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Changes of soil chemical, microbiological, and enzymatic variables in relation to management regime and the duration of organic farming in *Phaseolus vulgaris*

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In this study we investigated the effects of management regime (organic *vs* conventional) and the duration of organic farming on soil microbial and chemical variables (C- and N-microbial, C- and N-organic, N-inorganic, P-extractable, C- and N- mineralization rate) and on enzymes involved in N- and P-cycle. To meet this goal, sites cultivated organically with *Phaseolus vulgaris* (L.) for 2, 5, and 14 yrs as well as a conventional one were investigated. Samples were collected at two occasions coinciding to different stages of plant development. Both management regime and the duration of organic farming did not affect significantly the chemical and microbiological soil variables such as microbial C and N, rates of C- and N-mineralization, N-inorganic, P-extractable and C-organic. It seems that, for bean cultivations (bean is an annual plant), the mechanical disturbance of the soil induced yearly by plowing and tillage masked the effects of the repeated addition of organic amendments. On the contrary, enzymatic activities increased with increasing duration of organic farming (from 2 to 14 yrs) and they were higher in the oldest organic site compared to the conventional one. However, although most soil variables did not exhibit significant quantitative differences in various sites, these latter differed in respect to the temporal changes in these variables. The magnitude of temporal changes increased from conventional to the oldest organic site.

Key words: sustainable agriculture, amidohydrolases, phosphatases, plant stage.

INTRODUCTION

Organic farming has been considered an alternative to conventional agriculture, aiming at a better quality of products and at the sustainability of agriculture itself. Conventional agriculture involves intensive exploitation of soil reducing soil organic matter and aggregate stability (Bronick & Lai, 2005) and leads to the degradation of enzymes. Although specific enzymes have been used as indicators of soil quality, the idea that a series of enzymes must be measured in order this quality to be evaluated was only recently developed (Burns & Dick, 2002). In some cases, enzymes worked as good indicators of the effects of soil management because their activities do not exhibit seasonal oscillations like other parameters (e.g. microbial respiration). However, for assessing the effect of agricultural practices on soil quality a multifactorial approach is recommended. This approach involves physical, chemical, and microbiological soil parameters (Wander & Bollero, 1999; Widmer *et al*., 2006). The latter are related with the structure and the function of soil microbial community. Specifically, in organically managed systems, where the amounts of available nutrients depend exclusively on microbially mediated nutrient transformations, the attributes of microbial communities become even more important (Garbeva *et al*., 2004; Esperschüetz *et al*., 2007). Although comparisons between soils in organic and conventional cultivation systems are available (Clark *et al*., 1999; Gosling & Shepherd, 2005; Tu *et al*.,

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2006), a rarely investigated issue is how soil parameters are shaped in relation to the duration of organic farming (Monokrousos *et al*., 2006, 2008; Tsiafouli *et al*., 2006, 2007; Esperschüetz *et al*., 2007; Flieβbach *et al*., 2007), a fact that is related to the difficulties in establishing such an experimental design.

Our previous experience (Monokrousos *et al*., 2006, 2008) showed that although changes in soil biochemical variables were not related linearly to the duration of organic farming, there was a clear distinction between the newest transitional and the oldest certified organic system. However, the above findings referred to organic cultivation of a perennial plant, where no annual tillage or plowing took place. Thus, our aim in this paper was to detect whether these findings are also valid in the case of an annually cultivated plant. The hypothesis is that in the cultivations of annuals where tillage and/or plowing takes place yearly, the majority of soil variables exhibit insignificant differences among transitional and certified organic systems since the redistribution of incorporated manure induced by mechanical disturbance is expected to mask the effect of the duration of organic management. Specifically, the working hypotheses of this study are the following: (A) The management regime (conventional *vs* organic) and the duration of organic farming can affect: 1) the microbial biomass C and N, 2) the activities of microbial extracellular enzymes in the soil, 3) the mineralization rates of C and N, and 4) the available forms of N and P and the pools of C and N in the soil.

(B) The magnitude of seasonal changes in soil variables is differentiated between conventional and organic systems or among systems cultivated organically for different durations.

This hypothesis is based on our previous experience that showed that the magnitude of changes in the bacterial catabolic profiles was differentiated between organic and conventional systems (Papatheodorou *et al.*, 2008).

