SOIL ENZYME ACTIVITIES RECOVERY AFTER ORGANIC TREATMENTS OF DEGRADED AREAS WITHIN VINEYARDS

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Abstract

Soil enzymes were used to assess the impact of different treatments applied in four farms, each one with three vineyards as replicates, on soil functionality. 8 enzymes related to C, N, S and P cycling were measured and functional diversity index was estimated. Three treatments were compared: compost, green manure and dry mulching with respect to degraded and non-degraded soil. The four vineyards showed different enzymatic patterns and response to treatments. Vineyards with the largest difference between degraded and non-degraded soil have benefited more largely from the treatments. Among treatments, dry mulching and compost seemed to be effective to recover soil functionality in degraded vineyards. However, the effect might be limited in the short term.

Keywords: *soil enzymes, functional diversity, substrates decomposition, vineyards degradation*

Introduction

Soil enzyme activities are proximal driver of soil functioning, contributing to biogeochemical cycling, organic matter transformations and nutrient availability and are widely accepted as indicators of soil health, responding in a sensitive, quantitative and predictable manner to different land use and management (Aon et al., 2001; Badiane et al., 2001; Vepsäläinen et al., 2001). Soil enzymatic activities are closely related to microbial activity or biomass as they catalyse biochemical reactions and nutrient cycling in the soils. Furthermore, being synthesized by microorganisms, roots and soil micro- and meso-fauna such as earthworms or nematodes, enzymes can be a valid tool to present and manage complex information in a simple and informative manner.

The most studied group of soil enzymes that have ecological importance in soil are hydrolases, which are involved in the main biogeochemical cycling of elements and release C compounds as well as N, P and S. These enzymes exist in soil either intracellularly or extracellularly, free in soil solution or immobilized on the surface of organic and inorganic soil components.

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Several soil enzyme assays have been developed to detect the total potential activity against a specific substrate. Fluorometry has been proved to be more sensitive than are the colorimetric methods (Marx et al., 2001; Moscatelli et al., 2011) and has become more common since the adoption of microplates that facilitate the rapid measurement of a large number of enzymes and samples. In this context, measuring the activity of several soil enzymes could be useful to understand the organic matter turnover and the availability of inorganic nutrients and could give indications on the function and quality of an ecosystem and on the interaction among subsystems (Dick and Tabatabai, 1993).
Within this work, fluorimetric approach was used for the determination of hydrolase activities related to the main biogeochemical cycling. In particular, enzymes degrading cellulose (β-glucosidase, cellulose), hemicellulose (β-xylosidase), chitin (N-acetyl-β-D-glucosaminidase) phosphate (acid phosphatase) and sulphate (arylsulphatase) esters have been assessed, together with two unspecific endo-cellular enzymes (butyrate and acetate esterase).

Materials and methods

Soil sampling

Soil samples were collected in four farms, each one with three vineyards as replicates, before (2015) and after (2016 and 2017) organic treatments application. Two farms are located in France (Maison Blanche, Saint Émillion – MB and Pech Redon, La Clape - PR) and two in Italy (Fontodi, Panzano in Chianti – FON and San Disdagio, Civitella Marittima - SD). In each vineyard, an area characterized by soil degradation was selected. Each degraded area was subdivided into 4 plots, where different strategies of organic soil management were implemented: (COMP) composted organic amendment; (GM) green manure with winter legumes and cereal; (DM) reseeded legumes, mown and leaved on the ground as dry mulching; (CONTR) only tillage once per year. A reference plot, characterized by optimal soil functionality (ND, non-degraded) was selected in each vineyard. For further details on climate and pedological characteristics and for treatments type and application see D'Avino et al. (this issue).
Soils were sampled at 0-30 cm depth in French sites in 2015. In French sites in 2016 and 2017 and in Italian sites in the three years, they were sampled at 0-10 and 10-30 cm depths. Averaged activities at 0-30 cm depths are shown.

