

Carmen Trasar-Cepeda
Teresa Hernández
Carlos García
Carlos Rad
Salvador González-Carcedo *Editors*

Soil Enzymology in the Recycling of Organic Wastes and Environmental Restoration

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Editors

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Preface

Soil biological and related biochemical parameters, particularly enzymes, play a fundamental role in many soil processes such as the mineralization of organic matter, the synthesis of humic substances, the degradation of xenobiotics or the mechanisms involved in the biocontrol of plant pathogens. Their direct link with soil microorganisms gives them a key role as biomonitors of the evolution of soil quality, in the determination of their resistance to or their resilience against external environmental impacts, or in the monitoring of the application of organic amendments in the recovery of degraded, eroded or polluted soils. As a consequence of the importance of soil biological and biochemical parameters on soil processes, there is an increasing interest in their study, as well as in the application of new biochemical and molecular techniques, which, when applied to soil, are becoming indispensable diagnostic tools.

It is especially important to assess the evolution of soil quality after the introduction into the soil of exogenous materials which are produced as organic residues in the domestic, industrial or agronomic human activities. This form of waste reutilisation is extremely important in the Mediterranean environment due to the depletion in organic matter content of its soils and the consequent risk of soil erosion and environmental degradation. However, the role of organic amendments in the recovery of soil biodiversity has the counterpart of an increase in the risk of environmental pollution due to an excess or inadequate balance of soil nutrients or the presence of organic and inorganic pollutants. In this book, numerous research papers are presented concerning the effects of organic wastes addition to soil as a consequence of its generalised use in Southern European agroecosystems.

The research on soil enzymology, which has developed in Spain since the 1980s, has achieved a notably high level of relevance. However, its importance has not been recognised in the public and institutional domain and these studies have not been taken into account in terms of environmental monitoring of soil, nor in the design of the management of organic residues, nor environmental restoration. Therefore, at the beginning of December 2008, the Spanish Group of Soil Enzymology organised an international meeting, held in Burgos (Spain), with the intention of disseminating the research in soil enzymology. As a consequence of

this event, at which more than fifty research papers were presented, a collection of the most relevant works have been collected, updated and reviewed in this book presenting interesting topics in the research of applied soil enzymology and related parameters such as microbial biomass quantisation or the use of new molecular tools in soil biochemistry.

The book is divided in three main sections: the first one is dedicated to the discussion of the role of enzymes as indicators in environmental monitoring, the second one deals with the application of soil enzymology and related biochemical parameters to environmental restoration processes, most of them involving the recycling of organic wastes, and finally, the third section tries to introduce new molecular tools or new applications of enzymes to other technological applications of organic residues.

The book has an introductory chapter written by Prof. Nannipieri et al., which is an up to date revision of the history of soil enzymology, the future challenges for it and the emerging tools in molecular ecology. Furthermore, the first chapter of each section contains a deep analysis of its main purpose. The first section addresses the role of enzymes and other biochemical properties in soil and ecosystem monitoring, the second section revises the effect of heavy metal pollution on microbial activity and the changes induced in the microbial community structure, and in the third one, new advances in molecular fingerprinting are applied to the study of microbial communities that play a significant role in organic wastes treatments, such as anaerobic processes.

The book also includes interesting studies about the behaviour of enzymes in contrasting soils, such as those of Mexican *tetapetes*, andosols from the Canary Islands, minimally disturbed Mediterranean soils of Catalonia, grassland soils or eucalyptus plantations from Galicia or fire affected soils. Two chapters are related to the study of humus-enzyme complexes and the information that they give about soil quality or more general aspects related to soil biochemical properties such as the influence of pre treatments of soil samples and their influence in the consequent interpretation of the results.

In section two, after the general introductory chapter, particular aspects are included in several chapters such as the the effect of heavy metals on soil enzymes in soils contaminated after Aznalcollar spill (Seville, Spain) or agricultural soils contaminated with Zn. Other chapters are related to the effect of xenobiotics on soil enzymes such as those dedicated to study the effect of 2,4,5-trichlorophenol, Banvel or mixture of herbicides like oxyfluoren and glyphosate. Finally, the use of organic amendments in restoring degraded soils are treated in three different chapters: one of them is about the recovery of a forest nursery field soil using several organic amendments, another is the recovery of gypsiferous soils with deinking paper sludge, alone or co-amended with other organic residues, and, finally, the middle term effect of the use of compost of sewage sludge to agricultural soils in Catalonia.

