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Soil microbiome drives the recovery of ecosystem functions after fire

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ABSTRACT

Fire is an ecological disturbance that alters soil microbiomes and the functions they mediate in terrestrial ecosystems. Soil microbial diversity in Mediterranean Basin ecosystems shows resilience to fire following the restoration of plant-soil feedbacks. We hypothesised that microbial functions related to organic matter decomposition and nutrient cycling might show similar patterns of recovery. We quantified the rates of microbial respiration and enzymatic activities related to C, N and P cycling in three 20-year fire chronosequences including 150 transects in 50 burned and unburned plots (no historical fire registers) in a paired experimental design. Microbial functions, except for the hydrolysis of N compounds, were sensitive to fire but recovered the levels of unburned plots in approximately 20–24 years. The recovery of microbial functions responded to abiotic and biotic drivers. Total soil nitrogen concentration was overall strong predictor of microbial functions. In addition, fungal phylogenetic diversity significantly explained the post-fire trajectories of potentially mineralizable C, while bacterial diversity was involved in the restoration of organic C and P hydrolysis. Our results suggest that the long-term recovery of soil biodiversity in Mediterranean Basin ecosystems creates resilience to restore essential ecosystem functions after fire.

1. Introduction

Fires are widespread ecological disturbances that cause drastic changes in plant communities, modify the soil physical and chemical environment and ultimately alter soil microbiomes (Certini, 2005; Keeley et al., 2012). Combustion of organic matter and denaturation of enzymes caused by elevated temperature during fire directly impact microbially-mediated ecosystem functions (hereafter 'EFs'), including the decomposition of organic matter and the transformation of essential compounds related to carbon, phosphorous and nitrogen cycling (Certini, 2005; Knicker, 2007; López-Poma and Bautista, 2014). In parallel, shifts in the diversity and composition of soil microbiota can exert immediate changes in microbial EFs (Hart et al., 2005; Bárcenas-Moreno et al., 2011; Goberna et al., 2012; Graham et al., 2016).

In Mediterranean Basin ecosystems, where biological communities have co-existed with fire over evolutionary timescales, plants show high resilience to frequent fire (Lavorel, 1999; Keeley et al., 2012). Major groups of soil microbes show different levels of resistance - i.e. the degree to which microbial composition remains unchanged in the face of a disturbance - and resilience – i.e. the rate at which microbial composition returns to its original composition after being disturbed (Allison and Martiny, 2008; Griffiths and Philippot, 2013). Archaeal communities are the most resistant to high temperatures due to various heat-protection mechanisms such as ether (rather than ester) lipid membrane and DNA stabilization mechanisms (i.e. higher GC ratio) (Stetter, 1999). The extent of resistance and resilience of archaeal communities seem time- and context-dependent, as few available studies report from no fire-induced changes in community composition up to shifts that are not recovered after two years (Goberna et al., 2012; Mikita-Barbato et al., 2015; Pérez-Valera et al., 2018). Bacterial and fungal communities are more sensitive to fire, and show changes in community composition as well as reduced richness (Hart et al., 2005; Dove and Hart, 2017; Castaño et al., 2020; Sáenz de Miera et al., 2020). Soil bacteria are thought to be less sensitive than fungi to fire-induced changes in terms of biomass, richness and diversity (Pressler et al., 2019). Counterintuitively, richness reduction in both soil fungi and bacteria comes at increased levels of phylogenetic diversity (Rincón et al., 2014; Pérez-Valera et al., 2018). Opposing trends in taxonomic and

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phylogenetic diversity indicate that microbial communities after fire contain less taxa which are evolutionarily more distantly related. Plant recovery over time enriches the soil with organic matter (Johnson and Curtis, 2001), eventually restoring the naturally low levels of bacterial and fungal phylogenetic diversity (Fig. S1; Pérez-Valera et al., 2018). Drop of phylogenetic diversity during microbial community reassembly can result from the recovery of competitive hierarchies between deeply-branching lineages that operate under carbon-rich conditions (Goberna et al., 2014). Whether such a long-term restoration of soil microbial diversity drives the recovery of microbial EFs remains to be elucidated.

