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Physico-chemical and microbial perturbations of Andalusian pine forest soils following a wildfire

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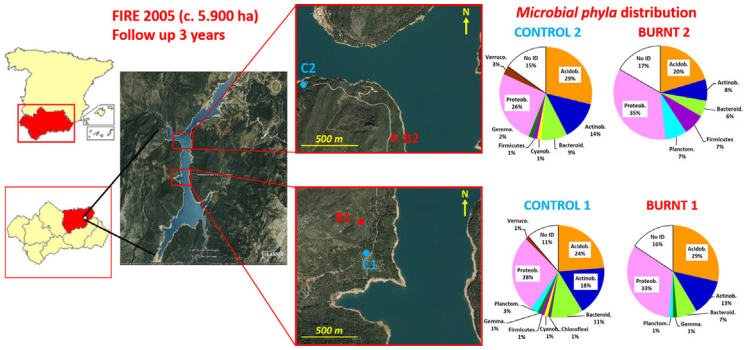
HIGHLIGHTS

- Physico-chemical and microbial characteristics of fire affected soils are studied.
- Fire increased soil N, soil OM and phosphatase activity.
- Proteobacteria and Firmicutes phyla increased in burnt soil.
- Acidobacteria and Bacteroidetes phyla decreased in burnt soil.
- Microbes respond to soil OM with higher diversity, activity and biomass production.

GRAPHICAL ABSTRACT



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ABSTRACT

Wildfires are a recurrent disturbance in Mediterranean forests, triggered by high fuel load, high environmental temperature and low humidity. Although, human intervention is behind the initiation of most fire episodes, the situation is likely to worsen in the future due to the effects of climate change in the Mediterranean "hotspot". Here we study chemical, physical and microbial characteristics of burnt soils from two well differentiated sites at Sierra de Cazorla, Segura and Las Villas Natural Park, Andalusia, (Spain) affected and unaffected by a wildfire, and followed their evolution for three years. The soils affected by a severe surface burn showed a significant increase in organic matter after 3 years from the fire. Viable bacteria and fungi also increased, especially 2–3 years post-burning. Substrate induced respiration (SIR) also increased significantly in burnt soil from site 1 (rendzina on carbonate) while a significant decrease was observed in the burnt soils sampled from site 2 (calcic luvisols) in samples taken one month after the wildfire. A recovery in both SIR and organic matter was observed after 2 and 3 years. Of seven soil enzymes studied, only phosphatase activity was significantly higher in most burnt soils over the three years. Analysis of bacterial community diversity using clone libraries showed a recovery in the number of *phyla* in burnt soils after 2 and 3 years in both sites, with an increase in *Proteobacteria* and *Firmicutes* and a decrease in *Acidobacteria phyla*. For *Bacteroidetes*, the percentages were lower in most burnt samples. This study reveals that if wildfire increases the organic matter availability, then the microbial community responds with increased activity and biomass production. Although fire exerts an initial impact on the soil bacterial community, its structure and functional profile soon recovers (after 2–3 years) contributing to soil recovery.

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1. Introduction

No single indicator that encompasses all aspects related to soil health has been accepted (Kibblewhite et al. 2008). In turn, a combination of soil variables such as soil respiration, physico-chemical characters and microbial biomass have been used to assess soil status. Microorganisms are able to react quickly to changes in soil ecosystem and exhibit a high degree of adaptability to the new environment. Therefore, soil microbial communities and microbial activities are also considered good indicators of soil health (Turco et al. 1994; Pankhurst et al. 1995). Among such indicators are those informing microbial biomass [commonly estimated by substrate induced respiration (SIR), analysis of polar lipid fatty acid (PLFA) and DNA soil quantification)], microbial activity (*e.g.* enzyme assays, BIOLOG systems) and microbial biodiversity [*e.g.* denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP)] (Kaiser et al. 1992; Kirk et al. 2004; Wright et al. 2004; Smith et al. 2008; Islam et al. 2011). Among the physico-chemical soil health indicators pH, conductivity, density, water retention and stability of aggregates are commonly assessed (Arias et al. 2005). The analysis of soil health is often most useful when applied to an environment which is being restored following perturbations such as pollution events and wildfires. Knowledge of soil health enables better restoration management and therefore a more effective recovery of degraded ecosystems (Mataix-Solera and Cerdá 2009b).

Extreme environmental conditions in the summer period (high temperatures and low relative humidity), together with other factors like the accumulation of easily flammable dried biomass and human intervention, responsible for the initiation of most fire episodes, makes wildfires the cause of important perturbations in Mediterranean bush/forest ecosystems. In addition, this situation is likely to worsen in the future due to global warming, with the Mediterranean region widely considered as a "hot-spot" for climate change (Giorgi 2006; Turco et al., 2017). The role of wildfires in the alteration of floral composition, tree regeneration and improving wood production has been widely studied (Mataix-Solera and Cerdá 2009a). Despite the fact that fires are considered a natural ecological factor in the Mediterranean ecosystem, anthropogenic activities in the region over the past 50 years *i.e.* grazing, cinegetic activities and tourism, has led to an increase in the occurrence of these perturbations. Wildfires produce changes in the physical, chemical and biological properties of soil (González-Pérez et al. 2004; Mataix-Solera and Cerdá 2009b; Gómez-Rey et al. 2013). Depending on severity, wildfires may exert soil nutrient depletion through processes including leaching and erosion, partial or complete combustion of organic matter and deterioration of the soil structure (Chandler et al. 1983; Carballas et al. 2009; Díaz-Raviña et al. 2010; Almendros and González-Vila 2012; Vega et al. 2013).

The effects of wildfires on the soil microbial community are also highly variable and dependent on multiple factors *i.e.* severity and intensity of fire as well as on the physico-chemical properties of the soil before and after the fire event. In many studies, it has been found that soil microbial biomass decreases following a wildfire and remains low for many years (Dooley and Treseder 2012). However, in other studies either an increase (Goberna et al. 2012) or no significant changes have been observed when comparing non-burnt and burnt soils (Hamman et al. 2007). In addition to the direct effects of fire and temperature reached in the soil, toxic compounds such as dioxins, hydrocarbons, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), which often inhibit the growth and survival of the living soil organisms may be produced (Kim et al., 2004a).

The analysis of the soil microbiota by means of culture-dependent techniques such as isolation plating has in the past provided useful but limited information because only a minor proportion of the soil microbes are cultivable. However, the recent application of culture-independent techniques to environmental microbiology studies, including analysis of polar lipid fatty acids (PLFA) and molecular techniques based on 16S and 18S rRNA sequences, have greatly improved our understanding of the activity and true diversity of soil microbial communities although molecular approaches also have known weaknesses (Rütters et al., 2002). Among the culture-independent techniques developed, denaturing gradient gel electrophoresis (DGGE) is one of the most widely applied techniques used to profile soil microbial communities (Nakatsu et al., 2000). This method is particularly useful when examining time series and population dynamics (Theron and Cloete 2000). Once the identity of an organism associated with any particular band has been determined, fluctuations in individual components of a microbial population, due to environmental perturbations can be promptly assessed (Head et al. 1998).

