Postprint of: Catena (158): 82-88 (2017)

WILDFIRE EFFECTS ON THE MICROBIAL ACTIVITY AND DIVERSITY IN A MEDITERRANEAN FOREST SOIL

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Abstract

A set of chemical, physical and microbial characteristics of different burnt soils from Sierra de Aznalcóllar (Sevilla, Spain) affected by one or two sequential fires, were analysed and compared with those of their respective control soils. A decrease in total organic carbon was observed in burnt soils, which could be attributed to the impact of the fires on vegetation cover. Biomass (estimated as viable and culturable microorganisms), substrate-induced respiration (SIR) and activity of different soil enzymes involved in carbon, nitrogen and phosphorus cycles were determined to assess

the effect of fire on total microbial populations and on soil activity. An increase in both bacterial and fungal biomass as well as respiratory activity was detected in most burnt soils. In terms of enzyme activity, no common pattern of behaviour was observed, except for the alkaline phosphatase activity, which showed increased levels in all the burnt soils. The effect of fire on microbial diversity was estimated for *Bacteria* and *Archaea* domains from DNA band patterns obtained in denaturing gradient gel electrophoresis (DGGE), as well as using 16S rRNA cloned sequences for *Bacteria*. Shannon index values obtained from the DGGE profiles showed higher diversity for both *Bacteria* and *Archaea* domains in burnt soils compared with the control ones. Variations in the number of different phyla present in burnt and control soils were inferred from the analysis of the 16S rRNA cloned sequences. However, in all areas the most important groups identified belonged to the *Proteobacteria*, *Acidobacteria* and *Actinobacteria* phyla. No differences between microbial communities present in burnt soils at the genus level were detected.

Keywords:

Wildfires, soil microbial activity, soil microbial diversity, DGGE, clone libraries

1. Introduction

Wildfires are considered one of the main disturbances in Mediterranean forest ecosystems (González-Pérez et al., 2008). They exert non-desirable effects on soil health and quality because of the destruction of vegetation cover and important changes in physical, chemical and biological properties of soil (González-Pérez et al., 2004; Gómez-Rey et al., 2013). The soil degradation induced by fire favours the occurrence of erosive processes and nutrient losses causing alterations in the normal biological cycling

of nutrients (Certini, 2005; Fernández et al., 2007). However, it is difficult to generalise about the effects of wildfires on soil due to the variability of factors involved (*i.e*. severity and frequency of fires, pre- and post-fire climatic conditions, topography of site, amount and nature of live and dead fuel). In some cases fire causes dramatic alterations in soil structure and function but in others the effect is reduced to a slight burning of the aerial parts of vegetation, resulting in "fertilisation" of the soil and a change in the trophic status of certain elements captured in vegetation. The study of the effects of wildfires on soils using different approaches is required to design effective rehabilitation strategies after the fire event (Mataix-Solera and Cerdá, 2009b).

Biochemical and microbiological soil properties are most responsive to disturbances caused by fire (Xu et al., 2012) and their analysis can be very useful in assessing the health of soils affected by fire (Mataix-Solera and Cerdá, 2009a; Kara and Bolat, 2009; Mataix-Solera et al., 2009). Microorganisms are central for ecosystem functioning; any reduction in microbial biodiversity not only reduces genetic resources, but also ecosystem productivity and alters its ability to buffer against disturbances. In addition, soil microbial communities mediate the decomposition of organic matter and nutrient cycling, playing an important role in the regeneration of degraded ecosystems. Therefore, the study of microbial soil communities allows greater understanding of soil health and thus provides valuable information that enables the effective restoration of degraded ecosystems (Arias et al., 2005). In addition, the understanding of the resistance and resilience of soil microbial communities to climate change and associated disturbances, such as wildfire, currently represents a subject of increasing interest.

Although several researchers have reported data about total microbial biomass and activity in burnt soils, there is confusing concerning the real impact of fire on microbial populations. While some studies demonstrate a decline in total soil microbial biomass

after a wildfire (Dooley and Treseder, 2012) others reported an increase in microbial biomass post-fire (Goberna et al., 2012) or no significant differences when comparing non-burnt and burnt soils (Hamman et al., 2007). However, most research about the effects of fire on microbial communities has focused on soil mineral horizons, not the organic horizons (Mikita-Barbato et al., 2015).

The main objective of this study was to evaluate the effect of wildfires on soil biological properties (microbial activity and diversity) from forest soils using both culture-dependent as well as molecular DNA-based techniques in order to assess the effect of fire on the distribution of specific bacterial communities within a complex ecosystem such as soil.

We hypothesised that the impact of wildfire on soil microbial communities would produce important changes in their activity and diversity allowing the use of these parameters as markers of soil health.

2. Materials and Methods

2.1. Studied site and sampling

The selected study site was located in Sierra de Aznalcóllar, Seville province, Southern Spain. The area is under a Mediterranean climate where forest fires are frequent during the summer time. The soils are Dystric Cambisols on schists (ISSS Working Group, 1998) developed under cork (*Quercus suber*) and pine (*Pinus pinea*) forests and with scarce Mediterranean bush vegetation. The climate in the area is Mediterranean; the average annual temperature ranges from 16 to 18 ^{0} C and annual rainfall is 559 mm. Precipitation occurs mainly from October to May with a very dry and warm period between June and September (<10 mm and >25 ^{0} C) that favours the occurrence of wildfires.

Soils affected by high severity wildfires and nearby unburnt soils with the same physiographic characteristics were selected for sampling. Two samples QQ1 and QQ2 (N 37º 34' 21'', W 6º 22' 20.3'' and N 37º 35' 32.9'', W 6º 22' 16'' respectively) correspond to burnt soils affected by the same sequential wildfire that occurred in August 1997 and in July 2004; the sample Q3 (N 37º 34' 18.3'', W 6º 18' 30.3'') corresponds to a soil burnt only once during the July 2004 wildfire event. A nearby soil with no recent history of forest fire (N 37º 34' 12.9'', W 6º 23' 34.1'') was used as control (C). Sampling was carried out one year after the last fire by taking three sub-samples 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 sub-samples from each site were dried at room temperature and sieved to fine earth (2 mm mesh size). Samples were maintained at room temperature for physical and chemical analysis, at 4º C for biological analysis and at -20ºC for genetic analysis.

2.2. Soil physical and chemical characterisation

Soil pH values were measured in a water slurry (1:2.5 solid:liquid ratio). Water holding capacity (WHC), total carbon and organic matter were determined following the standard methods described by Pérez-Leblic et al. (2012). The total nitrogen content was estimated by the Kjeldahl method (Kjeldahl, 1883).