Based on the multi-level resilience of Mediterranean Basin ecosystems to fire, and on the observation that main biological groups shaping the soil microbiome are recovered within approximately two decades (Fig. S1; Pérez-Valera et al., 2018), we hypothesised that microbial EFs might undergo a similar recovery rate. To test our hypothesis, we studied three 20-year fire chronosequences, i.e. a set of ecologically similar sites that differ in their time since fire, including 150 transects across 25 burned plots and their 25 unburned counterparts. We quantified the C mineralization potential and enzymatic activities involved in nutrient cycling. Then, we evaluated the fire-induced shifts in microbial EFs and linked them to changes in soil abiotic properties and the relative abundance of main fungal and bacterial lineages. We did not consider archaeal communities, since we previously described that archaeal diversity and community composition did not respond to fire in our study sites (Pérez-Valera et al., 2018). Finally, we sought whether the recovery of each EF responds to abiotic (soil properties) or biotic drivers (fungal and bacterial diversity). To do so, we used phylogenetic diversity since, by incorporating the evolutionary relationships between lineages, these metrics are able to capture shared functional abilities and are thus better proxies of microbial EFs (Martiny et al., 2015; Goberna and Verdú, 2016, 2018).

2. Material and methods

2.1. Study area and experimental design

We designed a space-for-time substitution experiment, in which we characterised three fire chronosequences in the north, centre and south of Valencia (E Spain). Each chronosequence contained eight to nine sites that had experienced a single wildfire event between 1994 and 2014 according to the historical fire registers provided by the Regional Government (Pérez-Valera et al., 2018). Based on the climatic conditions in the study area, ignition date (mostly in the hot and dry season) and fuel availability in unburned nearby sites, it can be assumed that fires were generally of high intensity. Chronosequences were located at (mean ± SE) 84 ± 22 km between each other, estimated as the pairwise mean distance between chronosequence centroids (i.e. the middle geographical point across sites). Within each chronosequence, sites were respectively located on average at 11.5 ± 0.8, 10.2 ± 1.2, and 10.8 ± 1.8 km between each other. Environmental heterogeneity across sites was reduced by selectively looking for areas that fulfilled similar criteria of land-use (forest soil), lithology (calcareous), slope orientation (N to E) and steepness (15±1°), as well as plant cover by using GIS with local maps and ortophotographs. Plant communities were generally consisted of evergreen shrublands with *Pinus halepensis* and varying abundance of *Quercus coccifera*, *Rosmarinus officinalis*, *Ulex parviflorus* and *Cistus* species. Site features were further validated *in situ* during an extensive field inspection. All details and UTM site locations can be found in Pérez-Valera et al. (2018).

To further account for environmental heterogeneity, we established a paired experimental design, each site having a burned and an unburned plot, according to historical fire registers (comprising up to 38 years before sampling; Table S1 in Pérez-Valera et al., 2018). In unburned plots we detected no signs of burning in the vegetation or soil profile during field inspections either. Paired burned and unburned plots (30 × 30 m each) had similar environmental conditions, land-use history and were located as close as possible but avoiding the fire edge (on average 435 ± 49 m separation). Supporting the environmental similarity between paired plots, soil abiotic properties (total organic C, total N, pH, humidity, electrical conductivity and NO_3^--N contents) showed spatial autocorrelation at short distances (<10 km), according to Mantel correlograms between soil dissimilarity (Bray Curtis) matrices and geographic distance matrices (see Fig. S4 in Pérez-Valera et al., 2018). In addition, total inorganic carbon, which is not expected to be affected by fire unless temperature exceeds 1000 °C (Certini, 2005), did not differ significantly between paired plots.

2.2. Soil sampling and sample analysis

Soil samples were taken at 0–5 cm depth over a 5-day period in May 2014. Three linear 25m-transects per plot were drawn in the direction of the slope, located in parallel 10 m apart, making a total of 150 samples (25 sites × 2 plots × 3 transects). Prior to sample collection, the ash layer, litter, mosses and stones were removed if present. Along each transect, ten subsamples (ca. 100 g each) were regularly taken every 2.5 m and pooled into one composite sample per transect. Soil samples were transported to the laboratory in an icebox containing cooling blocks, sieved through a 2 mm mesh upon arrival to the laboratory and kept at 5 °C during subsequent analyses. Soil physical and chemical properties, including pH, water content, electrical conductivity, total organic C, total N, ammonium (NH_4^+-N) and nitrate (NO_3^--N), were analysed following standard procedures, as described and published in Pérez-Valera et al. (2018).