Fire not only impacts the soil microbial community quantitatively, but also qualitatively through changes in the composition of the soil microbiota, availability of carbon and nutrients and microbial activity (Hernández et al. 1997; Jensen et al. 2001). Previous research has shown that the most significant differences in microbial diversity are found at lower taxonomical levels (*i.e.* at the species level) rather than at higher taxonomical levels (*i.e. phylum*, class, order) (Smith et al. 2008). Theodorou and Bowen (1982) observed that bacteria from the *Pseudomonas* genus were very sensitive to fire (*phylum Proteobacteria*) while other genera such as *Bacillus* or *Clostridium* (*phylum Firmicutes*) produce resistant spores which allow them to survive at 100–120 °C. In recent work, carried out in a Spanish Mediterranean area (Sierra de Aználcollar, Sevilla) affected by severe wildfires, higher diversity of both *Bacteria* and *Archaea* domains was found in burnt soils compared with the control soils, as assessed through DGGE analysis. In addition, based on analysis of 16S rRNA cloned sequences, several variations in the number of the different phyla present in burnt and control soils were reported (Rodríguez et al. 2017).

Most studies on the impact of wildfires have focused on short-term responses of the soil's physico-chemical properties or on its effect on specific organisms (Scharenbroch et al. 2012). Our limited knowledge of the consequences of wildfires on the activity and diversity of soil microbial communities over time, together with the increasing frequency of these events has enhanced research interest in this area. Improved understanding of the microbial community response to wildfires will undoubtedly contribute to improved biorestoration of affected soils and an enhanced understanding of the effects of wildfires on ecosystem services for which the soil microbial community play a vital role (Arias et al. 2005; Thom and Seidi 2016).

The main objective of this study was to assess the effects of a wildfire on the activity and diversity of the soil microbial population as well as following the response of the microbial population after the fire event For this, we studied two well differentiated locations and soils (rendzina on carbonate and calcic luvisol), affected and unaffected by a wildfire that occurred in 2005 in Cazorla, Segura and Las Villas (Jaén, Spain).

2. Materials and methods

2.1. Site description and sampling procedure

The study area is located in Sierra de Cazorla, Segura and Las Villas Natural Park (Jaén province, Andalusia, Spain), an area affected by a wildfire of a high severity that occurred between 7 and 10 August 2005 and burnt an area of >5000 ha, mainly of pine trees (*Pinus pinaster & P. nigra*) and Mediterranean shrubby vegetation. The topography of the area is abrupt with about 50% of the area at >1000 m above sea level (Molina et al. 2014). The area is characterized by Mediterranean climate with a mean annual temperature of 13.5 °C, and a mean annual rainfall of 1209 mm. Rainfall is abundant during the winter but from June to September severe drought and high temperatures raise the wildfire index to a critical level (Rivas-Martínez 1983).

In this area the soils are over a calcareous substratum and two well differentiated sites, 1 and 2 were selected. A map of the study site location is provided in the Supplementary Information (Fig. S1). The burnt

soils chosen (B: burnt) were affected by a wildfire that was evaluated in the field as causing severe surface burn (Keeley 2009) or a soil burn severity index (SBS) level 3 (Vega et al. 2013). Also control soils, not affected by the fire (C: control), were taken from a nearby area and with similar physiographic characteristics. The soils from site 1 (C1: N 38° 09′ 01.5″; W 2° 47′ 16.3″ and B1: N 38° 9′ 10.4″; W 2° 47′ 18.8″), situated in an upper part of the mountain chain, were classified as rendzina on carbonates; the soils from site 2 (C2: N 38° 10′ 00.6″; W2° 47′ 38.0″ and B2: N 38° 9′ 45.3″; W 2° 47′ 05.5″) situated in a lower part of the mountain chain on a bank of a road running parallel to "El Tranco de Beas" water reservoir, were calcic luvisols. The vegetation cover in the area is dominated by pine (*Pinus pinaster*) and an understory of Mediterranean bushes (*Cystus* spp., *Halimium* spp., *Ulex* spp., *Genista* spp., *Echinospartum* spp. and small aromatics *i.e. Rosmarinus* spp., *Thymus* spp., *Lavandula* spp.). Sampling was carried in September 2005, one month after the wildfire and before any rainfall occurred, then in January 2007 and finally in September 2008. Three samples were taken from each site (both control and burnt soils) within an area of approximately 20 m^2. The soil samples were taken from the A horizon (0–15 cm) after removal of the litter layer, collected in sterile flasks and transported to the laboratory on ice. The three samples from each site were dried at room temperature (23 °C) in a laminar flow cabinet for 18–24 h and sieved to fine earth (2 mm mesh size). Samples were maintained at room temperature for physical and chemical analysis, at 4 °C for biological analysis and at −20 °C for genetic analysis.

2.2. Soil physical and chemical analysis

The physical and chemical properties of the soil samples were determined as previously described (Guitián and Carballas 1976). Soil pH was measured in water (1:2.5 solid: water ratio); soil water holding capacity (WHC) at 33 kPa in a Richard's membrane-plate extractor; total carbon by potassium dichromate oxidation, and total nitrogen content by Kjeldahl digestion. The percentage of organic matter was calculated by multiplying the percentage of total carbon by the empiric factor 1.724 (Walkley 1947).

2.3. Biological properties

2.3.1. Bacterial and fungal quantification

Viable bacteria and fungi were isolated from soil by blending 10 g dry weight soil samples with 95 ml of phosphate buffer 0.1 M, pH 7. A dilution series down to 10^{-6} was made for each sample using phosphate buffer as diluent. Tryptic Soy Agar (TSA) and Sabouraud Agar (Merck) media were used for isolation. Plates were incubated for 7 days at 28 °C, and those dilutions with 30–300 colonies were counted. Counts corresponded to colony forming units (cfu) of bacteria and fungi respectively, calculated as means of three determinations and expressed as per gram of dry soil (cfu/g dwt).

2.3.2. Substrate-induced respiration determination

Substrate-induced respiration (SIR) was measured as described by Pérez-Leblic et al. (2012). Triplicate soil samples from each site (5 g each) adjusted to 50% water holding capacity (WHC) were incubated with glucose (0.45 mg/g soil) for 2 days at 30 °C in sealed flasks. CO_2 concentration was measured by an automated method (Bac-Trac) based on the changes of the impedance of a KOH solution (2%) in a μ-Trac 4200 analyser.

2.3.3. Enzyme activities

The effect of wildfire on soil microbial activity was carried out by assessing the activity of 7 key soil enzyme activities involved in the cycling of carbon (β-glucosidase, cellulase, invertase), nitrogen (urease and β-*N*-acetylglucosaminidase) and phosphorus (acid and alkaline phosphatases), in burnt and control soils. Acid and alkaline phosphatases, β-glucosidase and β-*N*-acetyl-glucosaminidase activities were analysed following the methods developed by Tabatabai (1982). The method described by Hoffmann and Pallauf (1965) modified by García-Álvarez and Ibáñez (1994) was used to determine invertase and cellulose activities. Urease activity was measured as previously described (Kandeler and Gerber 1988). All assays were performed in triplicate.

