



# Wildfire effects on diversity and composition in soil bacterial communities

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## ARTICLE INFO

### Article history:

Received 29 January 2020

Received in revised form 8 April 2020

Accepted 9 April 2020

Available online xxx

Editor: Paulo Pereira

### Keywords:

Wildfire severity

Soil bacterial community composition

Diversity

## ABSTRACT

In recent years, the Mediterranean area has witnessed an increase of both the frequency and severity of large fires, which appears to be intimately associated with climate and land use changes. To measure the impact of wildfires on living organisms, diverse indicators have been proposed. These indicators of fire severity traditionally rely on quantifying the damage caused to the vegetal component of ecosystems. However, the use of bacterial communities as severity indicators has received less attention. Here, we studied the differences between bacterial communities of three different Mediterranean ecosystems, two shrubby and one arboreal, two months after a large wildfire. Two levels of severity were compared to a control unburnt soil. The results showed that greater fire severity triggers a reduction in the diversity of soil bacterial communities. In high-severity fires, this reduction reached 40.6 and 58.6% of the control values for richness and Shannon's diversity, respectively. We also found that the greatest differences between communities could be attributed first to the severity of the fire, and second to the ecosystem from which they originated. Importantly, species of just five families of bacteria: Oxalobacteraceae, Micrococcaceae, Paenibacillaceae, Bacillaceae and Planococcaceae, became dominant in all three ecosystems. The average frequency increase for particular species was 100 times. However, due to random uncontrolled factors, the species that became dominant in each community were not always the same.

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## 1. Introduction

Forest wildfires represent one of the main threats to Mediterranean ecosystems, with important implications from an ecological point of view (Calvo et al., 2015; Van Drooge et al., 2016). Significant alterations in the historical wildfire regime have been observed in recent decades in the countries with a Mediterranean climate, including an increase in number, severity, and area affected (Moritz et al., 2014; Pausas et al., 2008). It is thought that in the upcoming decades, the increase in the number and severity of wildfires will be further aggravated by two projected factors. First, the ongoing and forecasted changes in climate worldwide (Hinojosa et al., 2016). And, second, the effects of global changes in the Mediterranean area as a consequence of the changes in the use of soil as laid out by prediction models (Moritz et al., 2014).

The severity of wildfires determines an ecosystem's post-fire resilience, or ability to come back to a pre-fire state, in the face of new fires (Holling, 1973; Fernández-Manso et al., 2016; Francos et al., 2016; González de Vega et al., 2016; Quintano et al., 2015, 2018), making this factor one of the essential components of a fire's aftermath. The severity of the wildfire is related to its intensity, and is defined as the impact caused in the ecosystems (González de Vega et

al., 2016; Keeley, 2009). The main advantage of the study of severity is that it can be measured after the fire has occurred, both in the short term (immediately after the fire) and in the medium term (one to two years later). Therefore, it allows for predicting the post-fire recovery of ecosystems (Dzwonko et al., 2015; Keeley, 2009; Miller et al., 2016).

Studies carried out in Mediterranean ecosystems have shown that they tend to have great resilience to fires of low recurrence and low severity (Calvo et al., 2008). However, this is not always the case. For instance, Fernández-García et al. (2019) found that changes in recurrence and, fundamentally, in severity, may cause the post-fire regeneration capacity of the vegetation to be altered or even lost. This poses an obvious challenge to an ecosystem's resilience. For example, in a Mediterranean forest dominated by *Pinus pinaster*, high levels of severity have a negative effect on the recovery of this typical re-sprouting species, favoring instead the species with a germinating strategy (Fernández-García et al., 2018).

These kinds of variations in the response of these two biological types, *re-sprouters* and *germinators*, in relation to fire severity could modify post-fire regeneration and resilience, significantly affecting ecosystem structure and post-fire management. In turn, the type of vegetation in the ecosystem affects the severity of the fire, mainly due to its effects on the amount of fuel that can be burned during the fire. Thus, the highest severities occur in shrubland ecosystems, while in forests the most frequent severities are low or medium (González de Vega et al., 2016).

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In addition to affecting the vegetation, the severity of wildfires also alters the abiotic properties of the soil (Certini, 2005; Hart et al., 2005; Pérez-Valera et al., 2019), and influences the post-fire regeneration of Mediterranean ecosystems (Fernández-García et al., 2019). For instance, polycyclic aromatic hydrocarbons, some of which are mutagenic, have been found to accumulate following a wildfire (Tsibart et al., 2014). With regards to biotic properties, some studies have also shown that microbial biomass is significantly affected by the severity of the fire (Hinojosa et al., 2016; Mayor et al., 2016; Pérez-Valera et al., 2017, 2018, 2019; Vega et al., 2013). Changes in microbial functionality have also been reported (Pérez-Valera et al., 2017, 2018, 2019). Microbial communities are an essential component of ecosystems, as they are involved in many biogeochemical processes and in an ecosystem's productivity (Bardgett and van der Putten, 2014; Heijden et al., 2008).

Soil bacteria are an extraordinary and diverse group of organisms with enormous functional capacities. They are essential to defining ecosystem performance, including mineral weathering, primary production and decomposition of organic matter (Bardgett and van der Putten, 2014; Heijden et al., 2008; Schimel and Schaeffer, 2012). Processes such as nitrification, denitrification or mineralization of N and C in the soil are crucial for the ecosystem, (Graham et al., 2014, 2016; López-Poma and Bautista, 2014), and can be better predicted by incorporating measures of the structure and diversity of the microbial community (Graham et al., 2014; Powell et al., 2015). A fire exposes the soil of microbial communities to extremely high temperatures and to changes in their abiotic environment, thus altering their taxonomic and phylogenetic composition (Pérez-Valera et al., 2018). Bacteria with heat-resistance capabilities (for example, spore-forming), as well as fast-growth strategies, tend to be less affected by wildfires (Bárcenas-Moreno et al., 2011; Ferrenberg et al., 2013; Goberna and Verdú, 2016; Smith et al., 2008).

The variation of diversity and its structuring as a result of wildfires have also been examined by several researchers in different ecosystems. The focus of these studies has predominantly been at the vegetation point of view (e.g. González de Vega et al., 2018; Mahood and Balch, 2019). There are also studies that emphasize the fungus and animal components (e.g. Ferreira et al., 2019). But very few studies have accomplished a classical ecological analysis of the structuring of diversity using data from soil bacteria following wildfires (Li et al., 2019). We aim to analyze the short-term effect of the severity of wildfires on the bacterial communities. Although it is assumed that the changes in diversity and composition of soil bacterial communities may not be the same for the different ecosystems, we have focused in finding patterns in common to them. Nevertheless, a key objective of the study is to identify bacteria taxa that could serve as indicators of severity for any ecosystem. The bacteria present in different ecosystems after an intense fire could be resistant species, migrants from the surrounding areas or colonizers that would launch the ecological succession into a totally altered environment. The present work, therefore, is a novel study on the ecological diversity of bacterial communities in Mediterranean ecosystems after a large wildfire.

## 2. Materials and methods

### 2.1. Area of study and sampling

The area of study is located in the Sierra de la Cabrera, in the Northwest of Spain. It is on the border of the Mediterranean biogeographic region (Rivas-Martínez et al., 2011). Soils are classified as Lithic leptosols and Humic cambisols, with sandy loam and sandy clay loam texture (ITAcYL, 2019). Soil is acidic, originating from

siliceous lithology. In 2017, between August 21st and 27th, a wildfire consumed 9939 ha of this area. A more extensive description of the area of study can be found (García-Llamas et al., 2019). Sixty-six samples were analyzed from three ecosystems: two shrublands and a forest, dominated by *Genista hystrix* (woadwaxen), *Erica australis* (Spanish heath) and *Quercus pyrenaica* (Pyrenean oak), respectively. Samples were classified into three categories according to the severity of the fire at each sampling point (C=control, L=low, and H=high). The number of samples oscillated between 4 (low severity, oak forest) and 10 (control, woadwaxen forest). The location of the 66 samples can be seen in Fig. 1. Sampling was not uniform. The numbers were as follows: (GC=10; GL=8; GH=9; EC=8; EL=8; EH=5; QC=8; QL=4; QH=6). G stands for *Genista*, E (*Erica*) and Q (*Quercus*). The letters C, L, and H reflect the severity levels.

Burnt-soil severity in the area of study had been previously evaluated two months after the wildfire and long before any rain event in a larger study that included the entire area of the fire (García-Llamas et al., 2019). To quantify severity, authors used an adapted version of the original Composite Burn Index (CBI) in which field plots of 30 × 30 m were considered. Only CBI values from the substrate stratum were contemplated (Fernández-García et al., 2018). Soil samples (approx. 1 L) from the top 0–10 cm were taken with a trowel, and mixed in plastic bags. After mixing, 50 mL were transferred to a falcon tube, stored in a cooler while in the field, frozen at −20 °C upon returning to the lab, and set aside for the analysis of bacterial components (also two months after the wildfire).