The third section concludes the book with chapters dedicated to the study of the extraction and characterisation of humus-enzyme complexes in vermicompost, the characterization of L-glutaminase in compost of urban refuse, the effect of dry

olive residues, after a fungal treatment or not, in the rhizosphere soil of lettuce and finally, the effect of biodisinfection processes on soil microbial populations.

The editors are grateful to Springer and to Ms. Oelschlaeger for the interest shown in publishing the results of this International Conference and to Springer Publishing Group for its help in the production of the book.

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Short-Term Effect of Fire Severity in Chemical and Biochemical Soil Properties in a Sandy Soil

María Belén Turrión, Francisco Lafuente and Rafael Mulas

Abstract The objectives of the study were to determine how fire severity influences some chemical and biochemical soil properties and to evaluate which properties are more sensitive to this factor. Two forest burned areas and their corresponding unburned areas in Valladolid Province (Spain) were selected. The vegetation in both areas was mixed forest plantation of *Pinus pinea* L. and *Pinus pinaster* Aiton, with *Albic Arenosols*. The fires occurred in June 2004 and the samples at 0–2 cm were taken five months later. Sampling was stratified on the basis of fire severity, defining three levels (high, moderate and low) based on pine canopy consumption, organic litter layer quantity and aspect, and ash quantity and color. In soil samples, pH, total soil organic C (SOC), Walkley–Black C (C_{W-B}), total nitrogen (N), available P (P_{Olsen}), microbial activity (C_{min}), microbial biomass C and P (MBC and MBP), and acid and alkaline phosphatase activity were determined. SOC and total N concentrations increased in burned soils from external inputs. Fire increased P_{Olsen} concentrations from their incorporation into the soil as ash. Our results showed a significant increase in C_{min} , MBC and MBP in burned plots, indicating that at least a part of the microbial community is favored by nutrient availability and pH increases. These fires caused a short-term fertilizing effect in the plots studied due to the very low soil fertility and the low temperature reached.

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1 Introduction

Wildfire disturbance has received a great deal of attention because of its critical role in forest ecosystems of the Mediterranean Basin (Kara and Bolat 2009). In modern forestry, wildfires are considered undesirable, because fire destroys vegetation cover and reduces soil fertility due to erosion and nutrient losses (Fernández et al. 2007). Fires affect physical, chemical and biochemical soil properties, primarily by transferring heat into soil. High soil temperatures kill soil microbes, kill or damage plant roots and seeds, destroy soil organic matter (SOM), and alter soil nutrient and water status. The degree of soil heating during fire depends on a variety of variables, including fuel characteristics, fire intensity and residence time, soil properties, such as moisture content, soil textures and organic matter content, and litter layer properties, such as moisture content, depth. Many of the fire-induced changes in soil are linked to SOM changes (Certini 2005).

There is some confusion in the literature over the concepts of fire intensity and fire severity (Pausas et al. 2003). Fire intensity is the rate of energy or heat release per unit length of fire front, kW/m; it is related to flame length. The relative amount of damage attributed to an area of vegetation is known as fire severity, which is most frequently described in discrete classes ranging from low to high or extreme (Hammill, Bradstock and van Wagendonk 2001; Pausas et al. 2003; Chafer 2008). Fire severity is thus the effect of fire on an ecosystem, as well as on the amount and location of organic matter consumed during a fire (Ryan and Noste 1985). Fire intensity and fire severity may or may not be related (Pausas et al. 2003). Knowledge on fire severity and intensity can be used by land managers to better predict the susceptibility of burned areas to post-fire soil erosion and its implications affecting water quality, forest regeneration, etc. (Chafer 2008; Pausas et al. 2003). Wildfires often appear as a chaotic mosaic of low affected areas alternating with severely affected areas (Rab 1996).