2.4. Molecular analysis from soil microbial communities

2.4.1. Denaturing gradient gel electrophoresis

To analyse soil prokaryotic populations, denaturing gradient gel electrophoresis (DGGE) separation of PCR-amplified *Bacteria* and *Archaea* 16S rDNA products was performed from soil DNA extracts. Soil DNA was extracted from two sets of 0.5 g of soil samples using a Power Soil DNA Isolation kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instruction. The concentration of extracted DNA was determined using a ND-100 Nanodrop (Thermo Fischer Scientific, USA). The PCR Primers 341F + GC clamp and 907R and 344F+ GC clamp and 915R were used to study *Bacteria* and *Archaea* communities, respectively. Taq polymerase (FideliTaq PCR Master Mix) from Invitrogen (USA) was used in all PCR amplifications. The PCR thermal cycle for *Bacteria* comprised an initial denaturing step at 94 °C for 7 min, followed by 32 cycles of 45 s at 94 °C, 45 s at 49 °C and 1 min and 30 s at 72 °C, and a final extension of 10 min at 72 °C. The *Archaea* PCR reaction was carried out with an initial denaturation step at 94 °C for 5 min, followed by 32 cycles of 45 s at 94 °C, 1 min at 54 °C and 1 min at 72 °C, and a final extension step of 10 min at 72 °C.

DGGE was performed with a D-code Universal Mutation Detection System (Bio Rad laboratories, Hercules, CA, USA). PCR products (between 800 and 1000 ng) were loaded onto 6% (wt/vol) polyacrylamide gels with a linear gradient of 55 to 60% or 50 to 60% denaturant for *Bacteria* and *Archaea,* respectively in 1 X TAE. One-hundred percent denaturant gradient was defined as 7 M urea and 40% (v/v) deionised formamide. Following electrophoresis at 60 V and 60 °C for 18 h, the gels were stained with ethidium bromide, exposed to UV light to visualise the bands and digitalised in a Gel Doc 2000 (BioRad laboratories, Hercules, CA, USA).

2.4.2. 16S rRNA library construction and sequence analysis

The phylogenetic affiliation of the *Bacteria* present in the samples was examined by partially sequencing the 16S rRNA gene. *Bacteria* 16S rRNA genes were amplified from soil DNA by PCR using the primers 27F and 1492R. The PCR thermal cycle for *Bacteria* comprised a hot start at 94 °C for 5 min, followed of 35 cycles of 1 min at 94 °C, 1 min at 55 °C and 2 min at 72 °C, and a final extension of 15 min at 72 °C. PCR-amplified fragments were purified using an Ultraclean 15 DNA Purification Kit (MoBio) and cloned into the pCR 2.1 vector of the Topo TA Cloning Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Competent *E. coli* cells were transformed and the resultant white colonies were screened for the expected size inserts using M13F and M13R primers. Clones were purified with an Ultraclean PCR clean-up Kit (MoBio, USA) and selected for sequencing at the Molecular Biology Service of the University of Alcalá (Madrid, Spain). The sequences obtained were compared to available database sequences using the Ribosomal Database Project for phylogenetic assignment (http://rdp.cme.msu.edu). Sequences with similarities >95% were considered to represent the same taxonomic group.

For *Bacteria* domain, a total of 635 *E. coli* clones (329 and 316 from sites 1 and 2, respectively) were subjected to sequence analysis followed by online homology searches using the RDP database. After discarding sequences that could not be aligned, 292 from site 1 and 263 from site 2 were valid. Only sequences that shared >95% identity with database sequences (C1.05, C1.07 and C1.08, 71, 46, and 36 clones, respectively; B1.05, B1.07 and B1.08, 59, 73 and 51 clones, respectively; C2.05, C2.07 and C2.08, 56, 54 and 32 clones, respectively; B2.05, B2.07 and B2.08, 45, 67 and 51 clones, respectively) were clustered into

operational taxonomic units (OTUs) at the phyla level and used for phylogenetic analysis.

2.5. Statistical analysis

The effect of wildfire on soils physico-chemical characteristics and microbial activity was statistically analysed using factorial ANOVA implemented in Statistica v8 software. An analysis for each dependent variable (pH, WHC, TOC, OM, total nitrogen, viable bacteria and fungi counts, SIR and enzyme activities) was performed. In all analyses, the same factors were included (control and burnt soil, site 1 and 2 and 2005, 2007 and 2008 sampling years). The dependent variables (viable bacteria and fungi) were logarithmically transformed before analysis. Significant differences were evaluated at the level of $p \leq 0.05$. The residuals of all ANOVA analyses had a normal distribution (Kolgomorov-Smirnov test, $p > 0.05$). Differences between groups were analysed using a post-hoc Fisher's test.

Unweighted pair group method (UPGM) cluster analysis using the PAST program (Hammer et al. 2001) was performed to analyse DGGE data, taking into account the Jaccard's similarity measure obtained from absence-presence of bands on the DGGE. Shannon indexes of general diversity were also calculated using the same program.

3. Results and discussion

3.1. Impact of wildfire on the physical and chemical properties of soils

Soil pH was not greatly affected by the wildfire (Table 1). Results obtained from a 3-year chronosequence show a slight but non-significant decrease in the pH values in the burnt soils of site 1 in comparison with unburnt soils. In site 2, pH differences between burnt and control soils were only significant in samples collected in 2008, for which an increase in the pH was detected. A slight reduction in pH could be attributed to organic denaturation, especially in soils that were exposed to temperatures up to 450–500 °C (Certini 2005). Generally, alkaline soils have a higher buffer capacity than acid soils; this may explain the lack of any significant differences in the pH of burnt and control soils. Generally, wildfires do not lead to an increase in pH in soils such as these which are buffered by carbonates (Certini 2005).

In contrast to pH, wildfires resulted in a significant increase in the water holding capacity (WHC) of burnt soils at both sites, even after 3 years when compared with their respective control soils (Table 1). In comparison the WHC of controls soils from both sites showed a significant decrease in 2008 samples, compared to those taken in 2005. In contrast a significant increase in WHC was observed when the burnt soils were compared (Table 1). From these results a positive effect of fire on WHC of soils from both sites could be inferred. The capacity of a soil to retain water is a property dependent on a number of factors. Mataix-Solera and Cerdá (2009a) reported that an increase in organic matter can lead to an increase in soil WHC; our results corroborate this fact; in Cazorla soils, a concomitant increase in both WHC and organic matter in the burnt soils with respect to unburnt soils was observed (Table 1). Wildfires may improve soil structure through "consolidation" of aggregates to form irreversible hydrogen bridges during soil heating. This increased WHC in burnt soils may be, in part due to the presence of wood ash (Pitman 2006).

The increase in both total organic carbon (TOC) and organic matter (OM) in the burnt soils of Cazorla remained 3 years post-burning, and was greatest in soils sampled after 2–3 years. This significant increase in TOC and OM in Cazorla burnt soils may be due to the incorporation into the soil of organic matter derived from partially or totally burnt plant material and/or the leaves of the plants that die as a result of the fire (Knicker et al. 2005). Both TOC and OM parameters were also dependent upon the topographic position of the sampling areas, with site 1 being at a higher elevation.