2.3. Soil biological characterisation

2.3.1. Viable microorganism's quantification

For this study 10 g dry weight soil samples were vigorously mixed with 95 mL of phosphate buffer 0.1 M, pH 7. Aliquots of this slurry were inoculated on 1:10 diluted

Tryptic Soy Agar (TSA) and on solid Oxytetracycline-Glucose-Yeast Extract (OGYE) media for colony-forming units (cfu) determination of bacteria and fungi, respectively (Elsas and Smalla, 1997; Tanner, 1997). Three plates per dilution were incubated for 7 days at 28 °C and plates showing 30 - 300 colonies were counted. Counts were estimated as the means of three determinations and expressed as colony forming units per gram of oven-dried soil (cfu/g dwt).

2.3.2. Soil substrate-induced respiration (SIR)

Soil respiration was determined in sealed 250 mL sterile flasks containing 15 g soil, 15 mL sterile distilled water and 0.35 g talcum and glucose (4 mg/g soil). The flasks were incubated at 28°C for 6 hours and the CO_2 evolved was measured in a CO_2 detector 1440 Gas Analyser at 0, 3 and 6 hours. The results were expressed as % CO_2 / h / 100 g of soil (Hernández and García, 2003).

2.3.3. Soil enzyme activities

Acid and alkaline phosphatases, ß-glucosidase and ß-N-acetyl-glucosaminidase activities were determined following the methods described in Tabatabai (1982). Invertase and cellulase activities were determined according to Hoffmann and Pallauf method (1965) modified by García Álvarez and Ibáñez (1994) and urease activity as described by Kandeler and Gerber (1988).

2.4. Soil microbial community analysis

2.4.1. *Bacteria* and *Archaea* fingerprinting

DNA was extracted from two sets of 0.5 g of soil samples using the MoBio Powersoil DNA extraction kit (MoBio Laboratories Inc., Carlsbad, CA, USA) according to the

152 manufacturer`s instructions. Extracted DNA concentration was determined in a
153 spectrophotometer ND-100 Nanodrop (Thermo Fischer Scientific, USA). *Bacteria* and
154 *Archaea* 16S rRNA genes were amplified from DNA samples by PCR and then
155 subjected to analysis by DGGE. Primers 341F + GC clamp and 907R and 344F+ GC
156 clamp and 915R were used to study *Bacteria* and *Archaea* community profiles,
157 respectively. The Taq polymerase (FideliTaq PCR Master Mix) used for all PCR
158 amplification was from Invitrogen (USA). The PCR thermal cycle for *Bacteria*
159 comprised a hot start at 94 °C for 7 min, followed of 32 cycles of 45 s at 94 °C, 45 s at
160 49 °C and 1 min and 30 s at 72 °C, and a final extension of 10 min at 72 °C. The
161 *Archaea* PCR reaction was carried out with an initial denaturation step at 94 °C for 5
162 min, followed of 32 cycles of 45 s at 94 °C, 1 min at 54 °C and 1 min at 72 °C, and a
163 final extension step of 10 min at 72 °C.
164 Denaturing Gradient Gel Electrophoresis (DGGE) was performed with a D-code
165 Universal Mutation Detection System (Bio Rad laboratories, Hercules, CA, USA). PCR
166 products (between 800 -1000 ng) were loaded into 6 % (wt/vol) polyacrylamide gels
167 with a linear gradient of 55 to 60 % or 50 to 65 % denaturant for *Bacteria* and *Archaea*,
168 respectively in 1 X TAE. The 100 % denaturant gradient was defined as 7 M urea and
169 40 % (v/v) deionised formamide. After electrophoresis at 60 V and 60 ºC for 18 h,
170 bands were visualised by staining the gels with ethidium bromide (50 µg/mL) for 20
171 min and destaining in deionised water for 40 min. The gels were exposed to UV light to
172 visualise the bands and digitalised in a Gel Doc 2000 (BioRad laboratories, Hercules,
173 CA, USA).
174 UPGM cluster analysis using the PAST program (Hammer et al., 2001) was carried out
175 using the DGGE banding profiles. The Jaccard´s similarity measure was obtained from
176 the absence-presence of bands. Similarities between the banding profiles were also

177 displayed graphically as a dendrogram. Shannon indexes of general diversity were also
178 calculated using the same program.
179
180 **2.4.2. 16S rRNA gene clone library and sequence analysis**
181 The phylogenetic affiliation of the *Bacteria* present in the samples was examined by
182 partially sequencing the 16S rRNA gene. *Bacteria* 16S rRNA genes were amplified
183 from soil DNA by PCR using the primers 27F and 1492R. The PCR thermal cycle for
184 *Bacteria* comprised a hot start at 94 °C for 5 min, followed by 35 cycles of 1 min at 94
185 °C, 1 min at 55 °C and 2 min at 72 °C, and a final extension of 15 min at 72 °C. The
186 PCR-amplified DNA fragments were cloned into the pCR 2.1 vector of the Topo TA
187 Cloning Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol.
188 Then, competent *E. coli* cells were transformed and plated. White colonies were
189 screened for inserts of the expected size by using primers M13F and M13R. Clones
190 were purified with an Ultraclean PCR clean-up Kit (MO BIO, USA) and selected for
191 sequencing at the Molecular Biology Service of the University of Alcalá de Henares
192 (Madrid), Spain. The sequences obtained were compared with available database
193 sequences using the Ribosomal Database Project (RDP) for phylogenetic assignment
194 (Cole et al., 2014). Sequences with similarities >95 % were considered to represent the
195 same taxonomic group. Shannon index of general diversity were determined using the
196 PAST program (Hammer et al., 2001).
197 For the *Bacteria* domain, a total of 327 *E. coli* clones were subjected to sequence
198 analysis followed by online homology searches using the RDP database. From those
199 only 257 were valid, once sequences that could not be aligned or produced alignments
200 that were too short or too long were removed. Only the sequences that shared more than
201 95 % of identity with those database sequences (C, 58 clones; QQ1, 60 clones; QQ2, 57

clones; Q3, 39 clones) were clustered into operational taxonomic units (OTUs) at the phyla level and used for phylogenetic analysis. To assess whether the number of studied sequences was sufficient to determine the microbial diversity of each sample (at phyla level) rarefaction curves were performed for each soil sample library. Because all communities contain a finite number of species, the curves tend to stabilise when all groups present have been detected. In this way we can compare the taxonomic diversity of different size samples.