MATERIALS AND METHODS

Study site, experimental design, sampling, and data analysis

The study was conducted in Prespa region located in the Northwestern part of Greece. It is one of the largest National Parks in Greece with a total area of 199 km^2, consisting of two connected lakes and the surrounding forested mountain region. The climate of the area is mild continental-central European with mediterranean features as well. The average annual precipitation ranges between 600 and 900 mm, and the wet season lasts from October to May (data from WWF-Greece 2002). A large portion of the area is cultivated with *Phaseolus vulgaris* (L.), which is the traditional product of this region. The replacement of conventional cultivations by organic ones is one of the key topics in Prespa management plans. In the middle of a topographically homogeneous area, three nearby sites cultivated organically for 2 (O2), 5 (O5), and 14 (O14) years and a conventional one, 500 m away, were selected. These sites were chosen to be close to each other to exhibit similar soil texture and physico-chemical properties (Table 1) in order to avoid bias induced by spatial heterogeneity. Thus, experimental areas differed only with respect to the duration of farming or the management regime (organic *vs* conventional). The size of the cultivated areas varied from 3000 to 5000 m^2 and their soils were characterized as sandyloam. All experimental sites were cultivated with the annual *Phaseolus vulgaris* (L.). In organic cultivations, 0.8 kg m^{-2} of cattle manure was added every two years. The conventional site (CF) received annually 0.02 kg m^{-2} of 11-15-15 (N-P-K) fertilizer.

Sampling followed a randomized complete design involving five randomly dispersed plots (1 m × 1 m) within each cultivated site. To account for intraplot spatial variability, in each plot 3 individual soil cores were collected from the upper 12 cm of the soil and average values were estimated. The diameter of each

TABLE 1. Soil physico-chemical characteristics recorded in the different sites

	Silt (%)	Clay (%)	Sand (%)	Bulk density (g cm^{-3})	Soil water filled pore space (%)	Water content (%)	pH
O2	23.8	17.8	58.4	1.36	47.88	9.34	6.61
O5	12.5	14.4	73.2	1.29	48.04	7.95	6.72
O14	30.5	15.7	53.8	1.28	40.56	8.10	6.69
CF	19.6	19.1	61.3	1.49	51.20	8.22	7.37

soil core was 7.5 cm. To consider the effect of abiotic variables, data were collected on two sampling occasions coinciding with different stages of plant development: 1) during the emergence of new seedlings in May 2004, 2) at the middle of the plant growth period in July 2004.

To study the effects of organic farming duration, sampling period and their interaction on soil variables, data from organic cultivations were analysed by a Multivariate ANOVA. To study the effect of management regime on soil variables, we compared samples from O14 and CF, by means of a t-test. Whenever the assumptions of normality and of homogeneity of variances were not met, data were transformed accordingly. In case of significant differences, *post-hoc* comparisons by a Bonferroni test were applied. The analyses were conducted with the SPSS software (version 15).

Biochemical analyses

Soon after sampling, fresh soil samples were sieved (mesh size 2 mm) and then stored at 10°C for 7 days to overcome the effects resulting from disturbance. Soil organic C was determined by a wet oxidation titration procedure using an acid dichromate system (Allen, 1974). Soil microbial biomass C was measured by the fumigation incubation method of Jenkinson & Powlson (1976), as modified by Ross (1990) in samples adjusted to 60% of their water holding capacity (WHC). The K_c factor equals 0.45 (Jenkinson & Powlson, 1976). Soil microbial biomass N was determined by the fumigation extraction method using a factor of 0.54 (Brookes *et al.*, 1985). Soil organic N was measured by the Kjeldahl method (Bremmer, 1960). NH_4-N and NO_3-N were determined in 2 M KCl extracts [1:10 soil dry weight (d.w.) solution], by distillation and subsequent titration (Allen, 1974). The N-mineralization rate was estimated by measuring the increase in mineral N after incubation of soil samples at field moisture (equivalent to 20 g d.w.) for 30 days at 25°C. In the same sample, the rate of C-mineralization was determined by titration after the incubation of samples over a period of 30 days. For extractable P, the method of Olsen *et al*. (1954) as specified by Allen (1974) was used.

