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Biochemical properties of two different textured soils (loam and clay) after the addition of two different composts during conversion to organic farming

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Abstract

The conversion to organic farming of two soils, a loam (Xerofluvent) under irrigation and a clay (Chromic Haploxerept) under dryland management, was studied through analysis of their biochemical properties. Soil biological status was evaluated by measuring microbial biomass carbon and the ratio of enzymatic activity to microbial biomass. Results were compared to those after application of inorganic fertilizer. At the end of the study, in both soil types the organic treatments had increased the organic matter and nitrogen content compared with the inorganic treatment. Total organic carbon (TOC) values in the clay were higher than in the loam. This could be related to a lower mineralization rate of soil organic matter under dry land management and/or with a protection effect, by the clay on the organic fraction. However, absolute ratios of some enzymatic activities (dehydrogenase, protease and alkaline phosphatase) by microbial biomass carbon in the clay were lower than in the loam indicating a lower rate of enzyme production by microbial biomass in the clay. In the clay soil there was a great increase in TOC, total N, microbial biomass and enzymatic activity, in all treatments, in the last crop cycle. This was mainly related to a previous legume crop. Generally organic management improved biomass and enzymatic activity in both soils. In the future further studies are required to confirm the positive long-term effect of organic fertilization on biochemical properties which maintain or improve soil quality.

Additional key words: compost, organic farming, soil enzymatic activity, soil microbial biomass.

Resumen

Propiedades bioquímicas de dos suelos de diferente textura (franca y arcillosa) tras la adición de dos composts diferentes en el proceso de reconversión a la agricultura ecológica

En este trabajo se aborda el estudio de la reconversión a la agricultura ecológica en dos suelos de diferente textura y régimen hídrico, un suelo franco (Xerofluvent) en regadío y un suelo arcilloso (Chromic Haploxerept) en secano. Se compara el manejo ecológico del suelo con respecto a un manejo convencional, desde el punto de vista biológico. Se evaluó el estado biológico del suelo a través del carbono de la biomasa microbiana y el cociente entre las actividades enzimáticas por biomasa microbiana. Se observó un incremento en el contenido de materia orgánica y nitrógeno en los suelos fertilizados orgánicamente de ambos experimentos de reconversión. No obstante, los valores más altos de los contenidos de carbono orgánico total se encontraron en el suelo arcilloso. Estos resultados pueden estar relacionados con la menor tasa de mineralización debido a baja humedad de este suelo y/o al mayor contenido en arcilla que tiene un efecto protector en la fracción orgánica. Las razones de las actividades enzimáticas (deshidrogenasa, proteasa y fosfatasa alcalina) por biomasa microbiana en el suelo arcilloso fueron más bajas que en el suelo franco, reflejando una menor síntesis de enzimas por los microorganismos en el suelo arcilloso. En el último ciclo de cultivo del experimento de reconversión en secano se observó un importante incremento del contenido en carbono orgánico total, nitrógeno total, biomasa microbiana y actividades enzimáticas, lo cual se atribuye al cultivo anterior (una leguminosa). Por lo tanto, serían necesarios estudios a más largo plazo para confirmar una mejora de las propiedades bioquímicas bajo manejo orgánico.

Palabras clave adicionales: actividades enzimáticas del suelo, agricultura ecológica, biomasa microbiana del suelo, compost.

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Introduction

Conventional agriculture systems have caused a progressive reduction in soil organic matter content, which contributes to desertification, increased soil erosion, and soil fertility losses in most Mediterranean soils. Organic farming offers the potential to maintain long-term soil structure, considering the soil as a living medium, where there are complex connections among soil inorganic components, microorganisms and plants.

The close relation between soil organic matter content and soil fertility is widely reported and universally accepted (Campbell, 1978; Smith *et al.*, 1993). Therefore, one of the most important ways to regenerate soil involves adding organic residues to maintain or enhance soil organic matter and therefore soil fertility. Organic amendments have numerous positive effects on soil physical, chemical and biological properties (Duggan and Wiles, 1976; Webber, 1978; Reganol *et al.*, 1993; Smith *et al.*, 1993).

Physical and chemical parameters have been used to measure soil quality (Parr and Papendick, 1997). Although these parameters change slowly and several years are necessary to obtain significant differences, biological and biochemical parameters are more sensitive and can provide earlier measurements of changes produced by soil management (Dick, 1994; Ndiaye *et al.*, 2000).

Many authors have studied soil biochemical and biological properties as indicators of changes caused by disturbances due to management. These are useful in the study of the regeneration of degraded soils (Garcia *et al.*, 2000). The quantity and activity of soil microbial biomass is influenced by such management factors as the addition of organic residues and tillage (Kaiser and Heinemeyer, 1993; Calderon *et al.*, 2001), cropping system (Kaiser and Heinemeyer, 1993; Ndiaye *et al.*, 2000) and pesticide and fertilizer application (Bossio *et al.*, 1998). Microbial biomass is one of the main agents of chemical transformation releasing essential nutrients to plants. Powlson and Jenkinson (1981) suggest microbial biomass is a valuable index of changes in soil organic matter and observed a close relationship between biomass and soil organic carbon content (Jenkinson and Ladd, 1981).

Soil enzyme activity also responds to agronomic practices such as fertilizers, organic amendments, vegetation cover, and pesticides (Gianfreda and Bollag, 1996). Enzymatic activity is stimulated by organic matter addition which contributes to retaining soil nutrients (Madejon *et al.,* 2003).

