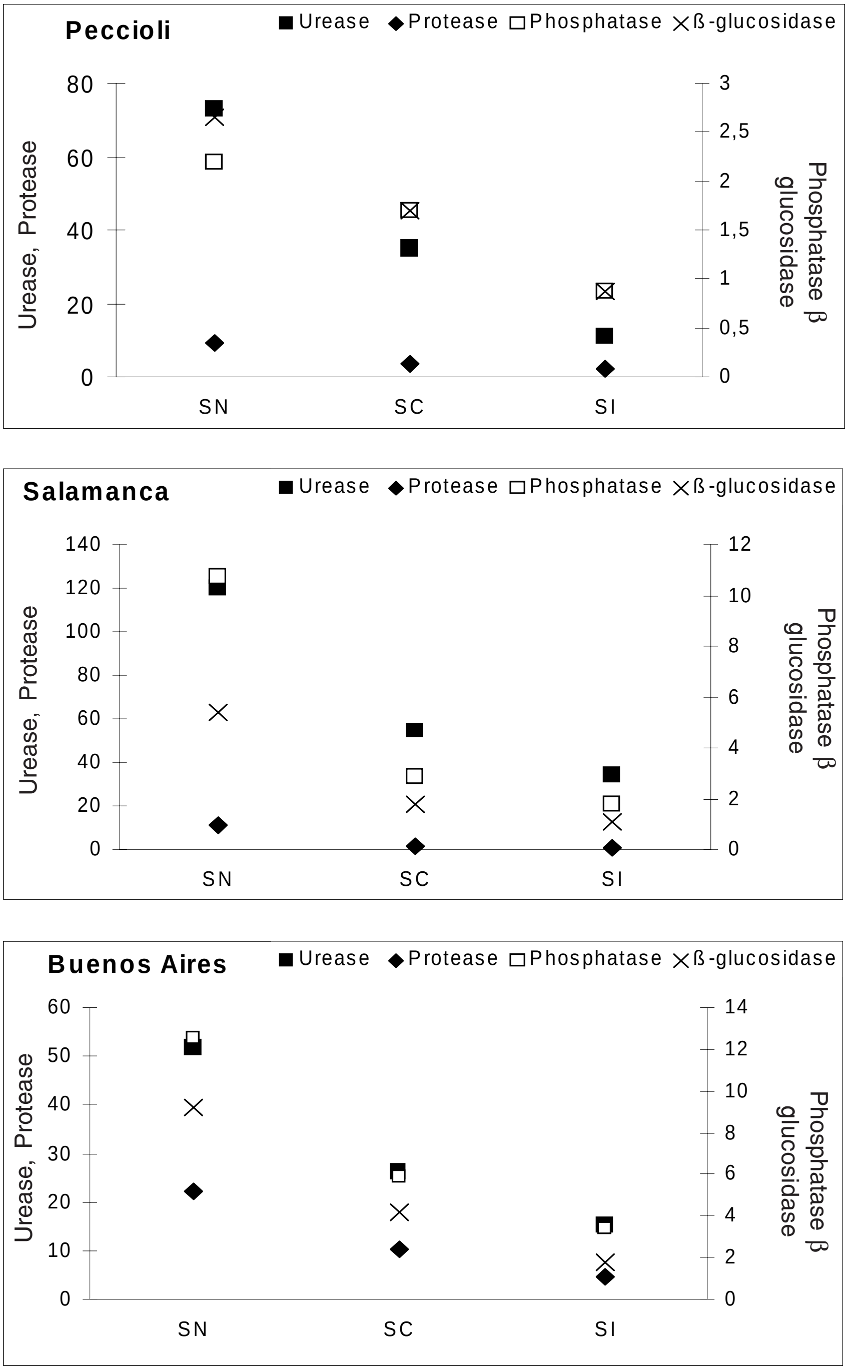
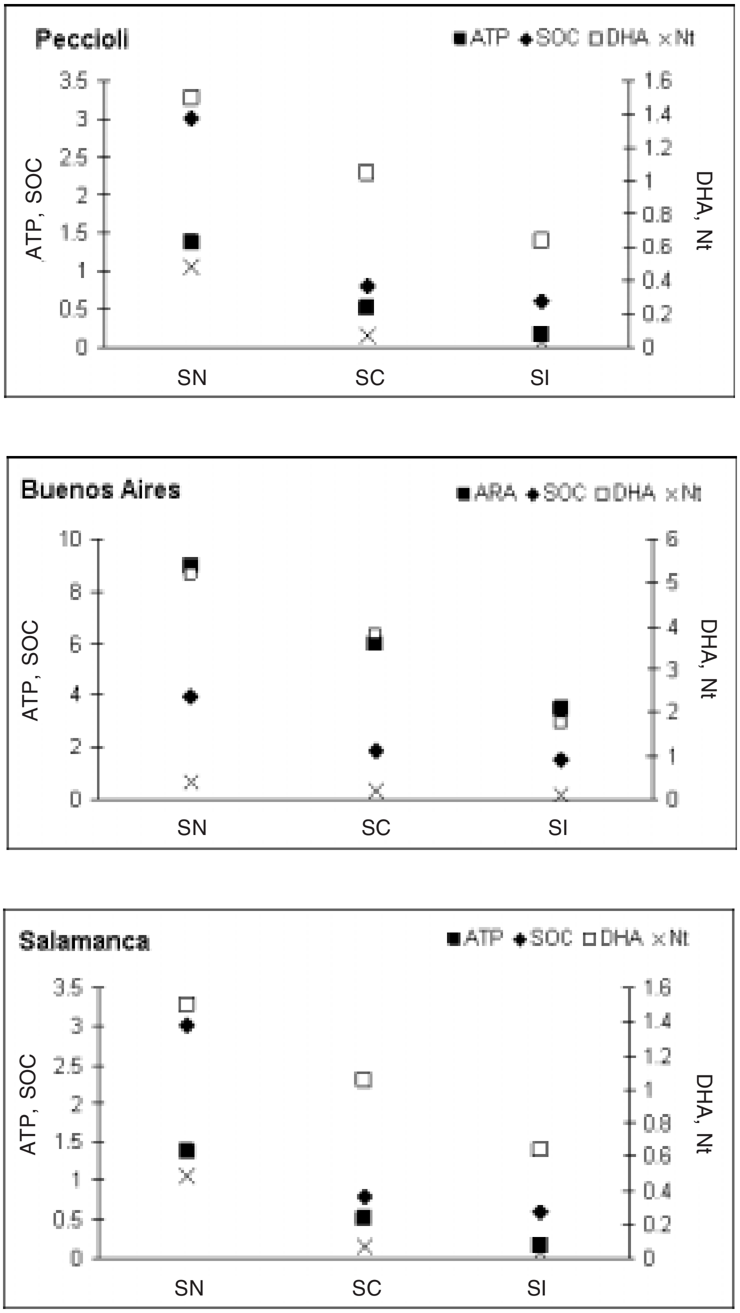