The human impact has resulted in the depletion of SOM of most semi-arid soils. Quantity and quality of SOM is vital not only for plant growth but also for the development of native microbial populations. Microorganisms have a fundamental role in the biogeochemical cycles and in the formation of soil structure, some works have indicated that a high level of microbial activity is needed to maintain adequate soil quality (Bastida et al. 2007; Llorente and Turrión 2010). It has been shown that different soil management regimes affect the structure and activity of the soil microbial community, via changes in the quantity and quality of the plant remains that enter the soil (Christensen 1996). However, the use of a single parameter to study the effect of soil disturbance on microbial populations and their activity is not valid because the spatial heterogeneity of soil, as well as the complex dynamics of the soil ecosystem, can affect different parameters in differing ways (García et al. 2005; Gil-Sotres et al. 2005). Many authors have proposed the combined use of a number of parameters as early indicators of stress or soil restoration (Dick et al. 1996; Gil-Sotres et al. 2005). Among the parameters related to biochemical and microbiological soil state, the indicators of soil

microbial activity have a great usefulness; the main indicators of such action are enzymatic activities, respiration, different C fractions in the soil, those related to the size of microbial populations (microbial biomass C, N and P), and those related to the availability of labile sources of C. These parameters can provide information on the microbiological quality of a soil (Bastida et al. 2008).

Soil studies in semi-arid areas have focused on the effects of a single fire, dealing with short-term nutrient dynamics in mineral soils (Carreira et al. 1997; Romanyà et al. 2001; Turrión et al. 2010), soil erosional responses, especially after the occurrence of rainfall events (Andreu et al. 2001; Pardini et al. 2004). Few studies have focused on the short-term effects of fire on semi-arid forest systems from the microbiological and biochemical point of view (Dumontet et al. 1996; Hernández et al. 1997; Hamman et al. 2007), and they have showed a depletion of soil microbial populations. An understanding of wildfire impact is needed to effectively manage forest ecosystems, including post-fire management decisions regarding seeding options, erosion control, and other managements. In this study, fire severity was classified according to the degree of canopy consumption after a crown fire. We hypothesized that soil properties could be related to fire severity (as defined above) and that the microbial activity will be depleted.

The objectives of the study were to evaluate the short-term effect of wildfires on some physical, chemical and microbiological characteristics of a forest soil under semi-arid climate, and to relate fire severity with these effects.

2 Materials and Methods

2.1 Study Site and Soil Sampling

The study sites were located near the city of *Valladolid* in Central-Northern Spain. Two burned forest areas (*Monte Arenas* and *Monte Llanillos*) and their corresponding adjacent unburned areas (Control) located in the southeastern section of Valladolid Province (Spain) at the villages of *Portillo*, *Aldeamayor de San Martín* and *La Parrilla* were selected. The altitudes were 766 m.a.s.l. in *Monte Arenas* and 842 m.a.s.l. in *Monte Llanillos*.

The soils were Albic Arenosols, characterized by high contents of sands, low C and N contents, low fertility, and absence of carbonates. Soil horizon properties of a typical profile of the Area are shown in the Table 1.

The vegetation in both plots was mixed forest plantation of *Pinus pinea* L. and *Pinus pinaster* Aiton. The climate of this region was semi-arid, characterized by dry warm summers. Based on climatological data from the past 30 years, mean annual temperature was 12.3°C and mean annual rainfall was 444 mm. The mean annual temperatures of the summer and autumn months were 21°C and 12.4°C, respectively. The mean summer precipitation was 63 mm and the mean autumn precipitation was 131 mm.

Table 1 Characteristics of the studied soils

Hor	Depth (cm)	pH H ₂ O	E.C. ^a (μS/cm)	SOC ^b (g kg ⁻¹)	Total N (g kg ⁻¹)	C/N	P _{0lsen} (mg kg ⁻¹)	CEC ^c (cmol _c kg ⁻¹)	Sand		Silt (%)	Clay (%)
									Coarse (%)	Fine (%)		
A1	0–10	6.0	34.4	6.4	0.44	14.5	2.37	4.75	68.9	24.2	2.5	4.4
A2	10–20	6.5	27.3	2.15	0.42	5.1	1.45	3.75	31.0	60.2	3.7	5.1
C	20–55	6.9	22.4	1.60	0.39	4.2	1.05	4.00	58.4	33.6	4.5	4.5

^a Electrical Conductivity^b Soil Organic C^c Cation Exchange Capacity

Coarse Sand (0.50–2 mm); Fine Sand (0.05–0.50 mm); Silt (0.05–0.002 mm); Clay (<0.002 mm)

Table 2 Fire severity classes judged from pine canopy damage; description and post-fire mortality of the adult pines (Pausas et al. 2003)

