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SOIL MICROBIOLOGY

Burning Fire-Prone Mediterranean Shrublands: Immediate Changes in Soil Microbial Community Structure and Ecosystem Functions

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Abstract Wildfires subject soil microbes to extreme temperatures and modify their physical and chemical habitat. This might immediately alter their community structure and ecosystem functions. We burned a fire-prone shrubland under controlled conditions to investigate (1) the fire-induced changes in the community structure of soil archaea, bacteria and fungi by analysing 16S or 18S rRNA gene amplicons separated through denaturing gradient gel electrophoresis; (2) the physical and chemical variables determining the immediate shifts in the microbial community structure; and (3) the microbial drivers of the change in ecosystem functions related to biogeochemical cycling. Prokaryotes and eukaryotes were structured by the local environment in pre-fire soils. Fire caused a significant shift in the microbial community structure, biomass C, respiration and soil hydrolases. One-day changes in bacterial and fungal community structure correlated to the rise in total organic C and NO_3^-–N caused by the combustion of plant residues. In the following week, bacterial communities shifted further forced by desiccation and increasing concentrations of macronutrients. Shifts in archaeal community structure were unrelated to any of the 18 environmental variables measured. Fire-induced changes in the community structure of bacteria, rather than archaea or fungi, were correlated to the enhanced microbial biomass, CO_2 production and hydrolysis of C and P organics. This is the first report on the combined effects of fire on the three biological domains in soils. We concluded that immediately after fire the biogeochemical cycling in Mediterranean shrublands becomes less conservative through the increased microbial biomass, activity and changes in the bacterial community structure.

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Introduction

Fire exposes soil microbes to extreme temperatures and changes in their physical and chemical environment [10]. Temperatures approaching 100°C denature biomolecules, decompose key metabolites such as ATP, and increase membrane fluidity thus threatening cell integrity and the control of solute and gas exchange, among other processes [13, 50, 56]. Microbes cope with high temperatures (1) through molecular mechanisms that either increase the stability of biomolecules and membranes to heating or repair damaged cell components, as reviewed in [13], and (2) by forming heat-resistance structures. Fungi produce spores and thick-walled aggregated hyphae (sclerotia) that withstand temperatures up to 80°C [31, 42, 50]. Many soil bacteria produce resistant structures (e.g. spores, cysts, akinetes, etc.) able to tolerate desiccation and slow temperature increases of up to 50–60°C [15]. Endospores are particularly resistant to extreme agents, including radiation, physical disruption and temperatures as high as 120°C for 20 min [15]. Spore formation has never been reported for archaea [37]. Still, the archaeal domain includes the most heat-resistant living cells [50] owing to their particular cell wall and membrane lipid structure [13].

Fire-induced changes in the physical and chemical soil properties depend on fire severity and soil type, as has been comprehensively reviewed [10, 42]. Burning induces the mineralisation of organic substances, causing a pulse of nutrients [10, 42, 47] that entails a temporary increase in resources for the soil microbiota [14, 27]. The release of cations to the soil solution can increase the soil pH [10], a parameter which strongly influences the microbial community structure [19, 43]. But such a pH spike does not occur in soils with a high buffering capacity, as those rich in carbonates [28]. These are only combusted at temperatures exceeding 1,000°C [10]. The transformation of soil organic matter also releases chemicals that can be toxic to microbes [10]. Furthermore, it can yield hydrophobic hydrocarbons that, together with the layer of ashes, limit the soil's water infiltration capacity and hence its moisture content [10, 42]. A severe combustion of the organic and inorganic soil components can eventually provoke the collapse of its structure [42]. This alters the microbial habitat through reducing the porosity [17] and hence the conduction of air, water and dissolved substances through the soil matrix.

Exposure to extreme temperatures has been proven to alter soil microbial biomass [11, 14, 28] and respiration [25, 28], bacterial and fungal growth [5], as well as the structure of the physiologic [1, 2, 9] and taxonomic groups of soil microbes [26, 33, 55, 64, 66]. Fire has also been shown to affect the activity of soil enzymes [17, 28], which are directly involved in decomposition and nutrient cycling [53, 57] and consequently in the performance of ecosystems [62]. However, no survey has explored the effects of fire on the community structure of the organisms that build the whole soil microbiota, that is, archaea, bacteria and fungi. Furthermore, it has rarely been investigated which environmental parameters govern the response of the soil microbial community structure to burning. Exceptions to this are the work of Smith et al. [55], who found the shift of the dominant bacteria to be driven by the fire-induced pH spike in brunisols and luvisols under a mixed wood. Hamman et al. [26] reported soil pH, total C and temperature to determine the change in the microbial community structure of burned soils developed from granite and covered with ponderosa pine and slimstem muhly. Also, Yeager et al. [66] found pH, together with the NH_3–N levels, to correlate with the composition of ammonia-oxidising bacteria after burning of acidic soils under ponderosa pines and Douglas firs. Finally, the literature lacks information on how the fire-induced changes in the soil microbial community structure correlate with microbial parameters that are linked to main ecosystem functions.

We speculated that fire immediately alters the community structure of soil microbes through direct heating and changes in the soil environment, such as desiccation and nutrient deposition [10, 27]. We expected that the community structure of microbes belonging to the three biological domains would show different responses to fire, based on their dissimilar resistance to high temperatures [50] and ecological niche occupation. Our objectives were to investigate (1) the community structure of soil archaea, bacteria and fungi thriving in the uppermost soil layer of a fire-prone Mediterranean shrubland and their immediate shifts due to burning, (2) the environmental variables determining the immediate changes (if any) of the microbial community structure after burning, and (3) the main microbial drivers of the change in the ecosystem functions related to biogeochemical cycling. With these purposes, we explored the spatial and environmental patterns of the community structure of soil microbes in unburned soils and its immediate changes after a prescribed burning. We did so by analysing the banding patterns after separating 16S rRNA and 18S rRNA gene amplicons through denaturing gradient gel electrophoresis (DGGE). We also examined main physical and chemical soil properties defining the microbial environment, as well as microbial biomass C, total activity (ATP), CO_2 production and hydrolases involved in the C, N and P cycles. To our knowledge, this is the first survey considering the effects of fire on all three biological domains of microbes.

Methods

Experimental Fire and Soil Sampling

An experimental fire was ignited on 22 April 2009 in a 500-m^2 area located in Teresa de Cofrentes (Valencia, Spain; UTM—30 N 676565.50, 4332416.06 m; 950 m a.s.l.; 20% NE facing slope). Mean annual rainfall is 446 mm and temperature 13.7°C. Soils are Humic Leptosols [18] and were completely covered by a dense shrubland (100% plant cover) dominated by *Rosmarinus officinalis* that regenerated from a wildfire in 1979. The fire completely reduced the shrub cover to ashes, but for some sparse *R. officinalis* stems. Temperature was measured using thermocouples and reached 611±94°C (average± SE; n=3) at 50 cm over the soil surface, 338±83°C (n=10) on the soil surface and 106±35°C (n=8) within the upper 2 cm below the surface.

