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▶ **To cite this version:**

Assunta Palese, Giacomo Giovannini, Sergio Lucchesi, Stefano Dumontet, Piero Perucci. Effect of fire on soil C, N and microbial biomass. Agronomie, 2004, 24 (1), pp.47-53. 10.1051/agro:2003061 . hal-00886242

HAL Id: hal-00886242
https://hal.science/hal-00886242

Submitted on 11 May 2020

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Agronomie 24 (2004) 47–53
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DOI: 10.1051/agro:2003061

Original article

Effect of fire on soil C, N and microbial biomass

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(Received 12 May 2003; accepted 1st December 2003)

Abstract – Fire is recognised as a potent ecological factor in woody and bushy environments. In this investigation the effect of experimental fire on soluble organic C and N, soil microbial biomass C and N, the bacteria/fungi ratio and soil enzymatic activities were measured 12 and 18 months after fire in a Lithic Xerocrept soil. The soil soluble C was changed both by fire and sampling time in burned soils, whereas soluble N did not show any clear trend. The enzymatic activities were decreased by fire, but a peak of activity was recorded in soil burned at 309 °C, which corresponded with the highest recorded soluble C. A quantitative reduction in soil microbial biomass C and N was observed.

experimental fire / Lithic Xerocrept soil / Mediterranean environment / soil microbial biomass

Résumé – **Effet des feux sur le C, N et sur la biomasse microbienne du sol.** Le feu est un facteur écologique notable dans les environnements arbustifs et boisés. Dans cet article nous avons étudié les effets d'un feu expérimental. En particulier, nous avons étudié le carbone et l'azote soluble, le carbone et l'azote de la biomasse microbienne, le rapport champignons/bactéries et les activités enzymatiques 12 et 18 mois après le feu dans un sol classé Lithic Xerocrept. Le carbone soluble a été modifié à la fois par le feu et par la période de prélèvement. L'azote soluble n'a pas été influencé de manière significative. Bien que les activités enzymatiques aient été diminuées par le feu, un pic d'activité a été noté pour le sol brûlé à 309 °C correspondant au plus haut niveau de carbone soluble. Une réduction du carbone et de l'azote de la biomasse microbienne a été observée.

feu experimental / sol Lithic Xerocrept / milieu mediterranéen / sol biomasse microbienne

1. INTRODUCTION

Fire is an important ecological factor in the development of forests and numerous studies have been devoted to the understanding of its effects on forest regeneration under different climates [33, 38]. Nevertheless, wildfires, which each year affect approximately 600 000 ha of bushy and woody environments in the Mediterranean basin, can pose a severe threat to the environmental equilibria of the region by impairing the relationships between the physical, chemical and biological components of the soil ecosystem.

Most wildfires are of an anthropogenic nature and affect an impressive extension of soil surfaces, threatening soil and vegetation resources in the area. Degradation of soil, which takes place after fire events, is driven by the deterioration of the soil structure [4, 16, 21], the loss of soil organic matter content [9, 12, 20, 34, 40] and the loss of soil mineral nutrients [10, 13, 14, 26]. Soil microbial biomass is also affected through size reduction, impoverishment of speciographic spectra and reduction in catalytic capabilities [1, 18, 25].

Several management practices consider the voluntary use of fire in order to reduce the litter and dry wood which can fuel forest fire [3, 14, 20, 40], to combat forest soil acidification by adding wood-ash to soil [2] and to ash-fertilise agricultural soil by burning crop residues [22, 30]. All these practices are currently utilised worldwide and they all have, to a different extent, a marked effect on the soil's physical, chemical and microbiological characteristics. An extended scientific literature is available on the effects of fire on soil properties; however, knowledge of its long-term consequences is still scarce.

The objective of this paper was to investigate the effects of experimental fires on some microbiological parameters of a Lithic Xerocrept soil, located in the central part of Italy (Tuscany), 12 and 18 months after a fire. The effect of the fire on the physical and chemical parameters of the same soil was reported by Giovannini and Lucchesi [23] and Giovannini et al. [24].