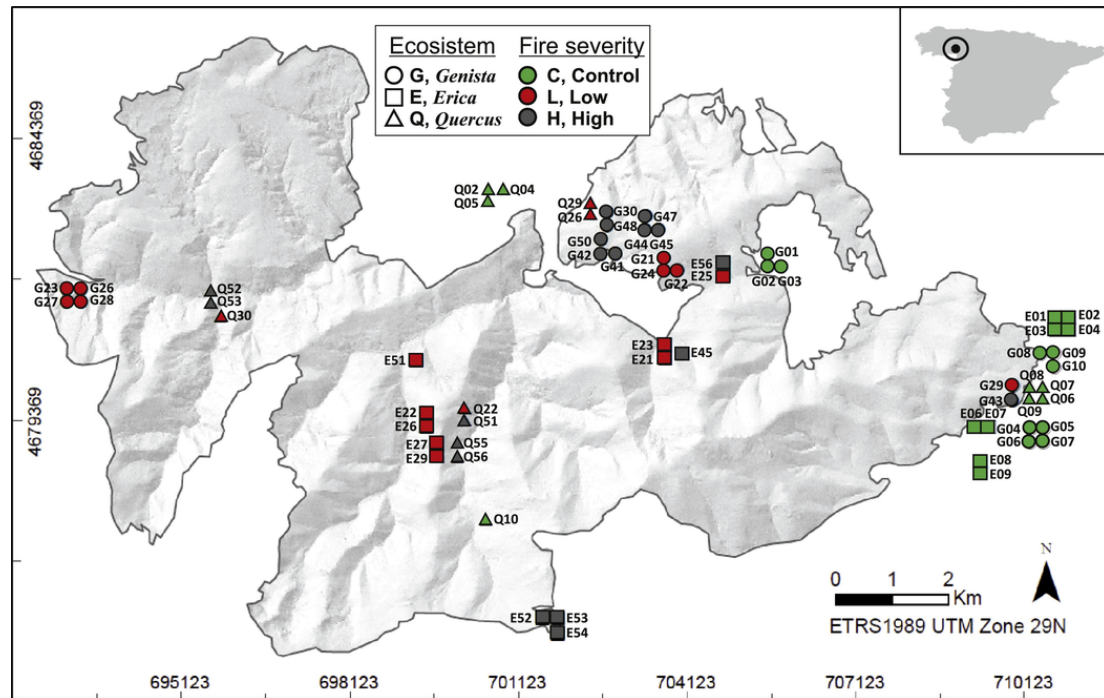
### 2.2. DNA extraction, PCR and sequencing

DNA was extracted from 0.25 to 1 g of soil for each sample. Since the amount of DNA is affected by the fire severity, different quantities of soil were required. Extraction was achieved using the Power Soil DNA isolation kit (MoBio Laboratories Inc., Carlsbad, CA), following the manufacturer's protocols. DNA concentration and quality were determined spectrophotometrically using a Nanodrop instrument (Thermo Fisher Scientific Inc., Waltham, Mass), and a Qubit 2.0 fluorometer (Invitrogen Inc., Carlsbad, CA). Additionally, DNA integrity was further confirmed by agarose gel electrophoresis. To amplify bacterial V3-V4 region of the 16S SSU rRNA, the 341F and 805R primers described by Herlemann et al., (2011) were used. Barcoded PCR libraries from each sample were quantified by real-time PCR in a Light-Cycler 480 (Roche, Basel, Switzerland), pooled with equimolar concentrations, and sequenced by paired-end sequencing (250 × 2) in the Illumina Miseq platform (Illumina Inc., San Diego, CA).

### 2.3. Sequence processing and statistical analysis

Bioinformatic processing of raw sequences was performed using Mothur and RDP tools (Cole et al., 2014; Schloss et al., 2009). After primer removal, overlapping paired-end sequences were merged into a unique longer fused read (termed herein *read*) and screened to remove low-quality reads and reads that diverge from the expected size (460 to 480 bp) using Mothur and in-house scripts. Reads were clustered in Operational Taxonomic Units (OTUs) at 97% identity using vsearch v.2.8.4 (Rognes et al., 2016). Taxonomic assignment of each OTU was obtained by means of CLASSIFIER (Wang et al., 2007).

Unique reads were determined with vsearch, and aligned using Infernal (Nawrocki et al., 2009), available on the RDP website (<https://rdp.cme.msu.edu/>). An approximate maximum-likelihood phylogenetic tree was constructed with FastTree (Price et al., 2010) using the *gtr* evolutionary model and edited with MEGA7 (Kumar et al., 2015).



**Fig. 1.** Location of the Sierra de Cabrera area that suffered the great wildfire of 2017. The communities were classified by the severity of the fire (C: Control; L: low severity; H: high severity) and by the ecosystem they came from, identified by the dominant plant species (G: *Genista hystrix*; E: *Erica australis*; Q: *Quercus pyrenaica*).

The phylogenetic tree and the table of frequencies of each OTU were used as input in the R/Vegan 2.5 package (Oksanen et al., 2010) of R to estimate alpha and beta diversities. The following alpha diversity indices were calculated: Rarefied Species Richness (using random subsamples of size equal to minimum sample size), Shannon's diversity index, and Simpson's dominance index.

Gamma diversity was calculated by merging all reads of the communities subjected to the same fire severities. Gamma diversity was estimated independently for each ecosystem and also for all ecosystems together. Beta diversity was calculated by the difference between gamma and alpha. Alpha was estimated as the average value for all communities in each treatment.

The phylogenetic Net Relatedness Index (NRI) (Webb et al., 2002) was computed using R/Spicy package (Kembel et al., 2010). NRI compares the metric Mean Pairwise Distance (MPD), which uses the phylogenetic standardized distances between all pairs of OTUs in the community under study, with the MPD of a null model. For the null model, MPD is computed by 1000 resampling of the total number of OTUs. NRI index considers, along with the richness, the phylogenetic distance among the OTUs that comprises each community. Values close to zero indicate a wide phylogenetic dispersion, which denotes a greater number of roles among the different species. Low values of NRI show that communities are formed by OTUs phylogenetically close, and thus, these OTUs are expected to perform the same ecological role.

Read counts were normalized to 100,000 per sample prior to mixed models. Fitting of mixed models was done on OTUs or specific taxa. Dissimilarities between pairs of samples were estimated by means of the phylogenetic UniFrac metric (Lozupone and Knight, 2005), and the ecological classic Bray–Curtis (quantitative) or Chao (qualitative) indexes. To identify statistical differences among bacterial communities in the different treatments (with severity and ecosystem factors), permutation tests of multivariate analysis of variance (PERMANOVA) were performed using dissimilarities matrixes.

Graphic exploration of the distances between communities was carried out by analysis of Principal Coordinates (PCoA) using the *pcoa* function included in the Vegan package.

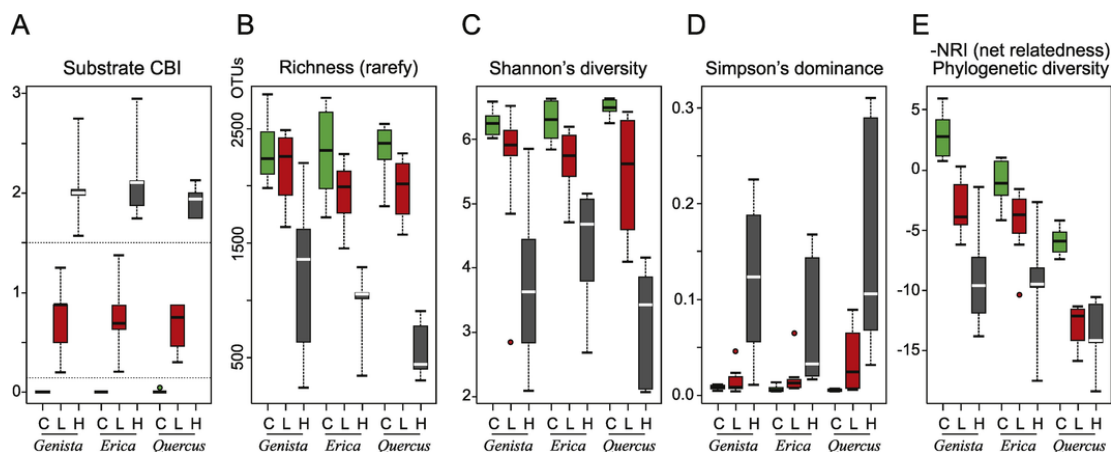
To explore differences in frequencies of OTUs and taxa, mixed models were evaluated with the R/lmerTest package (Kuznetsova et al., 2017). All response variables were evaluated considering fire as a fixed effect and ecosystem as random effect, which is able to introduce variability that cannot be attributed to the severity of the fire (Supplemental material S1). False discovery rate (FDR) correction was carried out using Benjamini and Hochberg, (1995) method. All statistics, including graphical explorations, mixed models, and PERMANOVAs, were performed with R statistical software.