Fire severity classes	Description	Post-fire mortality
LOW	Light fire; canopy trees retain >20% of green leaves (top of the canopy) Trees remain mainly green after the fire.	No
MODERATE	Most leaves (>80%) of canopy trees are scorched (dead) but not consumed. Green leaves may occur on the top (<5%). Trees are mainly brown (retained scorched leaves) after the fire.	Yes
HIGH	Severe fire; canopy trees with >80% of the leaves consumed and the rest (if any) scorched (top). No green leaves left.	Yes

A canopy fire occurred in these areas on June 19th, 2004. Both fires were caused by lightning storms, affecting 554.5 ha of forest in *Monte Arenas* and 155.4 ha of forest in *Monte Llanillos*. It lasted for less than a day. Measurements of fire intensity were not available because of the accidental nature of the fire. Areas little affected by fire alternating with areas seriously impacted could be observed, indicating different fire severity levels.

In the present study, we classified fire severity following the indications of Pausas et al. (2003), who provided a threefold classification of fire severity according to pine canopy damage: low, moderate and high (Table 2).

Three fire severity classes were differentiated in the *Monte Arenas* burned area (low, moderate and high). In the *Monte Llanillos* burned area only two fire severity classes were found (low and moderate). Only in the high fire severity class, the organic litter layer was consumed.

Soil samples were taken on November 2004, at 0–2 cm depth, after elimination of ashes and litter remains in disturbed soil samples. The precipitation between the dates of fire and the sampling was 99.0 mm. Fifteen sampling points were randomly selected along each experimental area in the burned area and fifteen in the adjacent unburned one. Every sample was made up of 10 sub-samples taken randomly around each sampling point to obtain a composite sample. In Table 3 it can be seen the data of composite samples analyzed.

Table 3 Physical and chemical properties of burned and unburned soils

	Fire severity	n ¹	WHC ² (%)	Porosity (%)	pH	P _{Olsen}	SOC (g kg ⁻¹ soil)	N (g kg ⁻¹ soil)	C _{W-B} ³ (mg kg ⁻¹ soil)	C _{labile} (mg kg ⁻¹ soil)	SOC/ N	C _{w-B} / SOC	C _{labile} / SOC (%)
Monte Arenas	Control	15	8.8 ^a	46.4 ^a	6.58 ^b	3.1 ^d	17.4 ^b	0.65 ^b	1.0 ^b	78.6 ^b	26.8 ^a	0.64 ^a	4.5 ^a
	L ⁴	5	9.5 ^a	48.2 ^a	7.82 ^a	19.0 ^c	16.3 ^b	0.54 ^b	1.1 ^b	84.8 ^b	29.4 ^a	0.66 ^a	5.0 ^a
	M ⁵	5	11.2 ^a	46.1 ^a	7.66 ^a	25.0 ^b	22.3 ^b	0.78 ^b	1.4 ^b	126.5 ^{ab}	26.6 ^a	0.64 ^a	5.7 ^a
Monte Llanillos	H ⁶	5	10.5 ^a	40.7 ^a	7.94 ^a	41.1 ^a	35.8 ^a	1.94 ^a	2.2 ^a	187.0 ^a	18.9 ^b	0.62 ^a	5.2 ^a
	Control	15	8.0 ^a	49.5 ^a	6.15 ^b	4.6 ^c	18.7 ^a	0.56 ^a	0.9 ^b	63.7 ^b	29.7 ^a	0.52 ^a	3.5 ^a
	L	7	9.3 ^a	50.9 ^a	7.96 ^a	18.9 ^b	15.4 ^a	0.55 ^a	0.8 ^b	103.4 ^a	31.0 ^a	0.54 ^a	5.3 ^a
	M	8	10.8 ^a	47.7 ^a	7.75 ^a	28.2 ^a	20.1 ^a	0.67 ^a	1.2 ^a	110.8 ^a	29.5 ^a	0.57 ^a	7.8 ^a

¹ Number of composite soil samples² Water holding capacity³ Easily oxidizable C determined by the Walkley and Black procedure⁴ Low fire severity⁵ Moderate fire severity⁶ High fire severityValues followed by the same letter are not significantly different ($P < 0.05$) within the same zone

2.2 Physical and Chemical Soil Characteristics

Water holding capacity (WHC) was determined gravimetrically. Soil porosity was calculated with bulk density (Blake 1965a) and the soil particle density was determined by the pycnometer method (Blake 1965b). Soil pH was determined in a 1:2.5 (w/v) soil:water suspension, easily oxidizable carbon (C_{W-B}) by the Walkley–Black method, and available P (P_{Olsen}) by the Olsen method. Labile organic C (C_{labile}) was extracted by 0.5 M K_2SO_4 . The C content in the extracts was estimated using a SKALAR FormacsHT Analyzer. Soil organic carbon (SOC) and total nitrogen were determined with a LECO CHN-2000 analyzer.