Prior to sampling, the ash layer and the surface cover including litter, mosses and stones were removed. Surface soil samples (0–2 cm) were taken from ca. 1×1 m georeferenced plots (n=10), which were randomly located at 1 to 3 m apart from each other within a 150-m^2 area. A single soil sample (approximately 300 g) was taken from each plot. Samples were collected immediately before (pre-fire), 1 day and 1 week after fire so as to avoid any variation due to seasonal changes. The pre-fire samples were taken as the unburned control. This considerably reduces the spatial heterogeneity in all microbial parameters that results from sampling an adjacent unburned area, which is the only option when wildfires are investigated. Geographic coordinates in the centre of the sampling plot were recording using a

GPS GeoExplorer® GeoXT™ (Trimble, CO, USA; maximum accuracy ±20 cm). Soils were sieved (<2 mm) and visible roots and animals removed. Soil samples were kept at 4°C.

Physical and Chemical Soil Variables

Soil texture was analysed using the Bouyoucos densitometer. Bulk density was determined as the ratio of the oven-dried (105°C) weight of unaltered samples to the volume of the sampling cylinder. Volumetric moisture content was determined as the amount of water contained in the same unaltered soil volume. Gravimetric moisture content was calculated as the loss of weight after oven-drying the sieved samples (105°C). Soil pH and electrical conductivity (EC) were measured in a soil suspension in water (1:2.5 and 1:5 w/v, respectively). Soil carbonates were titrated with HCl (1:1) and the volume of the CO_2 produced was measured using a Bernard calcimeter. Total organic carbon (TOC) was determined by dry combustion at 500°C. Extractable C with 0.1 M sodium pyrophosphate (pH 9.8), indicative of the recalcitrant C pool, in soil suspensions (1:10 w/v) was quantified colorimetrically after oxidation with $K_2Cr_2O_7$ [52]. Water-soluble C (WSC), indicative of labile C, was obtained from an aqueous solution (1:5 w/v), filtered (Albet 145 110) and quantified with a TOC analyser (TOC-5050A, Shimadzu). Water-soluble carbohydrates were extracted as WSC and measured with anthrone [7]. Total N (TN) was determined as Kjeldahl N [6]. Ammonium N (NH_4^+–N) and nitrate N (NO_3^--N) were extracted in 2 M KCl and determined colorimetrically (Helios Alpha, Thermo Fisher Scientific). Phosphorous was extracted as Olsen P and potassium with 2% Cl_2Ba and 2% triethanolamine. Both were quantified using an ICP emission spectrometer 6000 (Thermo Scientific).

Biochemical Soil Variables

Microbial biomass C (MBC), an indicator of microbial biomass, was determined by chloroform fumigation extraction [63] with some minor modifications, as in Ref. [22]. Briefly, 10 g soils were fumigated in air-tight containers with 0.5 ml ethanol-free $CHCl_3$ for 30 min. C was extracted by head-over-head shaking with 0.5 M K_2SO_4 (1:4 w/v). The extracts were centrifuged and the supernatant filtered. Filtrates were flushed with CO_2-free air to remove residual $CHCl_3$ and extractable C was measured by using a TOC analyser (TOC-5050A, Shimadzu). C content in unfumigated controls was analysed using the same procedure. Microbial biomass was calculated by subtracting the fumigated from the unfumigated samples and using a calibration factor K_{EC}=0.38 [63].

The ATP content, an indicator of total microbial activity when quantified under field moisture conditions [41], was extracted from soils using the procedure by Webster et al. [65] including the modifications described in Ref. [12]. The ATP content was quantified by means of the luciferine–luciferase system in a luminometer (Optocom 1, MM Instruments).

The CO_2 evolved under controlled conditions during an aerobic incubation was determined to estimate the soil's potential to mineralise organic C [41]. Soil samples (10–15 g) were moistened to 60% water-holding capacity prior to incubation at 28°C in 125-cm^3 airtight containers. The CO_2 (%) evolved in the containers was measured over 23 days at 3–4-day intervals with an infrared analyser (CheckMate II, PBI Dansensor). After each measurement, stoppers were removed for 1 h to balance the atmosphere inside and outside the bottles. Basal respiration, which is indicative of total microbial activity [41], was calculated as the average C content respired daily per kilogram soil.

Enzymatic Soil Variables

Soil β-glucosidase, alkaline phosphatase and urease activities are hydrolytic enzymes involved in main steps of the C, P and N cycles, respectively [57]. Their activity under optimal conditions was used as an indicator of microbially mediated biogeochemical ecosystem functions [53]. Soil β-glucosidase and alkaline phosphatase activities were determined colorimetrically as the amount of *p*-nitrophenol (PNP) produced after incubation of 0.5 g of soil (37°C, 1 h) in 2 ml of modified universal buffer (MUB; pH 6) and 0.5 ml of 0.025 M *p*-nitrophenyl-β-D-glucopyranoside or MUB (pH 11) and 0.025 M *p*-nitrophenyl-phosphate, respectively [16, 58]. Soil urease was quantified colorimetrically as the NH_4^+ produced after incubating (37°C, 2 h) 1 g of soil in 4 ml borate buffer (pH 10) and 0.5 ml of 0.48% urea [34].

DNA Extraction and PCR Amplification

DNA was extracted within 24 h after sampling from ca. 0.25 g soil using the PowerSoil DNA isolation kit (MO BIO Laboratories, Carlsbad, CA, USA). Extracted DNA was checked for quality by electrophoresis in 1% agarose gels run in 0.5× Tris–acetate–EDTA (TAE) buffer.

Partial sequences of the small subunit rRNA genes of fungi, bacteria and archaea were PCR amplified using the specific primers in Table 1. PCR reactions were performed in 25-μl volumes, with each standard reaction mix containing a final concentration of 1× reaction buffer [16 mM $(NH_4)_2SO_4$, 67 mM Tris–HCl pH 8.8, 1.5 mM $MgCl_2$, 0.01% Tween 20], 200 μM each dNTP, 0.2 μM each primer, 1 mM $MgCl_2$, 0.4 mg ml^{-1} bovine serum albumin, 0.63 U BioTherm™ DNA polymerase (GeneCraft, Germany) and sterile water. Thermal cycling with universal fungal primers consisted of 8 min at 94°C, 35 cycles including 30 s at 94°C, 45 s at 48°C and 2 min at 72°C, and a final elongation for 10 min at 72°C. Bacterial cycling consisted of 3 min at 94°C, 30 cycles of 1 min at 94°C, 1 min at 62°C and 2 min at 72°C,

Table 1 Primers used in this study

Primer	Target	Sequence 5′–3′	Reference
FR1[a]	Fungi	AICCATTCAATCGGTAIT[b]	[61]
FF390		CGATAACGAACGAGACCT	
968F[a]	Bacteria	AACGCGAAGAACCTTAC	[45]
1378R		CGGTGTGTACAAGGCCCGGGAACG	[29]
109 F	Archaea	ACKGCTCAGTAACACGT	[24]
934R		GTGCTCCCCCGCCAATTCCT	
357F[a]	Archaea	CCCTACGGGGCGCAGCAG	[67]
693R		GGATTACARGATTTC	

[a] GC clamp (CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGC ACGGGGGG) attached to the 5′-end of the primer [40]

[b] *I* inosine

and a final elongation for 15 min at 72°C. Archaeal amplification required a nested approach. The first PCR consisted of 5 min at 95°C, 31 cycles of 1 min at 95°C, 1 min at 55°C and 3 min at 72°C, and an extension for 15 min at 72°C. Nested PCR was the same as above, but included 40 cycles, annealing temperature was 40°C, and each elongation step had a length of 2 min. A volume of 2 μl DNA or PCR product was directly applied to the reaction mix. PCR products were checked for quality and length in 1% agarose gels and quantified with the Quant-iT™ PicoGreen® dsDNA Kit (Invitrogen, Carlsbad, CA, USA).