Figure 2. Changes in soil chemical and biochemical parameters in the three ecosystems studied (ATP expressed as µg g^{-1}; DHA as µg INTF g^{-1} h^{-1}; ARA as µg N-NH_4 g^{-1} h^{-1}; SOC as % and Nt as mg N g^{-1}) NS: native soil, CS: cultivated soil under rotation; IS: intensively cultivated soil.

Figura 2. Cambios en los parámetros químicos y bioquímicos en los tres ecosistemas estudiados (ATP expresado como µg g^{-1}; DHA acomo µg INTF g^{-1} h^{-1}; ARA como µg N-NH_4 g^{-1} h^{-1}; SOC como % and Nt as mg N g^{-1}) NS: suelo nativo, CS: suelo bajo rotación de cultivos; IS: suelo con agricultura intensiva



DISCUSSION

The results indicated that management practices that minimize the input of organic residues to soil decrease the activity of certain enzymes, affecting the capacity of the soil to recycle nutrients and to release them for their use by plants.

The ratio between the enzyme parameters (except DHA) and the SOC content were inconsistent (data not shown); while a definitive trend referring the variables to Nt content were found, being consistently lower in the NS soils (Table 2). The soil C/N ratio was also lower in NS with respect to CS and IS. Other ratios involving Nt content showed that the amount of N converted from organic to mineral forms (mineralization), on an annual basis, depends on the past management history, annual climatic variation, and inherent soil properties (Paul & Clark, 1989).