2.4.3. Statistical analysis

The results were statistically analysed using ANOVA. A post-hoc Tukey´s test was performed to establish significant statistical differences among groups of samples. Data were analysed using STATGRAPHICS program, and significant differences were considered at $p < 0.05$.

3. Results and discussion

3.1. Physical and chemical characteristics of burnt and control soils

The general physical and chemical characteristics of the soils are shown in Table 1. The pH of burnt soils was significantly higher than that of the control soil. This is a well-known effect after forest fires (Arocena and Opio, 2003; Gómez-Rey et al., 2013; Pourreza et al., 2014) and could be best explained by the liming effect known to be caused by the release of basic ions supplied by the ashes from the burnt vegetation. Organic acid denaturation could also contribute to this effect (Certini, 2005). In terms of soil water holding capacity (WHC), only the twice burnt soil sample QQ2 showed a significantly higher value than that of the unburned soil (C). Also, a significant decrease in organic matter (OM) and total organic carbon (TOC) was observed in burnt soils

227 compared with control soil; this could be attributed to the characteristics of the wildfire.
228 In fact, the effect of wildfire on these parameters is usually related to the intensity and
229 severity of the fire (Mataix-Solera and Cerdá 2009a) and the behaviour observed in the
230 Aznalcóllar burnt soils seems typical of soil samples where high-intensity fires or
231 successive fires occurred (*i.e.* QQ1 and QQ2 areas). It is also known that changes in
232 organic matter due to fires modify soil texture and in turn can affect the water retention
233 in soils (Weber et al., 2014) as observed in this study for the QQ2 area.
234 The significant increase in the percentage of nitrogen (N) in the burnt soil samples QQ1
235 and Q3 in comparison with the control soil (Table 1) could be due to the incorporation
236 of semi-pyrolysed materials (de la Rosa et al., 2008). The C/N ratio values were lower
237 in the burnt soils than in control soil. Similar results have been previously observed
238 (Almendros et al., 1984a, 1984b, 2003). Indeed, it was also reported that the decrease in
239 the C/N ratio was due to the formation and accumulation of new forms of recalcitrant
240 heterocyclic nitrogen and to volatilisation of organic carbon compounds (Almendros et
241 al., 2003; Knicker et al., 2005; De la Rosa et al., 2008).
242
243 **3.2. Biological characteristics of burnt and control soils**
244 For the estimation of microbial biomass culturable bacteria and fungi were counted and
245 for microbial activity substrate induced respiration (SIR) was performed (Table 2). In
246 burnt soil samples, the number of viable bacteria was always higher than in the control.
247 Concerning fungi counts, the number of cfu was significantly higher in QQ1 and lower
248 in Q3 compared with the control soil. The counts were not significantly different
249 between QQ2 and the control.
250 The observed shift in the microbial community towards bacteria in the high severity
251 burnt areas could be due to the domination of bacteria over fungi at higher pH (Rousk et

al., 2010) and lower C/N ratio (Sun et al., 2011) soils. In addition, bacteria have been reported to be more resistant to the direct effect of fire (Hart et al., 2005). SIR showed, in general, a slight increase in burnt soils. The addition of easily biodegradable plant material and the increase in exchangeable cations as result of the fire could enhance the growth of soil microbial populations and in turn increase SIR values. Concerning the effect of fire on both microbial biomass and SIR, no consistent trends are found in the recent literature. In fact, Mataix-Solera et al. (2009) when studying a number of different wildfires observed that fire exerted an increase, a decrease or no effect at all on soil biomass. This variability of soil biomass could be explained in terms of intensity and severity of the fire (Xu et al., 2012). A similar variable behaviour was described in Jiménez et al. (2007) for the effect of fires on SIR values.

The effect of wildfires on soil enzyme activities relating to the cycling of C, N and P are shown in Table 3. Two different patterns of enzyme activities were found. One corresponds to the areas affected by two successive fires (QQ1 and QQ2) in which most enzyme activities were lower in burnt soil samples than in control soil with the exception of alkaline phosphatase activity which presented higher values in burnt soils. The behaviour of this enzyme could be explained in terms of the increased pH in soils, affected by two sequential fires; alternatively, organic phosphorus from dead organisms and plants could induce the alkaline phosphatase activity in burnt soils (Ninnipieri et al., 2010). The second pattern was found in the Q3 soil, that was affected by one fire. Here, the enzyme activities were, in general, higher in the burnt soil than in control soil except ß-glucosidase and urease activities which were lower or similar, respectively than those in the control. Previous studies described the sensitivity of both enzymes for detecting the immediate and medium-term impact of fire (Barreiro et al., 2010; Basanta et al., 2004). Nonetheless, as described before for microbial biomass, there is no clear

277 relationship between enzyme activities and fires in the literature. While Eivasi and
278 Bryan (1996) and Boerner and Brinkman (2003) reported a decrease in acid phosphatase
279 activity in burnt soils, the opposite effect was described by Aiwa et al. (1999) and
280 Boerner et al. (2005). Vega et al. (2013) reported a decrease in acid phosphatase activity
281 for moderate and severely burnt soils, while no changes were found in low severity
282 burnt soil. In other studies carried out by Gutknecht et al. (2010) a 50-75% reduction of
283 β-glucosidase, β-N-acetylglucosaminidase and acid phosphatase activities in burnt soils
284 were found compared with control soils. Moreover, Boerner et al. (2000) described a
285 reduction in acid phosphatase activity, but did not find differences in β-glucosidase
286 activity in burnt soils.
287
288 **3.3. Genetic characterization of the microbial populations**
289 A combination of microbial community profiles and clone library approaches were used
290 to obtain information about the microbial biodiversity present in the soil samples.
291 Optimal conditions for DNA extraction and PCR amplification reactions were
292 previously established (Pérez-Leblic et al., 2012).
293
294 **3.3.1. *Bacteria* and *Archaea* community profiles**
295 The denaturing gradient of urea-formamide most suitable for the separation of DNA
296 fragments was found to be 55 to 60 % for *Bacteria* domain and 50 to 65 % for *Archaea*
297 domain. Different DNA bands were observed in DGGE; 21 corresponded to *Bacteria*
298 but only 7 to *Archaea*. For both *Bacteria* and *Archaea*, the lowest number of DNA
299 bands was detected in control soil (11 and 3 for *Bacteria* and *Archaea*, respectively)
300 while Q3 samples showed the highest number of bands (15 and 7 for *Bacteria* and
301 *Archaea*, respectively). Shannon indexes were calculated from the DNA banding