Denaturing Gradient Gel Electrophoresis

Denaturing gradient gel electrophoreses (DGGEs) were performed in an INGENY phorU® system (Ingeny International BV, Goes, The Netherlands). In all cases, 8% acrylamide–bisacrylamide gels were prepared, with various urea–formamide gradients (30–60% for fungi, 45–65% for bacteria and 45–60% for archaea, a 100% denaturant including 7 M urea plus 40% w/v deionised formamide). The same amount of PCR product was loaded on the gels for all samples (60, 100 and 150 ng for fungi, bacteria and archaea, respectively). All samples to be compared were run in the same gel to avoid differences due to inter-gel variation. In every gel, a molecular weight marker (Genecraft®, Germany) was loaded in four lanes, which were regularly distributed covering all samples. Gels were run in 1× TAE buffer (60°C) at 100 V for 16 h, silver-stained as in [21] and photographed (Power Shot A640; Canon, China). Pictures of gels were converted into the GelCompar® II software (Applied Maths BVBA, Sint-Martens-Latem, Belgium). The molecular weight markers were used as the reference to realign all lanes in each gel. Bands were automatically identified using a minimum profiling of 1%, 0.2% grey zone and assigned to a particular position using 1% tolerance. Bands were verified by visual screening of the gels. The number of bands in the DGGEs was taken as a broad indicator of microbial richness and the band profiles as reflecting the community structure. This should be interpreted with caution since it has been observed that a single species can generate bands migrating to different positions [45], and a single band can be composed of more than one taxon [51]. Binary occurrence matrices (band presence/absence) representing the occurrence of taxa in each plot were exported for further analyses.

Statistical Analyses

Under pre-fire conditions, the similarity of the microbial community structure between spatially closer plots was examined to test for spatial autocorrelation in the unburned soils as an indicator of dispersal limitation [23]. This was performed by calculating Mantel correlations between the spatial and occurrence distance matrices with 999 iterations [38]. Euclidean distances were used for geographic coordinates and a binary distance for occurrence (presence/absence of DGGE bands) matrices. We then checked whether unburned communities of soil microbes have a non-random structure mediated by the soil environment and, if so, characterised such a structure. To do this, we correlated occurrence with physical and chemical distance matrices using Mantel tests as above. Finally, we evaluated whether the similarity of the microbial community structure relates to the similarity in microbial biomass, activity and functioning in terms of hydrolysis of C, N and P compounds. In particular, we correlated occurrence with both biochemical and enzymatic distance matrices using Mantel tests as above. In all cases, when no significant spatial autocorrelations were found (i.e. for archaea and bacteria), correlations between microbial occurrence, environmental variables (physical and chemical soil variables), microbial biomass and activity (biochemical variables), and biogeochemical ecosystem functions (enzymatic variables) were sought by using Mantel tests as above. In case of significant spatial autocorrelations (i.e. for fungi), partial Mantel tests taking the spatial distance as a covariate were used instead to ensure that significant associations detected were not spurious relationships mediated by spatial proximity.

The effect of fire on physical, chemical, biochemical and enzymatic soil variables, as well as on microbial richness (number of DGGE bands), was tested by a generalised linear model, either based on a Gaussian or a Poisson (NH_4^+–N and microbial richness) error distribution, using ‘fire’ as an ordered factor with its three levels ranked (pre-fire–1 day–1 week). This allowed testing both linearised and quadratic contrasts, whose significance can be interpreted as follows. Significant linear contrasts with positive or negative *t* values indicate, respectively, an increase or decrease of the dependent variable in response to the experimental factor. Significant quadratic contrasts with positive or negative *t* values indicate, respectively, that the dependent variable reaches minimum or

maximum values at the intermediate treatment level (i.e. 1 day).

The effect of fire on the community structure of soil microbes was analysed by examining the correlations between each occurrence distance matrix and a binary treatment (e.g. 0= no fire, 1=fire 1 day) matrix using Mantel tests as above. This was performed separately for the periods pre-fire–1 day and 1 day–1 week. The soil parameters underlying the shift in the community structure in each time lapse were determined by correlating the distance matrices of fire-affected environmental variables and taxon occurrence. Likewise, the effects of such a shift on the microbial biomass, activity and biogeochemical ecosystem functions were sought. All analyses were performed using the MASS and Vegan packages for R 2.11.1 [48].

Canonical correspondence analysis (CCA) was used to help visualise the effect of the environmental variables (physical and chemical soil properties that were significantly affected by fire) on the structure of the whole microbial community (archaeal, bacterial and fungal DGGE band patterns). CCA based on inter-species correlation was performed with CANOCO 4.5 (Biometris—Plant Research International) [60]. An ordination plot was made with CanoDraw 4.0 (Šmilauer).

Results

Community Structure of Soil Microbes

The archaeal community structure under pre-fire conditions showed no spatial autocorrelation (r=0.222, p=0.093). It was significantly correlated with NH_4^+–N (r=0.442, p=0.009), humic-substance C (r=0.328, p=0.041) and marginally with pH (r=0.367, p=0.053). However, no correlation was found with either physical (r=0.118, p=0.298), biochemical (r= 0.062, p=0.348) or enzymatic (r=−0.365, p=0.958) variables.

Bacterial community structure in the unburned soils was not correlated to either spatial (r=0.092, p=0.28), chemical (r=−0.016, p=0.505), physical (r=0.288, p=0.109), biochemical (r=−0.182, p=0.817) or enzymatic (r=−0.099, p=0.646) distance matrices. But it was correlated to TOC alone (r=0.360, p=0.036).