Enzyme activities measurement

Enzyme activities were measured according to the methods of Marx et al. (2001) and Vepsäläinen et al. (2001). N-acetyl-β-glucosaminidase (NAG), β-glucosidase (βG), butyrate esterase (BUT), acid phosphatase (AP), arylsulphatase (ARYL), β-xylosidase (XYL), cellulose (CELL) and acetate esterase (AC) activity were measured using fluorogenic methylumbelliferyl (MUF) conjugated surrogate substrates (Sigma, St Louis, MO, USA). Briefly, 2 g soil sample was weighed into a sterile jar and incubated for 24 hours at 20% soil moisture. A homogenous

suspension was obtained by homogenizing samples with 50 mL deionized water with UltraTurrax at 9600 rev / min for 3 min. Aliquots of 50 µL were withdrawn and dispensed into a 96 well microplate (3 analytical replicates/sample/substrate). 50 µL of Na-acetate buffer pH 5.5 was added to each well. Finally, 100 µL of 1 mM substrate solution were added giving a final substrate concentration of 500 µM. Fluorescence (excitation 360 nm; emission 450 nm) was measured after 0, 30, 60, 120, 180 min of incubation at 30 °C with an automated fluorimetric plate-reader (Fluoroskan Ascent).

Statistical analysis

Analysis of variance was performed to assess the effect of treatments, years and their interactions on soil enzyme activities using Statistica package (StatSoft inc).
The order of magnitude of the values obtained for the different enzymatic responses varies considerably depending on the specific activity being determined, thus leading to some enzyme having more weight than others. To resolve this problem, the sum of the percentage of the maximum value found for a specific enzymatic response across all enzymes was used for the calculation of the sum of enzymes (SUM). From this percentage of maximum enzyme activities, the Simpson-Yule index was calculated following the equation $E = 1/\Sigma pi^2$, as indicated by Bending et al. (2004), where pi is calculated as the enzymatic response to a substrate as a proportion of enzymatic responses summed across all substrates for a soil. Discriminant function analysis (DFA) was performed using the percentage of maximum value for each enzyme to show separation among the four sites. Squared Mahalanobis distances between group centroids were determined. Two significant discriminatory roots were derived and the results of DFA were graphically presented in two dimensions.

Results and discussion

Overall, the four sites were significantly different in terms of soil enzymatic pattern (Fig. 1), with the greatest enzyme activities observed on average in Pech Redon and Fontodi, followed by San Disdagio and Maison Blanche. Differences among sites can be ascribed to several abiotic (climate, pH, carbonates, etc.), and biotic factors (organic matter, microbial biomass and activity, fauna and roots, etc.).
Greater enzyme activities were observed in ND soils with respect to CONTR in all sites along the three years of observations (Fig. 2 and Table 1).
Indeed, this difference was larger in the first year, as also reported in a previous work on the same sites before treatments application (Costantini et al., in press). In the second and third years the increase was reduced and remained significant in Maison Blanche and San Disdagio until the end of measurements.

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Table 1: *Mean activities of enzyme activities in the four sites in plots without treatments (***CONTR***), treated with compost (***COMP***), green manure (***GM***), mulching (***DM***) and non-degraded (***ND***) before (2015) and after (2016 and 2017) treatments.*

Site	Treatment	Year	nmol MUF g^{-1} h^{-1}							
			CELL	**AP**	**bG**	**NAG**	**XYL**	**BUT**	**AC**	**ARYL**
Maison Blanche	**CONTR**	2015	33	247	223	53	33	232	748	24
		2016	8	149	86	23	13	249	382	15
		2017	20	198	187	43	27	411	588	27
	COMP	2015	31	256	239	55	34	272	869	27
		2016	11	134	103	21	14	287	453	17
		2017	37	211	249	69	36	519	721	37
	GM	2015	19	224	179	56	16	228	749	24
		2016	11	159	99	24	14	267	482	18
		2017	29	181	205	43	28	398	545	32
	DM	2015	30	225	173	47	26	244	849	25
		2016	16	195	119	33	18	281	516	20
		2017	32	225	211	52	38	454	664	38
	ND	2015	36	249	378	76	39	331	1035	29
		2016	19	175	163	38	21	360	550	25
		2017	45	171	337	55	39	511	602	42
	ANOVA									
	Year		***	*	***	***	***	***	***	***
	Treatment		**	n.s.	*	*	**	n.s.	n.s.	*
	Y * T		n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.
Pech Redon	**CONTR**	2015	9	123	141	57	17	472	1101	20
		2016	7	96	107	21	14	365	667	11
		2017	33	84	173	49	35	563	1045	45
	COMP	2015	11	115	133	35	16	596	1048	14
		2016	5	80	58	17	8	302	513	8
		2017	32	80	171	46	32	505	1028	42
	GM	2015	20	133	215	46	23	685	1322	21
		2016	8	98	88	23	11	364	612	9
		2017	35	71	203	48	32	518	971	45
	DM	2015	12	111	110	41	13	536	991	12
		2016	7	93	93	18	10	352	635	10
		2017	33	68	214	53	36	580	1029	42
	ND	2015	17	123	198	39	31	690	1096	18
		2016	9	110	127	24	17	441	763	13
		2017	31	72	186	44	34	521	895	44
	ANOVA									
	Year		***	***	***	***	***	**	***	***
	Treatment		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Y * T		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 1 (to be continued)