At both sites, a significant increase in the percentage of total N present in burnt soils was observed (Table 1). The increase in soil total N in burnt soils was likely due to the incorporation of semi-pyrolysed organic materials into the soil (Mataix-Solera and Cerdá, 2009a). As a result of the increased N, the C/N ratio was lower in burnt soils during the first two years after fire but significantly increased in year 3. It is also important to take into account that fire may exert a mineralization effect on soil organic matter (Fernández et al. 1999). Similar results have been reported by a number of authors (Viro 1974; Vega 1986; Almendros et al.

Table 1
Physico-chemical characteristics in Cazorla, Segura y Las Villas soil samples.

	Site	2005		2007		2008	
		Control	Burnt	Control	Burnt	Control	Burnt
pH	1	8.16 ± 0.32 Aa	8.00 ± 0.34 Aa	7.77 ± 0.25 Aa	7.70 ± 0.37 Ab	8.24 ± 0.41 Aa	7.92 ± 0.10 Aa
	2	7.88 ± 0.32 Aa	7.44 ± 0.30 Aa	7.39 ± 0.32 Aab	7.60 ± 0.21 Aa	6.93 ± 0.34 Ab	7.60 ± 0.10 Ba
WHC[a] (%)	1	21.27 ± 1.40 Aa	29.00 ± 0.78 Ba	17.28 ± 0.88 Ab	39.73 ± 1.79 Bb	15.57 ± 0.63 Ab	34.77 ± 1.26 Bc
	2	28.67 ± 0.72 Aa	33.04 ± 1.12 Ba	31.66 ± 1.42 Ab	54.19 ± 0.53 Bb	23.53 ± 0.85 Ac	49.20 ± 1.53 Bc
TOC[b] (%)	1	0.84 ± 0.10 Aa	2.99 ± 0.09 Ba	1.23 ± 0.18 Ab	2.97 ± 0.16 Bb	2.47 ± 0.11 Ac	8.54 ± 0.42 Bc
	2	2.30 ± 0.02 Aa	2.23 ± 0.10 Aa	2.63 ± 0.03 Ab	4.39 ± 0.18 Bb	3.83 ± 0.15 Ac	10.24 ± 0.31 Bc
OM[c] (%)	1	1.45 ± 0.16 Aa	5.15 ± 0.15 Ba	2.13 ± 0.29 Ab	5.12 ± 0.28 Bb	4.25 ± 0.18 Ac	14.73 ± 0.69 Bc
	2	3.99 ± 0.03 Aa	3.84 ± 0.15 Aa	4.53 ± 0.05 Ab	7.56 ± 0.3 Bb	6.61 ± 0.26 Ac	17.66 ± 0.52 Bc
TN (%)	1	0.06 ± 0.01 Aa	0.20 ± 0.01 Ba	0.10 ± 0.01 Ab	0.35 ± 0.02 Bb	0.14 ± 0.01 Ac	0.47 ± 0.01 Bc
	2	0.22 ± 0.01 Aa	0.33 ± 0.01 Ba	0.14 ± 0.01 Ab	0.62 ± 0.01 Bb	0.20 ± 0.01 Aac	0.70 ± 0.02 Bc
C/N	1	14.00	12.92	12.30	8.48	17.64	17.05
	2	10.45	6.75	18.78	7.08	19.15	14.63

Means followed by the same letter are not significantly different. Uppercase compare control soils and burnt soils; lowercase compare the evolution of control soils or burnt soils along different sampling years. (means ± standard deviation; $n = 3$; $p < 0.05$).

[a] Water holding capacity.
[b] Total organic carbon.
[c] Organic matter.

2003). Almendros et al. (2003), Knicker et al. (2005) and De la Rosa et al. (2008) found that the observed initial decrease in the C/N was a result of the formation and accumulation of new recalcitrant heterocyclic N compounds and a more rapid volatilization of carbon compounds.

3.2. Biological characterization of burnt and control soils

Culturable microbial biomass (bacteria and fungi) and microbial activity (assessed through substrate induced respiration) were used as soil health indicators in order to evaluate the effect of wildfire on the soil microbial population (Table 2). The number of both viable bacteria and fungi increased significantly, especially 2–3 years post-burning (Table 2). This result could be attributed to the observed increase in carbon, organic matter and/or nitrogen content in burnt soils as a consequence of the accumulation of labile plant material into the soil (Mataix-Solera and Cerdá, 2009a; Weston and Attiwill 1990). In terms of fungi, the observed significant increase in cfu's in burnt soils may be attributed to the dispersion of spores rather than fungal mycelium, a phenomenon previously observed following thermal shock (Mataix-Solera and Cerdá, 2009a). Comparison among controls from different sampling years showed a significant increase in the number of viable bacteria along the three samplings for sites 1 and 2. Concerning fungi, a significant increase in cfu's was observed between controls from 2005 and 2007 for site 1 but in the case of site 2, a significant increase was detected over the three samplings. When burnt soils were compared a significant increase in viable bacteria and fungi was observed over the three years for site 1 but for site 2 the pattern observed was different for bacteria and fungi (Table 2). Although the number of viable bacteria and fungi in the control soils increased over the three years, this increase is significantly lower than that detected in burnt soils. This confirms that the resilient soil microbial population was able to adapt to the new environmental conditions caused by the wildfire, enabling the soil to deliver ecosystem functions quickly following the wildfire.

There is no consistency in the literature regarding the effect of wildfires on soil microbial biomass. Mataix-Solera and Cerdá (2009b), in a review of similar studies, described significant variations in the results obtained, citing examples where a fire resulted in increased soil microbial biomass, while also referencing work which described either a decrease or no significant variations in burnt soils. Similarly, Choromanska and DeLuca (2002), Bárcenas-Moreno et al. (2011) and Xu et al. (2012) observed a decrease in biomass in burnt soils compared to that recorded in control soils, while García-Villaraco Velasco et al. (2009) reported no significant changes in the bacterial biomass of burnt soils.

In terms of effect of wildfires on soil microbial activity, assessed through SIR significant differences were observed between control and burnt soils. SIR showed a significant increase in burnt soils with respect to the controls through the different samplings periods, with the exception of the first sampled soil from site 2 (Table 2). This result was consistent with the observed decrease in organic matter. A recovery in both SIR and organic matter was observed after two and three years after the wildfire (Table 2).

The increase in respiratory activity observed in the most of burnt soils, compared to the control soils, may be due to an increase in the organic matter content of the burnt soil from both sites. The incorporation of readily biodegradable plant material and the increase in interchangeable cations resulted in enhanced soil microbial growth and consequently, to an increase in SIR. Again in terms of the effect of wildfires on soil microbial activity no consistent trend has been reported. In a study by Wüthrich et al. (2002) on the effect of low and high severity fires on soil microbial biomass, no significant differences were observed between control soils and those burnt at low fire severity. However, in high severity fires, an increase in respiratory activity in soils taken 20 h after the fire, and in soils sampled several months later was reported. In contrast, in a study carried out by Jiménez et al. (2007) on the effects of prescribed fires, in half of the soil samples, respiratory activity was reduced in the burnt soils when compared to respiration rates recorded in control soils. In the remainder of the soil samples, no differences were observed in respiratory activity between the control and burnt soils.