### 3. Results and discussion

#### 3.1. Bacterial 16S sequencing and diversity structuration

The sixty-six soil samples were classified into 3 groups: control (C), with severity values zero or close to zero (CBI<0.10); low (L), with CBI between 0.15 and 1.50; and high (H), with CBI >1.50 (Fig. 2A). Read filtering yielded 9,223,774 usable reads (79.3% of the reads initially sequenced), after the removal of unpaired reads, reads that were too short, chimeras, and reads from chloroplasts. Unique reads were clustered into operational taxonomic units (OTUs), based on 97% pairwise identity. Rare OTUs, with <100 reads in the whole sample set, were also removed from subsequent analysis (16.3%). The remaining 7,723,331 reads were distributed among the sixty-six bacterial communities (Fig. 1), with about 117,000 reads per sample on average, and a range between 33,550 (G23, *Genista* ecosystem with L fire severity) and 232,929 reads (Q52, *Quercus* ecosystem with H severity).

OTUs are clusters of similar sequence variants. Each cluster is expected to represent a different taxonomic unit of a bacteria species, although this may not be always the case. The total number of reads



**Fig. 2.** Boxplots showing the variation in A) Composite Burn Index (CBI) or B–E) alpha diversity of bacterial communities, grouped by the ecosystem they came from or the range of severity into which they were classified. For all measures of alpha diversity, severity implied a decrease in diversity, and an increase in the dominance of some OTUs. C: Control; L: low severity fire; H: high severity fire.

were distributed in 5229 OTUs with more than 100 reads, with a mean of 1476 and median of 269 reads. The most frequent OTU is comprised of 483,587 reads. All reads were assigned to 21 bacterial phyla, being Proteobacteria the most frequent (37.87%), followed by Actinobacteria (17.23%), Acidobacteria (14.89%), Firmicutes (10.30%) and Bacteroidetes (5.41%). Within Proteobacteria, the majority are beta (56.08%), followed by alpha (32.67) and gammaproteobacteria (7.86%). These frequencies are consistent with those obtained by Delgado-Baquerizo et al. (2018) for acidic soils in their *Atlas of dominant bacteria found in soils* (see Fig. S8 in Delgado-Baquerizo et al., 2018). The authors found soils dominated by Proteobacteria, Actinobacteria and Acidobacteria, with frequencies similar to those we obtained. Nevertheless, the frequency of Bacteroidetes in our study corresponds more with that of low productivity soils in the *Atlas*. They also found that Firmicutes were only present in unclassified soils, while in our study we found a value of 10% in burned soils.

The richness of the different bacterial communities showed no relation to the ecosystem to which they belong ( $P=0.43$ ), but it did relate to the severity of the fire (Fig. 2B). That is, there are no significant differences that can be attributed to random effects, that is among ecosystems, although differences are observed depending on the severity of the fire when a mixed model was designed (Supplementary Table S1;  $P=1.1 \times 10^{-17}$ ), with differences among the three severity levels (between C and L,  $P=1.8 \times 10^{-2}$ ; between C and H,  $P=5.6 \times 10^{-18}$ , and between L and H,  $P=5.0 \times 10^{-13}$ ). Richness decreases from an average value of 2299 OTUs in control soil communities to 2023 in soils with low severity, and to 921 in soils with high severity. It is important to note that these numbers are relative and do not consider the lower bacterial mass in burned soils. Similar loss of richness was also found in controlled fires in an experiment that went from about 600 OTUs to about 520 one month after the fire (Pérez-Valera et al., 2017). The discrepancy in the amount of OTUs obtained by these authors and our analysis can be attributable to two factors: first, the fact that they worked with 454 pyrosequencing, which produces a smaller number of reads per sample and second, the observation that small fires in the plots they used are much less severe than the intense wildfire investigated in our work.

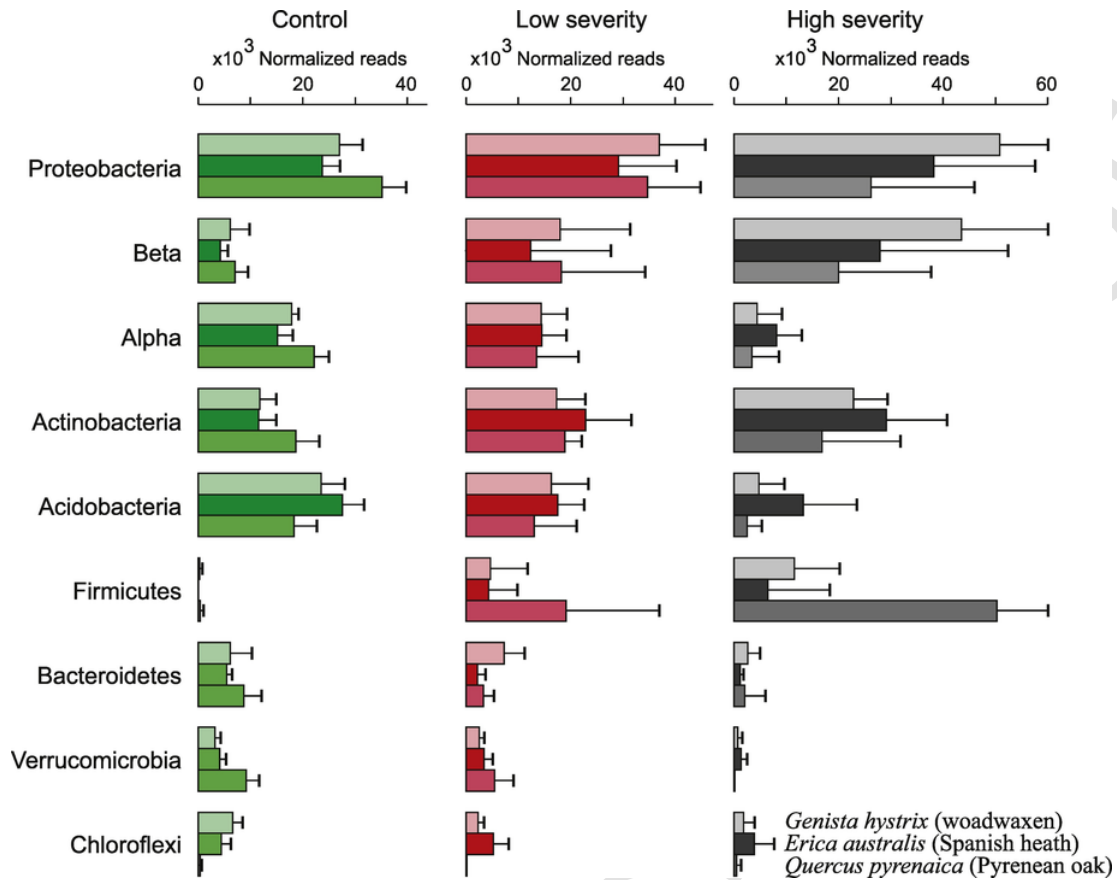
Shannon's diversity index ( $D'$ ) (Fig. 2C) illustrates how the loss of diversity in communities that have suffered a fire does not only imply a loss of OTUs, but also affects their frequencies ( $P=2.1 \times 10^{-17}$ , Table S1). Thus, results reveal that some OTUs have become domi-

nant in their respective communities after a fire. There are significant differences in  $D'$  index compared to the control both in communities affected by low ( $P=0.018$ ) and high severity ( $P<5.6 \times 10^{-18}$ ). However, the mixed model does not indicate that considering the ecosystem as a random factor has any effect. The increase in dominance (Simpson's index;  $P=1.7 \times 10^{-9}$ , without significant differences between ecosystems) only shows significant differences with the control in communities subjected to high severity ( $P=1.4 \times 10^{-9}$ ), but not in those subjected to low severity (Fig. 2D and Table S1).

Another diversity index is the net relatedness index (NRI, Fig. 2E), which quantifies phylogenetic diversity, and in some way, gives information on both the richness of species and their functionality. We found that the phylogenetic diversity decreased significantly with the severity of the fire for each ecosystem ( $P=8.2 \times 10^{-16}$ , Table S1). Using this index, differences between the control communities and both low ( $P=1.1 \times 10^{-7}$ ) and high severity ( $P=1.3 \times 10^{-16}$ ) were observed. The random effect attributed to the ecosystem cannot be obviated ( $P=4.7 \times 10^{-11}$ ), which indicates that there are differences among ecosystems. Interestingly, less phylogenetic diversity was observed in ecosystems dominated by *Quercus* than in ecosystems dominated by *Genista* or *Erica* (Fig. 2E). This observation suggests that phylogenetic, and possibly functional, diversity of bacterial communities in mature ecosystems could be lower than in previous stages in the ecological succession. Nevertheless, this is an aspect that was not intended to be investigated in this work. Pérez-Valera et al. (2017) quantified the NRI index in their small controlled fires in the same manner and found an increase in diversity after a fire. This increase of diversity showed by NRI arose progressively during the first 4.5 months after the fire, to steadily decrease afterwards. Those results, obtained in a contained fire with low severity, are in disagreement with the decrease in phylogenetic diversity of the communities found in our study.

