



# Dehydrogenase and mycorrhizal colonization: Tools for monitoring agrosystem soil quality



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## ARTICLE INFO

### Article history:

Received 15 June 2015

Received in revised form 17 December 2015

Accepted 18 December 2015

Available online 8 January 2016

### Keywords:

Soil biological indicators

Agriculture management

Organic inputs

Enzymatic activities

## ABSTRACT

In order to improve food production while reducing environmental impact, the redesign of agrosystems to incorporate monitoring tools for decision-making is a fundamental requirement. Chemical and physical indicators of medium and long-term soil quality have been developed in order to monitor changes in agroecosystems; however, it is crucial to develop parameters that can predict the short-term trajectory of the system. For this reason, the objective of the present study was to determine whether certain soil biological parameters, such as the activities of microbial enzymes (dehydrogenase (DH), acid phosphatase (ACP), urease (URE) and protease (PRO)) and mycorrhizal colonization, could be useful as indicators of soil biological quality, given their sensitivity to different agricultural management practices. An evaluation was conducted over two years in five different agrosystems of Valle de México in Mexico. The activity of DH presented greater sensitivity to changes in agricultural management produced by types of tillage and input (organic or synthetic) and topological arrangement, compared to that of URE, ACP and PRO, which did not present a clear pattern with respect to the different agrosystems or to sampling date (based on the agricultural practices). Mycorrhizal colonization was sensitive to the type of inputs used, but not to tillage type or crop rotation. It is therefore considered that DH and mycorrhizal colonization could represent useful parameters for measuring soil quality and the environmental impact of the use of agrochemicals in agriculturally managed soils. Based on DH and mycorrhization, the agrosystem with the highest quality soil was the Mesoamerican system known as “Milpa” (typified by minimum tillage, intercropping and organic inputs).

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## 1. Introduction

The biological components of the soil can be used as short-term agrosystem quality indicators, given their high sensitivity to any alteration of the system or change in the environment (Bending et al., 2004), as well as their close relationship with plant root systems, stress tolerance, productivity and adaptability, among other agrosystem characteristics (Rodríguez and Redman, 2008; Schnitzer et al., 2011; Lau and Lennon, 2011). Different agricultural management practices that imply various types of tillage, fertilization and weed control, among others, can generate physical and chemical conditions in the soil that affect the activity and composition of the microbiota and thus its enzymatic activity

(Acosta-Martínez et al., 2008; Schipanski and Drinkwater, 2012; Vasseur et al., 2013). The dynamics of release, flow and absorption of nutrients through the activity of extra- and intracellular enzymes are of great interest from an agronomic perspective (Ceja-Navarro et al., 2010; Kumar and Varma, 2011) and control the recycling of soil organic materials, thus dictating the availability of nutrients (Kohler et al., 2009).

Microbial enzymatic activities that are affected by the type of agricultural management include those of dehydrogenase, which is important for the decomposition of soil organic matter (SOM) and in N dynamics (García et al., 1993; Nannipieri et al., 2003; Vepsäläinen et al., 2004; De Varennes et al., 2007); protease and urease, which participate in the hydrolysis of peptide bonds and release of  $\text{NH}_4^+$  (Banik and Prakash 2004; Wang et al., 2008); and acid phosphatase, which catalyzes the hydrolysis of esters and anhydrides of phosphoric acid under different conditions of pH (Gianinazzi et al., 1992). Similarly, microbial enzymatic activity varies according to the associated plant species, since each has a

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different effect on the microbiota according to the nature and quantity of its root exudates (Nannipieri et al., 1990; Czarnes et al., 2000; Bending et al., 2004). There are microorganisms, such as arbuscular mycorrhizal fungi (AMF) (Nielsen and Winding, 2002), that are key to soil biological quality. These fungi associate with the roots of plants, contributing to nutrient (P) acquisition, resistance to pests and diseases and tolerance to drought and heavy metals, as well as improving soil structure (Gosling et al., 2006). Abundance of AMF in the roots can be a biological indicator of the impact of different agricultural management practices, since the degree of AMF colonization is related to practices such as type of rotation, proportion of organic material and intensity of tillage. It can also be an indicator of the physico-chemical characteristics of the soil (Hijri et al., 2006; Miller and Jackson, 1998).

Soil biological properties are closely related to agrosystem management and quality and monitoring of these properties can provide basic tools for evaluating these systems. As part of the task of redesigning these agrosystems from a holistic and co-innovatory perspective, an aspiration toward more closed cycles of energy and materials is required, while simultaneously reducing the use of synthetic products and considering renewable energy sources that respect the living organisms of the system (Baars and Baars, 2007; Guzmán and González de Molina, 2009).

The objectives of this study were: (i) to evaluate the biological quality of five agrosystems through measuring the potential of key soil enzymatic activities and the percentage of mycorrhizal colonization in production systems of native maize under different management strategies (type of tillage, fertilization, weed management and rotation), in the southeast of Valle de México, in Mexico; and (ii) to determine which of the assessed biological indicators could be monitored to obtain reliable info about the impact of management strategies on soil quality.