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2. MATERIALS AND METHODS

2.1. Site description, experimental design and soil sampling

The experimental area is located in Tuscany, Central Italy (43° 26’ N, 10° 31’ E), at an elevation of 90 m above sea level. The climate is typically Mediterranean and the mean annual precipitation is 700 mm. The studied soil has been described by Giovannini and Lucchesi [23]. Briefly, it was a Lithic Xerocrept constituting clay sediments with large quantities of round stones, primarily peridotites and basalt, limestone and jasper. The main soil characteristics in the 0–2.5 cm layer were: sand 330 g kg^{-1}, silt 330 g kg^{-1}, clay 340 g kg^{-1}, pH 7.7, C.E.C. 445.0 mmol (+) kg^{-1}, organic matter 260 g kg^{-1} and tota1 N 3.2 g kg^{-1} [27].

The studied area was covered by a typical Mediterranean vegetation mainly constituting a natural evergreen vegetation (*Pistacia lentiscus* L., *Myrtus communis* L., *Ligustrum vulgare* L., etc.), some herbaceous species (*Brachypodium pinnatum* P.B., *Onobrychis viciaefolia* Scop., *Teucrium* spp., etc.) and woody deciduous species (*Quercus pubescens* Willd., *Ulmus minor* Mill., *Prunus spinosa* L. and *Crataegus monogyna* L.).

The trial was carried out on 4 plots (4 × 20 m) [23]. The plots were separated from each other by fireproof tracks and individually fenced. Except the control plot, which was left undisturbed, the other plots were burned using natural standing vegetation or dry woody residues in order to obtain different fire intensities and different temperatures. Every plot, except the control, was equipped with thermocouples placed at the surface and at 2.5 cm and 5.0 cm below the soil surface to record the temperature during the fire.

The burning experiments were performed at the end of March in the absence of wind. The atmospheric pressure was 101.9 kPa; the air temperature was 20 °C and the relative humidity of the air was 80%. Triplicate soil samples were collected in the year following the experimental fire, in the months of April (in May only for the enzymatic analysis), from the 0–2.5 cm layer in the control plot and in burned plots near thermocouples recording temperatures of 102, 184, 210, 309, 395 457 and 558 °C on the soil surface. Plant and root residues were carefully removed from the soil by hand before sampling.

2.2. Microbiological and enzymatic analysis

After sampling, soil was immediately sieved at 2 mm and stored at 4 °C in plastic bags closed by a cotton wool cap, and microbiological analysis was carried out within 10 days. Before performing microbiological analysis the soil was brought to 60% of its water-holding capacity (WHC) and then equilibrated for 5 days at 22 °C [17]. Microbial biomass C (MBC) and N (MBN) were measured by the chloroform fumigation-extraction method (FE) according to Sparling and West [36], and soluble organic carbon and nitrogen were measured in the filtrate by the dichromate oxidation method [36] and by alkaline persulphate oxidation [8]. Acid and alkaline phosphatase, β-glucosidase and arilsulfatase activities were measured according to Page et al. [29]. Counts of bacteria and fungi were carried out according to Wollum [44].

Table I. Exposure time (s) of the top-soil (0 and 2.5 cm) to temperatures ≥ 60 °C.

T (°C)	Soil layer (cm)	Exposure time (s)
102	0	420
	2.5	0
184	0	780
	2.5	0
210	0	390
	2.5	0
309	0	1080
	2.5	0
395	0	1410
	2.5	30
457	0	1380
	2.5	1020
558	0	480
	2.5	150

2.3. Statistical analysis

All the analysis were carried out in triplicate. Means were classified using the Tukey’s multiple range test. The SYSTAT statistical program (SYSTAT Inc. Version 5.03, 1991) was used to perform the statistical analysis on the data.

3. RESULTS

3.1. Physical parameters related to fires of different intensity

Different fire temperatures and intensities in the burned plots were obtained because of the different quantities of fuel in the different plots. Temperatures ranging from 102 °C to 558 °C were recorded.

In Table I the values of soil temperatures during fire are reported. In addition, the time in seconds during which soils were exposed to a temperature ≥ 60 °C is also indicated. This parameter represents the time during which the soil and microbial populations were exposed to a temperature higher than that of pasteurisation (60 °C). It is worth noting that heat transferred in soil is related to fire intensity much better than to fire temperature [40].