Although there are some long-term studies on soil biological activity in organic farming systems (Schjonning *et al.*, 2002; Melero *et al.*, 2006) studies which focus on the conversion period are less common (Werner, 1997; Albiach *et al.*, 1998).

The object of this study was to determine the effect of two different composts (plant and animal), on the biochemical properties of two different textured soils (loam and clay) with different water regimes (the loam was irrigated and the clay was under dry land management). The results are compared with those obtained using inorganic fertilizer.

Material and Methods

Location and system management

The field studies were conducted in 2001. They were located on two farms; one located in the Guadalquivir River Valley (SW Spain) (37º 8' 33" N; 5º 16' 4" W at an altitude of 11 m), the IFAPA Centro Torres farm, Alcalá del Río (Seville). The other farm was at the IFAPA Centro Tomejil, Carmona (Seville) (SW Spain) (37º 24' 7" N; 5º 35' 10" W at an altitude of 79 m). The soil types (Soil Survey Staff, 1999) were a loam classified as a Xerofluvent and a clay classified as a Chromic Haploxerept respectively. The physical and chemical characteristics of the soils are given in Table 1.

The climatic characteristics of Torres farm are average annual rainfall 650 mm, 18°C average temperature and 4 mm average daily evapotranspiration. Tomejil farm has an average annual rainfall of 580 mm, 18°C

Table 1. Soil physical (0-15 cm depth) characteristics at the start of the experiment (n = 12)

Parameter	Torres	Tomejil
Sand (%)	44	4
Silt (%)	29.6	30
Clay (%)	26.4	66
pH (1:2.5)	8.04	7.70
EC (1:2.5) (dS m^{-1})	0.19	0.27
$CaCO_3$ (g kg^{-1})	249	223
TOC (g kg^{-1})	7.56	9.76
Kjeldahl-N (g kg^{-1})	0.88	1.40
Olsen-P (mg kg^{-1})	11.5	7.70
AAE-K (mg kg^{-1})	262	555

EC: electrical conductivity. TOC: total organic carbon. AAE-K: ammonium acetate extractable K.

Table 2. Chemical characteristics of the composts applied to organic plots in each crop cycle at Torres

Crop	Compost	Parameter						
		Moisture (g kg^{-1})	pH (1:5)	EC (dS m^{-1})	TOC (g kg^{-1})	Total N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})
Potato	Plant	247	7.80	1.30	148	9.60	3.20	3.80
	Animal	420	7.57	6.40	156	18.70	4.30	12.00
Lettuce	Plant	217	7.30	4.80	163	11.20	4.90	5.40
	Animal	175	7.90	4.10	124	7.60	4.00	6.00
Carrot	Plant	255	7.70	1.47	228	11.10	5.16	4.00
	Animal	248	7.60	5.72	183	6.43	5.00	5.10
Spinach	Plant	229	7.62	2.15	135	7.60	2.70	5.30
	Animal	271	7.64	5.56	148	8.20	4.30	8.00
Tomato	Plant	280	8.00	1.46	166	5.70	2.30	4.70
	Animal	364	8.95	11.50	266	18.50	7.80	18.20

Data are the mean of 3 samples. Data are expressed on an oven-dry compost weight. EC: electrical conductivity. TOC: total organic carbon.

average temperature and 3.3 mm average daily evapotranspiration.

Before the experiments, the previous crops on both farms were cotton and wheat, under conventional management, at Torres and Tomejil respectively.

Relevant characteristics of the two composts are shown in Tables 2 and 3. Organic treatment O_1 was fertilised with 30 Mg ha^{-1} of plant based compost (pruning waste and crop residues) and O_2 was fertilised with 30 Mg ha^{-1} of animal manure compost applied by surface tillage during each crop cycle.

The inorganic treatment was fertilised with chemical fertilizers at the normal doses used by farmers for these crops and are shown in Table 4. The doses of nutrients (N, P and K) applied per hectare in the inorganic and organic plots are shown in Table 5. Weed control in organic treatments was by mechanical tillage. In the inorganic treatment it was by herbicides.

The experimental design, in both experiments Torres and Tomejil, was a randomized complete block with four replicates. The three factorial treatments were inorganic fertilizer (I), plant compost (O_1) and animal manure (O_2). Plots were 10×20 m. The crop sequence is shown in Table 5.

The following crops were grown at Torres: potatoes (*Solanum tuberosum* cv. Spunta), lettuce (*Lactuca sativa* cv. Oreja de mulo), carrot (*Daucus carota* cv. Nantesa), spinach (*Spinacia oleracea* cv. Gigante de invierno)

Table 3. Chemical characteristics of the composts applied to organic plots in each crop cycle at Tomejil

Crop	Compost	Parameter						
		Moisture (g kg^{-1})	pH (1:5)	EC (dS m^{-1})	TOC (g kg^{-1})	Total N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})
Sunflower	Plant	247	7.80	1.30	148	9.60	3.20	3.80
	Animal	420	7.57	6.40	156	18.70	4.30	12.00
Wheat (2002)	Plant	217	7.30	4.80	163	11.20	4.90	5.40
	Animal	175	7.90	4.10	124	7.60	4.00	6.00
Lentils	Plant	330	8.00	3.53	115	6.74	1.76	5.30
	Animal	271	7.64	5.56	148	8.20	4.30	8.00
Wheat (2004)	Plant	453	7.57	1.25	209	14.40	4.50	3.10
	Animal	444	8.36	8.56	316	15.30	6.30	8.10

Data are the mean of 3 samples. Values are expressed on an oven-dry compost weight. EC: electrical conductivity. TOC: total organic carbon.