The activities of amidohydrolases were determined by the methods of Tabatabai (1994). Briefly, the methods are based on the determination of NH_4 released when soil is incubated at 37°C for 2 hrs with 0.1 M Tris (hydroxymethyl) aminomethane (THAM) buffer, toluene and L-asparaginase, L-glutaminase and urea for the determination of asparaginase, glutaminase and urease, respectively. Similarly, the activities of acid and alkaline phosphatases were assayed by the methods of Tabatabai (1994). The procedure involves extraction and quantitative determination of p-nitrophenol released when soil is incubated with p-nitrophenyl phosphate in modified universal buffer adjusted to pH 6.5 and 11 for acid and alkaline phosphatase, respectively. All enzyme activities were assayed on < 2 mm field-moist samples at optimal pH values in duplicates and in one control sample.

RESULTS

The duration of organic farming affected significantly the available forms of N and P (N-inorganic and P-

TABLE 2. Significant effects of sampling, treatment (duration of organic farming), and their interactions on soil variables according to the ANOVA results (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ns: not significant)

	Sampling	**Treatment**	**Sampling × treatment**
C-microbial	ns	ns	*
N-microbial	***	ns	ns
C-organic	***	ns	ns
N-organic	***	ns	***
C-mineralization rate	***	ns	*
N-mineralization rate	***	ns	***
N-inorganic	**	***	***
P-extractable	ns	***	ns
Acid phosphatase	***	***	***
Alkaline phoshatase	***	***	ns
Asparaginase	***	ns	ns
Glutaminase	***	***	ns
Urease	ns	***	*

TABLE 3. Mean values (s.e.) of soil biochemical parameters recorded in the various sites (in each case n = 10). Different letters correspond to statistically significant differences among the organic fields

	CF	O2	O5	O14
C-microbial (mg g^{-1})	0.91 (0.18)	0.72 (0.06)	0.78 (0.11)	0.76 (0.09)
N-microbial (mg g^{-1})	0.07 (0.01)	0.07 (0.01)	0.08 (0.02)	0.10 (0.02)
N-inorganic (μg g^{-1})	45.13 (2.05)	51.20^{a} (3.11)	61.09^{b} (3.64)	50.13^{a} (3.17)
N-mineralization rate (μg g^{-1} d^{-1})	0.36 (0.07)	0.85 (0.24)	0.49 (0.06)	0.54 (0.14)
C-mineralization rate (mg g^{-1} $month^{-1}$)	3.35 (0.27)	3.14 (0.22)	3.40 (0.29)	3.93 (0.45)
C-organic (mg g^{-1})	14.72 (1.42)	11.87 (1.62)	11.95 (1.74)	16.34 (2.34)
N-organic (mg g^{-1})	0.91 (0.04)	1.35 (0.09)	1.37 (0.05)	1.38 (0.12)
P-extractable (μg g^{-1})	23.81 (2.15)	24.11^{ab} (1.76)	28.41^{a} (0.96)	21.53^{b} (1.05)
Acid phosphatase (μg g^{-1} h^{-1})	249.72 (7.03)	206.3^{a} (8.89)	262.83^{b} (21.90)	290.52^{c} (17.41)
Alkaline phosphatase (μg g^{-1} h^{-1})	51.03 (12.69)	131.1^{a} (9.40)	189.00^{b} (15.57)	220.07^{c} (20.74)
Asparaginase (mg Kg^{-1} $2h^{-1}$)	15.63 (1.82)	28.98 (3.96)	24.02 (2.10)	24.53 (2.43)
Glutaminase (mg Kg^{-1} $2h^{-1}$)	43.83 (8.49)	309.02^{a} (34.25)	282.88^{a} (19.74)	367.28^{b} (31.39)
Urease (mg Kg^{-1} $2h^{-1}$)	22.77 (2.25)	47.98^{a} (3.71)	46.84^{a} (3.37)	68.13^{b} (3.65)

TABLE 4. Mean values (s.e.) of soil biochemical parameters in all organic sites recorded on the two sampling occasions (for each sampling n = 15). For variables presented in italics, the effect of sampling was insignificant