302 patterns and both the number of bands and the intensity were taken into account (Table
303 4). In terms of the *Bacteria* domain the lowest diversity index corresponded to the
304 control soil (2.40) while burnt soil samples Q3 and QQ1 exhibited the highest values
305 (2.71 and 2.59, respectively), although analysis of variance showed that p=0.625.
306 However, a significant increase in Shannon index for *Archaea* domain in burnt soils
307 compared with control soil was determined. The lowest value in the control soil was
308 1.04 and the highest in samples Q3 and QQ1 (1.89 and 1.56, respectively).
309 Hierarchical cluster analysis was performed in order to establish similarities between
310 the banding DNA patterns generated by PCR-DGGE of burnt and unburnt soil samples.
311 The dendrogram representation obtained from cluster analysis of the bands patterns
312 obtained by DGGE for *Bacteria* and *Archaea* domains are shown in Figure 1.
313 Concerning the *Bacteria* domain, soils appear distributed in two groups or clusters, one
314 corresponding to soil control and the other to burnt soils (Fig. 1A) with a Jaccard
315 similarity index of 0.5 between control and burnt soils. The burnt soils which showed
316 higher similarity index (0.6) were QQ1 and Q3. The dendrogram clearly shows that the
317 fire affected the pattern of bands and therefore soil bacterial communities. In fact, there
318 is a low similarity between the pattern of bands of burnt soils and that of the control and
319 higher similarity between the soils affected by fire. From this result it could be inferred
320 that an alteration in bacterial community structure is produced in these soils after the
321 wildfire, in a similar way to that described by Goberna et al. (2012).
322 For the *Archaea* domain (Fig. 1B), again the samples could be divided into two clusters,
323 one formed by the control and burnt soil QQ2 (with a similarity index between them of
324 0.75) and the other by the burnt soils QQ1 and Q3 (with a similarity index of 0.7). This
325 result highlights a different effect of the fire on bacterial and archaeal communities in
326 contrast to that described by Mikita-Barbato et al. (2015) which found a similar effect of

fire on both groups of microorganisms. One possibility is that the fire affected the bacteria and archaea communities differently because they occupy different locations within the soil profile.

3.3.2. Microbial clone library analysis

Rarefaction curves for *Bacteria* domain in control and burnt soils are shown in Figure 2. Curves corresponding to C, QQ2 and Q3 samples are stabilised, which indicate that even if we had studied a larger number of sequences, the probability to identify new *phyla* is low. However, the QQ1 rarefaction curve did not reach the stabilization which is indicative that the number of clones analysed was low.

The *phyla* distribution abundance (%) determined in Aznalcóllar soils is shown in Figure 3. The control sample (5 *phyla*) and the Q3 sample (4 *phyla*) showed lower numbers of *phyla* when compared with the double burnt samples QQ2 (6 *phyla*) and QQ1 (probably more than 10 *phyla*). In both, QQ1 and QQ2 areas *Actinobacteria* and *Firmicutes* phyla were detected. In previous studies using analysis of the 16S rRNA gene clone libraries the presence of *Firmicutes* in burnt soils (Belova et al., 2014) and an increase in *Actinobacteria* in burnt soils compared with control soil was reported (Ferrenberg et al., 2013; Prendergast-Miller et al., 2017). Concerning to the *Bacteroidetes* phylum its presence is very abundant in QQ1 in contrast with that in QQ2 in which it was not detected. It is important to consider the influence of the method used to analyse the diversity of *phyla* in the results obtained. In a mesotrophic peatland affected by fire both a decrease and an increase in *Bacteroidetes* was observed by 16S rRNA gene clone library analysis and fluorescence *in situ* hybridisation (FISH), respectively (Belova et al., 2014).

Together with the higher diversity detected in QQ1, it was also the sample in which the *phylum Acidobacteria* was not the major group and where *Chloroflexi* and *Cyanobacteria phyla* were detected. It is noteworthy that both, bacteria and fungi plate counts and SIR in QQ1 showed the highest values. This result provides evidence for a better recovery of this area which may have implications for ecosystem stability.

Shannon diversity index was calculated for all bacterial communities on the basis of the *phyla* identified. Diversity index were between 1.04 and 1.65, with the lowest values corresponding to the control C and the burnt soil Q3 (1.04 and 0.97) and the highest to the double burnt samples QQ1 and QQ2 (1.65 and 1.27).

The distribution of proteobacteria sub-groups are shown in Figure 4. In control soil, β- and α- *Proteobacteria* sub-groups are represented in the same percentage (36%). In the QQ1 soil, β-*Proteobacteria* was the predominant sub-group (50%), although sequences belonging to the δ-*Proteobacteria* and γ-*Proteobacteria* sub-groups were also identified. In the QQ2 soil, the most abundant sub-group was α-*Proteobacteria* (60%) and sequences belonging to the *δ-Proteobacteria* sub-group were also detected. In Q3 soil only α-*Proteobacteria* were identified. A higher diversity of *Proteobacteria* sub-groups in double burnt soils was observed in contrast with the scarce presence of this *phylum* in the soil affected only by one fire. From current literature a high variability in the behaviour of this group against fire was found.

For the *Archaea* domain the sequences analysed yielded equivalent results for all the samples with only *phylum Crenarchaeota*, class *Thermoprotei* identified. Therefore, it was not possible to proceed with any further phylogenetic analysis to differentiate between soil samples.

The dendrogram constructed for the *Bacteria* domain using the Bray-Curtis similarity index from the libraries is presented in Figure 5. QQ1 burnt soil showed greatest

differences compared to the other samples, while Q3 and C samples presented major phylogenetic relationships.

An attempt to identify genera from sequences analysis was also done. Valid genera were considered only when a similarity higher than 85% was found to those published in databases. In the control soil, *Sphingomonas* and *Mucilaginibacter* genera were identified belonging to the *Proteobacteria* and *Bacteroidetes phyla,* respectively. In the soils affected by fire, the genera *Massilia*, *Dokdonella, Bradyrhizobium* belonging to *Proteobacteria phylum* were identified, and the genera *Pseudolabrys*, *Flavisolibacter*, *Niastell*a, *Parasegetibacte*r, *Segetibacter*, *Mucilaginibacter*, *Fluviicola* and *Flavobacterium* were the most abundant in the *phylum Bacteroidete*.