2.3. Microbial respiration and enzymatic activities

We measured microbial heterotrophic respiration under optimal conditions in root-free sieved soil samples, and used it as an indicator of C mineralization potential (Nannipieri et al., 1990). CO_2–C production was measured during an aerobic incubation in the dark (60% water-holding capacity, 28 °C, 30 days) using a 6700 Headspace CO_2-analyzer (Illinois Instruments) as in Pérez-Valera et al. (2019). We fitted the curve of CO_2–C production over time to a density-dependent logistic growth equation:

$$CO_2 - C = \frac{CO_2 - C_{max}}{1 + e^{-r(t\ -\ s)}}$$

where CO_2–C $_{max}$ indicates the asymptote or maximum degree of CO_2–C production, *r* the exponential rate of CO_2–C production, *t* the time at which CO_2–C production was quantified and *s* the time at the midpoint of the exponential portion of the curve. We estimated the kinetic parameters of C mineralization potential in R 3.6.0 (R Core Team, 2019). CO_2–C $_{max}$ was the most responsive to fire and used for further analyses (details below).

Enzymatic activities related to C (β-glucosidase), P (alkaline phosphatase) and N (urease) cycling were quantified using standard procedures. Briefly, β-glucosidase and alkaline phosphatase activities were measured as the amount of p-nitrophenol (PNP) that 0.5 g of soil produced under controlled conditions of temperature (37 °C, 1 h) and pH (6 and 11, respectively) (Tabatabai and Bremner, 1969; Eiviazi and Tabatabai, 1988). Soil urease was quantified as the NH_4^+-N produced by 1 g soil after incubation for 2 h at 37 °C and pH 10 (Kandeler and Gerber, 1988).

2.4. Microbial composition and phylogeny reconstruction

We characterised the soil microbiome by extracting soil DNA and sequencing amplicons of fungal ITS regions and 16S rRNA genes (see Pérez-Valera et al., 2018 for a detailed description). Briefly, DNA was extracted in duplicates from ca. 0.25 g soil with the PowerSoil DNA

Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA). DNA amplicons based on ITS1F and ITS4R primers for fungi (Gardes and Bruns, 1993; White et al., 1990) and 515F and 806R primers for bacteria (Caporaso et al., 2012) were sequenced using Roche 454 FLX titanium instruments and reagents. After initial sequence processing (removal of sequences $<$150 bp, including Ns or homopolymers $>$6 pb), we obtained 1,080,311 (7202 ± 269 per sample) fungal and 1,280,728 (8538 ± 278) bacterial sequences. DNA sequences were clustered at a similarity of 97%, producing 6620 fungal and 7003 bacterial Operational Taxonomic Units (OTUs) in Qiime 1.9.1 (Caporaso et al., 2010a) after discarding singletons. DNA sequencing showed Good's coverage of 0.97 ± 0.001 for fungi and 0.90 ± 0.006 for bacteria as calculated with the *QsRutils* package for R (Quensen, 2020). Relative abundances were calculated as OTU fractions per transect and corrected by the estimated number of 16S rRNA gene copies for bacteria (Kembel et al., 2012).

Fungal phylogenies were reconstructed by grafting OTUs into a genus-level tree that we constructed based on the literature. Bacterial phylogenies where reconstructed using RAxML (Stamatakis, 2014) by using representative OTU sequences previously aligned with PyNAST (Caporaso et al., 2010b). Tree topology was constrained at the phylum level (class for Proteobacteria). Multiple phylogenies for fungi ($n = 5$) and bacteria ($n = 5$) were reconstructed to accommodate phylogenetic uncertainty. Standardized mean phylogenetic distance ($_{std}$MPD) was calculated as a metrics of phylogenetic α diversity in *picante* for R (Kembel et al., 2010). Further details about phylogeny reconstruction along with the phylogenetic trees used here can be found in Pérez-Valera et al. (2018).