Table 4. Doses of inorganic fertilizer applied in each crop cycle

	Deep fertilization		Top dressing	
	(Mg ha^{-1})	Fertilizer	(Mg ha^{-1})	Fertilizer
Torres				
Potato	1	N-8, P-15,K-15	0.3	Urea (46% N)
Lettuce	1	N-8, P-15,K-15	0.2 (two additions)	Ammonium nitrate (33.5% N)
Carrot	1	N-8, P-15,K-15	—	—
Spinach	1	N-8, P-15,K-15	0.1 (two additions)	Ammonium nitrate (33.5% N)
Tomato	1	N-8, P-15, K-15	0.162 (two additions)	Ammonium nitrate (33.5% N)
			0.187 (two additions)	Polyfeed: N-20, P-5, K-32
			0.142 (two additions)	Potassium nitrate: N-13, K-46
Tomejil				
Sunflower	0.3	N-15, P-15, K-15		—
Wheat (2002)	0.2	N-18, P-46	0.23	Urea (46% N)
Lentil	1	N-8, P-15, K-15	—	—
Wheat (2004)	0.24	N-18, P-46	0.25	Urea (46% N)

and tomatoes (*Lycopersicon lycopersicum* cv. Plato de Egipto). Four crops were grown in Tomejil plots: sunflower (*Helanthus annus* cv. Sanbro), winter wheat (*Triticum aestivum* cv. Astral), lentil (*Lens culinaris* cv. Rubia castellana) and winter wheat (*T. aestivum* cv. Simeto).

At Torres (loam) crops were irrigated three times by surface irrigation, except for the fifth crop cycle which was irrigated five times. Over the whole cropping period the total amount of water applied was 320, 80, 240, 40, and 880 mm for the first to fifth crop cycles. These crops also received total rainfall of 214, 296, 170, 458 and 2 mm, respectively. At Tomejil (clay), the crops were not irrigated and were grown under a dry land system. The crops at Tomejil received a total rainfall of 180, 307, 251 and 242 mm, respectively for the first to fourth crop cycle.

Table 5. Amount of nutrients (nitrogen, phosphorous and potassium) applied in inorganic and organic fertilizer in each crop cycle

Period	Crop	Inorganic fertilizer (kg ha^{-1})			Plant compost (kg ha^{-1})			Animal compost (kg ha^{-1})		
		N	P	K	N	P	K	N	P	K
Torres										
February-June (2001)	Potato	218	150	150	216	72	86	325	75	207
September-December (2001)	Lettuce	214	150	150	263	115	127	188	99	153
February-July (2002)	Carrot	80	150	150	248	115	89	145	113	115
October-February (2003)	Spinach	147	150	150	176	62.5	122	179	94	175
May-August (2003)	Tomato	300	169	400	123	49.7	101	353	149	347
Tomejil										
April-October (2001)	Sunflower	45	45	45	216	72	86	325	75	207
December- July (2002)	Wheat (2002)	142	92	0	263	115	127	188	99	148
January-July (2003)	Lentil	80	150	150	135	35	106	179	94	175
January-July (2004)	Wheat (2004)	158	110	0	236	74	51	255	105	135

Sampling and soil chemical analysis

Soils were sampled to a depth of 15 cm. In the experiment at Torres, soil samples were taken during flowering of each crop. At Tomejil, soil was sampled three months after sowing in each crop cycle. At each sampling, three soil cores, per plot, were randomly taken to provide a composite sample. Field moist soil was sieved (2 mm) and divided into two subsamples. One was immediately stored at 4ºC loosely tied plastic bags to ensure aeration and prevent moisture loss until microbiological and enzymatic activity assays. The other sub-sample was air-dried and used for total oxidisable organic C (TOC) analysis.

Soils texture and calcium carbonate content were determined using MAPA (1994) methods. The TOC was determined by the Walkley and Black (1934) wet dichromate oxidation method, total N by the method of Hesse (1971), available-P using the Olsen *et al.* (1954) extraction method and ammonium acetate extractable-K (AAE-K) by extraction with 1 M ammonium acetate at pH 7 (Richards, 1954).

Soil biochemical analysis

Microbial biomass C was determined by a fumigation-extraction method (Brookes *et al.*, 1985; Vance *et al.*, 1987). Soil samples were fumigated with ethanol-free $CHCl_3$. Non-fumigated control samples were also taken. After removal of the $CHCl_3$, fumigated and nonfumigated soil samples were extracted with 0.5 M K_2SO_4 and organic C quantified by oxidation with 66.7 mM $K_2Cr_2O_7$ with subsequent back-titration of unreduced dichromate. Microbial biomass C content was estimated as follows: microbial Bc = 0.38 Ec, where Ec is the difference between the organic C extracted from the fumigated and non-fumigated samples (Vance *et al.*, 1987).

Dehydrogenase was determined following Thalmann (1968) after soil incubation with 2,3,5 triphenyltetrazolium chloride (TTC) and measurement of triphenyl formazan (TPF) absorbance at 546 nm. Values of dehydrogenase activity are expressed as µg TPF mg^{-1} dry weight soil.

