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Enzyme activities in apple orchard agroecosystems: How are they affected by management strategy and soil properties

Carine Floch [a,*], Yvan Capowiez [b], Stéven Criquet [a]

[a] Institut Méditerranéen d'Ecologie et de Paléoécologie, UMR CNRS 6116, Equipe d'Ecologie Microbienne, Case 452, Université Paul Cézanne Aix-Marseille III, Faculté des Sciences et Techniques de Saint-Jérôme, 13397 Marseille Cedex 20, France
[b] Institut National de Recherche Agronomique, UR 1115 "Plantes et Systèmes Horticoles", Domaine Saint Paul, Site Agroparc, 84914 Avignon Cedex 9, France

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ABSTRACT

This study under field experimental conditions in apple orchard agroecosystems investigated the effects of pest management strategies (*i.e.* none, organic, conventional and integrated) on enzyme activities, in relation to soil properties. Enzyme activities chosen are implicated in the major biogeochemical nutrient cycles such as C (cellulase, fluoresceine diacetate hydrolase, β-galactosidase, β-glucosidase, phenol oxidase), N (arylamidase), P (acid and alkaline phosphomonoesterases, phosphodiesterase and phosphotriesterase) and S (arylsulfatase). Redundancy analyses and decomposition of the variances were performed to clarify how enzyme activities are affected by management strategy and soil properties. Results showed that the effects and their proportion attributable to management strategy and soil properties varied considerably depending on enzyme activity. Phenol oxidase activity was the only case where total variance was principally explained by management strategy (*i.e.* conventional and integrated) rather than by soil properties, and thus it seems to be an attractive potential indicator to assess soil quality in this agrochemical context.

1. Introduction

Soil is a dynamic, living, non-renewable resource that plays many key roles in terrestrial ecosystems (Doran and Parkin, 1994; Doran et al., 1996). Anthropogenic activities affect the quality of soil, which was defined by Doran and Parkin (1994) as "the capacity of soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health". In this context, agriculture is particularly challenged to develop appropriate strategies for sustainable land use and integrated crop productivity.

During the 20th century, conventional agricultural management (CONV) used synthetic fertilizers and pesticides to improve crop productivity. This intensive use of agrochemicals is known to reduce biodiversity, increase irreversible erosion of soil and deplete soil organic matter (Dick, 1992) and also to impact surface and groundwater quality, especially through leaching (Schiavon et al., 1995). Hence, over the last decades, organic management (ORG) has been introduced in order to preserve soil sustainability by allowing the maintenance and even the increase of soil fertility through the use of farmyard manure, the omission of synthetic fertilizers and pesticides, and the lower use of high energy-consuming foodstuffs (Fließbach et al., 2007). Although organic management is known to provide benefits for the soil environmement, it cannot always replace conventional management, which is often the only solution to certain local pest problems (Gewin, 2004). As an alternative to these two strategies, integrated pest management (IPM) involves a restricted use of chemicals to reduce environmental impacts (Denoyelle et al., 2007).

To evaluate the impact of management practices on the quality of soil, and thus to predict their consequences for the environment, several studies have attempted to determine the potential of microbial parameters as indicators (Schloter et al., 2003). Enzyme activities have been identified as possible indicators of the quality of soil because of their relatively rapid responses to changes in soil management (Dick, 1994; Bandick and Dick, 1999). However, one of the principal limitations to their use as indicators is their natural variability within and between soils (Trasar-Cepeda et al., 2000). For this reason, studies have often concluded that results obtained with one soil cannot be generalized to other soils differing in their intrinsic properties and characteristics (Gianfreda et al., 2005; Bielińska and Pranagal, 2007).

In this study, we have investigated the effects of different pest management strategies (*i.e.* none, organic, conventional and integrated) on some enzyme activities involved in the main biogeochemical nutrient cycles (*i.e.* arylamidase, arylsulfatase, cellulase, FDAse, β-galactosidase, β-glucosidase, phenol oxidase, alkaline and acid phosphomonoesterases, phosphodiesterase and

* Corresponding author. Tel.: +33 4 91 28 85 28; fax: +33 4 91 28 81 90.
E-mail address: carine.floch@univ-cezanne.fr (C. Floch).

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phosphotriesterase) in agricultural soils with various physico-chemical properties. Redundancy analyses (Ramette, 2007) and variation partitioning procedure (Borcard et al., 1992), were used to reduce data set complexity and to establish relationships between enzyme activities and environmental variables. In order to clarify the potential of enzyme activities as indicators of soil quality, we sought to answer the two following questions: i) to what extent are enzyme activities affected by management strategy and soil properties? and ii) how does such an effect vary among the different enzyme activities?

2. Materials and methods

2.1. Experimental site

The experimental site "La Petite Région" was approximately 60 km^2 and located in southeastern Avignon 43°56′7″N, 4°52′22″E (Vaucluse region, France). Sampling was performed in July 2007. The apple orchards, spread across the experimental site, were managed according to different pest management strategies. All soils from orchards originated from calcareous mineral parent material. Soils organically managed (ORG) mainly received copper/sulphur as fungicides and Carpovirusine 2000®, a granulosis virus used as biological control agent against the pest *Cydia pomonella*, in accordance with the French charter for apple production (Codron et al., 2003). Orchards conventionally managed (CONV) received chemical pesticides, essentially fungicides, including organophosphorus and carbamates, also in accordance with the French charter for apple production. The integrated management procedure (IPM) was a blend of the two previous management strategies, as provided by the regulatory Commission of the European Communities (2002). Major compounds used in each management strategy are given in Table 1. In every orchard, compound application began in March and ended in July or August. For organic, conventional and integrated managements respectively five, five and four apple orchards were studied. An abandoned apple orchard was chosen as control (NO) because it had not been subjected to pest management for over 20 years. For each apple orchard three rows were sampled, according to the AFNOR (1992) standard X 31-100. Each sample was obtained from sixteen subsamples randomly collected in the same row from a 0–20 cm soil depth, pooled, sieved through a 2 mm mesh and stored at 4 °C before use. Dry weight was determined after drying 1 g of soil at 100 °C in an oven for 24 h. Table 2 presents the physico-chemical characteristics of the soils, determined by the Soil Analysis Laboratory (LAS) from INRA (Arras, France), and the pest management strategy used in the corresponding orchards. Methods used to characterize the soils are described in AFNOR (1999a,b).