3.2. Chemical, microbiological and enzymatic characteristics of control and burned soils

The pH value of the soil was 7.7 before the fire [23]. One week after the fire, the pH of the burned soil ranged from 6.7 to 7.9. The highest pH values were recorded in plots in which the temperature reached 457 and 558 °C. Twelve and 18 months

Table II. Soluble C and N content in unburned (control) and burned soils (0–2.5 cm layer).

Months after fire	Depth (cm)	T (°C)	Soluble C (mg C kg^{-1} d.w.)	Soluble C as % of organic C %	Soluble N $N\text{-}NH_4^+ + N\text{-}NO_3^-$ (mg N kg^{-1} d.w.)	Org-N
12	0–2.5	control	$288^{ef}(\pm5)$	0.20	$43^{f}(\pm1)$	$49^{f}(\pm5)$
18	0–2.5	control	$72^{a}(\pm2)$	0.05	$29^{cde}(\pm2)$	$6^{a}(\pm1)$
12	0–2.5	102	$229^{d}(\pm15)$	0.17	$24^{b}(\pm1)$	$18^{bc}(\pm1)$
18	0–2.5	102	$66^{a}(\pm2)$	0.05	$24^{bc}(\pm2)$	$13^{ab}(\pm1)$
12	0–2.5	184	$179^{c}(\pm7)$	0.13	$60^{i}(\pm1)$	$49^{f}(\pm6)$
18	0–2.5	184	$145^{b}(\pm6)$	0.1	$8^{a}(\pm1)$	$24^{bcd}(\pm4)$
12	0–2.5	210	$179^{c}(\pm9)$	0.14	$25^{bcd}(\pm1)$	$40^{ef}(\pm1)$
18	0–2.5	210	$132^{b}(\pm2)$	0.15	$30^{de}(\pm3)$	$21^{bc}(\pm7)$
12	0–2.5	309	$306^{f}(\pm8)$	0.38	$49^{g}(\pm2)$	$42^{ef}(\pm3)$
18	0–2.5	309	$186^{c}(\pm12)$	0.14	$27^{bcd}(\pm2)$	$33^{de}(+4)$
12	0–2.5	395	$278^{e}(\pm4)$	0.37	$65^{i}(\pm1)$	$33^{de}(+3)$
18	0–2.5	395	$198^{c}(\pm11)$	0.25	$29^{cde}(\pm2)$	$16^{abc}(\pm1)$
12	0–2.5	457	$181^{c}(\pm2)$	0.22	$33^{e}(\pm3)$	$36^{e}(+8)$
18	0–2.5	457	$193^{c}(+5)$	0.22	$64^{i}(\pm1)$	$33^{de}(+2)$
12	0–2.5	558	$230^{d}(+12)$	0.26	$54^{h}(\pm3)$	$25^{cd}(+3)$
18	0–2.5	558	$224^{d}(\pm5)$	0.25	$29^{bcde}(\pm2)$	$21^{bc}(+1)$

Values are the mean of three samples, standard deviation is in brackets.
Values in the same column followed by the same letter are not different at $P < 0.05$ (Tukey's multiple range test).

after the fire the pH values ranged from 8.1 to 8.5 for all the burned soils [24].

The soluble C (extracted with K_2SO_4 0.5M) (Tab. II) seemed to be strongly influenced by sampling time. In samples collected 12 months after the fire, soluble C was nearly 4 times higher in the control plot and in plots in which the temperature reached 102 °C than in samples collected 18 months after the fire. The same trend was shown by soils exposed to temperatures ranging from 184 to 395 °C, even though the difference in soluble C content between the samples collected at 12 and 18 months after the fire was less pronounced. On the contrary, in soils in which temperatures reached 457 and 558 °C, the amount of soluble C did not show any statistically significant difference in samples taken at 12 and 18 months after the fire. In burned soils soluble C never reached values higher than the unburned plot, except for samples taken 12 months after the fire in soils in which the temperature reached 309 and 396 °C. These findings are not completely in agreement with those of Diaz-Raviña et al. [15], who found, after soil heating, 6 times more water-extractable organic C than in the control samples. Nevertheless, if soluble C is expressed as a % of organic C (Tab. II), an increase in soils submitted to the highest temperatures during the fire (309, 395, 457 and 558 °C) becomes evident, both in samples taken at 12 and 18 months after the fire. This result underlines an increase in easily decomposable C over total soil C, which was caused by the higher temperatures.