Table 2 Fire effects on the chemical and physical soil parameters

Variable[a]	Average (SE)[b]			*t* value[c]	
	Pre-fire	1 day	1 week	L	Q
pH	8.05±0.05	8.04±0.03	8.03±0.02	−0.51	−0.07
EC (μS cm^{-1})	230±11	287±14	341±9	**10.3*****	−0.18
TOC (g kg^{-1})	43.9±1.7	46.1±1.5	44.0±1.2	0.09	**−2.25***
HSC (g kg^{-1})	15.4±2.0	14.6±1.1	16.0±1.6	0.57	1.10
WSC (mg kg^{-1})	521±73	787±133	628±75	1.39	−1.95
CH (mg kg^{-1})	38.5±3.2	33.0±4.2	53.1±10.1	−0.18	0.69
$CaCO_3$ (%)	10.4±0.2	9.82±0.31	8.44±0.38	**−5.36*****	−1.35
TN (%)	0.39±0.04	0.43±0.04	0.43±0.05	0.70	−0.59
NH_4^+–N (mg kg^{-1})	1.70±0.16	6.65±1.99	5.64±1.27	**2.50***	**−2.39***
NO_3^-–N (mg kg^{-1})	34.6±5.0	96.8±8.2	77.6±6.9	**5.38*****	**−5.89*****
P (mg kg^{-1})	142±8	166±10	191±14	**4.87*****	−0.27
K (%)	0.29±0.01	0.27±0.01	0.30±0.02	−1.12	0.52
GH (%)	23.3±1.7	23.6±1.5	15.7±1.9	**−7.83*****	**−4.82*****
Coarse sand (%)	6.34±0.73	4.99±0.48	–	−1.69	–
Fine sand (%)	91.3±0.75	92.6±0.82	–	1.68	–
Silt (%)	2.29±0.32	2.70±0.43	–	0.50	–
δ_b (g cm^{-3})	0.79±0.09	0.69±0.04	–	−1.12	–
VH (%)	11.3±0.8	11.7±0.6	–	0.62	–

[a] *$CaCO_3$* carbonates, *CH* carbohydrates, *EC* electrical conductivity, *GH* gravimetric humidity, *HSC* humic-substance C, *K* potassium, *NH_4^+–N* ammonium-N, *NO_3^-–N* nitrate-N, *P* phosphorous, *TN* total N, *TOC* total organic C, *VH* volumetric humidity, *WSC* water-soluble C, *δ_b* bulk density

[b] Standard errors are given for n=10. All data are given on an oven-dried weight soil basis

[c] Statistical t values are given for linear (L) and quadratic (Q) contrasts taking `fire' as an ordered factor. Significant L contrasts (in bold) indicate an increase (positive t value) or decrease (negative t value) of the dependent variable in response to fire. Significant Q contrasts (in bold) indicate that the variable reached minimum (positive t value) or maximum (negative t value) levels at 1 day. Significant p values are indicated by * (p<0.05) or *** (p<0.001). Log transformations were applied to CH, K, P, TN, WSC and coarse sand prior to statistical analyses

Fungal community structure in the pre-fire state was more similar in spatially (r=0.287, p=0.044) and physically closer plots (r=0.326, p=0.044). It showed no correlation with the general chemical distance matrix (r=0.099, p=0.290), but it did with carbonates (r=0.394, p=0.015) and marginally with TOC (r=0.320, p=0.052). Similarities in the fungal community structure were unrelated to biochemical (r=−0.191, p=0.853) or enzymatic distances (r=0.073, p=0.337).

Effects of Fire on the Abiotic Environment

Fire caused a significant increase in the soil's electrical conductivity (EC), NH_4^+–N, NO_3^-–N and P (Table 2). Changes in EC and P during the first week followed an increasing trend, but NH_4^+–N and NO_3^-–N levels peaked 1 day after fire. Total organic C (TOC) reached maximum levels 1 day following fire. However, TOC contents after 1 week were not significantly different to those in the unburned control. Gravimetric humidity and carbonates significantly decreased during the week following fire (Table 2). No change was detected in any of the physical soil variables measured (Table 2).

Effects of Fire on the Microbial Community Structure

A total of 51, 91 and 63 band positions were identified in the archaeal, bacterial and fungal electrophoretic gels, respectively. Fire did not alter the number of bands in the DGGEs, corresponding to the dominant microbial taxa (Fig. 1). The number of archaeal bands did not decrease after burning (linear contrast, LC—t=−1.423, p=0.17; quadratic contrast, QC—t=0.164, p=0.87). Similar results were obtained for bacteria (LC—t=1.23, p=0.23; QC—t=−0.04, p=0.97). The number of fungal bands increased 1 day after the fire but returned to pre-fire conditions 1 week later (LC—t=0.162, p=0.87; QC—t=−2.156, p=0.04).

Burning altered the community structure of all archaea, bacteria and fungi, as proven by the significant correlations found between the taxon occurrence (DGGE band presence/absence) and binary treatment (pre-fire/1 day and 1 day/1 week) matrices. Archaeal community structure shifted 1 day after fire, but showed by far the lowest magnitude of change (pre-fire vs 1 day—r=0.089, p=0.043). Archaeal communities were not further altered in the following week (1 day vs 1 week—r=−0.033, p=0.73). Bacteria showed strong and significant shifts in both time lapses (pre-fire–1 day—r=0.441, p=0.001; 1 day–1 week—r=0.356, p=0.001), as fungi did (pre-fire–1 day—r=0.425, p=0.001; 1 day–1 week—r=0.445, p=0.001). Figures 2, 3 and 4 show the dendrograms constructed based on the number of different band positions in the DGGEs of archaea, bacteria and fungi, respectively. These dendrograms help visualise the patterns found using the matrix correlation analyses described above. First, the archaeal banding patterns were divided into two clusters, one of them