Site	Treatment	Year	nmol MUF g^{-1} h^{-1}							
			CELL	**AP**	**bG**	**NAG**	**XYL**	**BUT**	**AC**	**ARYL**
Fontodi	**CONTR**	2015	15	118	164	56	18	465	801	34
		2016	24	131	226	47	28	709	1041	32
		2017	11	51	122	33	18	390	562	34
	COMP	2015	21	126	185	76	21	605	984	33
		2016	38	156	236	71	31	823	1123	35
		2017	17	66	176	44	18	480	535	43
	GM	2015	24	133	165	77	23	556	893	38
		2016	37	160	270	53	33	770	1136	41
		2017	17	86	133	32	16	331	458	36
	DM	2015	22	142	204	76	26	678	1056	33
		2016	20	143	178	38	29	651	953	33
		2017	14	71	151	33	19	351	462	34
	ND	2015	21	134	184	85	30	559	934	37
		2016	43	165	285	51	31	788	1097	41
		2017	15	66	125	39	14	347	474	32
	ANOVA									
	Year		***	***	***	***	***	***	***	n.s.
	Treatment		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Y * T		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
San Disdagio	**CONTR**	2015	10	138	92	35	16	500	949	20
		2016	12	113	88	21	14	432	870	15
		2017	6	96	71	22	14	439	996	25
	COMP	2015	8	133	72	26	14	385	917	16
		2016	16	130	105	27	19	536	916	15
		2017	9	79	67	18	15	353	887	19
	GM	2015	11	119	87	30	15	416	816	17
		2016	19	148	189	37	28	608	1016	19
		2017	11	85	68	25	14	322	813	17
	DM	2015	10	106	63	27	12	348	713	12
		2016	17	160	167	40	25	593	1057	18
		2017	11	92	132	31	19	499	959	22
	ND	2015	22	171	177	55	23	595	1099	33
		2016	36	182	269	51	37	692	1166	40
		2017	21	84	117	33	16	360	568	29
	ANOVA		10	138	92	35	16	500	949	20
	Year		*	***	**	*	**	**	n.s.	n.s.
	Treatment		***	n.s.	***	*	n.s.	n.s.	n.s.	***
	Y * T		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

CELL=cellulose; **AP**=acid phosphatase**; βG**=glucosidase; **NAG**=N-acetyl-β-glucosaminidase; **XYL**= β-xylosidase; **BUT**=butyrate esterase; **AC**=acetate esterase; **ARYL**=arylsulphatase

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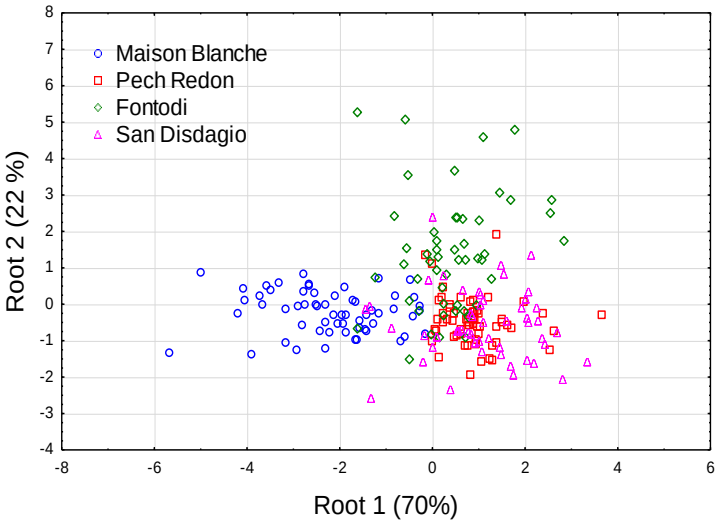