The organic N soil content extracted with K_2SO_4 0.5M (Tab. II) seemed to be affected by the sampling time and did not follow any clear trend in burned soil. The minimum amount of soluble organic N (6 mg kg^{-1} d.s.) was found 18 months after the fire in the control plot, while the highest value was recorded 12 months after the fire in the soil exposed to 184 °C (49 mg N kg^{-1} d.s.). The fire was responsible for the enrichment of the 0–2.5 cm soil layer in mineral N (NH_4^+ + NO_3^- extracted by K_2SO_4 0.5M). Such an enrichment was detectable only in some soils and not for both sampling dates. The mineral N soil content appears to be influenced both by fire and the season of sampling (Tab. II). The autumnal season was characterised by heavy rains, which were probably responsible for the leaching through the soil profile of the mineral salts produced by the fire and contributed to an irregular distribution of mineral N in the 0–2.5 cm layer of soil.

The increase in the labile fraction of soil organic C discussed above did not influence the microbial biomass C content of burned soil (Tab. III). The MBC of the control plot was quite high, ranging from 1830 mg C kg^{-1} dry soil 12 months after the fire to 1227 mg C kg^{-1} dry soil 18 months after the fire. These data are in agreement with the high organic C content of the studied soil (147 g kg^{-1} in the control plot). The MBC values recorded in all burned plots were always lower than those of the control, except for the plots heated at 558 °C, which showed, 18 months after the fire, a MBC value not statistically different from that of the control plot.

Table III. C and N microbial biomass and bacteria to fungi ratio in unburned (control) and burned soils (0–2.5 cm layer).

Months after fire	T (°C)	Biomass C mg C kg^{-1} d.w.	Biomass C as % of soil org C %	Biomass C/ Biomass N	Biomass N mg N kg^{-1} d.w.	Bacteria/Fungi
12	control	1830^{k}(±93)	1.24	6.6	276de(±21)	76
18	control	1227^{ij}(±36)	0.83	6.0	204^{bc}(±9)	n.d.
12	102	908^{def}(±1)	0.66	5.1	178^{ab}(±22)	36
18	102	769^{cde}(±23)	0.53	2.5	307^{def}(±2)	n.d.
12	184	550^{ab}(±22)	0.41	2.0	278de(±18)	335
18	184	438^{a}(±13)	0.33	3.2	138^{a}(±16)	n.d.
12	210	1304^{j}(±71)	1.00	3.8	342^{f}(±31)	80
18	210	1138^{hij}(±16)	0.81	3.9	293^{def}(±13)	n.d.
12	309	1085^{ghi}(±35)	1.34	3.7	296^{def}(±12)	381
18	309	753^{cd}(±8)	0.81	3.8	198^{b}(±7)	n.d.
12	395	883^{def}(±33)	1.16	5.3	167^{ab}(±4)	716
18	395	934^{efg}(±43)	1.18	2.9	325^{ef}(±18)	n.d.
12	457	670^{bc}(±11)	0.83	2.2	308^{def}(±40)	120
18	457	701^{c}(±20)	0.78	3.3	210^{bc}(±6)	n.d.
12	558	1032^{fgh}(±26)	1.19	4.1	254^{cd}(±19)	162
18	558	1238^{ij}(±55)	1.42	4.4	278de(±2)	n.d.

Values are the mean of three samples, standard deviation is in brackets; n.d. = not determined.
Values in the same column followed by the same letter are not different at $P < 0.05$ (Tukey's multiple range test).

In the control plot microbial biomass N was 276 mg N kg^{-1} dry soil and 204 mg N kg^{-1} dry soil 12 and 18 months after the fire, respectively (Tab. III). MBN did not seem to follow any clear trend regarding both temperature and exposure time at temperatures ≥ 60 °C (Fig. 1). A sharp reduction in the biomass C/N ratio (Tab. III) took place at all sampling dates and for all temperatures in burned soils. The C/N ratio decreased from 12 to 33% of control values.