	May	July
C-microbial (mg g^{-1})	0.70 (0.27)	0.81 (0.25)
N-microbial (mg g^{-1})	0.04 (0.03)	0.12 (0.04)
N-inorganic (μg g^{-1})	49.33 (9.48)	58.95 (11.17)
N-mineralization rate (μg g^{-1} d^{-1})	0.31 (0.16)	0.95 (0.57)
C-mineralization rate (mg g^{-1} $month^{-1}$)	4.24 (1.00)	2.74 (0.45)
C-organic (mg g^{-1})	9.96 (4.29)	16.82 (6.12)
N-organic (mg g^{-1})	1.12 (0.14)	1.61 (0.13)
P-extractable (μg g^{-1})	25.44 (3.61)	23.93 (6.00)
Acid phosphatase (μg g^{-1} h^{-1})	284.02 (62.87)	222.40 (46.78)
Alkaline phosphatase (μg g^{-1} h^{-1})	218.82 (56.07)	141.29 (37.95)
Asparaginase (mg Kg^{-1} $2h^{-1}$)	30.78 (9.14)	20.91 (6.51)
Glutaminase (mg Kg^{-1} $2h^{-1}$)	394.70 (59.66)	244.75 (58.72)
Urease (mg Kg^{-1} $2h^{-1}$)	57.08 (16.23)	51.55 (13.12)

TABLE 5. Comparison of soil variables between O14 and CF samples by a t-test

	t-value	df	significance
C-microbial	–0.29	18	ns
N-microbial	1.49	18	ns
C-organic	0.59	18	ns
N-organic	3.91	18	**
C-mineralization rate	0.84	18	ns
N-mineralization rate	0.44	18	ns
N-inorganic	1.32	18	ns
P-extractable	–0.61	18	ns
Acid phosphatase	2.03	18	ns
Alkaline phosphatase	6.74	18	***
Asparaginase	9.84	18	**
Glutaminase	9.95	18	***
Urease	10.56	18	***

**: $p < 0.01$, ***: $p < 0.001$, ns: not significant

extractable), as well as all enzymatic activities but that of asparaginase (Table 2). Higher values of available N and P were recorded in samples from O5 whereas the activities of acid and alkaline phosphatase, glutaminase and urease increased from O2 to O14 samples (Table 3). Sampling period had a significant effect on most variables, with the exception of C-microbial, P-extractable, and urease. All enzymatic activities and the rate of C-mineralization were higher in May, whereas parameters related to N-cycle such as N-microbial, N-inorganic, N-organic, and N-mineralization rate as well as C-organic exhibited the highest values in July (Table 4).

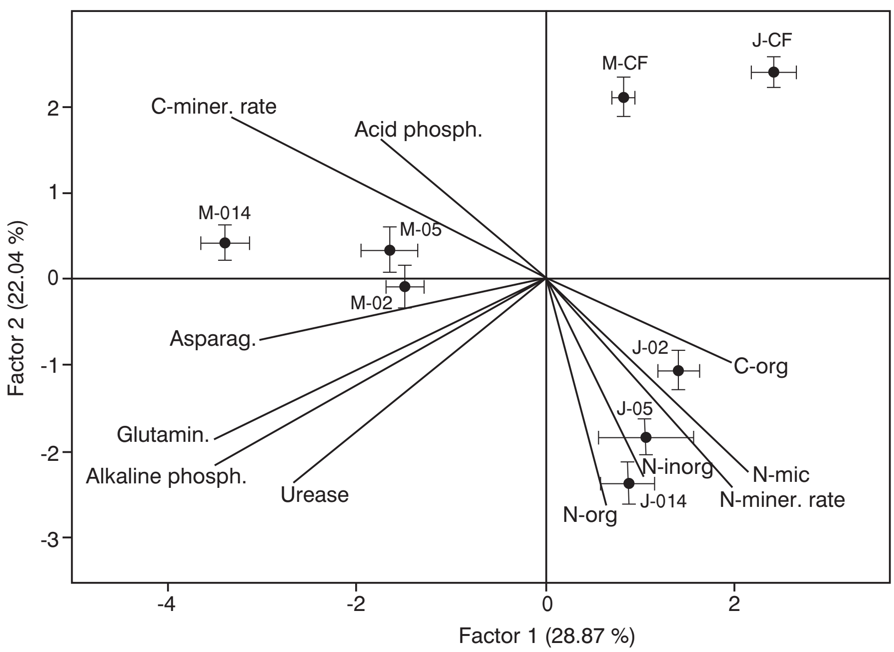

FIG. 1. Ordination of the samples and the soil biochemical variables on a PCA biplot. Each point corresponds to a sample collected from a specific site at specific time [1st symbol corresponds to time (M: May, J: July) and 2nd symbol corresponds to site (CF: conventional, O2: 2 years, O5: 5 years, and O14: 14 years organically cultivated sites)]. Error bars indicate standard errors.