2.3 Microbiological Soil Characteristics

Microbial biomass C (MBC) and microbial biomass P (MBP) were determined by the chloroform fumigation-extraction method, using 0.5 M K_2SO_4 as the extractant for MBC (Vance et al. 1987) and 0.5 M $NaHCO_3$ for MBP (Brookes et al. 1982). C contents in the fumigated and non-fumigated extracts were determined and P contents were determined by colorimetry (Murphy and Riley 1962). Carbon concentration in the fumigated sample extracted with 0.5 M K_2SO_4 was considered as labile C (C_{labile} ; Haynes 1999).

In order to measure the soil potential microbial respiration (C_{min}), fifty grams of moist soil sample (at 55% of water holding capacity) were placed in 500 ml stoppered glass jars and incubated at 28°C. The CO_2 evolved was collected, after 3 days of incubation, in 10 ml 0.5 M NaOH and determined by back-titration with 0.5 M HCl, after carbonate precipitation with $BaCl_2$ (Alef 1995).

The activity of microorganisms was expressed as metabolic quotient (qCO_2 : $mg\ CO_2-C\ mg^{-1}\ MBC\ d^{-1}$) which represents the microbial respiration per unit of biomass, and was calculated as reported by Anderson and Domsch (1993). The microbial quotient (MBC/SOC) represented the percentage of microbial biomass carbon with respect to the total organic carbon of soil (Anderson and Domsch 1993). The rate of organic carbon mineralization (C_{min}/SOC) represented the coefficient of endogenous mineralisation (Fierro et al. 2007) and it was expressed as $mg\ CO_2-C\ g^{-1}\ SOC\ h^{-1}$. As the available P concentrations in soil were low (Table 2), we considered interesting to know the enzymatic activities related to the P cycle, and acid and alkaline phosphatase activities were measured (Tabatabai and Bremner 1969).

2.4 Statistical Analyses

Normality and homocedasticity of the residuals were tested using the Kolmogorov–Smirnov and Levene tests, respectively. Since most of the variables did not satisfy these assumptions, and the transform of the variables using logarithm and square root

did not resemble a normal distribution, the non-parametric Kruskal-Wallis test was applied. When the Kruskal-Wallis null hypothesis was rejected, post-hoc pair wise comparisons were performed to investigate differences between pairs of means. All statistical analyses were performed using the Statistica 7.0 software package.

3 Results

3.1 Physical and Chemical Soil Characteristics

Physical and chemical characteristics of the soils studied are presented in Table 3. The WHC and porosity values were low and not significant differences were observed due to fire effect. The soil of unburned sites had low organic matter content, with organic C and total N concentrations of 18 and 0.6 g kg⁻¹ of soil, respectively. The mean value of P_{Olsen} in unburned soils was also low (3.8 mg P kg⁻¹). The low content of organic matter and mineral nutrients was also reflected in the C_{W-B} and labile C amounts.

The fires did not cause enough soil heating to produce significant changes to the soil physical properties studied. The pH in burned soils was significantly higher than in the control; however, no significant differences were observed in pH among fire severity levels. Olsen P concentrations increased significantly with the increase in fire severity. The concentrations of SOC, N, C_{W-B} and C_{labile} were significantly higher in high fire severity burned soils than in unburned soils. In high severity burned soil, SOC concentration was twice that of the control. The ratios of these parameters (N, C_{W-B} and C_{labile}) referred to SOC were less sensitive to the fire effect than the individual properties.

3.2 Microbiological Soil Characteristics

Microbiological characteristics of the studied soils are presented in Table 4. C_{min}, MBC and MBP, were significantly lower in unburned than in burned soils. Acid phosphatase showed significantly higher values in control than in burned soils. The mean MBC was 108 mg C kg⁻¹ in the unburned soils and 206 mg C kg⁻¹ in the burned soils. Only MBP showed significant differences among the fire severity levels in both studied areas. The higher the fire severity, the higher the BMP concentrations were obtained. The alkaline phosphatase concentrations were not significantly different either between burned and unburned soils, or among fire severity levels.

