Effect or organic amendments on soil enzymatic activities. Results of the "Bioindicators" French program

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Abstract
Soil enzyme activities are considered as indicators of the biological status of soils. They are an indicator integrating the entire soil biodiversity and are therefore representative of its biological functioning. The objective of this study is to evaluate the impact of the contribution of organic waste products of differents compositions on soil biological functions. Fourteen enzymes activities involved in the biogeochemical cycles of C, N, S and P were measured in 5 treatments including 4 differents composts treatments and a control without organic fertilizer. Seven enzymatic activities were stimulated by the addition of compost. Among them, lipase, laccase, arylamidase and alkaline phosphatase were particularly increased. Three multi-enzymatic published indexes were tested for their efficiency in our experimental conditions. Two of them showed the differences between the organic treatment and the control. No index difference could be noted between soils receiving organic inputs, contrary to what was observed when enzymes activities are considered individually.

Introduction
Biological tools are promising means to assess soil quality, and to reveal possible degradations due to agricultural practices. ADEME, the French Environment and Energy Management Agency, has set up in France a research program to develop biological tools suitable for multiple stakeholders. Among them, soil enzymatic activities reflect the functional biodiversity of soil, behave as relevant bioindicators. In addition, numeric indexes combining several enzymes have been developed using published data, as soil quality indicators improved [10, 7]. Unfortunately, the published enzymes activities differ greatly according authors both in kind of enzymes and in methodology utsed. In addition, most of the articles consider a limited number of enzymatic activities. Articles with many identical enzymatic activities are insufficient to develop and validate multi-enzymatic soil quality indexes. Here we studied the responses of 14 enzymatic activities In the present study, we thus aimed to (i) test the ability of fourteen enzymes, widely used in the literature, to indicate soil functioning in the long-term experiment Qualiagro-Feucherolles where soil received different types of organic amendments (ii) test the effectiveness of some published multi-enzymatic indexes.

Material and Methods
Field experiment
The Qualiagro-Feucherolles site is managed by INRA EGC and Veolia Environment. It is cultivated following a wheat–maize rotation. Four types of organic amendments are compared to a control treatment (F-CON): a biowaste compost (F-BIO), a compost issued from the co-composting of green wastes with sewage sludge (F-DVB), a farmyard manure (F-FUM), and a municipal solid waste compost issued from the composting of residual solid wastes (F-OMR). The organic amendments have been applied every two years in September since 1998 on wheat stubble.

Soil sampling

The sampling protocol followed recommendations from the European programme ENVASSO [2] and a previous framework from the national programme RMQS BioDi. In each plot, we designed a 10 m x10 m central subplot subdivided in four 5 m x 5 m frames. Within each frame, twelve soil cores were randomly collected during 2009 using 8-cm-diameter steel cylinder to a depth of 15 cm and mixed at the field to form one composite sample per frame. Soil samples were sieved at 2 mm and stored at field moisture at 4°C. Enzyme assays were performed on fresh sieved soils within 48h after sampling.

Enzyme assays

Fourteen enzymes activities were measured using spectophotometric method [1, 3]. See Table 1 for E.C. numbers). References of protocols used to measure each enzyme activity, as well as specific substrates, their concentrations and buffers used are listed in the table 1. Enzyme activity was expressed as nmole of hydrolyzed substrate per min and per g of dry soil.

Table1. Enzymes and substrates

Enzymes	Codes	E.C. number	Substrates (buffer pH and concentration)	References
Dehydrogenase	DEH	1.1.1.1	2,3,5 triphenyltetrazolium chloride ((pH 7; 0.12 M)	Schaefer et al, 963
Fluorescein diacetate	FDA	209.877.6	Fluorescein diacetate (pH 7.6; 1000 µg.mL^{-1})	Adam & Duncan, 2001
Lipase	LIP	3.1.1.3	p-NP-palmitate (pH 6.5; 1 mM)	Gupta et al, 2002
Cellulase	CEL	3.2.1.4	*p*-NP-β-D-cellobioside (pH 6; 10 mM)	Trap et al, 2012
Galactosidase	GAL	3.2.1.23	*p*-NP-β-D-galactopyranoside (pH 7; 0.02 M)	Eivazi & Tabatabai, 1988
N-acetyl glucosaminidase	NAG	3.2.1.30	*p*-NP-N-acetylglucosaminide (pH 6; 10 mM)	Trap et al, 2012
Xylanase	XYL	2.8.1.8	Xylan (pH 5.5, 12 g.L^{-1})	Schinner & von Mersi, 1990
Arylsulfatase	ARYLS	3.1.6.1	*p*-NP-sulfate (pH7; 25 mM)	Tabatabai & Bremner, 1970
β-glucosidase	GLU	3.2.1.21	*p*-NP-β-D-glucopyranoside (pH 6; 50 mM)	Eivazi & Tabatabai, 1988
Urease	URE	3.5.1.5	Urea (pH 7; 0,05 mM)	Sinsabaugh et al, 2000
Arylamidase	ARYLN	3.4.11.2	L-leucine b-naphtylamide (pH 8; 2 mM)	Martinez &Tabatabai, 2000
Acid phosphatase	ACP	3.1.3.2	*p*-NP-phosphate (pH 5; 50 mM)	Trap et al, 2012
Alkaline phosphatase	AKP	3.1.3.2	*p*-NP-phosphate (pH 9; 50 mM)	Trap et al, 2012