4. Conclusions

In spite of the difficulty in understanding the complex interactions caused by wildfires among physical and chemical characteristics of Dystric Cambisol Mediterranean soils and its microbial populations, several conclusions are inferred from this study. In burnt soils an increase in pH and total N and a decrease in total organic carbon, organic matter and C/N ratio was observed. Fires affected microbial community composition; however, the impact of fire on microbial communities did not seem to be straight-forward, dependent upon the number of fire episodes but rather perhaps to severity and duration. Shannon diversity indexes for all bacterial communities on the basis of the *phyla* identified corroborated the existence of higher diversity in double burnt soils. From this study we conclude that culture-dependent and culture-independent techniques are necessary to complement physical and chemical characterization in order to provide a holistic view of the effect of fire on soil.

Acknowledgments

This work has been supported by the MINECO project INTERCARBON (CGL2016-78937-R).

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HIGHLIGHTS

- Physico-chemical and microbial characteristics of fire affected soils are studied
- Microbial biomass and diversity were higher in burned than in control soils
- Impact of fire on soil microbiota was not directly dependent upon fire recurrence

Tables

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Table 1. Physical and chemical parameters in Aznalcóllar soil samples.

Sample	pH	WHC† (%)	TOC‡ (%)	OM§ (%)	Total N (%)	C/N ratio
C	4.8 ± 0.2*	26.9 ± 1.4*	2.3 ± 0.1*	3.9± 0.2*	0.14 ± 0.01*	16.20
QQ1	6.2 ± 0.2*	25.6 ± 1.1	2.0 ± 0.1*	3.4 ± 0.2*	0.17 ±0.01*	11.70
QQ2	6.0 ± 0.3*	30.4 ± 1.3*	1.9 ± 0.1*	3.3 ± 0.1*	0.15 ±0.01	12.90
Q3	5.7 ± 0.2*	28.0 ± 1.1	2.0 ± 0.1*	3.5 ± 0.1*	0.19 ±0.01*	11.28

†: Water holding capacity; ‡: Total organic carbon; §: Organic matter.
The analysis of variance showed $p<0.01$ for data. * Significant differences ($p<0.05$) between control and the different burnt samples using a Tukey´s test. Data ± STD; n=3 sampling points. C: control unburned soil; QQ1 and QQ2: Soils burnt twice in 1997 and 2004; Q3: Soil burnt once in 2004.

Table 2. Biological parameters in Aznalcóllar soil samples

Sample	Bacteria (cfu/g)	Fungi (cfu/g)	SIR†
C	$2.1 \times 10^6 \pm 1.1 \times 10^5$*	$1.13 \times 10^5 \pm 1.2 \times 10^3$*	0.11± 0.01*
QQ1	$14.4 \times 10^6 \pm 6.1 \times 10^5$*	$2.26 \times 10^5 \pm 2.5 \times 10^3$*	0.18 ± 0.02*
QQ2	$6.3 \times 10^6 \pm 2.3 \times 10^5$*	$1.1 \times 10^5 \pm 1.8 \times 10^3$	0.11 ± 0.01
Q3	$3.9 \times 10^6 \pm 3.3 \times 10^5$*	$0.7 \times 10^5 \pm 2.1 \times 10^3$*	0.14 ± 0.01

†: Substrate induced respiration (% CO_2/h/100 g dry soil). The analysis of variance showed a $p<0.001$ for data. * Significant differences ($p<0.05$) between control and the different burnt samples using a Tukey´s test. Data ± STD; n=3 sampling points. C: control unburned soil; QQ1 and QQ2 Soils burnt twice in 1997 and 2004; Q3: Soil burnt once in 2004.

Table 3. Enzyme activities (U†/g soil) in Aznalcóllar soil samples.

	Acid phosphatase	Alkaline phosphatase	ß-N-acetyl-glucosaminidase	ß-glucosidase	Cellulase	Invertase	Urease
C	3.03 ± 0.05*	0.44 ± 0.01*	0.65 ± 0.02*	1.14 ± 0.06*	0.15 ± 0.01*	24.06 ± 0.82*	0.93± 0.05*
QQ1	2.01 ± 0.03*	0.85 ± 0.02*	0.47 ± 0.01*	1.03 ± 0.06	0.10 ± 0.00*	17.86 ± 0.63*	1.92 ± 0.03*
QQ2	2.95 ± 0.06	0.80 ± 0.02*	0.56 ± 0.01*	0.74 ±0.04*	0.10 ± 0.00*	11.80 ± 0.45*	0.77 ± 0.01*
Q3	3.16 ± 0.06	0.70 ± 0.01*	1.01 ± 0.02*	0.73 ± 0.03*	0.23 ± 0.01*	26.12 ± 0.85*	0.92 ± 0.06

†: μmol/hour. The analysis of variance showed a $p<0.001$ for all data. * Significant differences ($p<0.05$) between control and different burnt samples using a Tukey´s test. Data ± STD; n=3 sampling points. C: control unburned soil; QQ1 and QQ2 Soils burnt twice in 1997 and 2004; Q3: Soil burnt once in 2004.

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

	Shannon index ($'H$)	
	Bacteria	*Archaea*
C	2.40	1.04*
QQ1	2.59	1.56*
QQ2	2.43	1.35*
Q3	2.71	1.89*

The analysis of variance showed $p<0.05$ just for *Archaea* data.
* Significant differences ($p<0.05$) between control and different burnt samples using a Tukey´s test. C: control unburned soil; QQ1 and QQ2 Soils burnt twice in 1997 and 2004; Q3: Soil burnt once in 2004.