Generally, enzyme activities were higher in soils from site 2 compared with those from site 1 (Table 3). This is consistent with the higher content of total carbon, organic matter and microbial biomass in the calcic luvisol from site 2.

In all soils, burnt and unburnt, alkaline phosphatase activity was higher than acid phosphatase, as expected given the alkaline nature of the soils (Table 3). However the activities of both phosphatases were significantly higher in burnt soils when compared to their respective control soils. Although similar results have been reported previously, generally in the literature no clear relationship between phosphatase activity and fires could be found. Thus, while Eivasi and Bayan (1996) and Boerner and Brinkman (2003) reported a decrease in acid phosphatase activity in burnt soils compared to control soils, Aiwa et al. (1999) and Boerner et al. (2005) reported increased acid phosphatase activity in burnt soils. An increase in alkaline phosphatase activity was reported in another Mediterranean burnt soils (Rodríguez et al. 2017); this could be explained in terms of a release of organic phosphorus from dead organisms and plants resulting in induction of this enzyme as previously described (Nannipieri et al. 2010).

The response to the wildfire in terms of soil β-glucosidase activity was significantly different between burnt and control soils but no defined pattern was found when comparing sites and sampling time. Gutknecht et al. (2010) reported a decrease in soil β-glucosidase in

Table 2
Biological parameters in Cazorla, Segura y Las Villas soil samples.*

	Site	2005		2007		2008	
		Control	Burnt	Control	Burnt	Control	Burnt
Bacteria (cfu/g) × 10^6	1	1.46 ± 0.09 Aa	1.5 ± 0.10 Aa	4.75 ± 0.18 Ab	13.2 ± 0.90 Bb	12.4 ± 0.11 Ac	31.8 ± 1.50 Bc
	2	4.25 ± 0.30 Aa	13.9 ± 0.87 Ba	1.85 ± 0.20 Ab	13.7 ± 1.00 Ba	5.9 ± 0.34 Ac	23.4 ± 6.30 Bb
Fungi (cfu/g) × 10^4	1	0.6 ± 0.03 Aa	2.4 ± 0.08 Ba	2.10 ± 0.11 Ab	12.3 ± 0.10 Bb	1.70 ± 0.13 Ab	9.72 ± 0.32 Bc
	2	1.7 ± 0.07 Aa	5.95 ± 0.30 Ba	2.6 ± 0.21 Ab	10.0 ± 0.3 Bb	7.70 ± 0.35 Ac	9.81 ± 0.40 Bb
SIR*	1	12.00 ± 1.50 Aa	42.60 ± 1.10 Ba	30.66 ± 3.05 Ab	45.96 ± 1.20 Bab	32.66 ± 6.43 Ab	50.60 ± 5.03 Bb
	2	51.20 ± 4.60 Aa	34.60 ± 1.90 Ba	45.66 ± 2.08 Ab	48.58 ± 2.05 Ab	36.00 ± 4.00 Ac	44.60 ± 1.95 Bb

Means followed by the same letter are not significantly different. Uppercase compare control soils with burnt soils; lowercase compare the evolution of control soils or burnt soils along different sampling years. (means ± standard deviation; $n = 3$; $p < 0.05$).

* Substrate induced respiration (mg CO_2/h/100 g dry soil).

Table 3
Enzyme activities (U[a]/g soil) in Cazorla, Segura y las Villas soil samples.

	Site	2005		2007		2008	
		Control	Burnt	Control	Burnt	Control	Burnt
Acid phosphatase	1	0.72 ± 0.01 Aa	1.63 ± 0.05 Ba	1.76 ± 0.06 Ab	1.11 ± 0.03	1.16 ± 0.08 Ac	1.67 ± 0.08
	2	2.32 ± 0.08 Aa	2.96 ± 0.09 Ba	1.99 ± 0.07 Ab	Bb 2.82 ± 0.09 Bb	1.8 ± 0.09 Ac	Ba 2.16 ± 0.07 Bc
Alkaline phosphatase	1	1.75 ± 0.09 Aa	3.65 ± 0.12 Ba	5.70 ± 0.19 Ab	2.26 ± 0.08	3.37 ± 0.20 Ac	4.22 ± 0.21
	2	4.70 ± 0.17 Aa	5.01 ± 0.18 Aa	4.79 ± 0.17 Aa	Bb 6.93 ± 0.25 Bb	3.56 ± 0.20 Ab	Bc 6.29 ± 0.30 Bc
β-glucosidase	1	0.56 ± 0.01 Aa	1.70 ± 0.07 Ba	3.99 ± 0.09 Ab	1.13 ± 0.11	2.82 ± 0.11 Ac	1.51 ± 0.09
	2	2.20 ± 0.07 Aa	1.40 ± 0.05 Ba	1.62 ± 0.10 Ab	Bb 1.78 ± 0.10 Bb	1.66 ± 0.06 Ab	Bc 1.80 ± 0.09 Ab
β-*N*-acetyl-glucosaminidase	1	0.09 ± 0.01 Aa	0.34 ± 0.01 Ba	0.38 ± 0.01 Ab	0.34 ± 0.01	0.20 ± 0.01 Ac	0.19 ± 0.01
	2	0.40 ± 0.01 Aa	0.59 ± 0.01 Ba	0.48 ± 0.01 Ab	Ba 0.52 ± 0.01 Bb	0.37 ± 0.01 Ac	Ab 0.24 ± 0.01 Bc
Cellulase	1	0.09 ± 0.01 Aa	0.08 ± 0.01 Aa	0.23 ± 0.01 Ab	0.12 ± 0.01	0.28 ± 0.01 Ac	0.39 ± 0.01
	2	0.13 ± 0.01 Aa	0.11 ± 0.01 Ba	0.21 ± 0.01 Ab	Bb 0.31 ± 0.02 Bb	0.17 ± 0.01 Ac	Bc 0.33 ± 0.01 Bc
Invertase	1	13.31 ± 0.5 Aa	26.37 ± 0.90	17.31 ± 0.42	4.64 ± 0.10	12.97 ± 0.51	7.16 ± 0.35
	2	17.16 ± 0.06 Aa	Ba 21.85 ± 0.70 Ba	Ab 6.85 ± 0.20 Ab	Bb 4.8 ± 0.10 Bb	Aa 12.55 ± 0.62 Ac	Bc 5.71 ± 0.28 Bc
Urease	1	0.58 ± 0.02 Aa	2.40 ± 0.10 Ba	6.78 ± 0.24 Ab	1.04 ± 0.05	8.03 ± 0.4 Ac	2.83 ± 0.10
	2	2.70 ± 0.10 Aa	2.30 ± 0.10 Ba	2.74 ± 0.13 Aa	Bb 2.03 ± 0.10 Ba	3.07 ± 0.15 Ab	Bc 3.18 ± 0.15 Ab

Means within a row followed by the same letter are not significantly different. Uppercase compare control soils with burnt soils; lowercase compare the evolution of control soils or burnt soils along different sampling years. (means ± standard deviation; $n = 3$; $p < 0.05$).
[a] μmol/h.