2.5. Statistical analysis

We tested the existence of short-term effects of fire on microbial functions, by comparing each EF (i.e. maximum degree of CO_2–C production, as well as β-glucosidase, alkaline phosphatase and urease activities) in plots that had burned 0–3 years before sampling and their unburned counterparts through paired t-tests in R.

To estimate the post-fire recovery of EFs, we used the difference (Δ) between paired burned and unburned transects as the dependent variable and time since fire as the fixed effect variable in Bayesian generalized linear mixed models (GLMM) with *MCMCglmm* for R (Hadfield, 2010). In all GLMMs we incorporated the geographic distance matrix between transects as a random variable to account for the non-independence of nearby transects as in Stone et al. (2011). Average recovery times were calculated by interpolation or extrapolation through the equation of the fitted model.

To visualise the effects of fire on soil abiotic properties, specific microbial lineages and EFs, we performed a principal component analysis (PCA) that included the differentials (Δ) of soil properties (i.e. TOC, TN, humidity, pH, electrical conductivity, NO_3^--N and NH_4^+-N), relative abundances of fungal and bacterial phyla and microbial EFs. The PCA was performed with the function *prcomp* in R with the *scale* argument.

Finally, we evaluated whether the recovery of microbial EFs was explained by the variation in soil abiotic properties and/or microbial phylogenetic diversity, through two consecutive Bayesian GLMMs. In the first model, we used each ΔEF as a dependent variable and soil abiotic properties (i.e. Δ in TOC, TN, humidity, pH, electrical conductivity, NO_3^--N and NH_4^+-N) as fixed factors, including time since fire and the geographic distance matrix as random variables as above. The second model had the same structure, but used the phylogenetic diversity of fungi and bacteria (Δ_{std}MPD) as fixed factors.

2.6. Accession numbers

Raw DNA sequences are available at the European Nucleotide Archive website (http://www.ebi.ac.uk/ena/data/view/PRJEB13469 and http://www.ebi.ac.uk/ena/data/view/PRJEB13853), as originally published in Pérez-Valera et al. (2018).

3. Results

Fire significantly decreased the maximum degree of microbial CO_2–C production, which we used as an indicator of soil organic C mineralization potential (Fig. 1, Table 1). Other kinetic parameters describing microbial respiration curves did not respond to fire, as was the case of the exponential rate of CO_2–C change (r) or the time at the midpoint of the curve (s, Fig. S2). Soil β-glucosidase and phosphatase activities significantly dropped after fire, while urease activity did not respond to the disturbance (Fig. 1, Table 1). Soil microbial CO_2–C production in burned plots increased with time since fire, and achieved the levels of unburned plots in 246.5 months (Fig. 1, Table 1). Similar patterns were observed for β-glucosidase (264.3 months) and phosphatase activities (293 months, Table 1).

Fire-induced shifts in microbially-mediated soil EFs correlated to changes in soil abiotic properties and microbial relative abundances, as shown by the PCA containing paired burned and unburned differences (Δ) for each variable (Fig. 2). In the figure depicting the first two principal components (PCs), recently burned plots tend to be located in the upper left portion of the graph while plots burned long ago are in the bottom right area (Fig. 2). The effect of time since fire overrode the environmental heterogeneity encompassed in all three chronosequences in determining the distribution of our samples in the same biplot, which did not show any clear pattern (Fig. S3). In particular, the first PC (22.5% variance) correlated to parameters that responded to fire and recovered with time such as pH (negative pole), and humidity, TOC and TN (positive pole, Fig. 2). High values in ΔTOC, ΔTN and Δhumidity, and low values in ΔpH associated with the positive pole of PC1 indicating similar levels in burned and unburned plots (according to the exploration of raw data). Thus, this axis can be interpreted as the post-fire recovery of soil organic matter. Microbial lineages such as Ascomycota and Firmicutes, among others, were favoured soon after fire, as shown by their negative loadings in PC1, while the recovery mainly promoted Basidiomycota and Glomeromycota (positive pole, Fig. 2; Figs. S4 and S5). The response of lineages such as Proteobacteria, Actinobacteria and Chytridiomycota, which showed higher dependence on changes in mineral N (i.e. NH_4^+-N and NO_3^--N) correlated with PC2 (12.7% variance), had complex and class-dependent fire responses and post-fire evolution (Fig. 2; Figs. S4, S5 and S6).

