

Artigo

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## Phosphomonesterase and dehydrogenase activities in a horticulture soil under integrated and organic management

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**Abstract** The effect of integrated and organic management on the levels of dehydrogenase and alkalyne phosphomonesterase (EC 3.1.3.1) activities was determined in a horticultural soil during a four year crop rotation, finding no statistically-significant differences. Since soil tillage and fertilization were similar, the absence of effects suggests that the well-reasoned use of small rates of agrochemicals in the integrated management for weed and pest control may have negligible effects on soil microbiological activity.

**Key words** soil enzymes · biological activity · ecological techniques · integrated and organic production.

### Introduction

Increasing concern about environmental protection and food quality has made possible a faster spread of organic farming throughout Europe. Despite undoubtable benefits for the environment, the practice of organic management is considered risky or troublesome for most traditional farmers, since no synthetic fertilizers can be used, and pests or diseases must be controlled by means of natural biocides, green covers, predators and manual weeding. Integrated production, a management system combining care about the environment and advanced agricultural practices, has thus appeared as an intermediate step between organic and conventional farming.

Soil biological activity is key in the correct functioning of these management systems, since nearly all fertilization relies on biological fixation of nutrients and degradation of the organic matter produced or added (Mäder et al., 2002). Most research studies on the subject have been focused on microbial biomass content and respiration measurements (Carpenter-Boggs et al. 2000; Nannipieri et al., 1990; Aoyama & Naguno, 1997). Soil enzymes, however, have long been acknowledged as good indexes of biological activity, and their role in the nutrient cycles (García & Hernandez, 2000; Leiros et al., 2000; Trasar-Cepeda et al., 1999), together with their high sensitivity to small changes of management or pollutants (Fliessbach et al., 1994) occurrence makes them a very useful tool in the diagnosis of fertility, both actual and potential, of soils managed under organic or integrated techniques. Enzyme activity measurements are usually faster and simpler than respirometric techniques, and less prone to interferences in the carbonate-rich soils typical of Mediterranean areas, and are likely to be a more stable and reliable index of biological activity than microbial biomass in soils under severe moisture and temperature fluctuations throughout the year.

Although not abundant, there is a number of research works comparing biological activity in soils under organic and conventional management. Some authors (Fraser et al., 1988), measured levels of dehydrogenase activity, microbial biomass content, respiration, N fixation, and denitrification in soils under organic and conventional management, obtaining different results depending on the parameter and management technique (crop rotation, fertilization, use of biocides) considered. On the contrary others works, reported higher levels of biodiversity and biological activity in the organic plots (Drinkwater et al., 1995). Similarly, another authors found higher levels of acid phosphatase activity in plots under organic and biodynamic management than those in conventional orchards (Oberson et al., 1993, 1996). In our Mediterranean area, an investigation compared pairs of citrus orchards with similar characteristics but different management system (conventional and organic), finding higher levels of all parameters related to biological activity

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(microbial biomass and phosphomonesterase, phosphodisterase, arylsulphatase, urease and dehydrogenase activities) in the organically-managed soils (Albiach, 1997). They reported similar results when compared phosphomonesterase and dehydrogenase activities after different fertilization (organic, organic-mineral and mineral) and management (organic and conventional) treatments (Albiach et al., 2000).

Continuing and complementing our previous efforts to get further information about the effects of management on soil biological activity, the objective of this work is to compare how two different, but environmentally-responsible, management systems (organic and integrated) may influence two indexes of biological activity in soils (phosphomonesterase and dehydrogenase activities).

## Materials and methods

This work was included in the large-scale EU project VEGINECO, which aimed at the development of sustainable systems of horticultural cropping harmonizing the production of high quality crops and the minimal environmental impact. This field experiment was carried out in the Experimental Farm of Fundación Caja Rural Valencia, at Paiporta (Valencia, Spain). Two adjacent plots were set up into a big horticultural orchard with clay-loam soil (Xerochrepts), and each plot was split in four subplots of 1100 m<sup>2</sup> on the average. In the past, these two plots were the same horticultural area, managed by conventional techniques.

The same crop rotation (Figure 1) was cultivated in both plots, but one was managed using integrated techniques, and the other by means of strict organic procedures, as regulated by Spanish normative (Reglamento (CEE) 2092/91). As well, each subplot started with a different year of the four-year rotation, in order to prove all crops, at the

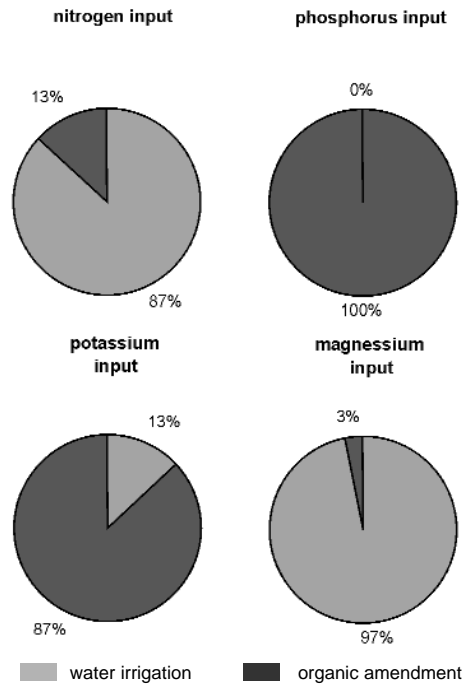
same time, in the three years experience, and to detect common problems on the rotation design. On the second year, it was necessary to change autumn lettuce per green bean due to an aphids problem. On the last year, it was removed summer lettuce to the rotation due to many pesticides problems.