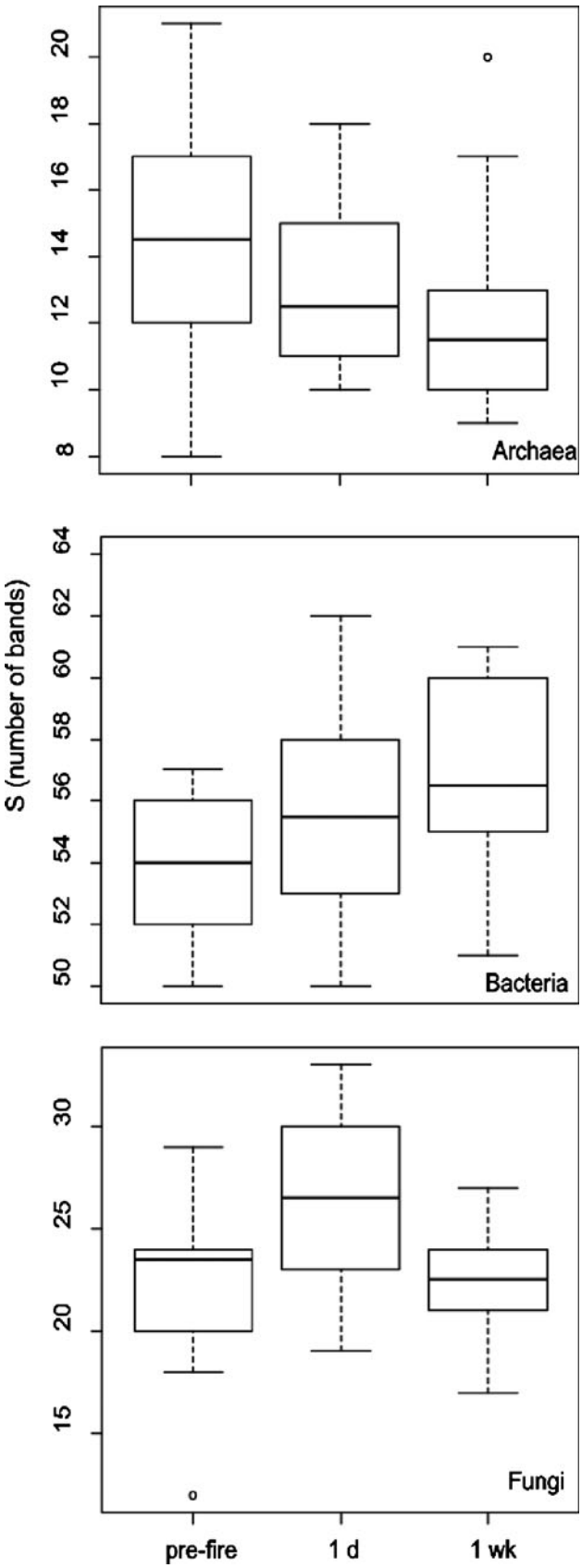

Figure 1 Richness of dominant soil archaea, bacteria and fungi, measured as the number of bands detected in DGGE profiles (n=10)

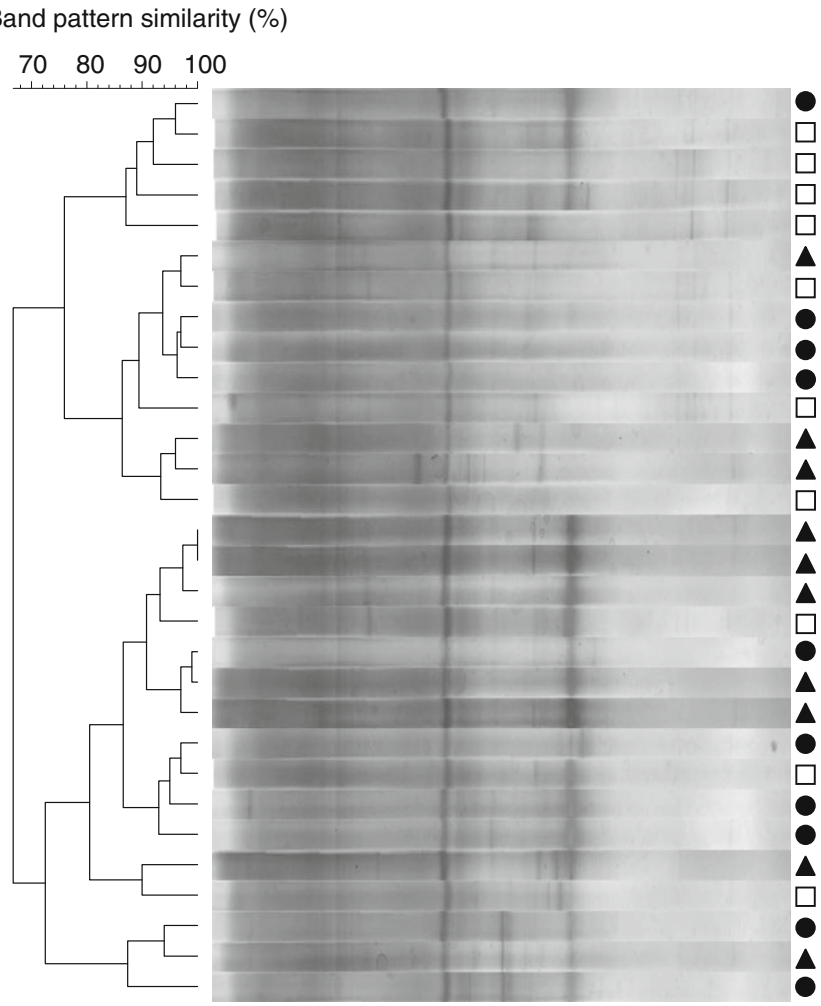

Figure 2 Band patterns after PCR amplification of the archaeal 16S rRNA gene and DGGE. Similarity was based in the number of different band positions and dendrogram was constructed using Ward's algorithm. Symbols indicate pre-fire (*squares*), 1 day (*circles*) and 1 week (*triangles*) samples

comprising seven out of 10 replicates of the pre-fire samples, and the other one the majority of the 1 day (six out of 10) and 1 week (seven out of 10) samples (Fig. 2). However, there was not a clear separation of the 1 day and 1 week samples (Fig. 2). Second, the bacterial banding patterns clustered in two clades, differentiating the pre-fire (eight out of 10 replicates) from the fire samples (Fig. 3). The latter clade was further divided in two, separating most 1 day from 1 week samples (seven out of 10 replicates in both cases; Fig. 3). Finally, the fungal banding patterns were grouped into two clusters, the first one comprising the pre-fire and 1 day samples. These were further assigned to two subclusters that separated most (six out of 10) pre-fire samples from all 1 day samples (Fig. 4). The second fungal cluster grouped all 1 week samples (Fig. 4).

Shifts in the archaeal community were uncorrelated to any of the fire-affected soil variables. One day shifts in bacterial community structure were correlated to changes in NO_3^-–N (r=0.276, p=0.002) and marginally TOC (r=0.175, p=0.052), while 1 week shifts were to those of gravimetric humidity (r=0.240, p=0.005), $CaCO_3$ (r=0.204, p=0.045) and marginally P (r=0.194, p=0.052). Fungal community structure was modified in the first day following changes in TOC (r=0.240, p=0.023) and NO_3^-–N (r=0.222, p=0.012), whereas shifts during the following week were unrelated to any of the chemical variables considered. Figure 5, which depicts the first two axes extracted from the CCA, shows the relationship between the environmental variables that were significantly affected by fire and the structure of the whole microbial community. This shows that overall NO_3^-–N, P, EC, TOC and NH_4^+–N contributed most to the separation of the pre-fire and post-fire samples, while the gravimetric humidity, $CaCO_3$, P and EC determined the differences between the post-fire 1 day and 1 week samples.

Effects of Fire on Microbial Biomass, Activity and Biogeochemical Ecosystem Functions

Burning significantly increased microbial biomass C (MBC), basal respiration (BR), as well as β-glucosidase