Protease activity was measured after soil incubation, with casein, and measurement of the absorbance of the extracted tyrosine at 700 nm following the procedure of Ladd and Butler (1972). Protease activity is expressed as µg tyrosine 2 h^{-1} mg^{-1} dry weight soil.

β-glucosidase activity was measured as indicated by Eivazi and Tabatabai (1988), after soil incubation with p-nitrophenyl-β-D-glucopyranoside and measurement of pnitrophenol absorbance at 400 nm.

Alkaline phosphatase was determined according to Tabatabai and Bremner (1969) after soil incubation with p-nitrophenyl phosphate disodium and the measurement of pnitrophenol absorbance at 400 nm. Values of β-glucosidase and alkaline phosphatase activity are expressed as µg p-nitrophenol h^{-1} mg^{-1} dry weight soil.

There were three replicates per plot of each biochemical analysis. Results are based on oven-dry soil weight.

Statistical analysis

Results were analysed by ANOVA, considering treatment as the independent variable. All statistical analyses were carried out using SPPS 11.0 for Windows. All values are expressed as means. Significant statistical differences of all variables among the different treatments were established using Tukey's test at $p < 0.05$.

Results

Total organic carbon, total N and C/N ratio

In both experiments there were statistical differences in TOC mean values between the organic and inorganic treatments from the second sampling. In soil fertilised organically TOC values were higher than in inorganically fertilised soil (Table 6).

In the last crop cycle of the experiment at Tomejil there was a great increase in TOC values in all treatments, especially in O_1 and O_2.

From the fourth crop cycle at Torres there were statistical differences in total N values between inorganically and organically fertilised soils (Table 6). At Tomejil, total N values in the O_2 treatment were statistically higher than in the other two treatments in the first and second crop cycles. In the last crop cycle a large increase in total N was observed in all treatments (Table 6).

At Torres the C:N ratios were only statistically different among treatments in the third crop cycle (Table 6). At Tomejil statistical differences among treatments were observed in some samplings (Table 6). Generally, C:N ratios showed slight random fluctuations.

Table 6. Mean soil TOC, Kjeldahl-N and ratios at Torres and Tomejil

Torres	Treatment	Potato	Lettuce	Carrot	Spinach	Tomato
TOC (g kg^{-1})	I	7.74 a	8.10 a	7.6 a	8.3 a	8.5 a
	O_1	7.80 a	9.78 b	9.1 b	12.2 b	13.5 b
	O_2	8.20 a	9.80 b	10.5 b	13.3 b	14.0 b
Total N (g kg^{-1})	I	0.86 a	0.97 a	0.99 a	0.90 a	0.96 a
	O_1	0.92 a	1.00 a	1.08 a	1.20 b	1.50 b
	O_2	0.93 a	1.10 a	1.20 a	1.20 b	1.60 b
C/N	I	8.97 a	8.45 a	7.72 a	9.0 a	8.80 a
	O_1	8.41 a	9.33 a	8.50 b	10.7 b	9.00 a
	O_2	8.81 a	8.97 a	8.80 b	9.8 ab	8.80 a

Tomejil	Treatment	Sunflower	Wheat (2002)	Lentil	Wheat (2004)
TOC (g kg^{-1})	I	9.87 a	10.1 a	9.63 a	13.2 a
	O_1	10.0 a	12.5 b	11.3 b	22.1 b
	O_2	13.0 b	12.8 b	12.0 b	23.5 b
Total N (g kg^{-1})	I	1.15 ab	1.07 a	1.06 a	1.23 a
	O_1	1.05 a	1.21 b	1.06 a	1.90 ab
	O_2	1.27 b	1.35 c	1.22 a	2.20 b
C/N	I	8.60 a	9.35 a	9.11 a	10.7 a
	O_1	9.53 ab	10.3 b	10.7 b	11.6 a
	O_2	10.1 b	9.50 ab	9.87 ab	10.7 a

I: inorganic fertilisation. O_1: Plant compost. O_2: animal compost. TOC: total organic carbon. Values of different parameters in the same column followed by the same letter are not significantly different ($p > 0.05$).

Microbial biomass

At Torres, microbial biomass C values in the O_2 treatment were higher than in the I treatment, except in the carrot and spinach crop cycle (Fig. 1A). However, at Tomejil, the microbial biomass C values were only statistically different between the organic and inorganic treatments during the last crop cycle (Fig. 1B).

In both experiments microbial biomass C values were similar, except for the fourth crop cycle.

Enzyme activity

Dehydrogenase activity increased progressively during the study, except for the fourth crop cycle of