It is also worth noting that 12 months after the fire the bacteria to fungi ratio (Tab. III) was higher in plots in which the highest pH values (8.4) were recorded. In comparison with the control, this ratio decreased in soil in which temperatures reached 102 °C, attained a level similar to the control in soil heated at 210 °C and dramatically increased in soils heated at 184, 309 and 395 °C. In soils which were heated at 457 and 558 °C this ratio decreased to a level of about 2 times higher than the control.

In Figure 2 the enzymatic activities in soil samples as modified by the fire are reported. There is a clear seasonal variation of measured enzymatic activity, as they were higher in May than in October in the control plot. The temperatures reached during the fire modified this trend. In May, acid phosphatase, alkaline phosphatase, β-glucosidase and arilsulphatase decreased strongly in samples heated at 102 °C, increased afterward and peaked in samples heated at 309 °C, which always showed values higher than the control, and lowered again at the highest temperatures. The peak in enzymatic activity recorded in May at 309 °C was not observed in October, when the values were more evenly distributed among different temperatures. Only the arilsuphatase showed the same trend in both sampling periods, although the activity was lower in October. It is worth noting that samples heated at 309 °C showed the maximum amount of soluble carbon (Tab. III).

4. DISCUSSION

The detrimental effect of fire on soil characteristics and microbial biomass is widely acknowledged. It is explained as a consequence of temperature, fire intensity and the modification of the physical and chemical soil environment [18]. The number of fire chemical and physical modifications which affect soil microbial biomass and enzyme activity can be identified in: (i) the reduction in soil organic matter content and the decrease in the labile fraction of organic C [19]; (ii) the higher conductivity due to the high content of ash-borne water-soluble inorganic ions [25], and (iii) the destruction or inactivation of hydrolytic enzymes [25]. All of these could account for the difficult recolonisation of burned soils, although they are heavily reinoculated by water, air and vegetal remains [41].

In this study, the bacteria/fungi ratio was higher than the control in soil heated at 184, 309, 395, 457 and 558 °C, indicating that bacterial decomposition was favoured over fungal decomposition. These findings are in agreement with Bisset and Parkinson [5], who pointed out that the taxonomic shift in

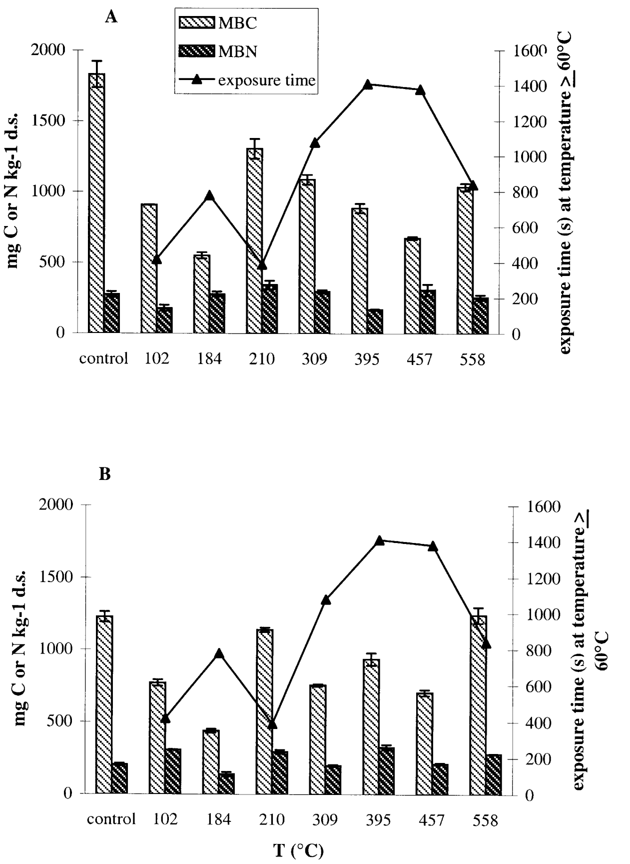

Figure 1. Microbial biomass C and N and top-soil exposition at temperatures 60 °C in unburned and burned samples. (A = 12 months after fire. B = 18 months after fire).