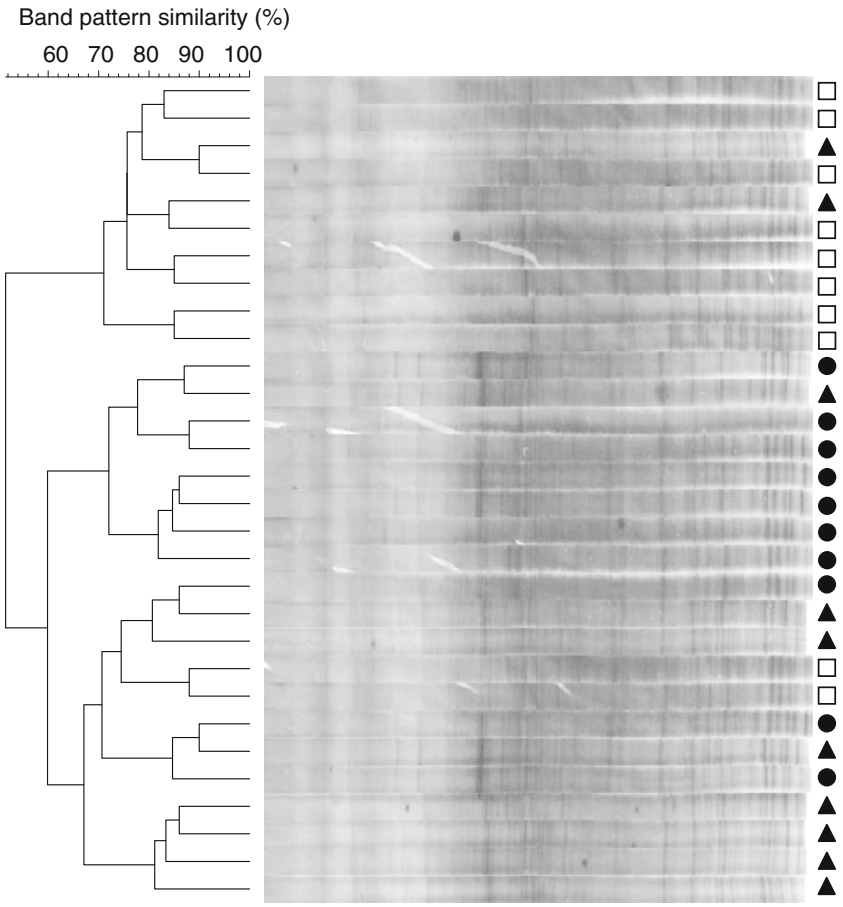

Figure 3 Band patterns after PCR amplification of the bacterial 16S rRNA gene and DGGE. Similarity was based in the number of different band positions and dendrogram was constructed using Ward's algorithm. *Symbols* indicate pre-fire (*squares*), 1 day (*circles*) and 1 week (*triangles*) samples

and phosphatase activities, while it reduced ATP and urease activity (Table 3).

Fire-related changes in MBC, ATP and BR were correlated to those in the bacterial community structure, marginally during the first day (r=0.144, p=0.065) but significantly within the subsequent week (r=0.305, p=0.005). On the contrary, changes in the biochemical parameters were unrelated to either the archaeal (pre-fire–1 day—r=0.099, p=0.209; 1 day–1 week—r=0.028, p=0.37) or fungal (pre-fire–1 day—r=−0.093, p=0.82; 1 day–1 week—r=−0.036, p=0.64) community structure.

Changes in the enzymatic activities measured were strongly correlated to the shift in MBC, ATP and BR (pre-fire–1 day—r=0.466, p=0.001; 1 day–1 week—r=0.723, p=0.001) and, to a lesser extent, to the 1 week changes in the bacterial community structure (pre-fire–1 day—r=0.067, p=0.25; 1 day–1 week—r=0.187, p=0.049). However, shifts in the enzymatic variables were not related to either the archaeal (pre-fire–1 day—r=−0.18, p=0.94; 1 day–1 week—r=0.019, p=0.40) or fungal (pre-fire–1 day—r=0.016, p=0.41; 1 day–1 week—r=−0.069, p=0.78) community structure.

Discussion

Our results confirm that fire immediately alters the community structure of soil microbes, their biomass and activity, and this is partly mediated through changes in the soil environment. This eventually affects the biogeochemical ecosystem functions driven by these organisms. Here, we experimentally demonstrate that organisms belonging to the three biological domains (archaea, bacteria and fungi) building the soil microbial community show different responses to fire.

Soil Microbial Community Structure in a Mediterranean Shrubland

Community structure of soil prokaryotes did not vary with geographic distance, suggesting no limitation for dispersal. Populations holding millions of individuals with micrometric sizes are expected to have a migratory flow independent on spatial distance, particularly at the local scale of our study but even at the global scale [19, 20, 39]. Dominant prokaryotes were shaped by the chemical (but not the physical) environment. In other words, local communities (in each plot) were not simply a random set of the global species pool, but

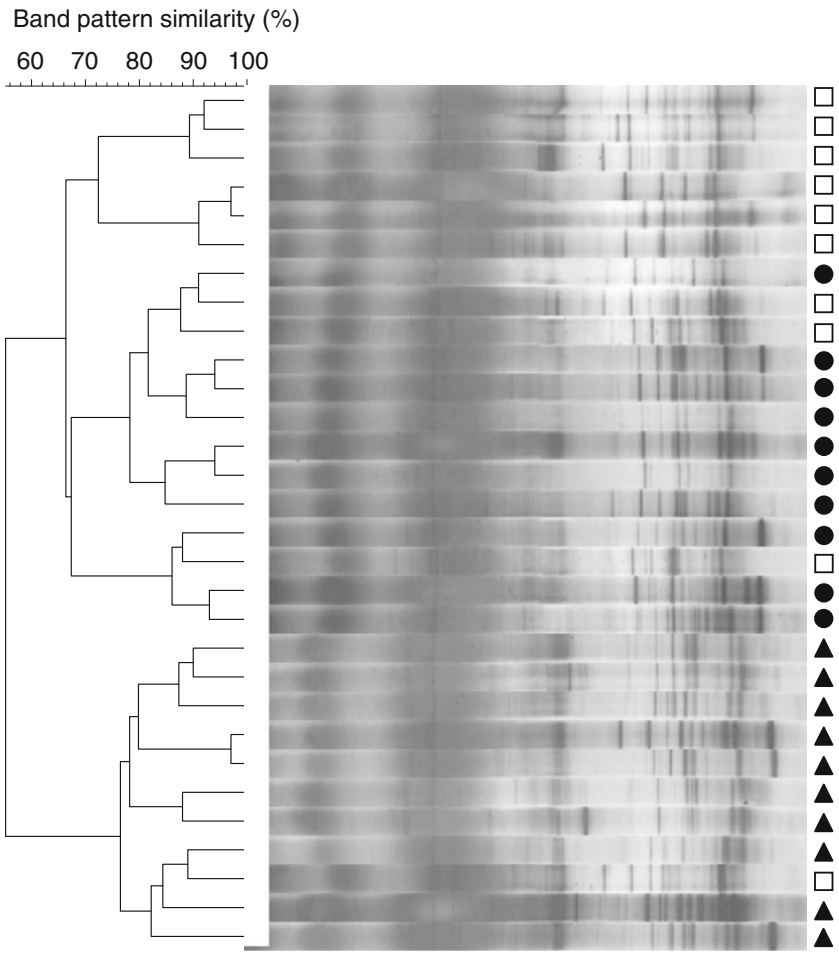

Figure 4 Band patterns after PCR amplification of the fungal 18S rRNA gene and DGGE. Similarity was based in the number of different band positions and dendrogram was constructed using Ward's algorithm. *Symbols* indicate pre-fire (*squares*), 1 day (*circles*) and 1 week (*triangles*) samples

depended on the particular environmental conditions. Bacterial community structure was determined by total organic C, suggesting a certain degree of specialisation based on the amount of oxidisable substances. Examples of local environment sorting of soil bacteria at spatial scales similar to ours, and relying on similar sample sizes and molecular methods, are available from other ecosystems—e.g. arid grasslands [35] or tropical forests [44]. Unfortunately, these authors did not report the environmental parameters underlying such non-random spatial patterns. The community structure of archaea was mostly determined by variations in the levels of ammonium N, suggesting a high abundance of archaeal ammonia oxidisers as commonly found in soils [32, 36, 43]. Soil pH was also a determinant of archaeal community structure in these basic soils, something that has been observed before for Crenarchaea and particularly for ammonia-oxidising Crenarchaea in acid and neutral soils [32, 43].