Alpha diversity is affected by the severity of the fire to which communities have been subjected. Severity causes a decrease in both taxonomic and phylogenetic diversity coupled with an increase in dominance and a reduction in the phylogenetic diversity associated with a decrease in the ecosystem roles. These changes affect the large taxonomic groups that make up each community (Fig. 3). Thus, severity produces a clear increase in the relative frequency of Proteobacteria, Actinobacteria and Firmicutes. However, there is a decrease in the other represented *fila*, that is, Acidobacteria, Bacteroidetes, Verrucomicrobia (which practically disappear) and Chlo-





**Fig. 3.** Number of reads (normalized to 100,000) of the most abundant bacteria fila. Alpha and Betaproteobacteria classes were also included. An increase in Betaproteobacteria, Actinobacteria and Firmicutes associated with the severity of the fire was observed, while for the rest of the fila, a reduction was seen. The communities have been grouped by the ecosystems they came from, which were characterized by the dominating plant species: *Genista hystrix*, *Erica australis* and *Quercus pyrenaica*.

roflexi. The increase in the frequency of Firmicutes is much larger in the ecosystem dominated by *Quercus*. Although Proteobacteria increase their relative frequency overall, this increase is due to an increase in Betaproteobacteria, while Alphaproteobacteria are greatly reduced. The increase in Firmicutes and Betaproteobacteria has been previously reported by Pérez-Valera et al. (2017).

The structuring of diversity for both the richness of OTUs and Shannon's index can be seen in Fig. 4. Differences among gamma diversities of each ecosystem compared to all ecosystems as a group is greater when considering richness than in Shannon's diversity. This indicates that many OTUs are not shared among ecosystems, but their frequencies are low and do not affect the value of the Shannon's index. Between the two factors (ecosystems and severity), communities constantly show a high beta diversity when considering richness, but it is relatively small when considering the Shannon's diversity. Again, differences in the composition of OTUs between samples are large, but only for low-frequency OTUs. Beta diversity does not seem to be affected by either the ecosystem or the severity of the fire in the different communities. However, alpha diversity decreases with the severity, as previously noted.

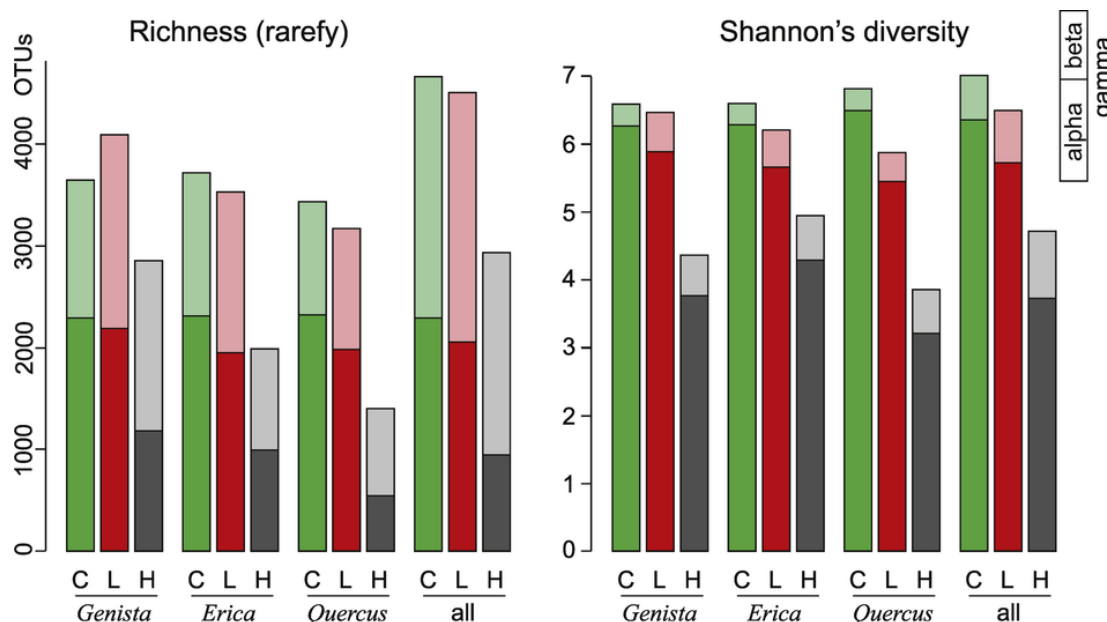
### 3.2. Distances between bacterial communities (Beta diversity)

Communities were compared using taxonomic (at OTU level) and phylogenetic indexes: the classical ecological indexes of Bray Curtis (BC, quantitative) and Chao (Ch, qualitative), and the phylogenetic indexes UniFrac weighted (wUF) and unweighted (uUF). The wUF

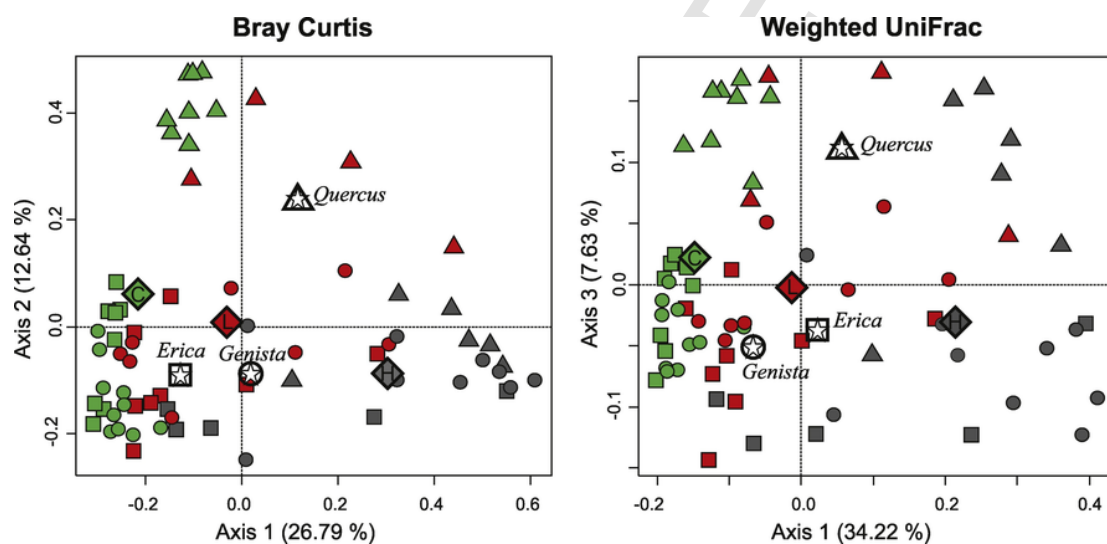
and uUF indexes take into consideration the proportion of branches shared by communities in a phylogenetic tree, which in our study is comprised of 5229 OTUs. Distance matrixes constructed with the components of the bacterial communities show a high association with both the severity of the fire and the ecosystem from which they originate. The values returned by PERMANOVA analysis for the four matrixes and the two factors were always significant ( $P < 5 \times 10^{-4}$ , with 10,000 permutations).

Principal coordinates analysis (PCoA) of the 4 beta diversity indexes (just the BC and wUF are shown in Fig. 5) indicates that, regardless of the chosen index, the first coordinate (first axis) is a good predictor of the severity of the fire. Mixed models of the projection of samples on the first axis were significant for the four indexes for both severity (Supplementary Table S1;  $P < 10^{-12}$ ) and random effect of ecosystem ( $3 \times 10^{-2} > P > 10^{-5}$ ). When comparing control (C) and high (H) severity levels, there were also significant differences with  $P < 10^{-14}$  (Supplementary table S1).

On the second axis, samples are clustered for the uUF, BC, Ch indexes, according to the ecosystem, with significant ecosystem effect ( $P < 10^{-10}$ ). Differences are mainly found between *Quercus* and any of the shrub ecosystems (Supplementary table S1). For wUF index, although ecosystem effect was not found in the second axis, it was in the third axis ( $P = 6.6 \times 10^{-15}$ ). Again, with appreciable shrub-*Quercus* differences (Fig. 5). Taken together, results suggest that fire severity is the principal factor for sample clustering, while for ecosystem maturity it is the second factor. In all situations, the samples of shrub ecosystems dominated by *Genista* or *Erica* remain clus-



**Fig. 4.** Structure of sample diversity considering the estimated richness by rarefaction and the Shannon index. Beta diversity is higher when analyzing the richness of the communities and decreases considerably when considering the Shannon index, which shows that the communities include numerous OTUs with very low frequency. In all three ecosystems, diversity was reduced with the severity of the fire. C: Control; L: low severity fire; H: high severity fire.