Table 1
Major compounds applied to organic, conventional and integrated managed apple orchards.

Presumed action	Compound	Management strategy		
		Organic	Conventionel	Integrated
Fungicide	Copper	×	×	×
	Sulfur	×	×	×
	Cypronidil		×	
	Difenoconazole		×	
	Dithianon		×	×
	Doguadine		×	
	Flonicamid		×	
	Mancozeb		×	
	Pyrimethanil		×	
	Tetraconazol		×	×
Insecticide	Abamectin		×	×
	Acetamiprid		×	×
	Azinphos methyl		×	×
	Chlorpyriphos ethyl		×	×
	Deltamethrin		×	×
	Endosulfan	×	×	×
	Mineral oil	×	×	×
	Granulosis virus	×	×	×
	Rotenon	×		
	Tebufenozid		×	

2.2. Enzyme assays

Arylamidase (ArylN) activity was assayed according to the method of Acosta-Martínez and Tabatabai (2000). A 1 g of soil sample was incubated 1 h at 37 °C with 3 ml of 0.1 M tris(hydroxymethyl)aminomethane (THAM) buffer pH 8.0 and 1 ml of 8.0 mM L-leucine β-naphthylamide hydrochloride. The reaction was stopped by adding 6 ml of ethanol (95%) and immediately centrifuged for 2 min at 12 000 *g*. After centrifugation, 1 ml of the supernatant was treated with 1 ml of ethanol, 2 ml of acidified ethanol, and 2 ml of *p*-dimethylaminocinnamaldehyde reagent. The resulting red azo compound was measured at 540 nm.

Arylsulfatase (ArylS) activity was assayed according to the method of Tabatabai and Bremmer (1970). A 1 g of soil sample was incubated 1 h at 37 °C with 4 ml of 0.5 M acetate buffer pH 5.8 and 1 ml of 5 mM *p*-nitrophenyl sulphate (PNS). The reaction was stopped by adding 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH, and immediately centrifuged for 2 min at 12 000 *g*. The amount of *p*-nitrophenol released from PNS was measured in the supernatant at 412 nm.

Cellulase (Cel) activity was assayed according to the modified method of Deng and Tabatabai (1994). 5 g of soil sample was incubated 4 h at 50 °C with 20 ml of 50 mM acetate buffer pH 5.5 and 2% of carboxymethyl cellulose. The mixture was centrifuged for 2 min at 12 000 *g* and the supernatant was treated with the Somogyi–Nelson reagent. The solution was centrifuged once as described above before the color measurement of reducing sugars at 520 nm.

Fluorescein diacetate hydrolase (FDAse) was assayed according to the modified method of Green et al. (2006). A 1 g of soil sample was incubated 1 h at 37 °C with 9 ml of 50 mM phosphate buffer pH 7.0 and 1 ml of 5 mM fluorescein diacetate (FDA). The pH buffer used was 7.0, instead of 7.6, recommended by Alarcon-Gutiérrez et al. (2008), to avoid potential non-enzymatic interferences. The reaction was stopped by adding 2 ml of acetone, and immediately centrifuged for 2 min at 12 000 *g*. The amount of fluorescein released from FDA was measured in the supernatant at 490 nm.

β-Galactosidase and β-glucosidase activities (Gal and Glu) were assayed according to Eivazi and Tabatabai (1988). A 1 g of soil sample was incubated 1 h at 37 °C with 4 ml of modified universal buffer (MUB) pH 6.0 and 1 ml of 5 mM *p*-nitrophenyl β-D-galactoside (PNGal) or *p*-nitrophenyl β-D-glucoside (PNGlu). The reaction was stopped by adding 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.1 M THAM pH 12, and immediately centrifuged for 2 min at 12 000 *g*. The amount of *p*-nitrophenol released from PNGal or PNGlu was measured in the supernatant at 412 nm.

Phenol oxidase (PO) activity was assayed according to the method of Floch et al. (2007). A 0.1 g of soil sample was incubated 5 min at 30 °C with 9 ml of MUB pH 2.0 and 200 µl of a 0.1 M 2,2′-azinobis-(-3-ethylbenzothiazoline-6-sulfononic acid) diammonium salt (ABTS) solution. The mixture was centrifuged at 11 300 *g* at 4 °C for 2 min and the oxidation rate of ABTS to $ABTS^{+\bullet}$ released in the supernatant was measured at 420 nm ($\varepsilon = 18\ 460\ M^{-1}cm^{-1}$).

Activities of acid and alkaline phosphomonoesterases (Pma and Pmb) were assayed according to the method of Tabatabai and Bremmer (1969). A 1 g of soil sample was incubated 1 h at 37 °C with 4 ml of modified universal buffer (MUB) pH 6.5 (for Pma) or pH 11.0 (for Pmb) and 1 ml of 5 mM *p*-nitrophenyl phosphate (PNP).