50–75% in soils burnt. In contrast Boerner et al. (2000) observed no significant differences in β-glucosidase activity between burnt and unburnt soils.

For cellulase activity, in the first sampling at both sites the activity in both control and burnt soils was similar. However, during the third sampling period the burnt soils showed a significant increase in the activity compared with that detected in the control soils. This may indicate a gradual recovery in the fungal populations as seen in Table 2.

For soil invertase activity, a significant increase in burnt compared with control soils was observed during the first sampling, while in the two following samples the activity of the burnt soils was significantly lower than that of the control soils.

In terms of the effect of wildfire on β-*N*-acetylglucosaminidase and urease activities (Table 3), significant differences were observed between burnt and control soils. In the case of β-*N*-acetylglucosaminidase activity, a significant increase in burnt soils was detected compared to the control in the first sampling but in the following samplings, when comparing sites and time of sampling, a different pattern was observed. β-*N*-acetylglucosaminidase may be involved in microbial N-acquiring activities (Sinsabaugh and Moorhead 1994). In addition, this activity has been proposed as a semi-quantitative indicator for soil fungal biomass (Miller et al. 1998) due to the fact that chitin is a major component of the fungal cell wall. However, the results obtained here do not show a clear relationship between fungal biomass (cfu's) and β-*N*-acetylglucosaminidase activity.

Urease activity was significantly decreased in most burnt soils compared to the respective controls; This is in line with previous studies that found a decrease in urease activity in Mediterranean forest soils affected by wildfires (Hernández et al. 1997; Boerner and Brinkman 2003; Ekinci 2006).

3.3. Effect of wildfire on soil microbial community through denaturing gradient gel electrophoresis (DGGE)

To assess the impact of fire on the soil microbial community together with subsequent changes following the fire, *Bacteria* and *Archaea* community profiles in burnt and control soils were studied through DGGE analysis of amplified 16S rDNA fragments. For both burnt and control soils, the denaturing gradient of urea-formamide most suitable for the separation of DNA fragments was found to be 55 to 60% for *Bacteria* domain and 50 to 60% for *Archaea* domain.

A total of 21 different *Bacteria* bands were detected. Fewer bands were detected in site 1 than in site 2; while the lowest number of bands correspond to the control soil from the first sampling of site 1 (8 bands), the highest number (17 bands) correspond to the final two samplings of control soil from site 2.

A total of 20 different *Archaea* bands were observed in the soil samples analysed. The number of bands observed in each of the analysed samples was similar (between 8 and 9 bands, on average). This result does not show a common effect of fire on bacterial and archaeal communities, in contrast to that described by Mikita-Barbato et al. (2015).

Shannon indexes were calculated from the DNA banding patterns, taking into account both the intensity as well as the number of bands (Table 4). An increase in the Shannon index for the *Bacteria*

Table 4
Shannon indexes (´H) calculated from *Bacteria* and *Archaea* denaturing gradient gel electrophoresis (DGGE profiles).

	Site	2005		2007		2008	
		Control	Burnt	Control	Burnt	Control	Burnt
Bacteria	1	2.02	2.25	2.51	2.25	2.51	2.51
	2	2.65	2.58	2.78	2.34	2.78	2.58
Archaea	1	2.02	2.20	2.37	2.20	1.95	2.20
	2	2.08	2.04	2.08	2.20	2.20	2.04

domain was observed in burnt soils compared with control soil, but only for the first sampling at site 1. Interestingly the lowest diversity was observed in control soil sample taken in 2005 (2.02) whilst a burnt soil sample taken during the final sampling year (2008) recorded the same diversity value than the corresponding control samples from the final two sampling years (2.51). At site 2, there was a decrease in the Shannon index in burnt soil; the lowest diversity recorded was 2.34 in burnt soil t sampled in 2007; the highest diversity was found in samples from the second and third sampling periods (Table 4). *Archaea* diversity was higher in burnt soils from site 1 than in the control soil, but only in the first and third samplings compared with their respective controls. In contrast, in site 2, the highest archaea diversity was recorded in the control from the third sampling and burnt soils from the second sampling (2.20) (Table 4). Overall, comparing bacterial diversity between the two sites, the diversity was higher at site 2 than site 1. In the case of *Archaea* the results obtained did not show a defined pattern.