The community structure of soil fungi was determined by all the spatial distance, the physical and chemical environment. Neighbouring plots were more similar in their fungal community structure, what could be attributed to dispersal decaying with spatial distance [4, 23]. This disputes previous ideas of ubiquity of eukaryotic microbes [20]. However, at the small spatial scale of our study, filamentous growth of soil fungi could also underlie the greater similarities found between spatially closer plots. The availability of organic compounds, the soil's density and capacity to store water also filtered the fungal communities irrespective of spatial distance.

Our results, which are summarised in Table 4, add further evidence to the existence of biogeographic patterns among prokaryotes [19, 39, 44] and microbial eukaryotes [59]. We have found so in a 150-m^2 square with a single soil type and completely covered by a homogenous shrubland, reflecting microbial adaptation to local environmental heterogeneity (i.e. physical and chemical soil variability). However, these observations are applicable only to the dominant community members due to our inability to sample the vast microbial diversity in soils [39].

Fire Effects on the Community Structure and Ecosystem Functions

Fire completely burned the plant cover. Despite the high density, flammability and growth type of the shrubland (with

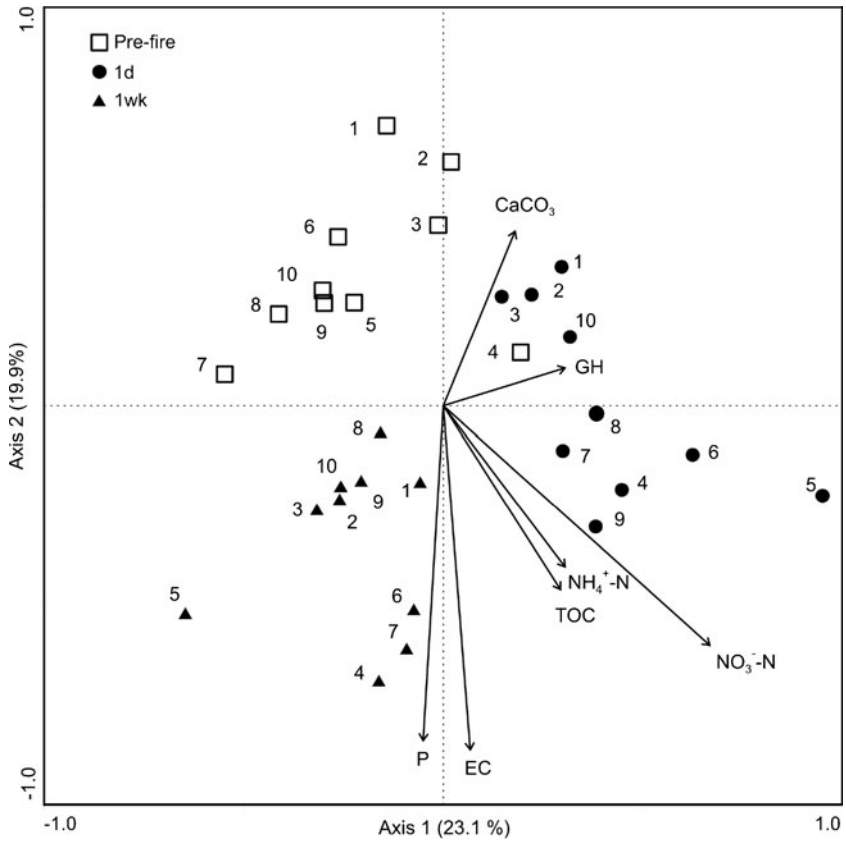

Figure 5 Biplot of the first and second axes obtained from canonical correspondence analysis of the pre-fire and post-fire samples, using 205 taxa (archaeal, bacterial and fungal DGGE bands) and the soil environmental variables that were significantly affected by fire. For each axis, the explained variation is given in *parentheses*. *Numbers* identify plots. *$CaCO_3$* carbonates, *EC* electrical conductivity, *GH* gravimetric humidity, *NH_4^+–N* ammonium-N, *NO_3^-–N* nitrate-N, *P* phosphorous, *TOC* total organic C

its biomass accumulated close to the ground), temperatures reached only 106°C on average in the upper centimeters belowground. Higher temperatures were likely prevented both by the presence of a mulching agent (i.e. the surface cover of litter, stones and mosses) and the high soil humidity [8]. However, high moisture conditions can be particularly lethal for soil microbes due to faster heat penetration and a smaller fraction of dormant organisms [11, 27, 42].

Fire did not alter the number of bands in the DGGEs, a broad indicator of richness of the dominant microbes, while it significantly impacted the community structure of all archaea, bacteria and fungi (Table 4). This demonstrates that some groups of microbes not only resisted burning but even benefited from the increased abundance in labile forms of C, N and other macronutrients. This idea is supported by the general rise in microbial biomass (MBC) and total activity

Table 3 Fire effects on the microbial biomass, activity, and C, N and P cycling enzymes

Variable[a]	Average (SE)[b]			*t* value[c]	
	Pre-fire	1 day	1 week	L	Q
MBC (mg C k g^{-1})	469±61	508±35	709±55	**3.71****	1.45
ATP (μg g^{-1})	2.19±0.15	2.07±0.13	1.58±0.26	**−2.98****	−1.06
BR (mg C–CO_2 kg^{-1} day^{-1})	15.8±2.0	23.8±2.5	24.6±2.4	**4.19*****	−1.98
GA (μmol PNP g^{-1} h^{-1})	2.93±0.26	3.67±0.37	3.94±0.43	**2.92****	−0.77
PA (μmol PNP g^{-1} h^{-1})	15.7±1.9	21.2±2.5	23.0±2.6	**3.60****	−1.07
UA (μg NH_4^+–N g^{-1} h^{-1})	1.06±0.11	0.91±0.08	0.80±0.09	**−2.29***	0.07

[a] *BR* basal respiration, *GA* β-glucosidase activity, *MBC* microbial biomass C, *PA* alkaline phosphatase activity, *UA* urease activity

[b] Standard errors are given for $n=10$. All data are given on an oven-dried weight soil basis

[c] Statistical t values are given for linear (L) and quadratic (Q) contrasts taking 'fire' as an ordered factor. Significant L contrasts (in bold) indicate an increase (positive *t* value) or decrease (negative *t* value) of the dependent variable in response to fire. Significant Q contrasts (in bold) indicate that the variable reached minimum (positive *t* value) or maximum (negative *t* value) levels at 1 day. Significant *p* values are indicated by * ($p<0.05$), ** ($p<0.01$) or *** ($p<0.001$). Log transformations were applied to BR, GA, PA and UA prior to statistical analyses