To analyse the drivers of the shifts in microbial EFs we performed statistical models that used as predictors soil abiotic properties and microbial phylogenetic diversity, as a means to account for the complexity of microbial responses. Both abiotic and biotic drivers explained the variation in all microbial EFs, except for urease activity that responded exclusively to abiotic factors. Recovery of total nitrogen was an overall predictor of microbial EFs, while restoration of the levels of pH, NO_3^--N and electrical conductivity partly explained maximum CO_2–C production and β-glucosidase activity (Table 2). Urease activity also responded to TOC and soil humidity. Importantly, our models showed that microbial phylogenetic diversity also explained the restoration of essential EFs. While fungal phylogenetic diversity was a good predictor of the trajectories of potentially mineralizable C, bacterial diversity was involved in the restoration of the hydrolysis of organic C and P compounds (Table 2). In all cases, the lowest the levels of phylogenetic diversity, the highest the microbial EF rates (Table 2).

4. Discussion

Fire significantly decreased the C mineralization potential as well as the enzymatic hydrolysis of organic C and P compounds, while it did not alter that of N compounds. The decline in microbial activity, and particularly heterotrophic respiration and C- and P-related EFs in soils is a common observation following high-intensity fires (e.g. Fritze et al., 1993; Hernández et al., 1997; Bárcenas-Moreno et al., 2011; Uribe et al., 2013; Fernández-García et al., 2019). Such a decrease is due to the thermal denaturation of enzymes, drop in microbial biomass and altered