Values of the MBC/SOC and C_{min}/SOC corresponding to unburned soils were low; the highest values corresponded to low fire severity soils, while severely burned and control soils presented similar values for these ratios. Microbial biomass was enriched in P, MBC to MBP ratios being two times lower in soils

Table 4 Microbiological properties of burned and unburned soils

Fire severity	MBC ¹	MBP ²	C _{min} ³	Phos. ⁴ acid	Phos. ⁴ alk.	MBC/SOC ¹ (%)	MBC/MBP ¹	C _{min} /SOC	γCO ₂ ⁵	Phos.			
										acid/MBP	Phos. alk		
Monte	Control	118.6 ^b	23.5 ^d	36.02 ^b	0.861 ^a	0.490 ^a	0.66 ^c	5.1 ^b	2.18 ^{ab}	0.015 ^a	7.28 ^a	36.86 ^a	6.34 ^a
Arenas	L ⁶	229.8 ^a	30.9 ^c	49.76 ^{ab}	0.214 ^b	0.415 ^a	1.40 ^a	7.4 ^a	3.02 ^a	0.009 ^a	0.94 ^b	6.97 ^b	0.52 ^b
	M ⁷	229.6 ^a	73.0 ^b	61.56 ^a	0.215 ^b	0.339 ^a	1.07 ^b	3.1 ^c	2.92 ^a	0.010 ^a	0.94 ^b	2.95 ^b	0.63 ^b
	H ⁸	227.6 ^a	107 ^a	69.98 ^a	0.104 ^b	0.382 ^a	0.64 ^c	2.1 ^c	1.96 ^b	0.014 ^a	0.45 ^b	0.98 ^b	0.27 ^b
Monte	Control	97.0 ^b	20.9 ^c	30.32 ^b	0.749 ^a	0.267 ^a	0.64 ^b	4.6 ^a	1.76 ^b	0.011 ^a	7.56 ^a	35.37 ^a	2.74 ^a
	Llamillos L	171.4 ^a	46.7 ^b	65.96 ^a	0.418 ^b	0.553 ^a	1.22 ^a	3.7 ^{ab}	3.92 ^a	0.014 ^a	2.45 ^b	8.96 ^b	0.37 ^b
	M	172.8 ^a	55.0 ^a	60.21 ^a	0.170 ^b	0.458 ^a	0.88 ^b	3.1 ^b	3.19 ^a	0.016 ^a	0.99 ^b	3.09 ^b	0.76 ^b

¹ Microbial biomass C in mg kg⁻¹ soil; MBC/SOC and MBC/MBP in %.

² Microbial biomass P in mg kg⁻¹ soil

³ C mineralizable in mg CO₂-C kg⁻¹ soil d⁻¹; C_{min}/SOC in mg CO₂-C g⁻¹ SOC d⁻¹

⁴ Phosphatase acid or alkaline in μmol pNP g⁻¹ soil h⁻¹; Phos. acid/MBC in μmol pNP g⁻¹ MBC h⁻¹; Phos. acid/MBP in μmol pNP g⁻¹ MBP h⁻¹

⁵ Metabolic quotient in mg CO₂-C mg⁻¹ MBC h⁻¹

⁶ Low fire severity

⁷ Moderate fire severity

⁸ High fire severity

Values followed by the same letter are not significantly different ($P < 0.05$) within the same zone

affected by high severity fire than in control soils. A significant decrease in acid phosphatase to MBC and to MBP ratios was observed after fire occurrence. These ratios were not significantly different among fire severity levels. The metabolic quotient $q\text{CO}_2$ did not show significant differences either between burned and unburned soils, or among fire severity classes. There were significant differences in the acid phosphatase to alkaline phosphatase ratio between unburned and burned soils, but not among fire severity levels.

4 Discussion

The WHC and porosity values of the control are commonly found in sandy soils (Marshall and Holmes 1988), and fire did not affect them.