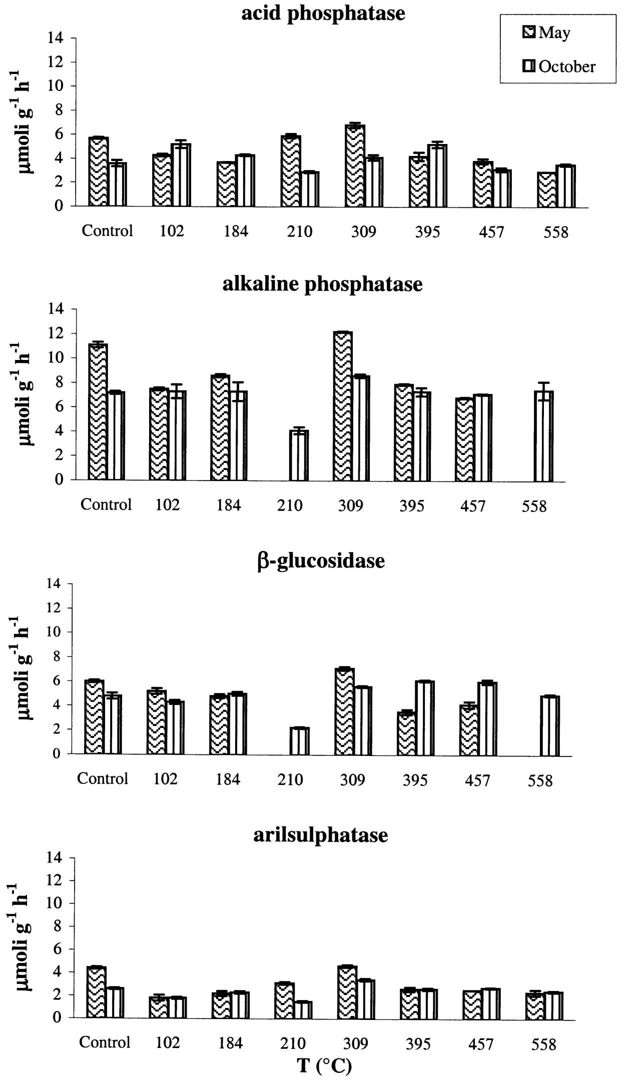

Figure 2. Enzymatic activities in unburned and burned samples. (Alkaline phosphatase and β-glucosidase were not recorded in May).

the composition of soil microbial biomass after fire leads to fundamental changes in soil energy pathways. Bååth et al. [2] used phospholipid fatty acids (PLFAs) analysis to detect the bacteria/fungi ratio in burned and wood-ash fertilised soils. They found that both treatments seriously affected the fungal biomass, but also demonstrated that pH was not the main factor in determining such a shift in the taxonomic composition of the microbial biomass. As our burned and unburned soils showed the same pH, even 12 months after the fire, the shift in the bacteria/fungi ratio could be explained by taking into account the hypothesis of Widden and Parkinson [43], who found a water-soluble substance in soil after a wildfire, which was toxic for fungi.

Theodorou and Bowen [37] found, after 4 weeks of a bushfire of moderate intensity, an increase in microbial numbers in the burned soil in comparison with the control. These authors explained such an increase by considering that pH rose from 4.6 to 5.8 and that the mild heat released easily-available energy sources from the soil organic matter. Bauhus et al. [3] found that fire could promote autotrophic bacteria over chemiotrophic bacteria because of the soil enrichment in mineral salts. These authors also found a higher bacteria/fungi ratio in burned soil caused by the rise in pH after fire.

Coles and Morrison [11] explained the decrease in pH after fire by pointing out that soil heating exposes new surfaces and dehydrates colloids, with the subsequent decrease in the soil buffer capacity. Inversely, when soil is exposed to temperatures higher than 700 °C the loss of OH^- groups from clay minerals and formation of oxides caused by the carbonate disruption can account for pH increase [22]. In soil rich in organic matter, fire can increase the pH by lowering the organic acid contents and producing hydroxides and carbonates added to the soil with ashes [39]. The liming effect of ashes in increasing soil pH seems to be of less importance until the rainfall leaches ash mineral constituents into the soil profile.