The activity of soil enzymes is related to SMA (Dick, 1992) and, therefore, the enzymes could be used as integrated indices into the biological and biochemical changes produced by different types of management. Soil enzymes could be considered an integrative index of past soil biological activity (Dick *et al*., 1996). Thus, the activity of enzymes remains catalytic because such enzymes are probably bind and protected in soil humic or clay complexes, whereas N could decrease with intensive management practices. The above explanation is not valid for DHA enzyme because this enzyme is an integral part of the viable cell of the micro-organisms, so it is not stable outside the cell (Nannipieri *et al*., 1990).

The study of different hydrolase enzyme activities is important since they indicate the potential of a soil to carry out specific biochemical reactions, and these hydrolytic enzymes are important in maintaining soil fertility (Burns, 1982). URA and PTA act in the hydrolysis of organic N producing inorganic N, the former using urea-type substrates and the latter simple peptidic substrates. PHA catalyses the hydrolysis of organic-P compounds to phosphates and GCA hydrolyses GCA in soil or in decomposing plant residues (Pascual *et al.,* 2000). In all the situations the biggest changes were presented in URA and PHA, while PTA seemed to show the smallest sensibility to the disturbances (Fig. 1). The overall levels of hydrolytic enzymes detected in the cultivated soils were low compared with the soil of the same area, which has not suffered human intervention. These results agreed with those found where continuously cultivated soils have lower levels of microbial biomass, lower enzyme activities, and lower potential to mineralize organic sources of nutrients than uncultivated soils (Dick, 1992; Chander *et al.,* 1997).

The changes of SOC, Nt, DHA activity and ARA (Fig. 2) suggests that these variables may be used as potential indicators, since these properties were significantly affected by the different management regimes of the soils. For the SOC and Nt contents, significant differences are only seen between the cultivated and NS soils ($P < 0.01$). Higher SOC contents improve structural stability and increase water retention (Gregorich *et al.,* 1993), both conditions favouring microbiological activity as shown by DHA activity and ARA. These latter two biochemical parameters allowed the identification of differences not only with the NS ($P < 0.01$) but also among the soils under different management regimes ($P < 0.05$). Thus, DHA activity and ARA would be more sensitive indicators than SOC and Nt measurements. Similar results were reported by Gupta *et al*. (1994), Ladd (1994), and Pankhurst *et al*. (1995), who found that microbial parameters responded to soil management changes at short-term, whereas the SOM-content changes at long term (Nannipieri, 1984).

In order to gain an overall understanding of the results, a correlation matrix was constructed for each of the ecosystems studied, taking into account the differences among the different cultivation regimes (Table 3). Two levels of significance were considered ($P < 0.01$ and $P < 0.05$) to evaluate better the association among the variables determined.

DHA was correlated ($P < 0.05$) with ATP content and with ARA in the Spanish and Argentinean ecosystems, respectively, while no correlation with ATP was found in the Italian ecosystem. The good correlation between DHA and ARA corroborated that the liberation of ammonia from arginine is due to the activity of microbial cells, not by extracellular enzymes (Alef & Kleiner, 1987). ATP content and the hydrolases were significantly correlated ($P < 0.05$) in the European soil ecosystems. In Argentine a similar correlation was found between AR and the hydrolase activities. Therefore, the ATP content and the ARA seem to be good indicators of microbial processes in soils undergoing different management strategies. A high and positive correlation also occurs among all the total hydrolase activities, suggesting an equilibrium between the cycles of the principal nutrients.

The lack of correlation between DHA and SOC would be probably due to the fact that this enzyme is an integral part of micro-organisms and can not accumulate in a complex form in soils. Therefore, it is recommendable to use the DHA as an indicator of the viable microbial populations (Dick *et al*., 1996).

CONCLUSIONS

The present results, obtained from soils in different temperate ecosystems belonging to three countries, confirmed the utility of the biochemical parameters studied as potential indicators of soil degradation caused by some cultivation practices. Among the variables assessed, enzymatic activities (ureases, protesases, phosphatases, and dehydrogenases) and arginine ammonification may be sensitive indicators to detect changes occurring in soils under field conditions. These variables are associated with biochemical processes and they are relatively easy to measure and interpret.

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