Given the overall objectives of the EU project, the fertilization programme was designed in both cases (organic and integrated) taking into account not only the crop needs but also the nutrient loads of irrigation water, organic amendments, crop residues and green manures, to avoid any lack or excess of fertilizers. The contribution of each external input is displayed on Figure 2. Tillage and fertilization being nearly identical, (30 t/ha of a mixture of cattle and sheep manure in each plot) the main difference between both management techniques was biocide use. Only copper, neem oil, *Bacillus thuringiensis*, metaldehyde, rotenone, sulphur, mineral oil and soap were used for pest control in the organic system, as required by the normatives, but small rates of more than 30 additional authorized chemicals were used in the integrated plot (chlorpyrifos, lambda cyhalothrin, pencicuron, oxifluorfen, bentazone, procimidone, pendimethaline, pirimicarb, etc.).

Soil was sampled in six different times when no crops were being grown during the rotation, with some exceptions, for example before oat-vetch or fennel, where sampling soil was impossible (see Figure 1). The values for each sampling corresponded to the mean obtained of the four subplots, and it was taken 20 subsamples from the 0-30 cm layer of each subplot. Since analysing fresh samples was non-viable given the workload the project involved, all subsamples corresponding to the same subplot were carefully mixed, air-dried, ground and passed through a 2-mm sieve. Although all forms of soil storage are known to affect enzyme activity (Arias et al., 1997), drying is probably

First year													
Sub plots	Winter			Spring			Summer			Autumn			
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
I						lettuce					fennel		
II									artichoke				
III									lettuce		onion		
IV						watermelon			cauliflower				
Second year													
Sub plots	Winter			Spring			Summer			Autumn			
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
IV	potato						lettuce		fennel				
I	lettuce					oat-vetch		artichoke					
II	artichoke						Green bean			onion			
III	onion					watermelon			cauliflower				
Third year													
Sub plots	Winter			Spring			Summer			Autumn			
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
III	potato									fennel			
IV	oat									Artichoke(seed)			
I	artichoke						Green bean			onion			
II	onion						watermelon			cauliflower			

Figure 1.- Crop rotation followed during the three years of study



**Figure 2.-** Relative contributions of each resource of macronutrients

the most suitable for arid Mediterranean soils, which are naturally dry most of the time during the year. Once in dry form, and before analysis, all samples were stored during more than six months, to reach a stable level of enzyme activity, like some studies showed (Bonmatí, 2001). Soil chemical and physicochemical properties were determined according to the Official Methods of the Spanish Ministry of Agriculture (MAPA, 1986). Irrigation water was quite salty and very rich in nitrates, reducing the significance of biological activity in N soil-plant relationships, and thus alkaline phosphomonoesterase was selected as index of nutrient-related enzyme activity and measured using one of the methods (Tabatabai & Bremner, 1969, modified by Eivazi and Tabatabai, 1977). This method is based in the addition of one substrate to the soil, p-nitrophenil disodic phosphate, and the later measure of the p-nitrophenol (PNF) produced by the enzymatic activity by spectrophotometry at 400 nm. Dehydrogenase activity is widely considered as a good index of general soil metabolism activity (Moore & Russell, 1972), and was therefore determined by means of the methodology (Casida et al., 1964), where the substrate is the 2,3,5-triphenyltetrazolium and the produced product is the triphenylformazan (TPF), determined by spectrophotometry at 485 nm. All determinations were replicated at least three times. The statistical significance of the results was assessed by means of the software packages Statgraphics 4.0 (Manugistics Inc.) and R-base (Ihaca & Gentleman, 1996).

## Results and discussion

Tables 1 and 2 show the values of some selected soil properties for the full span of the experiment, and the realized yields during the crop rotation, respectively. As

Parameters	Targets	Results		
		1 <sup>st</sup> year	2 <sup>nd</sup> year	3 <sup>rd</sup> year
<b>Integrated</b>				
Available Nitrogen	<70 kg N/ha	-	410	252
Available Phosphorus	30-45 mg P/kg	122	97	87
Available Potassium	150-300 mg K/kg	599	471	353
Available Magnesium	80-120 mg Mg/kg	348	387	361
Organic matter	<1%	1.84	1.76	1.61
<b>Organic</b>				
Available Nitrogen	<70 kg N/ha	-	324	223
Available Phosphorus	30-45 mg P/kg	118	92	87
Available Potassium	150-300 mg K/kg	733	513	353
Available Magnesium	80-120 mg Mg/kg	322	419	361
Organic matter	<1%	1.89	2.13	1.72