It is well known that the organic C content is dramatically reduced and its quality is strongly modified by fire [9, 12, 20], which leaves in the soil the most resilient organic fractions [42] with a consequent negative effect on the kinetics of the mineralisation processes driven by the microbial biomass. As highlighted by Giovannini et al. [22], in soil after fire the most important transformations are the modification which organic matter undergoes from 220 to 460 °C. Such temperatures are able to strongly modify the quality of organic matter and change, in the meantime, the chemical environment in which the microbial biomass expresses its catalytic performances. In this range of temperatures, potentially detrimental changes in mineral soil composition can also occur, as reported by Saa et al. [32].

The analysis of the data recorded 12 and 18 months after the fire and reported in Table III does not allow the identification of any trend describing the effect of temperature on the microbial biomass. The analysis of the changes in MBC as a function of the time at which the soil microbial population was exposed to temperatures ≥ 60 °C during the fire is much more informative (Fig. 1). The data plotted in Figure 1 clearly show that two superposing effects of the fire influenced soil microbial biomass. One is the maximum temperature reached during burning in the 0–2.5 cm soil layer, and the second refers to the exposure time at temperatures 60 °C. The exposure time seems to be of great utility in depicting the overall effect of heating on soil microflora, together with temperature. It is worth noting that MBC, when expressed as a percentage of soil organic C, decreased in soils heated at 102 and 184 °C in comparison with the control (Tab. III). Fire lowered the microbial biomass more than organic C in these soils, mantained such a value close to that of control in soils heated at 309 °C and slightly lowered it afterwards.

In May, soil heated at 309 °C showed the highest amount of soluble C. This relevant amount of "high quality substrate", as defined by Bosatta and Ågren [7], could be responsible for the stimulation of the microbial metabolism with a subsequent increase in exo-enzyme production. Although Miller and Dick [28] demostrated that β-glucosidase in soil increases according to the augmentation of fungi biomass, we did not observe a relationship between the β-glucosidase activity and the bacteria to fungi ratio and MBC to MBN ratio at any of the recorded temperatures.

Three different factors could be influencing the enzymatic activity: season, time elapsed from the fire and temperature reached during the fire. Dumontet et al. [18] reported that time elapsed from the fire plays an important role in modulating the biochemical patterns of burned soil, while Bolton et al. [6] underlined the importance of season in soil enzymatic activity and Hernández et al. [25] stressed the effect of fire on the activity of soil enzymes in soils. The evaluation of the effect of fire on both the stability of enzymes in soil and the microbial production of them should be, therefore, carefully interpreted, taking into account all the mentioned variables.

The use of fire as a manangement practice is therefore questionable, as underlined by Gillon et al. [20] who compared the supposed beneficial effects of prescribed burning (stimulation of diazotrope activity) with the evident detrimental ones (N and P decline). Theodorou and Bowen [37] pointed out that one of the more serious fire threats is the burning of the unincorporated organic layer, with subsequent exposition of the soil to erosion and leaching of nutrients for several years.

5. CONCLUSIONS

Fires, including those of moderate intensity, have quite a long-lasting effect on the chemical and microbiological soil parameters. The fires we studied here took place in a bushy environment, which was quickly re-colonised by the indigenous vegetation, while 18 months after the fire the chemical and microbiological characteristics of the burned soils still did not reach the control values.

Although microorganisms account for 1 to 5% of soil organic matter [30], they act both as a sink of mineral nutrients and a catalyst during the decomposition of organic material [35]. These microbial features are greatly influenced by environmental stresses, fire included, as demonstrated by the quantitative reduction in MBC and MBN caused both by heating and time for which soil microbes were exposed to a temperature ≥ 60 °C. Fire affected soil microbial biomass not only quantitatively, but also by modifying its species composition and, consequently, its catalytic performances and the soil energetic pathways [31]. The increase in the concentration of "high-quality substrate" for the microbial metabolism, which we observed in May in soil heated at 309 °C, seems to be quite ephemeral and cannot support a hypothetical beneficial effect of fire of moderate intensity on soil microbial parameters.

A more detailed study on the effect of fire on: (i) global microbial catalytic activities; (ii) nutrient cycling and soil fertility, and (iii) possible detrimental effects on the microbial biomass caused by toxic substances released by organic matter combustion is needed.

Acknowledgements: We thank Gianfranco Lucamante for technical assistance in all the operative work in the experimental area and in the soil sampling.

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