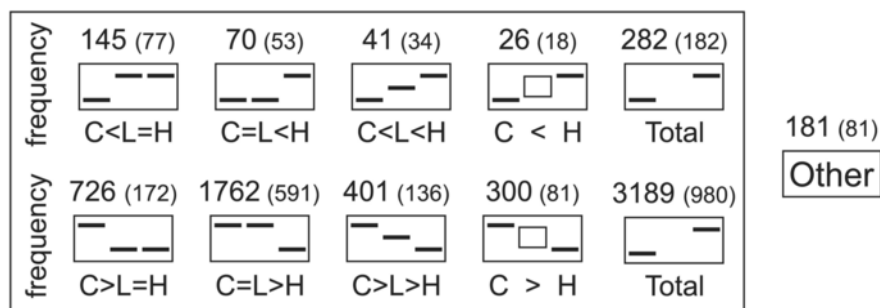
**Fig. 5.** Principal coordinates analysis (PCoA) plots derived from pairwise Bray Curtis and Unifrac distances between bacterial communities. Stars indicate the centroids of the ecosystems and the diamonds the centroids of the severities of the fire experienced by the samples (C: Control; L: low severity fire; H: high severity fire). The shape and color of the centroids serve as the legend of the figures and coincide with those of Fig. 1.

tered together. This observation was previously seen when analyzing the alpha diversity index -NRI (Fig. 2E), where *Quercus* communities displayed less phylogenetic diversity and their OTUs were closer in the phylogenetic tree.

Quantitative indexes (BC and wUF) have the greatest amount of variance explained in its first axis (26.79 and 34.22% respectively, Fig. 5). This observation suggests that differences among communities affect both the frequency of OTUs and their position in the phylogenetic tree, as shown by the Unifrac metric (Lozupone and Knight, 2005).

### 3.3. Differences at the OTUs and taxa level among bacterial communities

In the mixed models, when considering fire severity as principal factor, a total of 3652 of 4696 normalized logarithmic abundance of OTUs were significant after FDR correction (Benjamini and Hochberg, 1995) (Fig. 6). For most of them, 3189 OTUs, there is less abundance in burned soils; whereas for 282 there is an increase in the relative abundance of the reads. Within these 282, the increase in abundance occurs in soils subjected to both low and high severity for 145 OTUs, whereas for 67 the change in abundance is progressive.



**Fig. 6.** Number of OTUs with at least 100 reads that show significant differences between treatments (larger bold numbers). Solid bands indicate the treatments for which these differences were found. For the cases C < H and C > H, the L severity was not significantly different from C or H. In parentheses the number of OTUs for which no significant differences between ecosystems were found for each particular case. C: Control; L: low severity fire; H: high severity fire.

Moreover, for 26 of these 67 OTUs, there are no significant differences between low severity and both control and high severity. For 182 of the OTUs that increase their abundance (between brackets in Fig. 6), they do so without significant ecosystem random effect. Our results are in contrast to (Whitman et al., 2019), who found 160 OTUs raising their frequency in burned soils versus 133 that decrease. This discrepancy could be attributed to the less fire severity in the samples they studied.

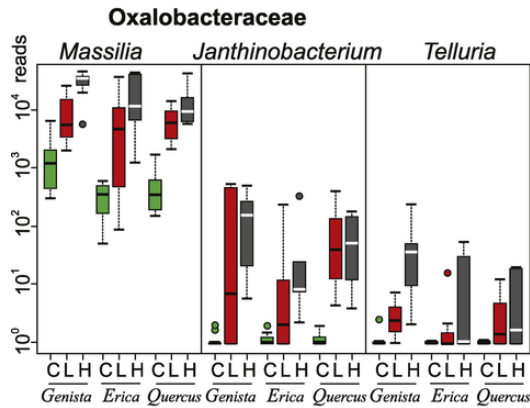
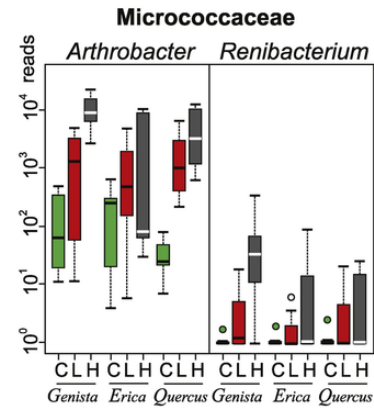
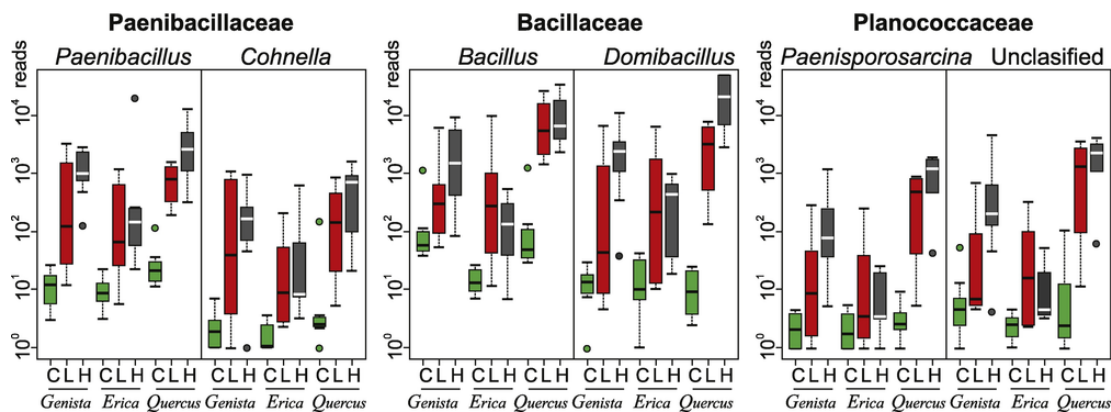
Many of the OTUs whose abundance is favored by fire belong to the Phylum Firmicutes, specifically to the Paenibacillaceae, Bacillaceae and Planococcaceae families. Various OTUs belong to the genus *Paenibacillus*. Another family that includes several of the OTUs positively associated to fire severity is Oxalobacteraceae, which belongs to the class Betaproteobacteria. Among Betaproteobacteria, the genus *Massilia* stands out. The increase in Firmicutes and *Massilia* was also found in another study (Pérez-Valera et al., 2017). Their abundance was attributed to the forms of resistance (spores) of the Firmicutes, and the ability to be an early root-colonizer of *Massilia*. In our study, the third group most represented among the significant OTUs were the Actinobacteria, mainly of the Micrococcaceae family (3 of the OTUs belong to the genus *Arthrobacter*). Significant differences in OTUs were also detected for other underrepresented groups, such as *Pseudomonas* or *Pedobacter* (Bacteroidetes).

Some of the OTUs that significantly increased their relative abundance became dominant in their communities. An example is OTU\_2 (*Massilia suwonensis*) with more than 10,000 (10%) normalized reads in 3 communities after fire. Nevertheless, this was not the case in 9 out of the 20 communities with high severity. The following OTUs were comprised of more than 10,000 normalized reads: OTU\_3 (*Massilia solisilvae*) in 6 communities, OTU\_8 (family Oxalobacteraceae) in 5 communities, OTU\_6 (*Arthrobacter humicola*) in 7 communities, OTU\_5 (*Bacillus gossypii*) in 2 communities, and OTU\_1 (*Domibacillus tundrae*), in 5 communities. For the latter, the frequencies were higher than 50% in 2 communities. Together, results suggest that just a few OTUs were responsible for the greatest reduction in diversity observed in soil bacterial communities after a wildfire. These OTUs belong to only a few families of 3 bacterial phyla, which explain the decrease in phylogenetic diversity of these communities. It was not possible to point out specific OTUs that could serve as indicators of the severity of the fire, since they may be different for the different communities. Nevertheless, these OTUs are included in just a few families, so the increase in the frequencies of these taxa would in fact be good severity indicators.