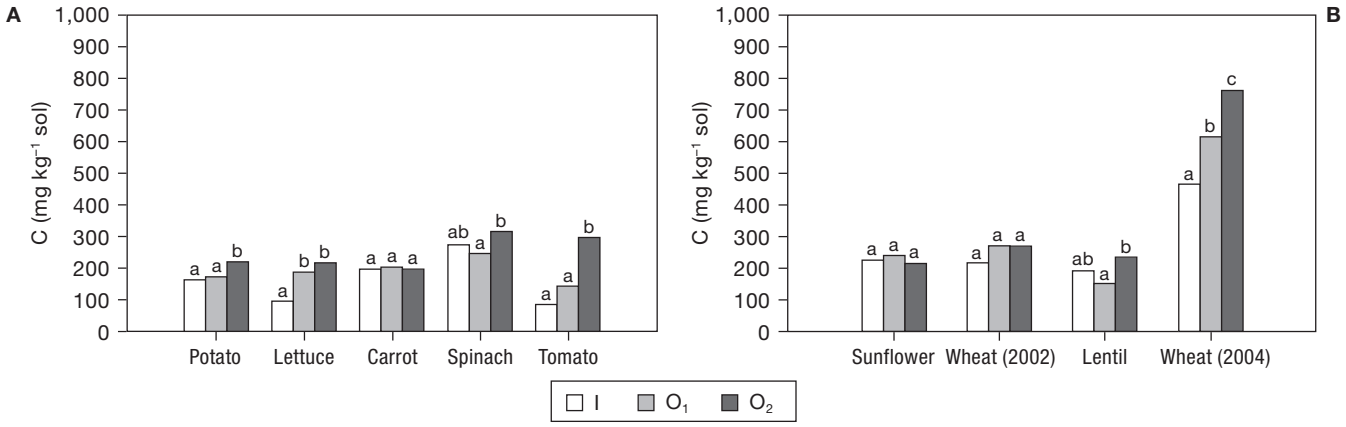

Figure 1. Soil microbial biomass C at Torres (A) and Tomejil (B). Significant differences ($p < 0.05$) among treatments are indicated by different letters.

Table 7. Mean soil enzymatic activity at Torres and Tomejil

Torres	Treatment	Potato	Lettuce	Carrot	Spinach	Tomato
Dehydrogenase	I	23.6 a	29.2 a	44.0 a	36.0 a	34.0 a
	O_1	20.5 a	40.0 a	53.0 b	30.0 a	67.0 b
	O_2	18.0 a	38.7 a	68.0 c	37.2 a	98.7 c
Protease	I	59.5 a	66.0 a	60.0 a	82.0 a	70.0 a
	O_1	70.7 a	151 b	97.7 b	177 b	113 b
	O_2	124 b	102 a	100 b	103 c	111 b
β-glucosidase	I	63.3 a	50.7 a	67.2 a	83.6 a	93.0 a
	O_1	63.0 a	53.5 a	72.0 a	78.0 a	118 b
	O_2	76.6 a	52.5 a	65.0 a	118 b	157 c
Alkaline phosphatase	I	293 a	307 a	450 a	595 a	465 a
	O_1	328 ab	336 a	636 b	829 b	1,290 b
	O_2	350 b	351 a	650 b	869 b	1,283 b

Tomejil	Treatment	Sunflower	Wheat (2002)	Lentil	Wheat (2004)
Dehydrogenase	I	33.3 a	51.4 a	33.0 a	34.0 a
	O_1	29.4 a	51.3 a	38.1 ab	40.0 ab
	O_2	34.4 a	56.0 a	45.7 b	51.0 b
Protease	I	15.7 a	39.0 a	47.4 a	107 a
	O_1	30.0 b	50.0 ab	62.4 ab	216 b
	O_2	14.7 a	70.1 b	67.2 b	307 c
β-glucosidase	I	110 a	169 ab	142 a	330 a
	O_1	110 a	155 a	150 a	349 a
	O_2	122 a	181 b	158 a	343 a
Alkaline phosphatase	I	242 a	525 ab	390 a	645 a
	O_1	255 a	468 a	551 b	995 b
	O_2	282 a	602 b	607 b	891 b

I: inorganic fertilisation. O_1: plant compost. O_2: animal compost. Dehydrogenase: mg TPF dwt kg^{-1}. Protease: mg Tyrosine kg^{-1} dwt $2h^{-1}$. β-glucosidase: p-nitrophenol (mg kg^{-1} dwt h^{-1}). Phosphatase alkaline: p-nitrophenol (mg kg^{-1} dwt h^{-1}). Values of different parameters in the same column followed by the same letter are not significantly different ($p > 0.05$).

the experiment at Torres (Table 7). There were only statistical differences between the organic and inorganic treatments in the third and fifth crop cycles. Generally dehydrogenase activity in the O_2 treatment was statistically higher than in the inorganic treatment from the third crop cycle (Table 7).

At Torres protease and phosphatase activity in organically fertilised soil was statistical higher than in inorganically fertilised soil from the third crop cycle (Table 7). However, there was no statistical difference in β-glucosidase until the fifth crop cycle (Table7).

At Tomejil, protease activity values were only statistically different among the different treatments at the last sampling (Table 7). Generally, there was no difference in β-glucosidase activity among treatments (Table 7). Alkaline phosphatase levels were higher in organically fertilized soil than in the inorganically fertilized soil (Table 7) from the third cycle.

In general, dehydrogenase, protease and phosphatase activities were similar at both sites. However, soil β-glucosidase activity at Tomejil (clay) was higher than in soil at Torres (loam).

Ratios of enzyme activity to microbial biomass

The ratios of enzyme activity to microbial biomass are shown in Figures 2, 3, 4 and 5. At Torres, ratio of dehydrogenase activity to microbial biomass fluctuated during the experiment. Values were higher in inorganically fertilised soil for the two first cycles. No differences were recorded between organically and inorganically fertilised soil in the ratio of dehydrogenase activity to microbial biomass at Tomejil (Fig. 2B). In the fourth crop cycle, the ratio of dehydrogenase activity to microbial biomass was very low in all treatments.