The effect of fire on SOM amount is highly variable, and depends on several factors, including fire type (canopy or aboveground *versus* underground fires), intensity, slope. These effects may range from almost the total destruction of the SOM to increases that may reach 30% in the surface layers as a consequence of external inputs, mainly from dry leaves and incompletely burned plant materials (González-Pérez et al. 2004). Generally, low severity fires increase total N and SOC concentrations (Fierro et al. 2007; Kara and Bolat 2009; Gray and Dighton 2009) and a decrease of SOC and N is observed after highly severe wildfire (González-Pérez et al. 2004). Our results showed no significant differences in total N and SOC between unburned and low or moderate affected soils and only the areas affected with high severity fire showed significantly higher concentrations than the unburned soils. This fact could indicate that the wildfires studied did not reach high temperatures, although the classification based on the visual inspection of the remnant vegetation allowed differentiating among low, moderately and highly impacted areas. Besides the external inputs of SOM from plant necromass, it is also necessary to consider that litter turns, after fire, into particulate (fine-earth sized particles), which mix with the whole soil material in the organic horizon. This, in turn, causes a net increase in the C content, friable charred organic matter and particulate charcoal (González-Pérez et al. 2004).

Klopatek et al. (1991) stated that one of the greatest initial effects of fire is the reduction of the SOC/N ratio due to the increased mineralization process; however, our results do not support such a statement for low and moderate severity fire level.

Significant increases in easily oxidisable and labile C due to fire effect were observed. Pardini et al. (2004) found that fire tends to affect short-term organic carbon content by converting inaccessible organic forms into usable labile forms via combustion of the litter layer. Fierro et al. (2007) pointed out that fire can supply easily decomposable organic matter in the short time both due to incorporation of partially burned aboveground biomass and to drying and killing of belowground and microbial biomass, being less probable the second if only low soil temperature values have been reached during the fire.

Significant higher pH were found in the burned samples than in the control probably due to the so-called “ash bed effect” of fire on acidic soils (Certini 2005). The soils studied had low available P, and the P added in ash due to the fire increased the availability of P for plants and microorganisms. Phosphorus availability was probably increased not only due to incorporation of nutrients to the soil as ash, but also to increased P mineralization due to higher soil pH (Romanyà et al. 1994). Several authors have reported an increase of soil mineral nutrients following fires (DeBano 1991; Klopatek et al. 1991). Such an increase was explained by an enrichment of soil by ash, which would represent a reservoir for mineral N, P, Ca, K, and Mg (Dumontet et al. 1996). Saá et al. (1993) reported a meaningful 5-fold increase in inorganic soluble P in the 0–5 cm layer of a humic cambisol under *Pinus pinaster* and *Ulex europaeus* one month after wildfire, as compared with the unburned sites. In our study, the P_{Olsen} and pH surface layer increases could be explained by an enrichment of soil by ash and an absence of lixiviation due to the semi-arid climate, characterized by relatively low annual precipitation (less than 450 mm), summer drought and mesic temperature. After fire events, mineral nutrients may temporarily remain in the ash layer, but eventually they may be solubilised, brought downward through the soil profile, and utilized by the growing plants and soil microorganisms (Belillas and Rodá 1993). Although leaching of mineral nutrients could be expected from the pedological characteristics of the soils studied, such leaching was limited in time and intensity by the semi-arid climate and by the low rainfall on the area from fire event to sampling date.

The control MBC and MBP values found in the present study are very low as compared to the values reported by other authors for forest soils. They are even lower than the values found by Dumontet et al. (1996), who obtained a mean of 176 mg C kg⁻¹ soil for MBC in unburned soil from a dunal Mediterranean environment.

Biological and biochemical parameters are sensitive to the slight modifications that soil can undergo in the presence of any degrading agent (Nannipieri et al. 1990). Microbial biomass C represents the living component of the organic matter of soil, excluding animals and plant roots. Although microbial biomass usually makes up less than 5% of SOM (Dalal 1998), it carries out many critical functions in the soil ecosystems, among which the following could be pointed out: microbial biomass is both a source and sink for nutrients, it participates in the C, N, P and S transformations, it plays an active role in the degradation of xenobiotic organic compounds and in the immobilization of heavy metals, it participates in the formation of soil structure, etc. (Nannipieri et al. 2002). The MBC/SOC has been proposed as a more sensitive index of soil changes than total organic C (Bastida et al. 2008), since the microbial biomass of a soil responds more quickly to changes than organic matter does. In the soils studied, MBC only represented around 0.65% of SOC for unburned soils and around 1% for burned soils. Wildfire is reported to have variable effects of forest soil microbial biomass. The hypothesis that fire depleted the microbial activity was not supported by the data. The N and P_{Olsen} enrichment and the increase in soil pH observed in the surface layer may be