Reads were also clustered by first assigning each read to its corresponding taxa at the genus level. The same genera that increased their frequencies with the severity, found in the analysis with OTUs, were

also found with this clustering (Fig. 7 and mixed model results are included in Table S1). Each of the genera showed a distinct behavior in the different communities. The genus *Massilia* has been associated with early stages of microbial succession (Ofek et al., 2012), and, thus, it can be used as a reference (Fig. 7A). *Massilia* has an average abundance of 963 normalized reads in the control communities, with a range from 49 to 6510 (Mixed model:  $P=1.3 \times 10^{-13}$ ,  $P(C=L)=1.9 \times 10^{-8}$ ,  $P(C=H)=6.3 \times 10^{-15}$ ,  $P(L=H)=7.7 \times 10^{-4}$ , and low ecosystem effect 0.028, Table S1). They are bacteria for which no resistance forms have been described, so their presence in burnt soils could only be explained by their arrival from unburned areas. However, the relative abundance of *Massilia* in soils subjected to low fire severity is nine times that of the controls (8864 normalized reads), with a very wide range, especially in ecosystems dominated by *Erica* (87 to 36,920). In contrast, in soils subjected to high severity, the abundance is 24 times that of the control (24,058 with range of 1243 to 46,937). These results indicate that *Massilia* becomes dominant in some communities after wildfires, but not in all. Besides, the dominant species within the genus *Massilia* are not always the same. For instance, OTUs from at least *M. suwonensis* and *M. solisilvae* were found. A different scenario was observed for two other phylogenetically close genera of the Oxalobacteraceae family, which *Massilia* also belongs to: *Janthinobacterium* and *Telluria*. These two are basically non-existent in the control communities (<1.6 normalized reads), but reach up to 530 and 236 reads respectively in some burnt soils (Fig. 7A, statistics in Table S1). However, the great variation in abundance of particular taxa or OTUs in each community suggests that it could be a stochastic process. Taken together, results reveal that the species that initiate the post-fire ecological succession belong in many cases to these three close genera, although the particular species within each genus may differ.

Several polycyclic aromatic hydrocarbons from the organic matter accumulate in the soil during the fires. For instance, in a burned woodland soil, Andreolli et al. (2015) detected phenanthrene, benzo(a)anthracene, chrysene, benzo(k)fluoranthene and benzo(a)pyrene. They are toxic to humans, but some bacteria, such as species of the genera *Arthrobacter* and *Bacillus*, are capable of degrading them (Seo et al., 2009). Numerous *Arthrobacter* have been examined for their potential to degrade xenobiotics and other harmful substances. For instance, a strain has been found capable of growing using naphthalene steam as the sole source of carbon (Dore et al., 2003). Moreover, several benzoate- and phenanthrene-degrading *Arthrobacter* strains have been isolated after long-term exposure to polycyclic aromatic hydrocarbons (Bodour et al., 2003; Kotoučková et al., 2004). With regard to abundance, *Arthrobacter* displays a similar pattern to that of *Massilia* (Fig. 7B; Mixed model in Table S1:  $P=5.3 \times 10^{-9}$ ,  $P(C=L)=1.4 \times 10^{-4}$ ,  $P(C=H)=7.6 \times 10^{-10}$ ,

**A. Betaproteobacteria, Burkholderiales****B. Actinobacteria, Actinomycetales****C. Firmicutes, Bacillales**

**Fig. 7.** Number of reads (normalized to 100,000) of some of the bacteria genera that increase their frequency significantly depending on the severity of the fire in the three ecosystems analyzed. The genera can be grouped by their taxonomic classification in the families Oxalobacteraceae (A), Micrococcaceae (B), or in the families Paenibacillaceae, Bacillaceae and Planococcaceae of the order Bacillales (C). C: Control; L: low severity fire; H: high severity fire.

$P(L=H)=3.7 \times 10^{-3}$ , and without ecosystem effect 0.69). Thus, *Arthrobacter* is present in control bacterial communities, but the burning of the ecosystem triggers an increase in their relative abundance. Similar increment in *Arthrobacter* following a fire was observed by Whitman et al. (2019). They recorded a change from 0.09% in control soils to 4% in burnt soils. Another genus of the Micrococcaceae family, *Renibacterium*, is almost non-existent in unburnt soils ( $<1.5$  reads), but its abundance increases up to 300-fold, although just in some communities. The ecological function of Micrococcaceae in the ecosystem could be to metabolize aromatic compounds present in the burned organic matter, and thus, it benefits from something that is toxic to other organisms, such as bacteria and plants. As seen in Oxalobacteraceae, the abundance of a particular Micrococcaceae species (or OTUs) appears to be the result of a stochastic process. This process is suggested by the wide range of variation in their respective abundance in communities from soils subjected to equal severities and from the same ecosystems (Fig. 7A and B).

Firmicutes proliferated in all burned soil communities. This growth occurs from the relatively low abundances seen for the control communities, and is significant for 23 different Firmicutes OTUs, mainly belonging to the genera included in Fig. 7C (statistics in Table S1). Lucas-Borja et al. (2019) found an increase in the frequency of Firmicutes of the genus *Paenibacillus* in experimental outdoor fires. In some cases, they become the dominant species of the community,

as it can be seen with some *Bacillus* and *Domibacillus* of the Bacillaceae family. However, the species within the Paenibacillaceae family are the ones that show the most significant OTUs. Once more, this different alterations in the abundance of OTUs among communities suggests an important stochastic factor. Nonetheless, the overall increase in Firmicutes should be attributed to their capacity for thermo-resistant endospore formation (Smith et al., 2008). In this regard, the ability to metabolize complex organic compounds and fix nitrogen that numerous species of Firmicutes have shown is also important (Yeager et al., 2005).

**4. Conclusions**

After a wildfire, the bacterial communities occupying the most external layers of the soil, close to surface, are subjected to substantial changes. Some OTUs become dominant. These dominant OTUs belong to three specific taxonomic groups, organized according to how they take advantage of the ecosystem's new conditions: a) Oxalobacteraceae close to the *Massilia* genus, which act as pioneers of the ecological succession; b) Micrococcaceae, which are able to use aromatic carbon sources; and c) several genera of the order Bacillales, which survive fire by means of endospores of resistance. Interestingly, within each taxonomic group, the OTUs that become dominant are not always the same. There appears to be a random component caused by the specific species present in nearby areas at a certain pe-



riod of time. This implies a loss of alpha diversity in the communities, both Shannon's diversity as well as richness. However, Shannon's beta diversity remains unaltered, since the dominant OTUs in the different samples are not always the same, even though they have been exposed to the same fire severity. Despite the fact that dominant OTUs are not always the same in the different communities under high severity, the large relative frequencies of reads belonging to the Oxalobacteraceae families (largely *Massilia*), Micrococcaceae (predominantly *Arthrobacter*), and of the order Bacillales (including bacteria of the genera *Paenibacillus*, *Bacillus* and *Domibacillus*), suggest that they are the best bacterial indicators of fire severity.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2020.138636>.

## Uncited reference

Herlemann et al., 2011

## CRediT authorship contribution statement

**Luis E. Sáenz de Miera:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Visualization, Writing - review & editing, Project administration, Funding acquisition. **Rayo Pinto:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Visualization, Writing - review & editing, Project administration, Funding acquisition. **Juan J. Gutierrez-Gonzalez:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Visualization, Writing - review & editing, Project administration, Funding acquisition. **Leonor Calvo:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Visualization, Writing - review & editing, Project administration, Funding acquisition. **Gemma Ansola:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Visualization, Writing - review & editing, Project administration, Funding acquisition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

This study was financially supported by the Spanish Ministry of Economy and Competitiveness, the Spanish Ministry of Science, Innovation and Universities, and the European Regional Development Fund (ERDF), in the framework of the GESFIRE (AGL2013-48189-C2-1-R) and FIRESEVES (AGL2017-86075-C2-1-R) projects; and by the Regional Government of Castile and León in the framework of the FIRECYL (LE033U14) and SEFIRECYL (LE001P17) projects.