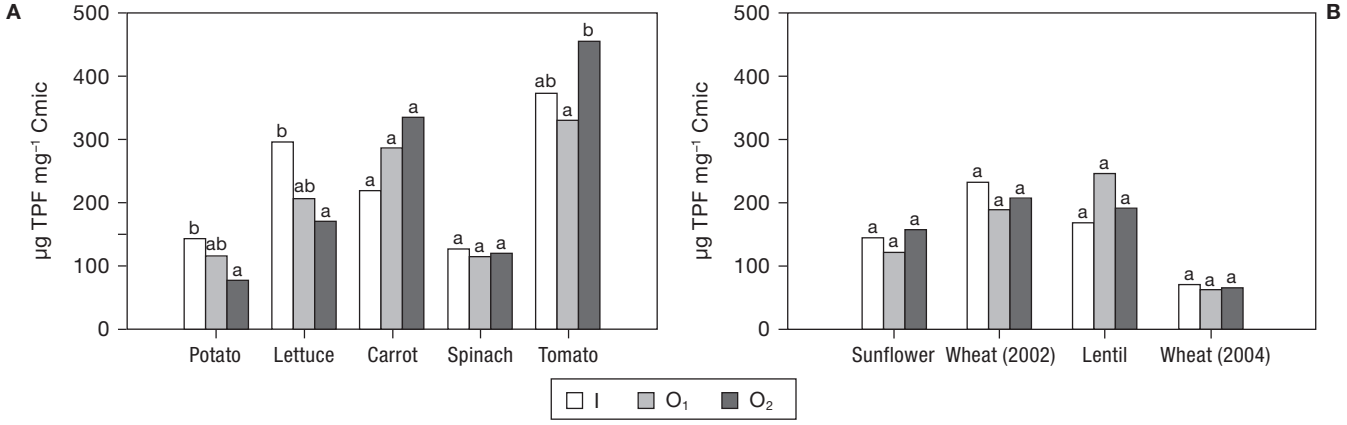

Figure 2. Ratios of dehydrogenase activity to microbial biomass C (Cmic) at Torres (A) and Tomejil (B). Significant differences ($p < 0.05$) among treatments are indicated by a different letter.

In both experiments, values of the ratios of hydrolytic enzymes to microbial biomass fluctuated during the experiment and can not be attributed to treatment effects.

In both experiments, statistical differences in the ratio of protease activity to microbial biomass were observed between treatments in the fourth crop cycle. The highest ratios were in the organically fertilised soils (Fig. 3A and 3B).

Ratios of β-glucosidase activity to microbial biomass were only statistically different among treatments at the second crop cycle of the experiment at Torres and in the last crop cycle at Tomejil. The highest β-glucosidase activity to microbial biomass ratios were in treatment I (Fig. 4A and 4B).

The ratio of phosphatase activity to microbial biomass was statistically significant between O_2 and I in the second, forth and fifth crop cycles at Torres (Fig. 5A). However, significant statistical differences between organic and inorganically fertilised soils in the ratio of phosphatase activity to microbial biomass were only seen in the fourth crop cycle at Tomejil (Fig. 5B).

Generally, ratios of dehydrogenase, protease and phosphatase activity to microbial biomass in the clay at Tomejil were lower than in the loam at Torres.

Discussion

Although during conversion to organic farming increases in soil organic matter occur slowly (Werner, 1997), in this study increased TOC was obtained from the second crop cycle at both sites. Several authors report increased soil organic carbon after the addition of compost (Marschner *et al.*, 2003; Melero *et al.*, 2006). This is important in Mediterranean soils, where

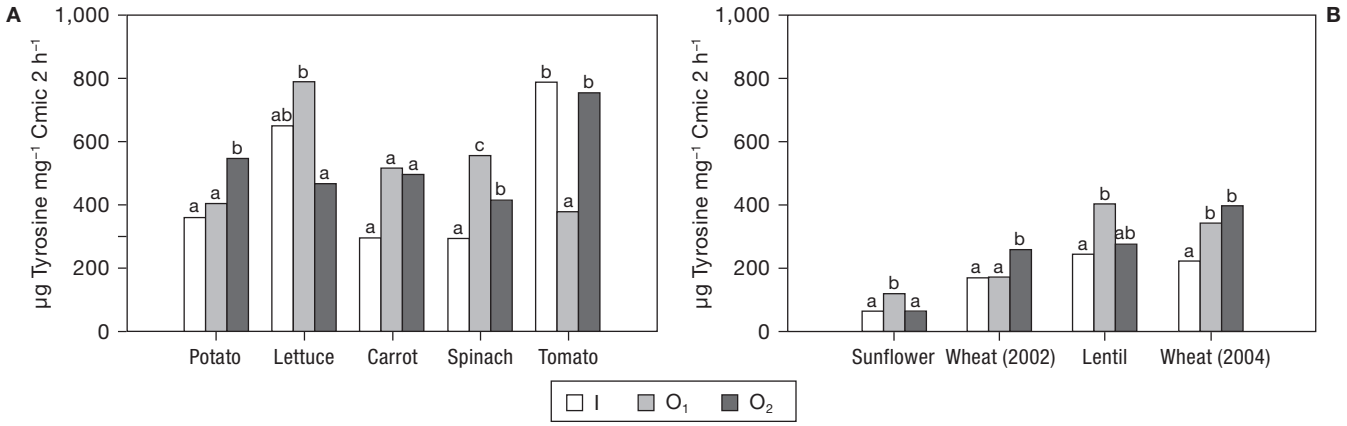

Figure 3. Ratio of protease activity to microbial biomass C (Cmic) at Torres (A) and Tomejil (B). Significant differences ($p < 0.05$) among treatments are indicated by a different letter.