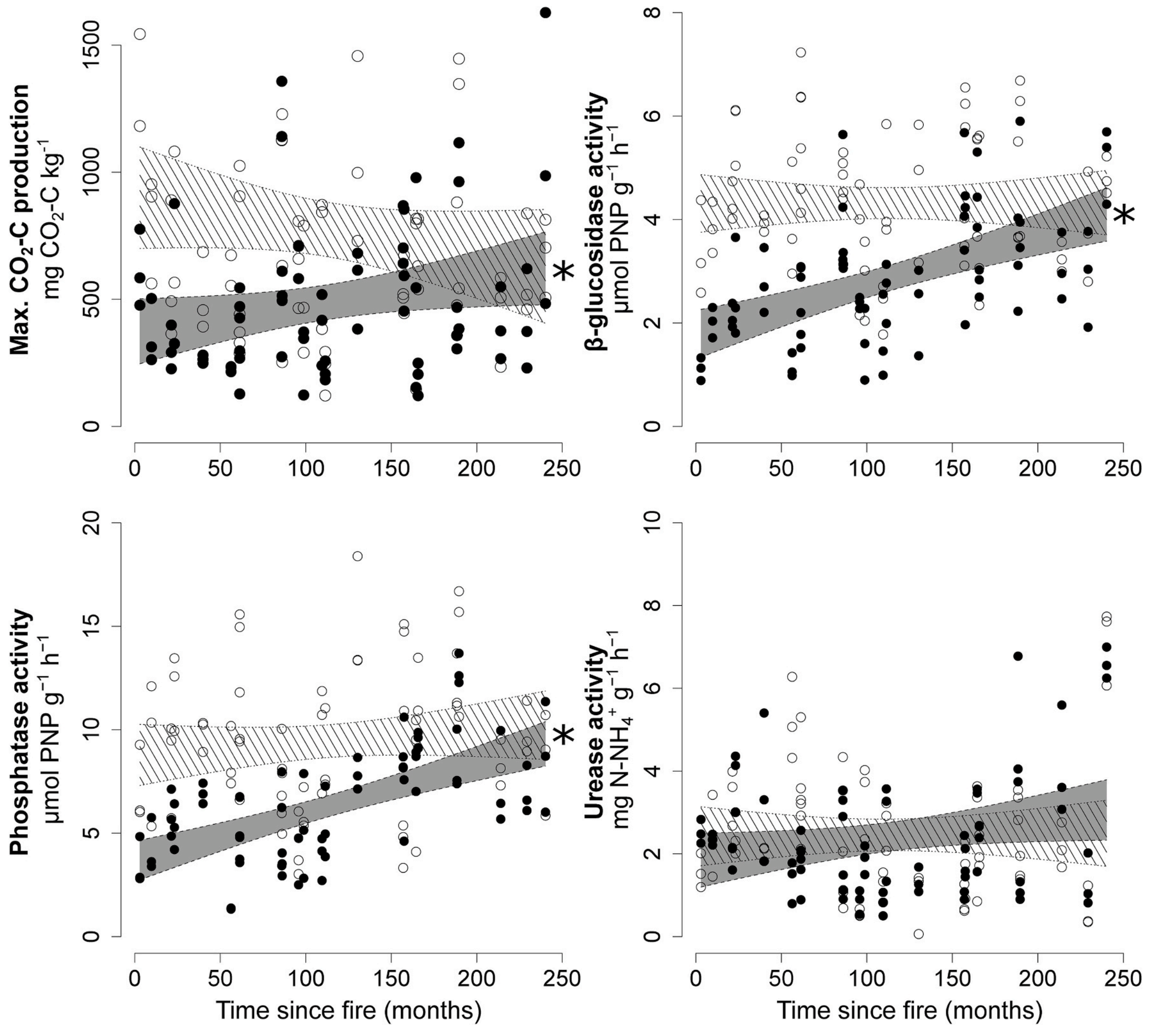

Fig. 1. Post-fire trends of maximum degree of microbial CO_2–C production, β-glucosidase activity, alkaline phosphatase activity and urease activity. Filled circles indicate burned transects and unfilled circles unburned transects. Shaded and hatched areas show the confidence intervals of linear regressions between burned and unburned transects, respectively. Asterisks indicate the existence of a significant post-fire temporal trend of the studied parameter measured as the paired difference (Δ) between burned and unburned transects ($p < 0.05$). Data are expressed on a dry weight basis.

Table 1

Statistical analysis showing: a) Short-term (0–3 years) fire effects on microbial EFs based on paired t-tests comparing burned and unburned transects; b) Effect of time since fire on microbial EFs measured as the paired difference (Δ) between burned and unburned transects; Bayesian post-mean estimates and their 95% expected credible intervals (in brackets) are shown. Significant differences are shown in bold type. *$p < 0.001$.

Microbial EFs	a) Short-term fire effects (t_{14})	b) Effect of time since fire
Max. CO_2–C production	**−4.36 ***	**2.14 [0.69, 3.72]**
β-glucosidase activity	**−9.40 ***	**0.009 [0.006, 0.014]**
Phosphatase activity	**−6.23 ***	**0.017 [0.006, 0.028]**
Urease activity	1.15	0.005 [-0.0002, 0.010]

microbial community composition (Knicker, 2007; Holden and Treseder, 2013; Fernández-García et al., 2019). However, these results differ from reported increases in microbial EFs following low to medium intensity burning (Bárcenas-Moreno and Bååth, 2009; Goberna et al., 2012; Pérez-Valera et al., 2019). Such enhancement might be caused by an incomplete combustion of organic matter that releases labile forms of C, N and other macronutrients, and thus potentially induces microbial recolonization and activity in the short term (Certini, 2014; Muñoz-Rojas et al., 2016). The lack of response of urease activity to fire adds complexity to reported decreases (Hernández et al., 1997; Goberna et al., 2012; Fontúrbel et al., 2012; Xue et al., 2014; Pérez-Valera et al., 2019) and increases (Ajwa et al., 1999) under post-burning scenarios. The observation that urease activity is unrelated to time since fire supports previous work in Mediterranean Basin ecosystems burned 3, 15 and 21 years ago (Moya et al., 2018). Contrasting results suggest that urease activity could be driven by differences in environmental (e.g. climatic, edaphic) conditions along with variations in fire severity (Moya et al., 2018, Fernández-García et al., 2018, 2019) that determines the extent of urease inhibition owing to the pulse of ammonium-N that typically follows the combustion of organics (Hoare and Laidler, 1950).