responsible for the stimulation of microbial biomass growth, resulting in an increase in MBC and MBP concentrations and C mineralization (C_{\min}). The effects of fire on soil microbial biomass depend on the intensity and duration of the fire; such effects can range from complete sterilization to little or no effect (Kara and Bolat 2009) and can also be increased, so Fierro et al. (2007) have indicated that the few data concerning Mediterranean-type ecosystems provide evidence of an increase of soil microbial activity and thus mineralization after fire. Microorganisms have a fundamental role in the biogeochemical cycles and in the formation of soil structure, some works have indicated that a high level of microbial activity is needed to maintain adequate soil quality (Bastida et al. 2007; Llorente and Turrión 2010). However, it is not always in this way, a clear example of the opposite situation is the effect of wildfire, which can increase microbial activity and not improve soil quality as can be observed in the present study.

The hypothesis that soil properties could be related to fire severity was supported by the data in the case of chemical properties. The soil in the burned areas was nutrient enriched and this enrichment was higher when high fire severity was reached and no significant differences in MBC and C_{\min} were observed with the fire severity, we can conclude that the three fire severity levels studied had a similar short-term effect on microbial parameters.

The lowest values of the MBC/SOC and C_{\min} /SOC corresponded to high fire severity. The increase of MBC and C_{\min} due to fire, but not influence of the fire severity level, and the SOC increase with the fire severity due to a higher addition of organic materials could explain this pattern.

The qCO_2 values found in the soils studied are high in comparison with the data reported by other authors (Wardle and Ghani 1995), indicating low microbial efficiency. The qCO_2 values were not affected by the fire-produced disturbance. In the soils studied, qCO_2 was not a sensitive indicator of short-term fire effect and, as Sojka and Upchurch (1999) point out, the use of one or two biochemical properties is not sufficient to demonstrate the complexity of the functioning of this soil system. In general, the qCO_2 value is greater in a distorted ecosystem than in a stable ecosystem (Dalal 1998). However, the significance of this increase is unclear, because it may be due to a drop in efficiency of substrate utilization by the microbiota, to a microbiota response to adverse conditions, to the predominance of zymogene microorganisms over the autochthonous microorganisms, or to the alteration of the bacteria/fungi ratio as they have different carbon use strategies (Dilly and Munch 1998).

Fire often results in effects on organic matter and microbial community structure that can be detected by quantifying enzyme activity. For example, acid phosphatase activity, as an indicator of overall microbial activity, often decreases as a consequence of fire (Saá et al. 1993; Eivazi and Bryan 1996; Boerner et al. 2000). Our results corroborate this affirmation. In the unburned soils studied, acid phosphatase activity was higher than alkaline phosphatase activity; however, both concentrations were similar in burned soils.

Our results showed that acid phosphatase to MBC and to MBP ratios were indexes very sensitive to fire effect, but were not sensitive to fire severity. These ratios decreased significantly with the fire. Landi et al. (2000) indicated that the

ratio between activity parameters and microbial biomass represents a single combination of two different measurements in a single criterion that can give some indications of changes occurring in microbiological activity and ecological information on specific aspects of microbial activity. An increasing ratio may indicate either increasing enzyme production and enzyme release by microorganisms or enhanced release of enzymes immobilized on clay or humic colloids to the soil solution (Kandeler and Eder 1993). On the other hand, rising pH and P content has found to decrease acid phosphatase activity (Haynes and Swift 1988).

5 Conclusions

Although the classification based on the visual inspection of the remnant vegetation after fire allowed differentiating three fire severity levels (low, moderate and high), however the results showed that in all cases the reached temperature was low.

Our results showed that low-impact burning in very poor soils can cause short-term increases in the availability of plant and microbial nutrients, and these mineral mobilizations increased microbial activity, although the soil quality was not improved.

The labile carbon fractions (C_{labile} and $C_{\text{W-B}}$) were more sensitive to fire severity than SOC. P_{Olsen} and MBP were the most sensitive of the studied parameters to the effects of fire and fire severity.

The metabolic quotient did not appear as an adequate indicator of microbial response to fire disturbance.

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