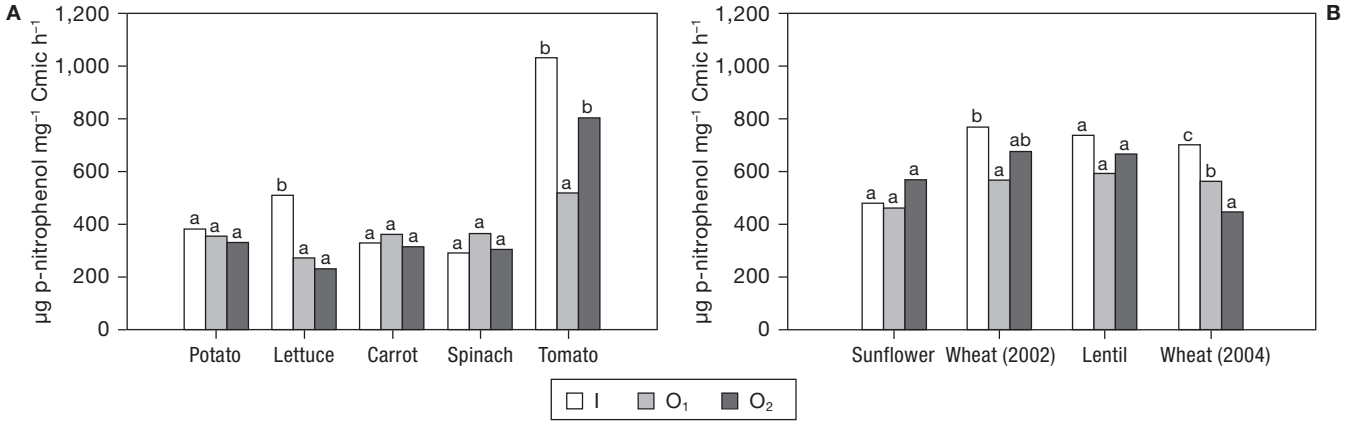

Figure 4. The ratio of β-glucosidase activity to microbial biomass C (Cmic) at Torres (A) and Tomejil (B). Significant differences ($p < 0.05$) among treatments are indicated by a different letter.

the level of organic matter in agricultural soils is normally < 10 g kg^{-1} (Costa *et al.*, 1991).

In the final crop cycle at Tomejil there was a large increase in TOC in all treatments. This increase may be related to the previous crop cycle with a legume. Legumes add both organic matter and N to the soil (Omay *et al.*, 1997; Ashraf *et al.*, 2004) and increase soil fertility. Further, crop rotations that included high-residue-producing crops, wheat in our study, increased soil organic carbon and N content (Omay *et al.*, 1997).

At the end of the experiment, at both sites there was an increase in TOC compared with values at the start of the study in all treatments. The trend was $O_2 > O_1 > I$. However increases in TOC values in the clay (Tomejil) were greater than in the loam (Torres). This could be related to differences in water supply and soil texture between the sites. A lower soil water content can decrease metabolic activity of soil microflora (Gianfreda and Bollag, 1996), affecting mineralisation of soil organic matter. On the other hand, the capacity of soil to protect mineralization of soil organic matter is related to clay content (Muller and Hoper, 2004). Formation of clay-humic complexes protects structured clay colloids and lowers organic matter mineralization.

Although N inputs to soil were similar in the organic and inorganic treatments (I = 959 g kg^{-1}; O_1 = 1,027 g kg^{-1}; O_2 = 1,191 g kg^{-1}) at Torres, addition of organic N favoured the increased soil N reserves. Marschner *et al.* (2003) also reported increased soil N in soils which had organic residue inputs.

At Tomejil soil N content increased in the organically fertilised soil. These results were related with N inputs added to soil in compost. Apart from this addition, the legume crop could also have had an important role in increased soil N (Ashraf *et al.*, 2004; Dinesh *et al.*, 2004) in last crop cycle.

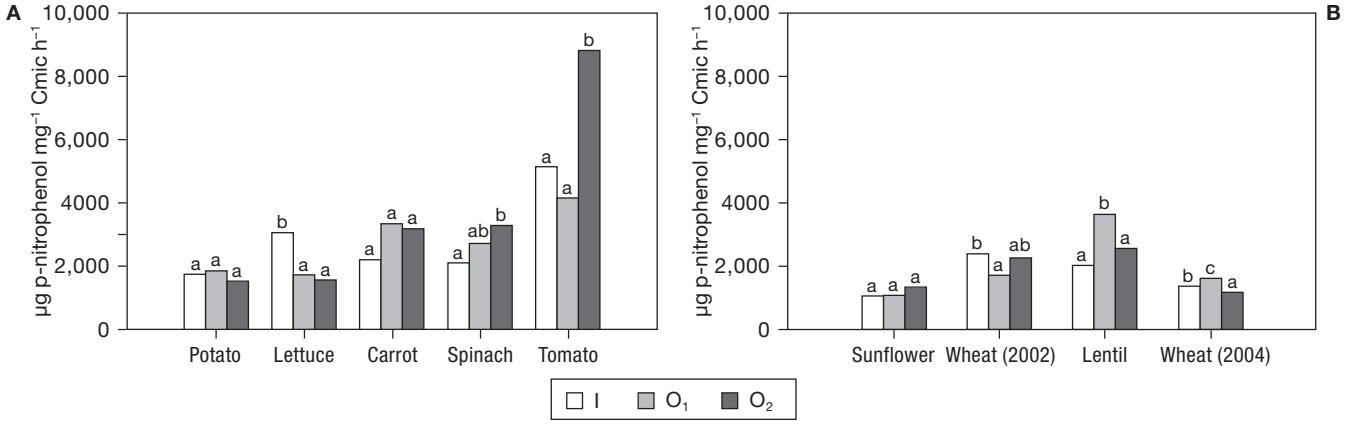

Figure 5. The ratio of alkaline phosphatase activity to microbial biomass C (Cmic) at Torres (A) and Tomejil (B). Significant differences ($p < 0.05$) among treatments are indicated by a different letter.