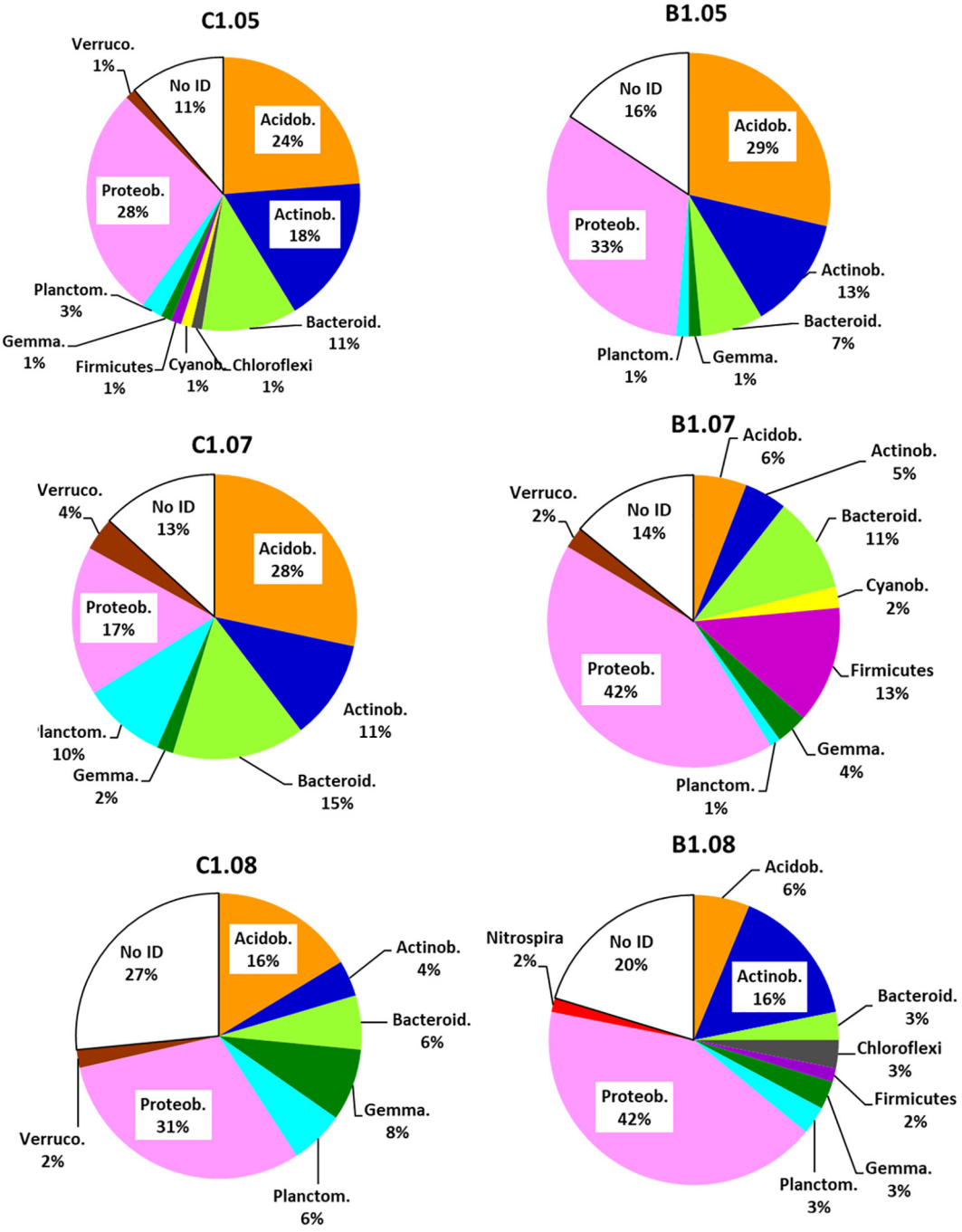

Fig. 1. Percentage distribution of *phyla* obtained from clone libraries analysis from the Cazorla, Segura and Las Villas Natural Park soil samples. C1.05, C1.07, C1.08: control soils from site 1 sampled in 2005, 2007 and 2008, respectively. B1.05, B1.07 and B1.08: burnt soils from site 1 sampled in 2005, 2007 and 2008, respectively.

3.4. Microbial clone library analysis

To further assess the impact of wildfires on the soil microbial community, analysis of the bacterial 16S rRNA gene clone libraries was carried out. Distribution abundance (%) of *phyla* identified in burnt and control soils from both sites is presented in Figs. 1 and 2.

At site 1 (Fig. 1), 10 different *phyla* were identified in the control sample (C1) while in the two following control samplings only 7 were identified. In burnt soils a notable decrease in the number of phyla was observed in the first sampling (B1.05), but after two and three years an increase in the number of *phyla* compared with their respective controls were detected (C1.07 and C1.08, 7 phyla; B1.07, 9 phyla and B1.08, 9 phyla).

Most of bacterial sequences were classified as *Proteobacteria* (28–42%), *Acidobacteria* (6–29%), *Actinobacteria* (5–18%) *phyla,* and in sample B1.07 and B1.08 also *Firmicutes* (13 and 2%, respectively). Interestingly, in the *phyla* from burnt soil samples taken one month after the wildfire (B1.05) *Chloroflexi*, *Cyanobacteria*, *Firmicutes* and *Verrucomicrobia* could not be detected while a significant increase in the percentage of *Proteobacteria* and *Acidobacteria* was observed. A recovery in the number of phyla was observed in samples taken 2 and 3 years after the fire. Most notable was an increase in *Proteobacteria*

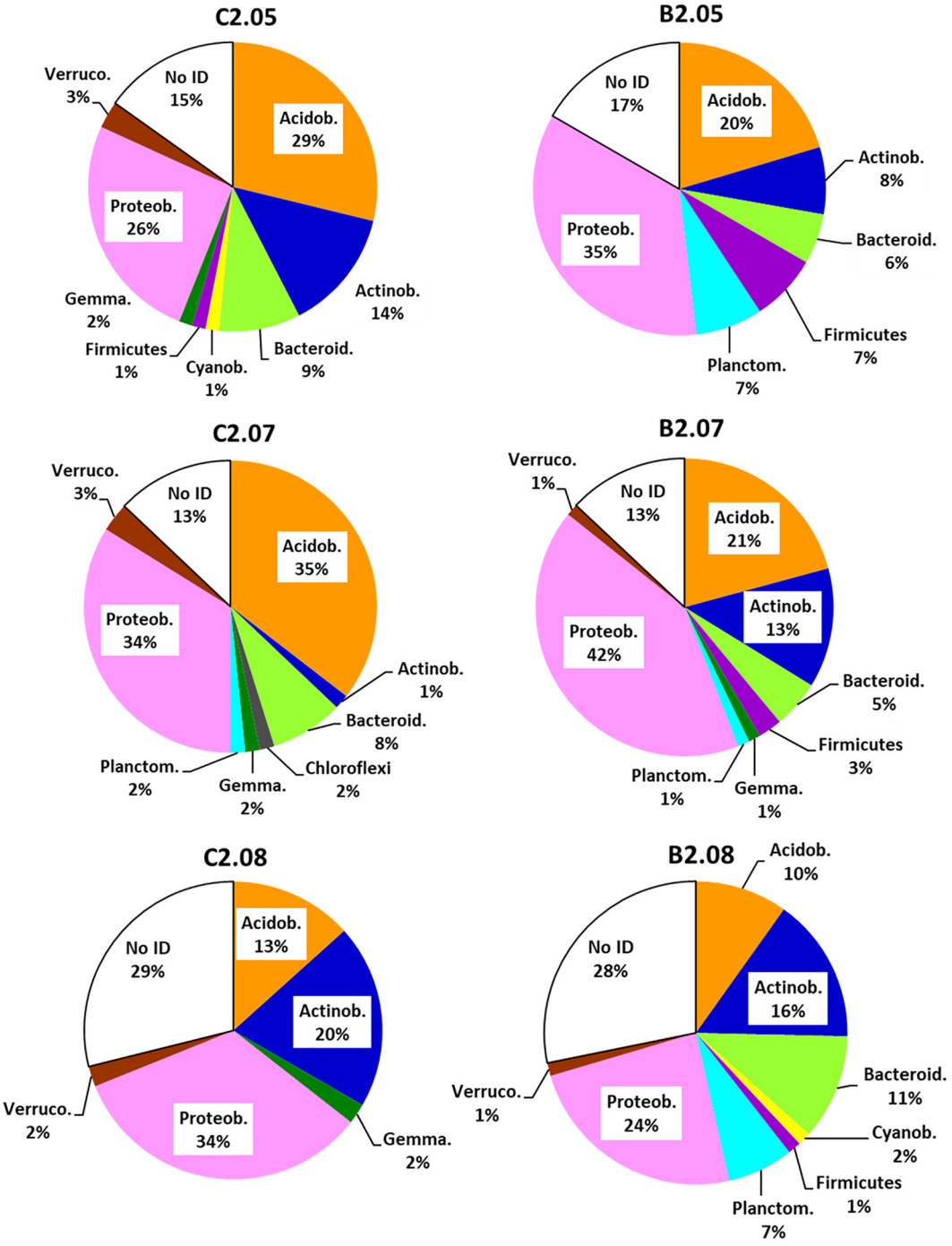

Fig. 2. Percentage distribution of *phyla* obtained from clone libraries analysis from the Cazorla, Segura and Las Villas Natural Park soil samples. C2.05, C2.07, C2.08: control soils from site 2 sampled in 2005, 2007 and 2008, respectively. B2.05, B2.07 and B2.08: burnt soils from site 2 sampled in 2005, 2007 and 2008, respectively.

after 2 and 3 years of the wildfire (17 to 42% for C1.07 and B1.07, respectively; 31 to 42%, respectively) and a decrease in *Acidobacteria* (28 to 6% for C1.07 and B1.07, respectively). *Firmicutes* were only detected in burnt soils corresponding to sample B1.07 (13%) and B1.08 (2%). The *Actinobacteria* phylum was detected in all samples, but showed increased presence in C1.05 and B1.08 (18 and 16%, respectively) (Fig. 1). In previous studies also using analysis of 16S rRNA gene clone libraries an increase in *Firmicutes* in burnt soils (Belova et al. 2014) compared with control soil was also reported (Ferrenberg et al., 2014; Prendergast-Miller et al. 2017).