Figure 1 *Discriminant Function Analysis showing separation among the four sites on the basis of enzyme activities (percentage of maximum value for each enzyme).*

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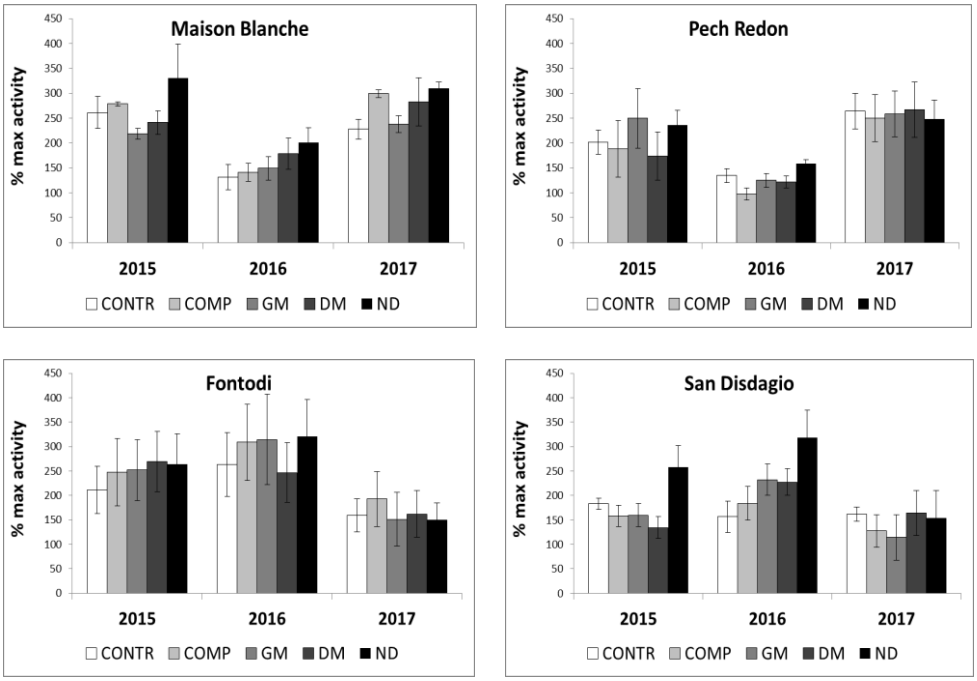

Figure 2. *SUM of enzyme activities in the four sites in the three sampling years before (2015) and after (2016 and 2017) treatments. Error bars are reported.*