At both sites the addition of compost and input of crop residues slightly increased the C:N ratio. However at Tomejil this increase was higher and could be related to lower organic matter mineralization of soil with a higher clay content.

At the end of study, in both soil types the highest microbial biomass C values were in organically managed soils. Schjonning *et al.* (2002) and Melero *et al.* (2006) observed similar results. The increase in microbial biomass in organically fertilised soils might be due to either the protective capacity of organic matter over microbial biomass (Pascual *et al.*, 1997) or the microbial biomass incorporated, through organic amendments, or the addition of substrate-C (García-Gil *et al.*, 2000). Schjonning *et al.* (2002) also reported these effects of organic amendment.

The animal based compost was more effective at increasing soil organic matter and microbial biomass than plant compost. Plant compost, with a high lignin and cellulose content probably needs more time to degrade these compounds.

At Tomejil, microbial biomass C showed a greater increase, in all treatments in the fourth crop cycle. This increase, observed at the last sampling, could be due to higher growth of microbial biomass, induced by the legume crop (Dinesh *et al.*, 2004). Crop rotations, which included a legume crop, had higher microbial biomass development than in monocultures (Anderson and Domsch, 1989). This is related to the higher inputs and different crop residues in the crop rotation.

Except for the fourth crop cycle at Tomejil, in our experiment, the microbial biomass C values in both soils were similar. Therefore neither soil texture nor the different water supply had influenced this parameter. On the contrary, Muller and Hoper (2004) observed a close correlation between clay content and microbial biomass based on the hypothesis of the protective capacity of clay on microbial biomass. Here, we hypothesise that in the clay (under a dry land system) this protective capacity could have been countered by decreased soil water that might have led to osmotic desiccation of microbial cells (Gianfreda and Bollag, 1996).

In both experiments organic fertilizer had a positive effect on enzymatic activity. Marschner *et al.* (2003) and Melero *et al.* (2006) observed an increase of enzymatic activity following the addition of organic amendments indicating micro-organism activation through carbon inputs, although organic residues can also directly increase enzyme content.

The lower dehydrogenase activity, in the fourth crop cycle (winter), at Torres could be related to low temperatures that could decrease activity. Saratchandra *et al.* (1989) also observed lower dehydrogenase activity in the winter.

At Tomejil the increase in protease, β-glucosidase and phosphatase activities, in all treatments at the last sampling, could be related to a legume crop. Legume crops increase microbial activity and enzyme synthesis due to increased carbon turnover and nutrient availability (Dinesh *et al.,* 2004).

Generally, the inputs of the different crop residues could have influenced enzyme synthesis in the microorganisms. Perucci *et al.* (1984) showed that crop residues type had a clear influence on enzymatic activity. Bending *et al.* (2002) and Acosta-Martínez *et al.* (2004) also observed that both the quantity and quality of crop residue can affect soil microbial properties.

As with microbial biomass C, dehydrogenase, protease and phosphatase activity was similar in both soil types. Therefore neither soil texture nor the different water supply influenced these biochemical properties.

We hypothesise that protective capacity and stabilization of the clay, under dry land conditions on enzymatic activity could have been countered by the decrease in soil water content that may have lead to lysis of microbial cells and the liberation of enzymes into the soil, which may be partly or totally reduced because of deactivating processes (e.g., immobilization on soil colloids, proteolysis) (Gianfreda and Bollag, 1996). However at Tomejil values for β-glucosidase were higher than at Torres. These results could be attributed to the higher soil TOC contents at Tomejil.

As discussed previously, the lower ratios of dehydrogenase activity to microbial biomass in the fourth crop cycle (winter) at Torres, could be related to low temperatures.

At Tomejil the small ratios of dehydrogenase activity to microbial biomass in the fourth crop cycle can be related to high microbial biomass C content at that sampling.

The ratios of enzymatic activity (dehydrogenase, protease and phosphatase) of microbial biomass in the clay at Tomejil were lower than in the loam at Torres. These results indicate a lower rate of enzyme production by microbial biomass in the clay. However, clay minerals through their stabilisation and protective effect on enzymes contribute to the accumulation and survival of soil enzymes (Gianfreda and Bollag, 1996). At Tomejil the low water input could have had a negative effect

on the ratio of enzymatic activity of microbial biomass. Our results could also be attributed to degradation, or inactivation, of released enzymes and/or extracellular enzymes before their stabilization in the soil (Acosta-Martínez *et al.*, 2004).

In summary, there was an increase in the quantity and activity of microbial biomass, which is of great importance in organic matter turnover and nutrient availability for micro-organism and plants. The ratios of enzymatic activity to microbial biomass fluctuated during the experiment. Thus, further studies are necessary to confirm the positive effect of organic management on biochemical soil quality.

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