Table 4 Factors determining the community structure of soil microbes in unburned Mediterranean shrublands and its immediate shifts after burning

	Unburned soil		Burning effects on	
	Spatial autocorrelation[a]	Environmental patterns[b]	Community structure (1 day | 1 week)[c]	C, P, N cycling[d]
Archaea	No	NH_4^+>pH≈HSC	Yes | No	No
Bacteria	No	TOC	NO_3^->TOC | GH>P≈$CaCO_3$	Yes
Fungi	Yes	$CaCO_3$≈TOC≈phys	TOC≈NO_3^- | Yes	No

$CaCO_3$ carbonates, *GH* gravimetric humidity, *HSC* humic-substance C, *NH_4^+* ammonium-N, *NO_3^-* nitrate-N, *P* phosphorous, *phys* combination of five physical variables (coarse sand, fine sand, silt, volumetric humidity and bulk density), *TOC* total organic C

[a] *No* community structure in the unburned soil was not more similar between neighboring plots, *Yes* community structure in the unburned soil was more similar between neighboring plots

[b] Community structure in the unburned soil was correlated to the listed environmental parameters

[c] *No* community structure did not change after burning, *Yes* community structure changed due to burning but underlying environmental parameters were not found. If found, environmental parameters are listed instead

[d] *No* changes in C, P and N cycling were unrelated to changes in community structure, *Yes* changes in C, P and N cycling were significantly related to changes in community structure

(CO_2 production) measured under optimal conditions of humidity and temperature. The ATP content, another general marker of microbial activity [41], did not follow the same trend probably owing to small molecules being easily degraded by temperatures approaching 100°C (half-life 30 min in vitro; cited in [56]). Furthermore, ATP levels are highly dependent on soil moisture [22], and its extraction efficiency from soils most likely decreases with increasing levels of organic matter as was the case here. Several authors have reported that burning (or artificial heating) results in increased microbial biomass and respiration in Mediterranean soils due to higher concentrations of oxidisable carbon and nutrients [5, 14, 25]. Forest fires that depleted the soil organic C pool of Mediterranean pine forests diminished the levels of microbial biomass and basal respiration [28], while those without an effect on organic C did not influence the microbial biomass [17].

Archaea showed the smallest change in community structure, which is consistent with archaeal higher resistance to high temperatures [50] due to their particular cell wall and membrane lipid structure [13]. It should be considered that we used a nested amplification approach, with a total of 71 cycles, due to the low abundance of archaeal templates in our samples. This might have modified the archaeal richness through the formation of PCR artefacts and the differential amplification of templates depending on their initial abundance [3]. We failed to find the environmental parameters underlying the archaeal change among the 18 physical and chemical variables studied. Despite ammonium-N was the strongest predictor of the undisturbed community suggesting high abundance of ammonia oxidisers among archaea, dramatic fire-induced changes in ammonium-N were not correlated to changes in archaea. Jia and Conrad [32] found that ammonium fertilisation did not increase the number of copies of the archaeal ammonia monooxygenase gene in an agricultural soil. They suggested that under such conditions soil ammonia-oxidising archaea have a heterotrophic or mixotrophic metabolism [32] what could explain the lack of correlation between the ammonium-N levels and the post-fire archaeal community structure.

Immediate shifts in bacterial and fungal community structure were determined by the increase in the amount of oxidisable substances (TOC) and inorganic nutrients (nitrates and phosphorous), as well as factors controlling their availability (soil moisture and carbonates). Several groups of microbes could have benefited from such post-fire conditions. Firstly, microbes bearing heat-resistance structures that germinate when triggered by high temperatures and increased nutrient abundance [15]. Indeed, bacterial endospore formers recover quickly after fire and show abnormally high levels at least up to 1 year after burning [55, 64, 66]. Also, fungi forming heat-resistant spores or sclerotia easily colonise burned or heated soils [31, 46, 49]. Secondly, both nitrifiers and denitrifiers could have increased their biomass and/or activity in response to the burst in mineral N. Ammonium-N increases immediately after fire due to the mineralisation of organic N compounds [47], while the peak in nitrate-N is believed to be the result of nitrifier activity [27]. The composition of ammonia-oxidising bacteria has been shown to change after a wildfire in a semi-arid mixed conifer forest soil [66]. Also, our preliminary quantification of several functional genes involved in nitrification and denitrification support the immediate growth of both microbial guilds (unpublished data).

Changes in the community structure of soil bacteria, but not those of archaea or fungi, correlated with the increased microbial biomass and total activity. Furthermore, the shifts in the bacterial community, as well as the microbial biomass and activity, determined the altered biogeochemical ecosystem

functions (Table 4). Immediately after the fire, we detected an increased potential activity of β-glucosidases that break down the β-glucoside bonds of carbohydrated chains [57]. This might have been connected to the rise in the levels of water-soluble carbohydrates (although this was not statistically significant due to the large spatial variability of carbohydrate levels in the post-fire soils). Similarly, the potential activity of alkaline phosphatases was induced by burning. These enzymes catalyse the hydrolysis of phosphoric esters, rendering an alcohol and orthophosphoric acid [57], thus replenishing the soil pool of inorganic P. The enhanced hydrolysis of organic C and P compounds was therefore probably related to the increased abundance of oxidisable forms and the higher nutrient demand of an activated community. This initial peak in enzymatic activity seems to disappear as labile organic compounds return to their original levels several months after fire [28].

In contrast to the above, the potential hydrolysis of urea into carbon dioxide and ammonia was immediately reduced by burning, as has been previously reported [17]. Such a reduction most likely responds to the burst in ammonium-N, which is a main inhibitor of urease activity [30]. Still, the fire-induced availability of the substrates for nitrification (NH_4^+) and denitrification (NO_3^-) might enhance N cycling [27]. This remains to be further investigated.

Higher CO_2 production as well as hydrolysis of glycosides and phosphorylated compounds after burning indicate faster exchange rates between soil microbes and the environment: more organic compounds were mineralised per unit of time and, thus, nutrient turnover was less conservative. Therefore, our results suggest that even moderate fires can interrupt the conservative cycling typical of mature ecosystems [54] through shifts in the bacterial community structure, microbial biomass and total activity. To which extent these changes persist over time should be evaluated in long-term studies.

Conclusions

The community structure of the dominant soil microbes shifted immediately after a moderate fire following changes in oxidisable C, mineral N, phosphorous, carbonates and desiccation. Also, microbial biomass and total activity changed, as did ecosystem functions related to biogeochemical cycling. Presuming that eventually the original functions will be restored, it would be interesting (1) to investigate the resilience and rate of recovery of the microbial community structure and ecosystem functions, particularly those of N cyclers, and (2) to reveal the identity of those microbes responsible for changes in ecosystem functioning after burning. Linking microbial community structure with the performance of ecosystems is essential for predicting the consequences of the increasing wildfire frequency caused by the global temperature rise.

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