**Table 1.-** Evolution of some soil properties in the integrated and organic plots

Crop	1 <sup>st</sup> year		2 <sup>nd</sup> year		3 <sup>rd</sup> year	
	Integrated	Organic	Integrated	Organic	Integrated	Organic
Watermelon	70.3	68.3	85.7	65.9	109.9	96.9
Cauliflower	28971	28473	24672	28333	27279	23250
Potato	--	--	56.40	49.4	49.8	44.4
Lettuce 1	--	--	32434 <sup>(1)</sup>	0 <sup>(2)</sup>	--	--
Lettuce 2	23581	20037	0 <sup>(2)</sup>	0 <sup>(3)</sup>	--	--
Lettuce 3	29900	23557 <sup>(1)</sup>	--	--	--	--
Fennel	15.6	18.0	32.1	31.9	39.6	39.6
Green bean	--	--	7.30	7.30	8.81	6.49
Artichoke	--	--	17.6	17.1	17.6	14.0
Onion	--	--	0 <sup>(3)</sup>	0 <sup>(3)</sup>	60.6 <sup>(3)</sup>	49.7 <sup>(3)</sup>
Vetch barley	--	--	--	--	--	--

**Table 2.-** Realised yield per crop in the integrated and organic systems (t/ha, save cauliflower and lettuce, expressed in number of pieces). (1) infection by *Nassonovia ribisnigris*; (2) Bad planting conditions; (3) infection by likely *Delia platura*

expected, soil management did not have a strong influence in them, since a carefully-balanced fertilization taking into account all sources of nutrients (crop residues, irrigation water, etc.) was one of the overall objectives of the VEGINECO project. It was observed the same tendency in all soil parameters studied, decreasing and approaching them to the target defined at the beginning of the experience. Integrated and organic plots were thus very similarly fertilized, and whatever possible change must be accounted for the different use of biocides.

Figures 3 and 4 show the levels of dehydrogenase and alkaline phosphomonoesterase activities. The observed values fall within the ranges reported by other authors for other soils managed under ecological cropping techniques, and can thus be considered as typical (Hassink et al., 1991; Oberson et al., 1993, 1996; Robertson & Morgan, 1996; Albiach et al., 2000).

No significant effect of the management system on the enzymatic activities measured was found. Temporal and spatial variability of data was rather important, but for both systems the levels of activity averaged for the full plot and span of the experiment were very close. This was particularly remarkable in the case of dehydrogenase activity, since the mean values for both management systems were nearly the same. Given that fertilization and

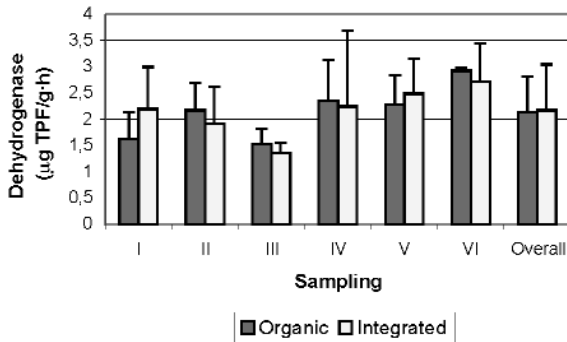


Figure 3.- Levels of dehydrogenase activity

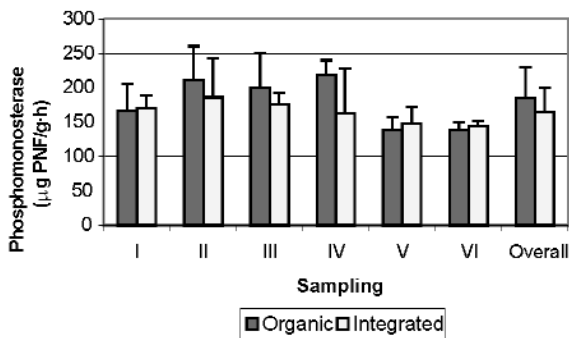


Figure 4.- Levels of phosphomonosterase activity

tillage practices were similar in both cases, the main conclusion of this lack of effects is that a carefully-managed pest and weed control using reasonable rates of chemical agents does not necessarily have a negative effect on soil biological activity.

No significant linear relationship between dehydrogenase and phosphomonosterase activities was observed in any of the data collections used in the calculations (data corresponding to the whole plot, or to the organic and integrated subplots). This could be considered somewhat surprising because of the importance of biological activity in the nutrient release in organically-fertilized soils and the general acknowledgment of dehydrogenase as a suitable index of overall biological activity (Moore & Russell, 1972). In fact, the existence of a significant relationship between dehydrogenase and phosphomonosterase activities in Mediterranean organically-fertilized soils has been reported (Albiach et al., 2000), but cases in which this relationship was not observed can also be mentioned (Albiach et al., 1999, 2001).

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