## References

- Andreolli, M., Lampis, S., Brignoli, P., Vallini, G., 2015. Bioaugmentation and biostimulation as strategies for the bioremediation of a burned woodland soil contaminated by toxic hydrocarbons: a comparative study. *J. Environ. Manag.* 153, 121–131. <https://doi.org/10.1016/j.jenvman.2015.02.007>.
- Bárcenas-Moreno, G., García-Orenes, F., Mataix-Solera, J., Mataix-Beneyto, J., Bååth, E., 2011. Soil microbial recolonisation after a fire in a Mediterranean forest. *Biol. Fertil. Soils* 47, 261–272. <https://doi.org/10.1007/s00374-010-0532-2>.
- Bardgett, R.D., van der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. *Nature* 515, 505–511. <https://doi.org/10.1038/nature13855>.
- Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Wiley R. Stat. Soc.* 57, 289–300.
- Bodour, A.A., Wang, J.M., Brusseau, M.L., Maier, R.M., 2003. Temporal change in culturable phenanthrene degraders in response to long-term exposure to phenanthrene in a soil column system. *Environ. Microbiol.* 5, 888–895. <https://doi.org/10.1046/j.1462-2920.2003.00481.x>.
- Calvo, L., Santalla, S., Valbuena, L., Marcos, E., Tárrega, R., Luis-Calabuig, E., 2008. Post-fire natural regeneration of a *Pinus pinaster* forest in NW Spain. *Plant Ecol.* 197, 81–90. <https://doi.org/10.1007/s11258-007-9362-1>.
- Calvo, L., Huerta, S., Marcos, E., Calvo-Fernández, J., Taboada, A., 2015. The role of prescribed fire in the provision of regulating ecosystem services of Spanish heathlands. *Ecol. Quest.* 21, 71. <https://doi.org/10.12775/eq.2015.012>.
- Certini, G., 2005. Effects of fire on properties of forest soils: a review. *Oecologia* 143, 1–10. <https://doi.org/10.1007/s00442-004-1788-8>.
- Cole, J.R., Wang, Q., Fish, J.A., Chai, B., McGarrell, D.M., Sun, Y., Brown, C.T., Porras-Alfaro, A., Kuske, C.R., Tiedje, J.M., 2014. Ribosomal database project: data and tools for high throughput rRNA analysis. *Nucleic Acids Res.* 42, 633–642. <https://doi.org/10.1093/nar/gkt1244>.
- Delgado-Baquerizo, M., Oliverio, A.M., Brewer, T.E., Benavent-González, A., Eldridge, D.J., Bardgett, R.D., Maestre, F.T., Singh, B.K., Fierer, N., 2018. A global atlas of the dominant bacteria found in soil. *Science* (80-) 359, 320–325. <https://doi.org/10.1126/science.aap9516>.
- Dore, S.Y., Clancy, Q.E., Rylee, S.M., Kulpa, C.F., 2003. Naphthalene-utilizing and mercury-resistant bacteria isolated from an acidic environment. *Appl. Microbiol. Biotechnol.* 63, 194–199. <https://doi.org/10.1007/s00253-003-1378-4>.
- Dzwonko, Z., Loster, S., Gawroński, S., 2015. Impact of fire severity on soil properties and the development of tree and shrub species in a Scots pine moist forest site in southern Poland. *For. Ecol. Manag.* 342, 56–63. <https://doi.org/10.1016/j.foreco.2015.01.013>.
- Fernández-García, V., Santamarta, M., Fernández-Manso, A., Quintano, C., Marcos, E., Calvo, L., 2018. Burn severity metrics in fire-prone pine ecosystems along a climatic gradient using Landsat imagery. *Remote Sens. Environ.* 206, 205–217. <https://doi.org/10.1016/j.rse.2017.12.029>.
- Fernández-García, V., Fulé, P.Z., Marcos, E., Calvo, L., 2019. The role of fire frequency and severity on the regeneration of Mediterranean serotinous pines under different environmental conditions. *For. Ecol. Manag.* 444, 59–68. <https://doi.org/10.1016/j.foreco.2019.04.040>.
- Fernández-Manso, A., Fernández-Manso, O., Quintano, C., 2016. SENTINEL-2A red-edge spectral indices suitability for discriminating burn severity. *Int. J. Appl. Earth Obs. Geoinf.* 50, 170–175. <https://doi.org/10.1016/j.jag.2016.03.005>.
- Ferreira, D., Pinho, C., Brito, J.C., Santos, X., 2019. Increase of genetic diversity indicates ecological opportunities in recurrent-fire landscapes for wall lizards. *Sci. Rep.* 9, 1–11. <https://doi.org/10.1038/s41598-019-41729-6>.
- Ferrenberg, S., O'Neill, S.P., Knelman, J.E., Todd, B., Duggan, S., Bradley, D., Robinson, T., Schmidt, S.K., Townsend, A.R., Williams, M.W., Cleveland, C.C., Melbourne, B.A., Jiang, L., Nemergut, D.R., 2013. Changes in assembly processes in soil bacterial communities following a wildfire disturbance. *ISME J* 7, 1102–1111. <https://doi.org/10.1038/ismej.2013.11>.
- Francos, M., Úbeda, X., Tort, J., Panareda, J.M., Cerdà, A., 2016. The role of forest fire severity on vegetation recovery after 18 years. Implications for forest management of *Quercus suber* L. in Iberian Peninsula. *Glob. Planet. Change* 145, 11–16. <https://doi.org/10.1016/j.gloplacha.2016.07.016>.
- García-Llamas, P., Suárez-Seoane, S., Fernández-Guisuraga, J.M., Fernández-García, V., Fernández-Manso, A., Quintano, C., Taboada, A., Marcos, E., Calvo, L., 2019. Evaluation and comparison of Landsat 8, Sentinel-2 and Deimos-1 remote sensing indices for assessing burn severity in Mediterranean fire-prone ecosystems. *Int. J. Appl. Earth Obs. Geoinf.* 80, 137–144. <https://doi.org/10.1016/j.jag.2019.04.006>.
- Goberna, M., Verdú, M., 2016. Predicting microbial traits with phylogenies. *ISME J* 10, 959–967. <https://doi.org/10.1038/ismej.2015.171>.
- González de Vega, S., de las Heras, J., Moya, D., 2016. Resilience of Mediterranean terrestrial ecosystems and fire severity in semiarid areas: responses of Aleppo pine forests in the short, mid and long term. *Sci. Total Environ.* 573, 1171–1177. <https://doi.org/10.1016/j.scitotenv.2016.03.115>.
- González de Vega, S., de las Heras, J., Moya, D., 2018. Post-fire regeneration and diversity response to burn severity in *Pinus halepensis* Mill. forests. *Forests* 9, <https://doi.org/10.3390/f9060299>.
- Graham, E.B., Wieder, W.R., Leff, J.W., Weintraub, S.R., Townsend, A.R., Cleveland, C.C., Philippot, L., Nemergut, D.R., 2014. Do we need to understand microbial communities to predict ecosystem function? A comparison of statistical models of nitrogen cycling processes. *Soil Biol. Biochem.* 68, 279–282. <https://doi.org/10.1016/j.soilbio.2013.08.023>.
- Graham, E.B., Knelman, J.E., Schindlbacher, A., Siciliano, S., Breulmann, M., Yannarell, A., Beman, J.M., Abell, G., Philippot, L., Prosser, J., Foulquier, A., Yuste, J.C., Glanville, H.C., Jones, D.L., Angel, R., Salminen, J., Newton, R.J., Bürgmann, H., Ingram, L.J., Hamer, U., Siljanen, H.M.P., Peltoniemi, K., Potthast, K., Bañeras, L., Hartmann, M., Banerjee, S., Yu, R.Q., Nogaro, G., Richter, A., Koranda, M., Castle, S.C., Goberna, M., Song, B., Chatterjee, A., Nunes, O.C.,