For site 2, a recovery in the number of phyla over time was observed in burnt soils, as noted for site 1 (Fig. 2). These results suggest that fire exerts an initial impact on the soil microbiota, although the structural and functional profile of the microbial communities seems to be recovered after the fire along the period considered.

In burnt soil from site 2 (Fig. 2), the phylum *Proteobacteria* was more dominant that in control soils in samples taken in 2005 and 2007 (Fig. 2). In contrast, a significant decrease in the percentage of *Acidobacteria* in burnt soils compared to the respective control soils was detected. An initial decrease in the percentage of *Actinobacteria* in burnt soil B2.05 compared with the control was observed (14 to 8% for C2.05 and B2.05, respectively), although this percentage increased in later years in both control and burnt soils.

Concluding, in most burnt soils (sites 1 and 2) an increase in both *Proteobacteria* and *Firmicutes* was observed, together with a decrease in *Acidobacteria* phyla compared to their respective control soils. In terms of *Bacteroidetes*, the percentages were generally lower in most burnt samples compared with their abundance in the control samples. Previous studies using 16S rRNA gene clone library analysis, on soils from a mesotrophic peatland affected by fire also reported a decrease in *Bacteroidetes* (Belova et al. 2014).

The percentage distribution of *Proteobacteria* groups is presented in Fig. 3. *α-Proteobacteria* and *β-Proteobacteria* were the most abundant groups in both control and burnt soils from sites 1 and 2; γ- and δ-*Proteobacteria* represent <10% of the phylum. This result has been also reported by Rodríguez et al. (2017) in other Mediterranean burnt and control soils from Andalusia. At site 1 a more detailed analysis reveals a decrease in *β-Proteobacteria* from burnt soil sample taken during the first sampling (22%) in comparison with the control (29%), but an important recovery in the percentage of *β-Proteobacteria* was observed in samples taken during the following samplings (B1.07, 58%; B1.08, 48%). At site 2, a similar pattern was observed but related to *α-Proteobacteria* (B2.05, 27%; B2.07, 56%; B2.08, 57%) in comparison with the control soil (45%). Increased variability in *Proteobacteria* in some of the analysed burnt soils was observed as previously described (Rodríguez et al. 2017) for other Andalusian burnt area, but greater variability in the behaviour of this group towards fire has been also reported (Kim et al., 2004b; Smith et al. 2008; Belova et al. 2014).

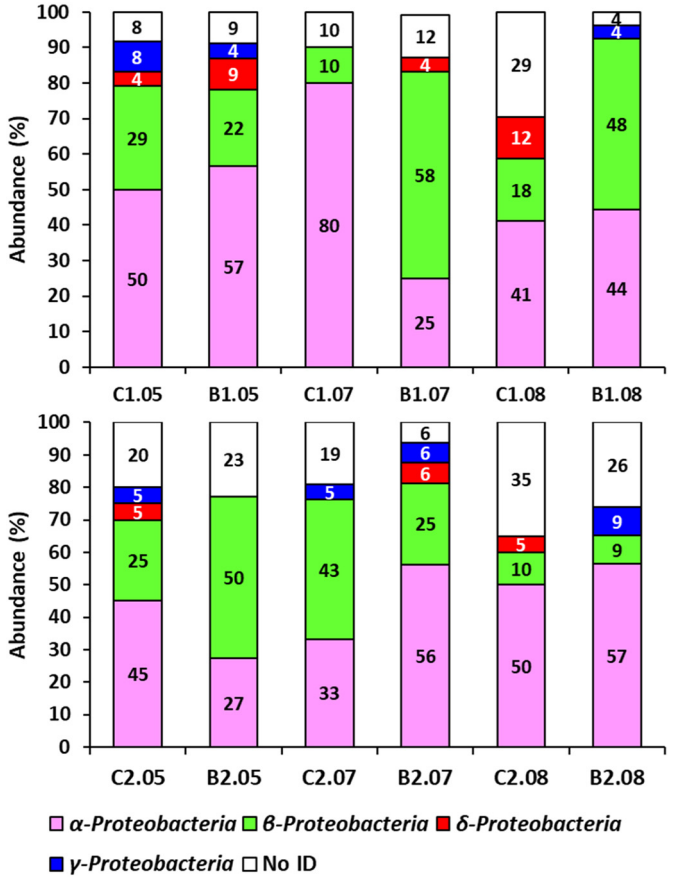

Fig. 3. Percentage distribution of Proteobacteria sub-groups from the Cazorla, Segura and Las Villas Natural Park soil samples. C1.05, C1.07, C1.08: control soils from site 1 sampled in 2005, 2007 and 2008, respectively. C2.05, C2.07, C2.08: control soils from site 2 sampled in 2005, 2007 and 2008, respectively. B1.05, B1.07 and B1.08: burnt soils from site 1 sampled in 2005, 2007 and 2008, respectively. B2.05, B2.07 and B2.08: burnt soils from site 2 sampled in 2005, 2007 and 2008, respectively.

4. Conclusions

The impact of a wildfire at two differentiated sites at the Cazorla, Segura and Las Villas Natural Park, Andalusia, Spain revealed a significant increase in total organic carbon, total nitrogen and organic matter in the burnt soils, which remained 3 years post-burning, suggesting that in spite of the severity of the wildfire, the impact on the soil physico-chemical characteristics was not high. The microbial community responded to fire by increased biomass and SIR, with both viable bacterial and fungal counts rising especially 2–3 years post-burning. In terms of soil enzyme activities, of the seven enzymes studied only phosphatase activity was significantly higher in most burnt soils over the three years. Analysis of the bacterial community diversity using clone libraries showed a recovery of the number of phyla in burnt soils one year after the fire onwards, in both sites. In addition at both sites an increase in *Proteobacteria* and *Firmicutes* was observed together with a decrease of the *Acidobacteria phyla* compared to the control soils. The response to wildfire by the soil microbial community was not found to be solely site dependent. Nevertheless, this study has revealed that if the wildfires lead to increased availability of organic matter the microbial community will respond with increased activity and biomass production. Our results suggest that soil microbial population has the ability to rapidly adapt to the soil environmental changes caused by fire by modulating both its composition and metabolic functions. Soil microbiota plays a key role on ecosystem resilience and, although further research effort is needed, we foresee that rehabilitation practices directed to SOM restoration may greatly benefit post fire soil recovery.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:https://doi.org/10.1016/j.scitotenv.2018.04.028. These data include Google map of the most important areas described in this article.

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