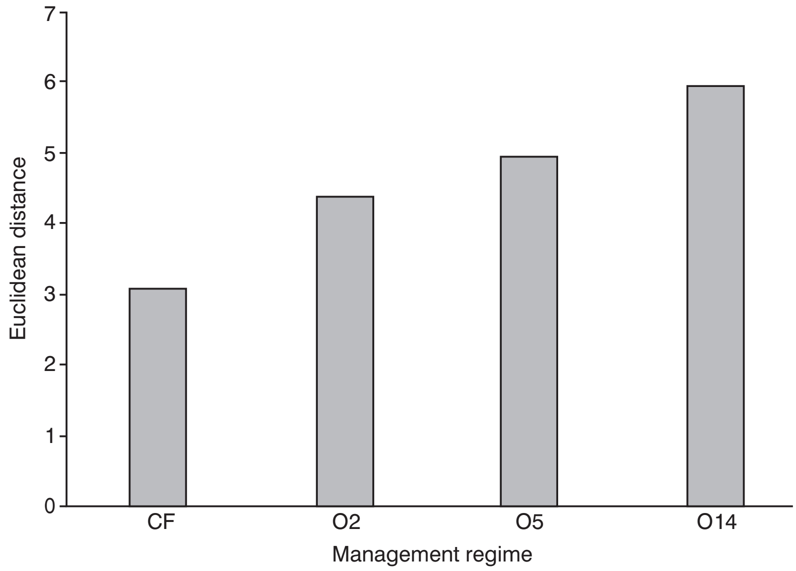

FIG. 2. The magnitude of changes in soil variables between the two samplings in various sites. The estimation of Euclidean distances is based on the scores of the first and second PCA axes.

The comparisons between the organic and the conventional samples are presented in Table 5. Significant differences between O14 and CF samples were recorded only for enzymatic activities and for the amounts of N organic and relevant values were always higher in organic samples.

In Figure 1 the ordination of samples in a PCA biplot is presented. The first two axes of the analysis explained almost 50% of data variability. Although there was a general tendency of samples from May to occupy the upper left part of the graph while those from July the bottom right one (indicating the effect of the sampling period), this did not hold for all samples. The samples from CF were ordinated towards the upper right part of the graph regardless the time of collection. On the contrary, organic samples collected during the two sampling periods were clearly distinguished and this was more obvious for O14 samples. The ordination of soil variables in the same biplot indicated that the ordination of July samples was driven by parameters related to N-cycle (N-organic, N-inorganic, N-microbial, and N-mineralization rate) while that of May samples was related with the rate of C-mineralization and the activity of acid phosphatase. To depict the magnitude of temporal changes in soil variables in the various sites, we estimated the Euclidean distances between centroids on the first two PCA axes. As shown in Figure 2, the distance between the centroids of the samplings increased from CF to O14 samples.

DISCUSSION

In most reported cases of comparisons between organic and conventional systems, the former consist of complex rotations including perennial and annual crops in order to ensure the best performance of soil functions (Dawson *et al*., 2008). Our study sites were cultivated exclusively with *P. vulgaris*, a leguminous plant, with a fallow period from October to April. After harvesting, the residues of the aboveground biomass are left on the soil surface until the end of the fallow period and then they are incorporated into soil by plowing. This farming practice is applied in conventional as well as in organic bean cultivations. The comparison between O14 and CF sites showed that the activities of all enzymes were lower in the conventionally cultivated site, a finding that is in accordance with the relevant literature (Satpathy & Behera, 1993; Jordan *et al*., 1995; Monokrousos *et al*., 2006). The differences between the two management systems were non-significant when other soil variables were considered. Emmerling *et al*. (2001) reported also no differences in microbial biomass between an integrated farming system and a conventional one during an observation period of 10 years. The lack of differences reported herewith could be probably related to the incorporation of the decomposed plant residues in the soil and/or to the fertilizer application in CF. The concentrations of N-P-K of the fertilizer were almost similar and as Chu *et al*. (2007) mentioned, balanced fertilization could promote the biomass and the activity of microorganisms.