NP: nitrophenyl

Validation of multi-enzymatic indexes of soil quality

Three published multi-enzymatic indexes were tested in this study. The first index was proposed by Jimenez et al (2002). They developed a linear model with the minimum number of biological variables predicting the content of organic carbon in soil

Organic C = – 0.4008 arylsulphatase + 0.4153 dehydrogenase + 0.4033 acid phosphomonoesterase + 0.4916 β-glucosidase.

We also test the “alteration index” (AI3) developed by Puglisi et al (2006). This index was developed from studies investigating enzymes measurements in soils subjected to different management and treatment regimes: AI3 = 7.87 β-glucosidase – 8.22 phosphatase – 0.49 urease

Lastly, we tested the index proposed by Garcia Ruiz et al (2008). This third index is basically the geometric mean of all enzyme activities values:

$$\text{MGea} = \sqrt[14]{\text{B-GLU} \times \text{PAC} \times \text{PAL} \times \text{URE} \times \text{DES} \times \text{LIP} \times \text{XYL} \times \text{CEL} \times \text{NAG} \times \text{LAC} \times \text{ARYLN} \times \text{ARYLS} \times \text{FDA} \times \text{GAL}}$$

Statistical analyses

All tests were computed with the R freeware (R Development Core Team, 2008) and statistical significance was set at P < 0.05. Kruskal-Wallis rank sum and *post-hoc* multiple comparison tests (package “pgirmess”) were used to verify whether multi-enzymatic indexes varied significantly between control treatment compared to the four types of organic amendments.

Results and discussion

Response of enzymatic activities to organic amendment

The activities of ACP, FDA, DEH, CEL and ARYLS were not affected by organic amendment (Table 2). In contrast, URE values recorded in the amended plots (F-FUM, F-BIO, F-DVB and F-OMR) were significantly higher compared to control (F-CON). We found the highest AKP, LIP, GLU and ARYLN activities values in F-OMR plot compared to control, while GAL and XYL activities showed their highest values in F-FUM plot. The F-OMR plot exhibited in average the highest enzymatic activity values, while control exhibited the lowest ones. NAG and LAC were the only enzymes whose highest activities were recorded respectively in F-DVB and F-BIO plots.

Table 2. Effect of organic amendment on soil enzyme activities

Enzymes	**Plots**														
	F-CON			F-FUM			F-BIO			F-DVB			F-OMR		
C cycle															
Dehydrogenase (DEH)	0.09	(0.03)	a	0.07	(0.03)	a	0.07	(0.02)	a	0.05	(0.03)	a	0.07	(0.02)	a
Fluorescein diacetate (FDA)	1.26	(0.37)	a	1.92	(0.66)	a	1.65	(0.68)	a	1.69	(0.50)	a	1.47	(1.13)	a
Lipase (LIP)	0.35	(0.14)	**b**	1.34	(0.08)	**a**	1.14	(0.42)	**a**	0.53	(0.17)	b	2.03	(0.78)	**a**
Cellulase (CEL)	0.52	(0.37)	a	1.08	(0.17)	a	0.80	(0.30)	a	0.56	(0.08)	a	0.90	(0.32)	a
Galactosidase (GAL)	7.27	(0.53)	**b**	9.35	(1.48)	**a**	7.51	(0.73)	b	8.10	(1.88)	b	7.95	(1.10)	b
Glucosidase (GLU)	3.41	(0.26)	b	4.60	(0.29)	a	3.82	(0.62)	b	4.11	(0.48)	b	4.67	(1.09)	a
N-acetyl glucosaminidase (NAG)	0.92	(0.42)	**b**	0.98	(0.18)	b	1.41	(0.28)	b	1.50	(0.06)	**a**	1.19	(0.22)	b
Xylanase (XYL)	0.01	(0.01)	**b**	0.05	(0.02)	**a**	0.01	(0.02)	b	0.00	(0.00)	b	0.01	(0.01)	b
Laccase (LAC)	1.6855	(0.26)	**c**	4.03	(0.45)	**b**	5.84	(0.89)	**a**	3.04	(1.68)	abc	5.11	(0.37)	**a**
N cycle															
Arylamidase (ARYLN)	1.69	(0.26)	**b**	2.41	(0.53)	b	0.99	(0.17)	**c**	1.78	(0.34)	b	3.62	(0.64)	**a**
Urease (URE)	19.32	(3.37)	**b**	28.00	(4.56)	**a**	29.12	(1.63)	**a**	27.95	(1.49)	**a**	28.53	(3.11)	**a**
S cycle															
Arylsulfatase (ARYLS)	7.05	(0.49)	a	7.16	(0.72)	a	6.54	(0.73)	a	6.78	(0.41)	a	7.43	(0.20)	a
P cycle															
Acid phosphatase (ACP)	0.57	(0.41)	a	0.52	(0.50)	a	0.62	(0.45)	a	0.75	(0.36)	a	1.34	(0.64)	a
Alkaline phosphatase (AKP)	9.48	(2.69)	**b**	15.87	(1.20)	**a**	15.05	(2.56)	**a**	12.86	(1.15)	b	16.17	(1.53)	**a**