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150 **Table 2.** *Percentage difference of enzyme activities with respect to Control in the four sites*
151 *after treatments application in 2016 and 2017. Significant differences are reported in bold.*
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Site	Year	Treatment	% difference with respect to control									
			CELL	**AP**	**bG**	**NAG**	**XYL**	**BUT**	**AC**	**ARYL**	**SUM**	**S-Y**
Maison Blanche	**2016**	**COMP**	**37**	-10	20	-7	8	15	18	17	8	14
		GM	**39**	7	15	8	6	7	26	21	14	3
		DM	**99**	**31**	**38**	**46**	**36**	12	**35**	**34**	**36**	7
		ND	**140**	17	**89**	**67**	**61**	**44**	**44**	**72**	**53**	17
	2017	**COMP**	**86**	7	**33**	**61**	**33**	**26**	**23**	**35**	**31**	8
		GM	**46**	-8	10	1	3	-3	-7	18	5	1
		DM	**61**	14	13	20	**39**	10	13	**39**	24	3
		ND	**128**	-14	**80**	**29**	**44**	**24**	2	**55**	**36**	3
Pech Redon	**2016**	**COMP**	-33	-17	-46	-19	-43	-17	-23	-4	-27	-4
		GM	6	2	-18	8	-24	0	-8	-1	-7	-1
		DM	2	-3	-14	-16	-33	-4	-5	-1	-10	-1
		ND	**30**	**15**	**18**	**15**	**20**	**21**	14	3	**18**	3
	2017	**COMP**	-3	-5	-1	-6	-10	-10	-2	2	-5	2
		GM	6	-16	18	-2	-8	-8	-7	2	-2	2
		DM	1	-20	**24**	8	1	3	-2	1	1	1
		ND	-8	-15	8	-10	-4	-7	-14	0	-6	0
Fontodi	**2016**	**COMP**	**55**	19	4	**50**	8	16	8	3	17	3
		GM	**52**	22	20	12	17	9	9	-1	19	-1
		DM	-16	9	-21	-19	4	-8	-8	-6	-6	-6
		ND	**78**	**26**	**26**	8	9	11	5	1	22	1
	2017	**COMP**	**45**	28	**45**	**31**	5	23	-5	-6	**21**	-6
		GM	**49**	**69**	9	-6	-10	-15	-19	-19	-5	-19
		DM	**24**	**38**	**24**	-1	9	-10	-18	-8	1	-8
		ND	**36**	**29**	3	16	-22	-11	-16	-2	-6	-2
San Disdagio	**2016**	**COMP**	**31**	**15**	**20**	**32**	**33**	**24**	5	6	17	6
		GM	**61**	**30**	**116**	**78**	**97**	**41**	17	13	**48**	13
		DM	**39**	**41**	**90**	**95**	**75**	**37**	21	8	**45**	8
		ND	**197**	**61**	**207**	**146**	**154**	**60**	**34**	19	**103**	19
	2017	**COMP**	**55**	-19	-6	-20	2	-19	-11	-8	-21	-8
		GM	**78**	-12	-5	13	-5	-27	-18	-16	-29	-16
		DM	**73**	-4	**85**	**41**	**32**	14	-4	0	1	0
		ND	**249**	-13	**64**	**47**	9	-18	-43	-6	-5	-6

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These two sites showed also the largest impact of treatments (Table 2), however a different response was observed in the four vineyards (Table 1 and 2):

Maison Blanche

In the first year DM showed to be the most effective treatment, able to increase most of the enzyme activities considered. This effect decreased in the second year, and was maintained for enzymes related to cellulose and hemicellulose degradation and arylsulphatase only, suggesting a short-term effect of this treatment application, more evident and permanent for C-cycling enzymes. In the second year COMP showed the maximum increase with respect to CONTR, for all enzymes. GM increased cellulase activity only, in both years.

Pech Redon

The treatments did not affect significantly enzyme activities, with the exception of β-glucosidase in the second year after dry mulching. This vineyard showed also the lowest difference between CONTR and ND soils, suggesting that soil functionality was i) less responsive to degradation or ii) degradation was not so strong.

Fontodi

In the first year GM increased cellulolytic enzymes and acid phosphatase and this effect persisted in the second year. However, other enzymes were not affected by this treatment. In the second year COMP application positively affected enzyme activities related to C and P cycling, and also N cycling with DM. This vineyard seemed to be slower in the response to treatments, even if after the second year of treatments the activities were comparable to those of ND soil.

San Disdagio

This vineyard showed the highest percentage effects of treatments, in particular in the first year, when GM and DM almost doubled enzyme activities with respect to CONTR, though without reaching the values of ND soils. This effect was evident for most enzymes of C, N, S, and P cycling. In the second year the effect persisted for cellulase with all treatments and also for chitin and hemicellulose degrading enzymes with DM.

Conclusions

Overall, treatments application showed to improve soil enzyme activities, although to different extent depending on vineyard type and treatment. Maison Blanche and San Disdagio were the two vineyards most responsive to treatments, possibly as a consequence of the largest difference between degraded and non-degraded soil found in these two sites. Among treatments, DM and Compost seemed to be effective to recover soil functionality in degraded vineyards. However, the effect might be limited in the short term.

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