Rates of C mineralization potential in burned plots, as well as enzymatic hydrolysis of C and P compounds, increased with time since fire, and achieved the levels of unburned plots in 246.5–293 months. Altogether our results indicate that most of the studied functions were sensitive to fire, but resilient in a period of approximately 20–24 years. This observation is in line with previous reports indicating that soil microbial communities are generally sensitive to disturbance both in composition and function (Mataix-Solera et al., 2009), but can be resilient particularly to pulse (short-term intense) disturbance (Allison and Martiny, 2008; Shade et al., 2012). Resilience is seldom reported, probably due to bias in sampling intensity or duration (Shade et al.,

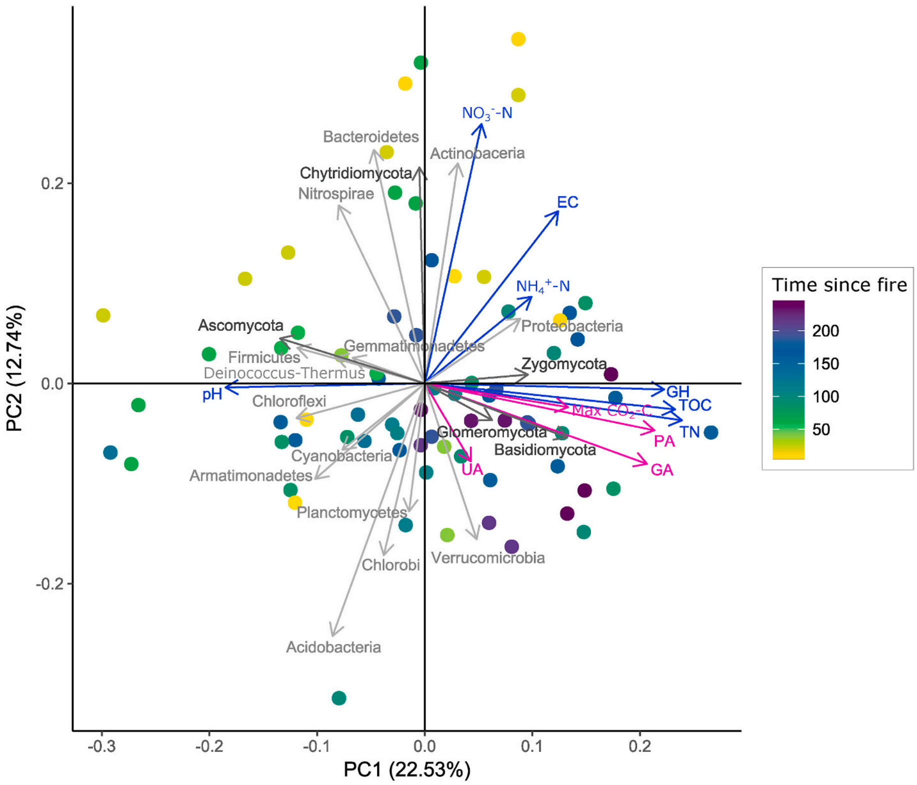

Fig. 2. Biplot of the first and second axes obtained from a principal component analysis that included the paired difference (Δ) between burned and unburned transects of i) main soil abiotic properties (blue arrows; GH *gravimetric humidity*; TOC *total organic carbon;* TN *total nitrogen*; EC *electrical conductivity*; pH; NO_3^--N *nitrate-N*; NH_4^+-N *ammonium-N*), ii) EFs (purple arrows; Max. *CO_2–C production*; GA *β-glucosidase activity*; PA *phosphatase activity*; UA *urease activity*) and iii) fungal (dark grey arrows) and bacterial abundances (light grey arrows) at the phylum level. Arrows indicate the factor loadings on each axis. Transects are coloured according to time since fire (in months). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Results of two Generalized Linear Mixed Models showing Bayesian post-mean estimates and their 95% expected credible intervals (in brackets) of the effect of i) physical and chemical soil parameters and ii) fungal and bacterial phylogenetic diversity (stdMPD) on C mineralization potential (Max. CO_2–C production) and enzymatic activities. Dependent and independent variables were measured as the paired difference (Δ) between burned and unburned transects. Significant differences (i.e. credible intervals not including zero) are shown in bold type.