## 2. Materials and methods

### 2.1. Experimental site and soil sampling

The experimental site is located in the municipality of Cocotitlán in the east of Estado de México, in Mexico (19°12'18"–19°14'33"N; 98°49'46"–98°52'52"W: 2300 masl). The climate is of type C(w1)(w), temperate sub-humid, with summer rains (García, 2004). The wet season extends from May to October at the experimental site, with a mean annual precipitation of 784 mm. The soil is Vitric eutric epiarenic (WRB classification). In 2011, the area in which the experimental plots were established (1 ha) was subdivided into plots of  $6.6 \times 30$  m ( $198 \text{ m}^2$ ), in which five different treatments were established (Table 1). In the case of the treatments with rotation (ZTRO+r and ZTRQ+r), we have presented two versions in order to visualize the performance of both crops (maize = m and oats = o), using a random block experimental design with three replicates, giving a total of 15 experimental plots. All of the management models featured

the production of native maize ("chalqueño"), and were based mainly on tillage type (minimum and conventional), crop management (rotation, monoculture and intercropping) and use of organic and synthetic inputs. Organic management in 2012 consisted of the following treatments: ZTIO+r and ZTRO+r, which were fertilized with composted cow dung added to each plant ( $4.4 \text{ t ha}^{-1}$ ); CTMO30r, in which two fertilizations were carried out, the first consisting of composted cow dung ( $1.166 \text{ t ha}^{-1}$ ) and the second with a different composted manure ( $2.733 \text{ t ha}^{-1}$ )—in 2013, however, the first fertilization in the ZTIO+r and ZTRO+r treatments consisted of an application of mountain microorganisms and  $500 \text{ kg ha}^{-1}$  basalt rock dust ( $187.5 \text{ kg}$ ), while the second consisted of composted sheep dung ( $3.3 \text{ t ha}^{-1}$ ) and chicken manure ( $1.1 \text{ t ha}^{-1}$ ); CTMO30r, in which the first fertilization consisted of dry sheep manure ( $3.3 \text{ t ha}^{-1}$ ) and basalt rock dust ( $0.5 \text{ t ha}^{-1}$ ), while the second used chicken manure ( $1.1 \text{ t ha}^{-1}$ ). Weeds were removed manually. In 2012 and 2013, synthetic management consisted of fertilization with urea ( $243.5 \text{ kg ha}^{-1}$ ) and calcium triple superphosphate ( $50 \text{ kg ha}^{-1}$ ), while herbicide (Hierbamina (2,4-D)) was applied at a rate of  $12.51 \text{ g ha}^{-1}$ .

Composite soil samples were obtained from each experimental plot at depths 0–10 and 10–20 cm of the soil profile. Samples were stored at  $4^\circ\text{C}$  until subsequent analysis. In order to determine enzymatic activity, sampling was conducted on seven different dates in the years 2012 (10th of July and 8th of September) and 2013 (14th of January, 3rd of May, 4th of June, 11th of September and 2nd of October). There were five sampling dates in the case of the mycorrhizae: June 4th, July 19th, September 11th and October 2nd, all in 2013. The dates were chosen according to both the occurrence of rain and the different treatments in the plots (Table 2) in order to evaluate the potential of enzymatic activity and mycorrhizal colonization in both dry and wet periods, before and after the application of agrochemicals or organic inputs, according to treatment.

### 2.2. Enzymatic activities

The enzymatic activities considered for evaluation of potential were those of dehydrogenase (DH), acid phosphatase (ACP), urease (URE) and protease (PRO). All of these enzymes participate in processes of SOM decomposition (Das and Varma, 2011). Measurement of the potential of each enzyme activity was performed following the spectrophotometric methods described by García et al. (2003). The activity of dehydrogenase, the only intracellular enzyme considered in this study, was determined by measuring the iodinitrotetrazolium formazan (INTF) formed after incubating 1 g of soil for 20 h at  $20^\circ\text{C}$  in darkness. A Shimadzu dual-beam spectrophotometer was used at wavelength 490 nm (García et al., 2003). Acid phosphatase activity was determined following the method of Tabatabai (1994), which is based on quantification of the *p*-nitrophenol released after incubating 1 g of soil at  $37^\circ\text{C}$  for 1 h in a buffered solution of *p*-nitrophenyl phosphate. The

**Table 1**  
Different agro-management experimental treatments conducted in Cocotitlán, México, Mexico.

Treatment	Tillage	Residues management	Fertilization	Weed management	Rotation
ZTIO+r	Minimum	Retention (100%)	Organic	Manual Forage crop	Intercropping (maize, squash, beans, vetch)
ZTRO+r	Minimum	Retention (100%)	Organic	Manual	Rotation (maize -m-, oats -o-)
ZTRQ+r	Minimum	Retention (100%)	Chemical	Chemical	Rotation (maize -m-, oats -o-)
CTMO30r	Conventional	Incorporate (30%)	Organic	Manual	Monoculture (maize)
CTMQ-r	Conventional	Without residues	Chemical	Plow and chemical	Monoculture (maize)

ZTI = minimum tillage with intercrop, ZTR = minimum tillage with rotation, CTM = conventional tillage with monoculture, O = organic inputs, Q = chemical inputs, +r = with residues, -r = without residues, 30r = with 30% residues.

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