Figure1

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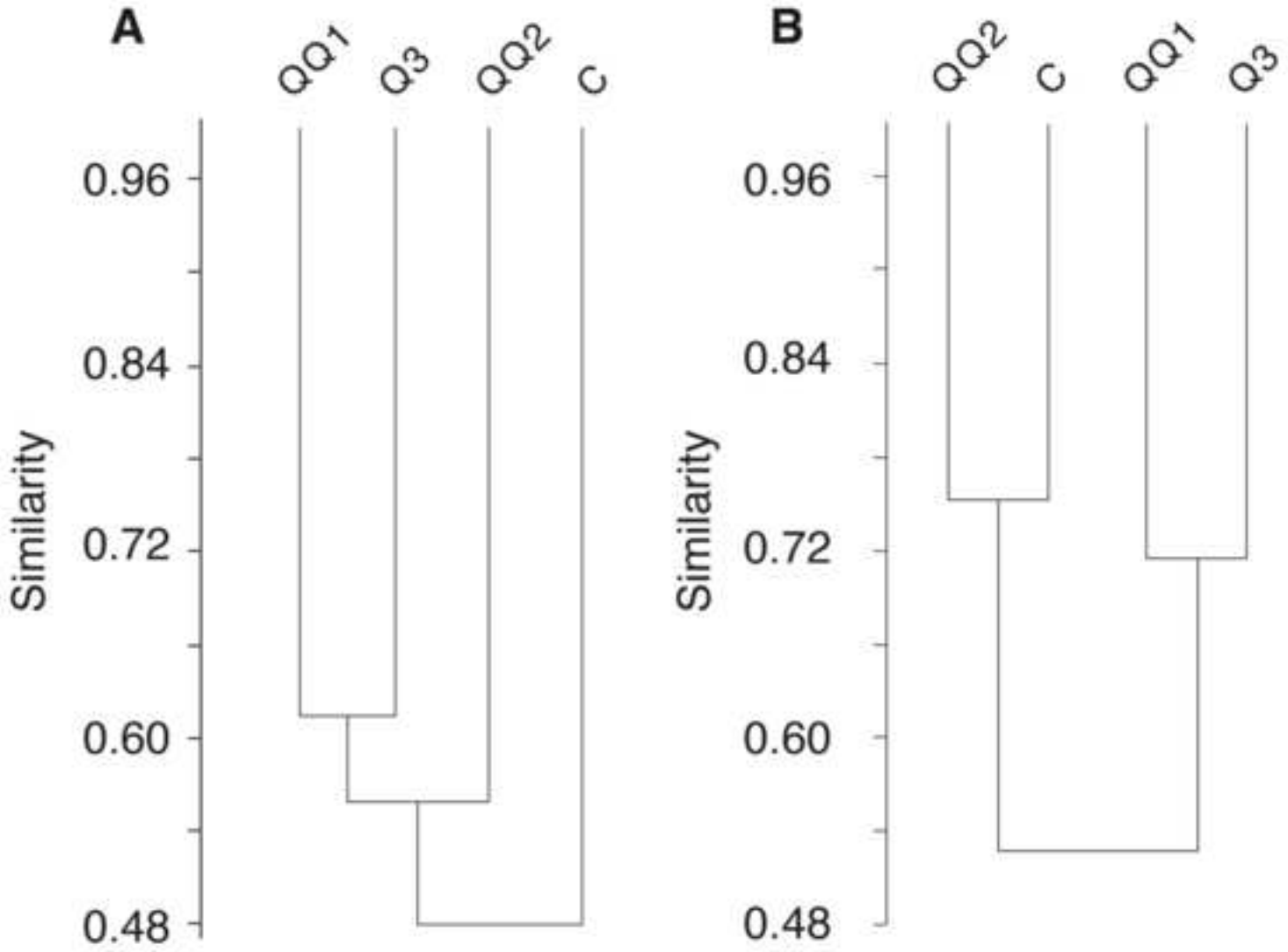

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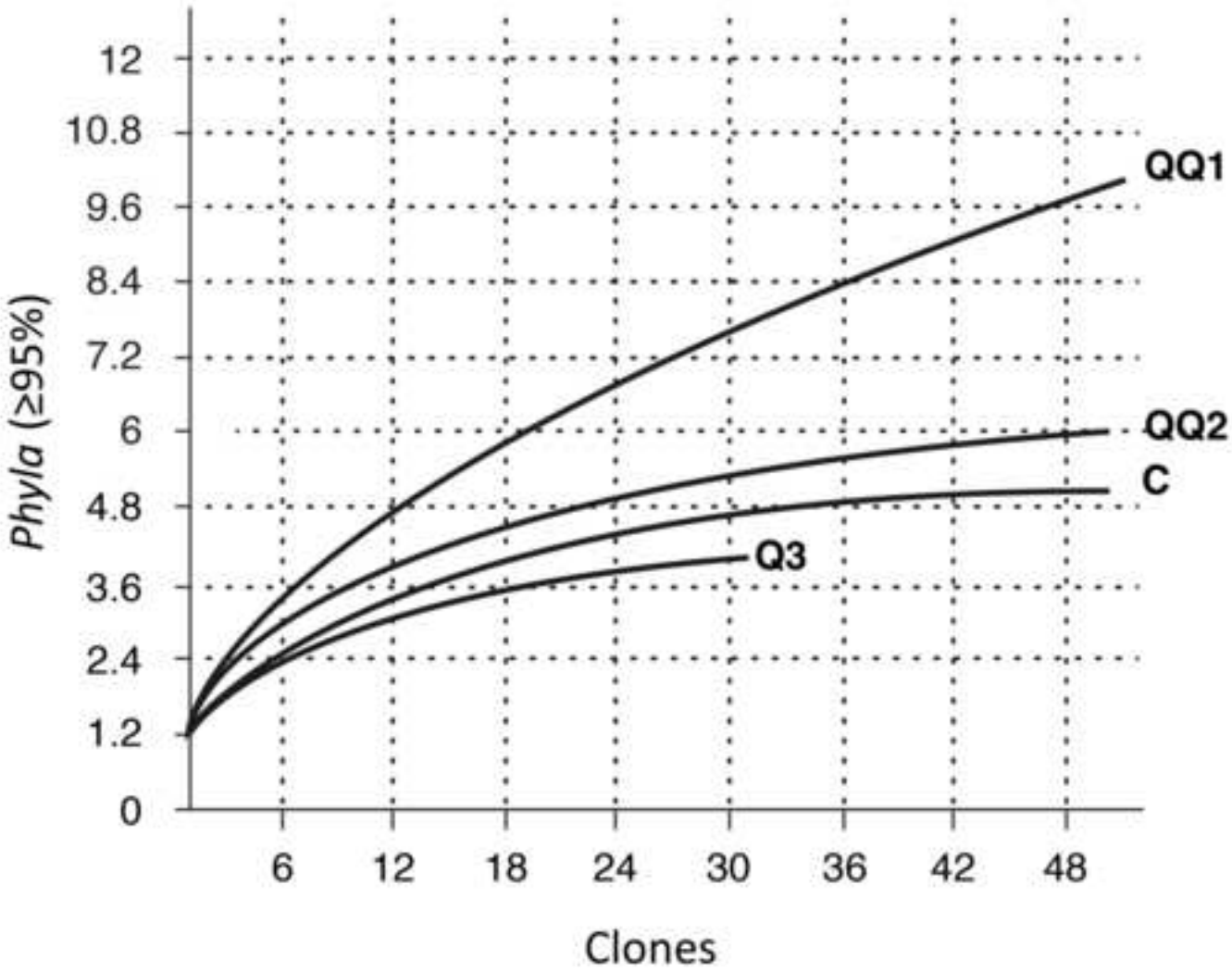