Mean (SD)

Letters (a and b) indicate significant differences btween controls and plots with organic amendment according to Kruskal-Wallis ($P < 0.05$,

Among the 14 enzymatic activities measured, only 7 (namely lipase, β-glucosidase, N-acetyl-glucosaminidase, laccase, arylamidase, urease and alkaline phosphatase were modified according to the spreading of organic amendments onto the soil (Figure1). The Kruskall-Wallis test revealed particularly the higher significance of lipase, laccase, arylamidase and alkaline phosphatase responses to amendments, 4 enzymes involved in the main biogeochemical cycles CNP

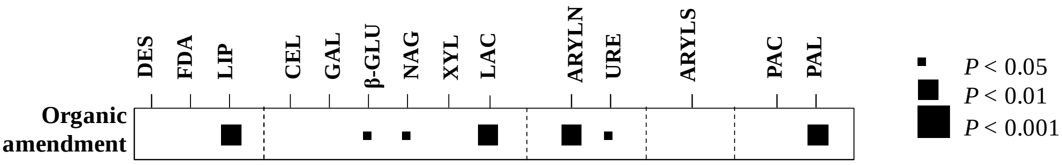


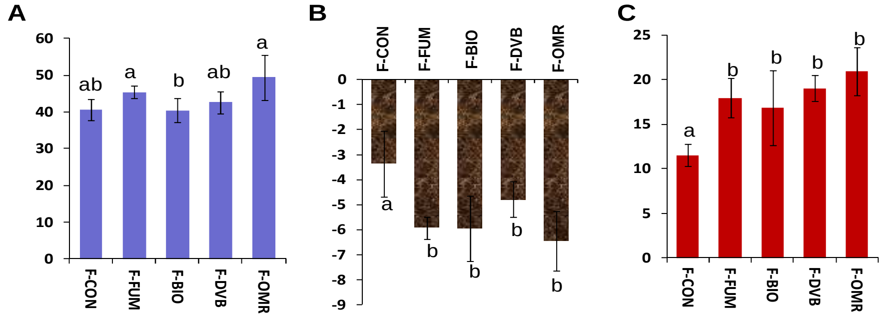
Figure 1. Synthesis of enzymatic activities response to organic amendment according to Kruskal-wallis test n=4

These findings corroborate others studies [8, 6]. For instance, Kandeler et al., (1999)showed that farmyard manure enhance alkalin phosphatase activity in soils. Saha et al., (2008b) concluded that organic amendments applications enhanced organic matter contents and microbial biomass in soil and this provided better potential for higher enzyme activities. The results show that the response of the enzyme activities to the addition of organic waste products varies according organic amendment nature. Finally, we can conclude that the addition of organic amendments improves soil biological state by increasing soil organic matter content and stimulating biological activity, especially enzymes involved in C, N and P cycles.

Validation of multi-enzymatic indexes of agricultural soil quality

Enzyme activities have been proposed by several authors as suitable soil properties for use it in the assessment of soil quality [10]. The equation proposed by De la Pas Jimenez et al (2002) to interpret changes in organic carbon content by taking into consideration the activity of three enzymes was able to discriminate soil quality under organic amendments (Figure 2). The index developed by Puglisi et al (2006) significantly discriminate soil organic amendments. Although the index was developed on three different agricultural sites differing in pedology, climate, cropping, and subjected to different forms of alteration, it remains highly effective. Nevertheless, it was ineffective to discriminate soil without contrasting organic amendment types. This index was thus not sensitive enough to take into account the effect of different organic amendment types with contrasting carbon quality on soil state. The GMea index was able to discriminate control plot from other organic amendment. Contrary to what can be observed in regard to the individual enzyme activities, the multi-enzymatic index discriminate only the control (F-CON) receiving no organic inputs. Indeed, the nature of the organic waste does not significantly affect the enzymatic index while the organic carbon content of the soil is modified according to the initial stability of these products.