- Lopes, A.R., Cao, Y., Kaisermann, A., Hallin, S., Strickland, M.S., Garcia-Pausas, J., Barba, J., Kang, H., Isobe, K., Papaspyrou, S., Pastorelli, R., Lagomarsino, A., Lindström, E.S., Basiliko, N., Nemergut, D.R., 2016. Microbes as engines of ecosystem function: when does community structure enhance predictions of ecosystem processes? *Front. Microbiol.* 7, 1–10. <https://doi.org/10.3389/fmicb.2016.00214>.
- Hart, S.C., DeLuca, T.H., Newman, G.S., MacKenzie, M.D., Boyle, S.I., 2005. Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. *For. Ecol. Manag.* 220, 166–184. <https://doi.org/10.1016/j.foreco.2005.08.012>.
- Heijden, C.S., van der M.G.A., Bardgett, R.D., Straalen, van N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett.* 11, 296–310. <https://doi.org/10.1111/j.1461-0248.2007.01139.x>.
- Herlemann, D.P.R., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J.J., Andersson, A.F., 2011. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME J* 5, 1571–1579. <https://doi.org/10.1038/ismej.2011.41>.
- Hinojosa, M.B., Parra, A., Laudicina, V.A., Moreno, J.M., 2016. Post-fire soil functionality and microbial community structure in a Mediterranean shrubland subjected to experimental drought. *Sci. Total Environ.* 573, 1178–1189. <https://doi.org/10.1016/j.scitotenv.2016.03.117>.
- Holling, C.S., 1973. Of ecological systems. *Source Annu. Rev. Ecol. Syst.* 4, 1–23.
- ITACyL, 2019. Portal de Suelos. Visor de datos de suelos. [WWW Document]. URL [http://suelos.itacyl.es/visor\\_datos](http://suelos.itacyl.es/visor_datos) (accessed 6.7.19).
- Keeley, J.E., 2009. Fire intensity, fire severity and burn severity: a brief review and suggested usage. *Int. J. Wildl. Fire* 18, 116. <https://doi.org/10.1071/wf07049>.
- Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D., Blomberg, S.P., Webb, C.O., 2010. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26, 1463–1464. <https://doi.org/10.1093/bioinformatics/btq166>.
- Kotoučková, L., Schumann, P., Durnová, E., Spröer, C., Sedláček, I., Neča, J., Zdráhal, Z., Némec, M., 2004. *Arthrobacter nitroguajacolicus* sp. nov., a novel 4-nitroguajacol-degrading acinobacterium. *Int. J. Syst. Evol. Microbiol.* 54, 773–777. <https://doi.org/10.1099/ijs.0.02923-0>.
- Kumar, S., Stecher, G., Evolution, K.T.-M. biology and, 2016, U, 2015. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 1–11. <https://doi.org/10.1093/molbev/msw054>.
- Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. lmerTest package: tests in linear mixed effects models. *J. Stat. Softw.* 82, <https://doi.org/10.18637/jss.v082.i13>.
- Li, W., Niu, S., Liu, X., Wang, J., 2019. Short-term response of the soil bacterial community to differing wildfire severity in *Pinus tabulaeformis* stands. *Sci. Rep.* 9, 1–10. <https://doi.org/10.1038/s41598-019-38541-7>.
- López-Poma, R., Bautista, S., 2014. Plant regeneration functional groups modulate the response to fire of soil enzyme activities in a Mediterranean shrubland. *Soil Biol. Biochem.* 79, 5–13. <https://doi.org/10.1016/j.soilbio.2014.08.016>.
- Lozupone, C.A., Knight, R., 2005. UniFrac: a new phylogenetic method for comparing microbial communities UniFrac: a new phylogenetic method for comparing microbial communities [see notes, compare to Bray-Curtis]. *Appl. Environ. Microbiol.* 71, 8228–8235. <https://doi.org/10.1128/AEM.71.12.8228>.
- Lucas-Borja, M.E., Miralles, I., Ortega, R., Plaza-Álvarez, P.A., Gonzalez-Romero, J., Sagra, J., Soriano-Rodríguez, M., Certini, G., Moya, D., Heras, J., 2019. Immediate fire-induced changes in soil microbial community composition in an outdoor experimental controlled system. *Sci. Total Environ.* 696, <https://doi.org/10.1016/j.scitotenv.2019.134033>.
- Mahood, A.L., Balch, J.K., 2019. Repeated fires reduce plant diversity in low-elevation Wyoming big sagebrush ecosystems (1984–2014). *Ecosphere* 10, <https://doi.org/10.1002/ecs2.2591>.
- Mayor, Á.G., Goirán, S.B., Vallejo, V.R., Bautista, S., 2016. Variation in soil enzyme activity as a function of vegetation amount, type, and spatial structure in fire-prone Mediterranean shrublands. *Sci. Total Environ.* 573, 1209–1216. <https://doi.org/10.1016/j.scitotenv.2016.03.139>.
- Miller, J.D., Safford, H.D., Welch, K.R., 2016. Using one year post-fire fire severity assessments to estimate longer-term effects of fire in conifer forests of northern and eastern California, USA. *For. Ecol. Manag.* 382, 168–183. <https://doi.org/10.1016/j.foreco.2016.10.017>.
- Moritz, M.A., Batllori, E., Bradstock, R.A., Gill, A.M., Handmer, J., Hessburg, P.F., Leonard, J., McCaffrey, S., Odion, D.C., Schoennagel, T., Syphard, A.D., 2014. Learning to coexist with wildfire. *Nature* 515, 58–66. <https://doi.org/10.1038/nature13946>.
- Nawrocki, E.P., Kolbe, D.L., Eddy, S.R., 2009. Infernal 1.0: inference of RNA alignments. *Bioinformatics* 25, 1335–1337. <https://doi.org/10.1093/bioinformatics/btp157>.
- Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., O'Hara, R., Simpson, G.L., Al, E., 2010. *Vegan: Community Ecology Package*. R Package Version 1.17-4. 2010.
- Pausas, J.G., Llovet, J., Rodrigo, A., Vallejo, R., 2008. Are wildfires a disaster in the Mediterranean basin? - a review. *Int. J. Wildl. Fire* 17, 713. <https://doi.org/10.1071/wf07151>.
- Pérez-Valera, E., Goberna, M., Faust, K., Raes, J., García, C., Verdú, M., 2017. Fire modifies the phylogenetic structure of soil bacterial co-occurrence networks. *Environ. Microbiol.* 19, 317–327. <https://doi.org/10.1111/1462-2920.13609>.
- Pérez-Valera, E., Verdú, M., Navarro-Cano, J.A., Goberna, M., 2018. Resilience to fire of phylogenetic diversity across biological domains. *Mol. Ecol.* 27, 2896–2908. <https://doi.org/10.1111/mec.14729>.
- Pérez-Valera, E., Goberna, M., Verdú, M., 2019. Fire modulates ecosystem functioning through the phylogenetic structure of soil bacterial communities. *Soil Biol. Biochem.* 129, 80–89. <https://doi.org/10.1016/j.soilbio.2018.11.007>.
- Powell, J.R., Welsh, A., Hallin, S., Allison, S.D., 2015. Microbial functional diversity enhances predictive models linking environmental parameters to ecosystem properties. *Ecology* 96, 1985–1993. <https://doi.org/10.1890/14-1127.1>.
- Price, M.N., Dehal, P.S., Arkin, A.P., 2010. FastTree 2 - approximately maximum-likelihood trees for large alignments. *PLoS One* 5, <https://doi.org/10.1371/journal.pone.0009490>.
- Quintano, C., Fernández-Manso, A., Calvo, L., Marcos, E., Valbuena, L., 2015. Land surface temperature as potential indicator of burn severity in forest Mediterranean ecosystems. *Int. J. Appl. Earth Obs. Geoinf.* 36, 1–12. <https://doi.org/10.1016/j.jag.2014.10.015>.
- Quintano, C., Fernández-Manso, A., Fernández-Manso, O., 2018. Combination of Landsat and Sentinel-2 MSI data for initial assessing of burn severity. *Int. J. Appl. Earth Obs. Geoinf.* 64, 221–225. <https://doi.org/10.1016/j.jag.2017.09.014>.
- Rivas-Martínez, S., Rivas-Sáenz, S., Penas-Merino, A., 2011. Worldwide bioclimatic classification system. *Glob. Geobot.* 1, 1–638. <https://doi.org/10.5616/gg110001>.
- Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. *PeerJ* 4, e2584 <https://doi.org/10.7717/peerj.2584>.
- Schimel, J.P., Schaeffer, S.M., 2012. Microbial control over carbon cycling in soil. *Front. Microbiol.* 3, 1–11. <https://doi.org/10.3389/fmicb.2012.00348>.
- Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* 75, 7537–7541. <https://doi.org/10.1128/AEM.01541-09>.
- Seo, J.S., Keum, Y.S., Li, Q.X., 2009. Bacterial degradation of aromatic compounds. *Int. J. Environ. Res. Public Health* <https://doi.org/10.3390/ijerph6010278>.
- Smith, N.R., Kishchuk, B.E., Mohn, W.W., 2008. Effects of wildfire and harvest disturbances on forest soil bacterial communities. *Appl. Environ. Microbiol.* 74, 216–224. <https://doi.org/10.1128/AEM.01355-07>.
- Tsibart, A., Gennadiev, A., Koshovskii, T., Watts, A., 2014. Polycyclic aromatic hydrocarbons in post-fire soils of drained peatlands in western Meschera (Moscow region, Russia). *Solid Earth* 5, 1305–1317. <https://doi.org/10.5194/se-5-1305-2014>.
- Van Drooge, B.L., Sicard, M., Stohl, A., Fontal, M., Bravo, N., Muñoz, A., Lange, D., Fernández, P., Grimalt, J.O., 2016. Detection and simulation of wildfire smoke impacting a Mediterranean urban atmosphere. *Atmos. Pollut. Res.* 7, 494–502. <https://doi.org/10.1016/j.apr.2015.12.003>.
- Vega, J.A., Fontúrbel, T., Merino, A., Fernández, C., Ferreira, A., Jiménez, E., 2013. Testing the ability of visual indicators of soil burn severity to reflect changes in soil chemical and microbial properties in pine forests and shrubland. *Plant Soil* 369, 73–91. <https://doi.org/10.1007/s11104-012-1532-9>.
- Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* 73, 5261–5267. <https://doi.org/10.1128/AEM.00062-07>.
- Webb, C.O., Ackerly, D.D., McPeck, M.A., Donoghue, M.J., 2002. Phylogenies and community ecology. *Annu. Rev. Ecol. Syst.* 33, 475–505. <https://doi.org/10.1146/annurev.ecolsys.33.010802.150448>.
- Whitman, T., Whitman, E., Wooley, J., Flannigan, M.D., Thompson, D.K., Parisien, M.A., 2019. Soil bacterial and fungal response to wildfires in the Canadian boreal forest across a burn severity gradient. *Soil Biol. Biochem.* 138, <https://doi.org/10.1016/j.soilbio.2019.107571>.
- Yeager, C.M., Northup, D.E., Grow, C.C., Barns, S.M., Kuske, C.R., 2005. Changes in nitrogen-fixing and ammonia-oxidizing bacterial communities in soil of a mixed conifer forest after wildfire. *Appl. Environ. Microbiol.* 71, 2713–2722. <https://doi.org/10.1128/AEM.71.5.2713-2722>.