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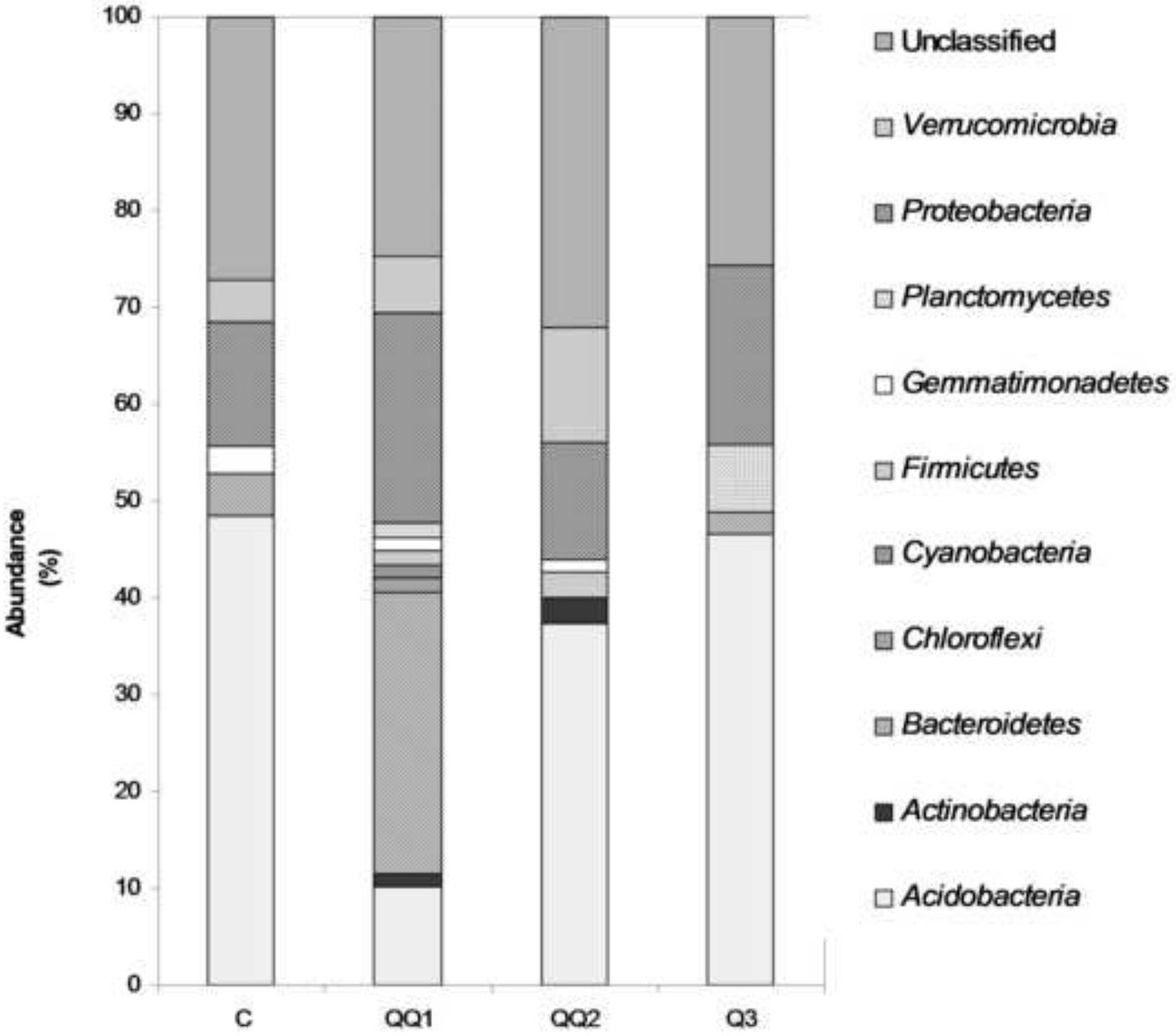

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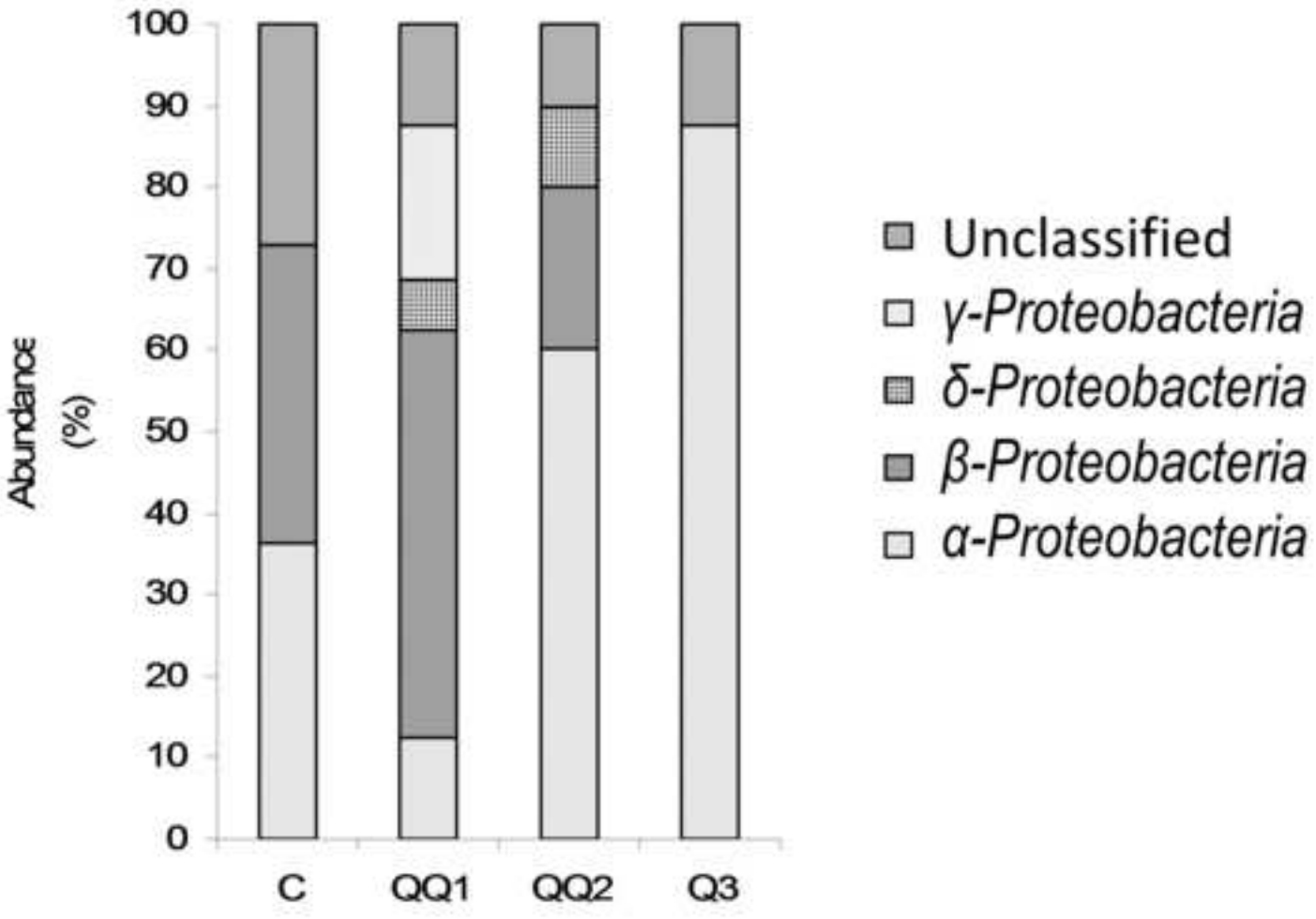