Figure 2. Validation of published multi-enzymatic indexes calculated on our data.



(A)The multi-enzymatic index organic carbon of De la Paz Jimenez et al (2008), (B) The alteration index of Puglisi et al. (2006), (C) The multi-enzymatic index (geometric mean) of Garcia Ruiz et al (2008). Bars represent SD. Letters indicate significant differences according to Kruskal-Wallis test ($P<0.05$).

Conclusion and perspectives

Our results show that successive intakes of organic amendments strongly affect several specific enzymatic activities in a long-term experiment.

Our study revealed that the most sensitive enzymes reflecting soil functioning under organic amendment were lipase, β-glucosidase, N-acetyl-glucosaminidase, laccase, arylamidase, urease and alkaline phosphatise. They demonstrate that the activities to measure need to be selected according to the agricultural practice to assess. Few soil state indexes using only enzymatic activities are available in the literature. Among the 3 indexes tested, two were found to be able to discriminate soils receiving organic inputs compared to control in our experimental conditions. Index values suggest a better state of biodiversity in soils receiving organic amendments. Nevertheless, it seems that the enzymatic index does not allow to discriminate the effects of different types of amendments which suggests a low sensitivity of index with respect to observations made from single activities. Our results showed very relevant other enzymes widely used in the literature that can be used to develop new multi-enzymatic indexes to evaluate soil quality and monitor soil status under different conditions.

References

[1] Lebrun JD, Trinsoutrot-Gattin I, Vinceslas-Akpa M, Bailleul C, Brault A, Mougin C, Laval K, 2012. Assessing impacts of copper on soil enzyme activities in regard to their natural spatiotemporal variation under long-term different land uses. Soil Biology & Biochemistry 49, 150-156.

[2]Cluzeau D, Guernion M, Chaussod R, Martin-Laurent F, Villenave C, Cortet J, Ruiz-Camacho N, Pernin C, Mateille T, Philippot L, Bellido A, Rouge L, Arrouays D, Bispo A, Peres G (2011) Integration of biodiversity in soil quality monitoring: Baselines for microbial and soil fauna parameters for different land-use types. European Journal of Soil Biology

[3] Floch C, Capowiez Y, Criquet S, 2009. Enzyme activities in apple orchard agroecosystems: How are they affected by management strategy and soil properties. Soil Biology & Biochemistry 41, 61-68.

[4] Garcia-Ruiz R, Ochoa V, Hinojosa MB, Carreira JA (2008) Suitability of enzyme activities for the monitoring of soil quality improvement in organic agricultural systems. Soil Biology & Biochemistry 40:2137-2145.

[5] Saha, S., Prakash, V., Kundu, S., Kumar, N., Mina, B.L., 2008b. Soil enzymatic activity as affected by long term application of farm yard manure and mineral fertilizer under a rainfed soybean-wheat system in N-W Himalaya. European Journal of Soil Biology 44, 309-315.

[6] Melero, S., Madejon, E., Herencia, J.F., Ruiz, J.C., 2007. Biochemical properties of two different textured soils (loam and clay) after the addition of two different composts during conversion to organic farming. Spanish Journal Of Agricultural Research 5, 593-604.

[7] Puglisi, E., Del Re, A.A.M., Rao, M.A., Gianfreda, L., 2006. Development and validation of numerical indexes integrating enzyme activities of soils. Soil Biology and Biochemistry 38, 1673

[8] Bell, M., Seymour, N., Stirling, G.R., Stirling, A.M., Van Zwieten, L., Vancov, T., Sutton, G., Moody, P., 2006. Impacts of management on soil biota in Vertosols supporting the broadacre grains industry in northern Australia. Australian Journal Of Soil Research 44, 433-451.

[9] Jimenez MD, de la Horra AM, Pruzzo L, Palma RM (2002) Soil quality: a new index based on microbiological and biochemical parameters. Biology and Fertility of Soils 35:302-306.

[10] Trasar-Cepeda, C., Leiros, C., Gil-Sotres, F., Seoane, S., 1998. Towards a biochemical quality index for soils: An expression relating several biological and biochemical properties. Biology And Fertility Of Soils 26, 100-106.
[11] Kandeler, E., Tscherko, D., Spiegel, H., 1999. Long-term monitoring of microbial biomass, N mineralisation and enzyme activities of a Chernozem under different tillage management. Biology And Fertility Of Soils 28, 343-351