The duration of organic farming had no significant effect on the microbial variables (C- and N-microbial), the rates of C- and N-mineralization, and the carbon and nitrogen soil pools, although the values of C-organic exhibited an increasing trend from O2 to O14 samples. It is often reported that organic C is a weak indicator of changes induced by organic farming (Gosling & Shepherd, 2005; Marinari *et al.*, 2006). Martini *et al.* (2004) examined soil quality variables in transitional (< 1 year managed organically) and organic (> 5 years) tomato cultivations and found differences only regarding total N and extractable P. On the other hand, in the study of Monokrousos *et al.* (2006) regarding organic cultivations of the perennial *Asparagus officinalis* (L.), the transitional cultivation (2 years) exhibited lower values of N- and P-microbial and of N-mineralization rate than the older organic (5 years). In the present study, the lack of remarkable effects of organic farming duration could be related to the specific agricultural practices that farmers apply in annual cultivations, e.g. plowing at the end of winter. Moreover, at the beginning of spring, farmers use tillage to incorporate and distribute manure into soil and this creates conditions suitable for mineralizing nutrients (Peigné *et al.*, 2007). According to Green *et al.* (2007) these mechanical disturbances could be strong enough to eliminate any possible effect of the repeated addition of organic material. Also, Calbrix *et al.* (2007) concluded that the effect of organic amendments is less intense than that of the mechanical disturbance of soil whereas van Diepeningen *et al.* (2006) found significant effects of plowing and mechanical weeding on soil characteristics. On the other hand, the duration of organic farming affected significantly the enzymes phosphatase, glutaminase, and urease. Their activities increased linearly from O2 to O14 site. Organic amendments seemed to be more effective than mechanical disturbance on enzymatic activities as well as on the functioning of the soil bacterial community in these sites. Indeed, when the catabolic profiles of the bacterial metabolism were examined, the various organic fields were clearly distinguishable (Papatheodorou *et al.*, 2008).

To examine the magnitude of changes in soil variables, between May and July we estimated Euclidean distances based on the PCA scores of samples. The magnitude of changes between the two samplings increased from CF to O14 samples. Analogous were the reports by Papatheodorou *et al*. (2008), regarding the changes in bacterial functional diversity over time in conventional and organic cultivations. The authors discussed the existence of a unique microflora in conventional area that remains stable over year. Similarly, Tu *et al*. (2006) found that microbially mediated N supply remained unchanged throughout the year in conventional plots but showed temporal changes in organic systems. According to these authors, a better synchronization between plants and microbes concerning nutrient release was achieved in organic systems.

PCA showed that May organic samples were characterized by the high values of C-mineralization rate and of acid phosphatase. Moreover, the amounts of alkaline phosphatase, asparaginase, and glutaminase decreased significantly from May to July. It seems that the incorporated, before seeding, organic inputs constitute available carbon and nutrient sources for the microbial community, resulting in an immediate increase of both enzymatic activities and C-mineralization rate which was not followed by a corresponding increase of microbial biomass. On the contrary, July samples were characterized by high values of all variables associated with the N-cycle, such as N-inorganic, N-organic, N-microbial, and the potential N-mineralization rate. This indicates that in the middle of the growth period N was not limited for crop production in organic fields, a result that was reported also by Martini *et al*. (2004) and Tu *et al*. (2006) for tomato cultivations. The amount of N in July, in our sites, might be due to the fact that the N-fixing bacteria living symbiotically on the roots of the bean plants have been completely developed. Moreover, since in the process of N fixation adequate amounts of P are essential (Johansson *et al*., 2004) and in our study the amounts of P-extractable remained stable between samplings, we conclude that even in May adequate amounts of available P exist in these sites.

To sum up, in relation to the working hypotheses of this study we concluded that (A) the microbial parameters and the rates of C- and N-mineralization were unaffected by the management regime or the duration of the organic farming. On the contrary, enzymatic activities increased with the duration of cultivation and they were higher in the oldest organic site compare to the conventional one. Regarding the available and the accumulated amounts of nutrients in soil, only N-organic differed between the two management systems. Finally, (B) the magnitude of temporal changes in soil variables increased from the conventional to the oldest organic site.

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