Figure5

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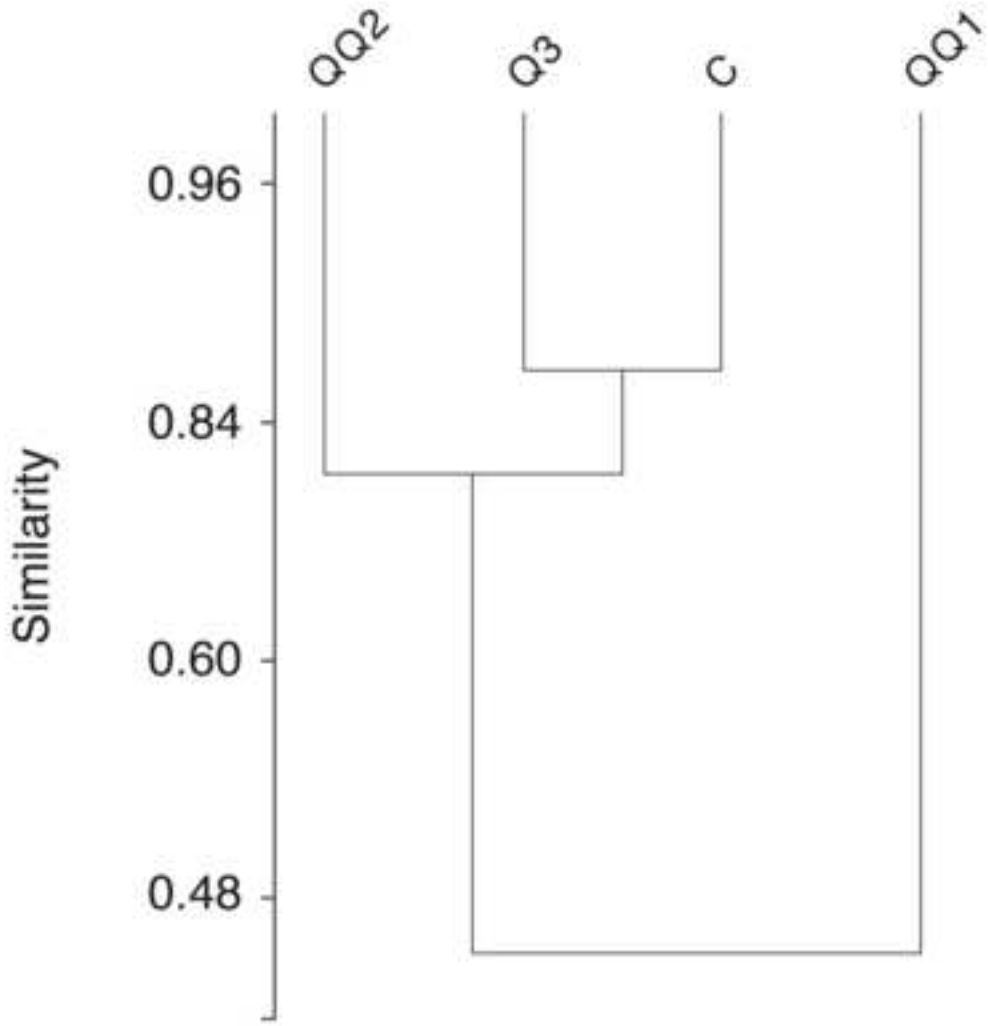

FIGURE CAPTIONS

Figure 1. Dendrograms of hierarchical analysis inferred from DGGE results using Jaccard´s similarity coefficients. A) *Bacteria*; B) *Archaea*. C: control unburned soil; QQ1 and QQ2: Soils burnt twice in 1997 and 2004; Q3: Soil burnt once in 2004.

Figure 2. Rarefaction curves for 16S rRNA clone libraries from the Aznalcóllar soil samples. C: control unburned soil; QQ1 and QQ2: Soils burnt twice in 1997 and 2004; Q3: Soil burnt once in 2004.

Figure 3. Percentage distribution of phylogenetic groups (*Phyla*) obtained from clone libraries analysis from the Aznalcóllar soil samples. C: control unburned soil; QQ1 and QQ2: Soils burnt twice in 1997 and 2004; Q3: Soil burnt once in 2004.

Figure 4. Percentage distribution of *Proteobacteria* sub-groups from the Aznalcóllar soil samples. C: control unburned soil; QQ1 and QQ2: Soils burnt twice in 1997 and 2004; Q3: Soil burnt once in 2004.

Figure 5. Dendrogram of hierarchical analysis inferred from clone libraries for the *Bacteria* domain. C: control unburned soil; QQ1 and QQ2: Soils burnt twice in 1997 and 2004; Q3: Soil burnt once in 2004.