Variable Predictor	Max. CO_2–C production	β-glucosidase activity	Phosphatase activity	Urease activity
Total Organic C	−0.02 [-0.05, 0.01]	−0.09 [-0.2, 0.04]	−0.07 [-0.4, 0.24]	**−0.31 [-0.5, -0.13]**
Total Nitrogen	**0.59 [0.1, 1.14]**	**3.37 [0.67, 5.83]**	**8.3 [2.7, 13.5]**	**9.17 [5.84, 12.6]**
Humidity	−0.02 [-0.044, 0.0004]	0.05 [-0.05, 0.15]	0.21 [-0.02, 0.47]	**−0.14 [-0.29, -0.004]**
pH	**−0.42 [-0.64, -0.24]**	**−1.2 [-2.01, -0.4]**	−0.12 [-2.19, 2.18]	0.76 [-0.4, 2.06]
Electrical Conductivity	**0.002 [0.0005, 0.004]**	0.004 [-0.004, 0.01]	0.001 [-0.02, 0.02]	−0.01 [-0.01, 0.003]
NO_3^--N	**−0.002 [-0.004, -0.001]**	**−0.01 [-0.02, -0.002]**	−0.01 [-0.02, 0.01]	0.01 [-0.005, 0.02]
NH_4^+-N	−0.005 [-0.014, 0.005]	−0.01 [-0.05, 0.04]	0.06 [-0.04, 0.15]	0.02 [-0.04, 0.08]
Fungal phylogenetic diversity	**−0.029 [-0.057, -0.001]**	−0.10 [-0.24, 0.05]	−0.34 [-0.69, 0.05]	0.15 [-0.01, 0.32]
Bacterial phylogenetic diversity	−0.032 [-0.096, 0.026]	**−0.41 [-0.76, -0.16]**	**−1.23 [-2.03, -0.32]**	−0.11 [-0.18, 0.41]

2012). A review on 131 studies did not find evidence of recovery trends of microbial community composition within the first ten years after fire, although most studies monitored on average only the first two years (Pressler et al., 2019). A few experimental studies and meta-analyses support the recovery of microbial respiration and carbon cycling activities to pre-disturbance levels over periods ranging from ca. 3–15 years (Bárcenas-Moreno et al., 2011; Dooley and Treseder, 2012; López-Poma and Bautista, 2014; Yang et al., 2020). Our results in water-limited ecosystems suggest slower recovery EF rates. Differences across studies can originate from natural variation across biomes, as well as fire intensity and recurrence, which can significantly impact the response of soil microbial communities (Egidi et al., 2016; Pressler et al., 2019). We previously found that, in Mediterranean Basin ecosystems, the resilience to fire of plant communities is essential for the restoration of interrupted plant-soil feedbacks (Pérez-Valera et al., 2018). It has been traditionally thought that the development of plant-soil feedbacks is extremely slow in drylands, but this idea has seldom been tested (Navarro-Cano et al., 2015). Our results suggest that, under dry conditions, litter inputs and improved resource availability in mineral soils during secondary succession may take at least two decades to effectively counteract the initial negative response. These results are in line with previous reports on primary succession in nearby areas, where we described significant increments in soil fertility (e.g. six-fold rise in TOC) and microbial mediated functions during the first two decades after plant establishment (Navarro-Cano et al., 2015).

The recovery of microbial EFs responded both to abiotic and biotic drivers. The post-fire trajectories of C mineralization potential, and rates of C, P and N cycling showed complex linkages to soil abiotic properties as well as to the relative abundances of fungal and bacterial lineages. However, two main lessons can be extracted from our results. First, the recovery of total soil nitrogen was an overall predictor of microbial EFs, which fits well with the notion that nitrogen tends to be a limiting resource under Mediterranean conditions (Hooper and Johnson, 1999). Second, the recovery of soil microbial phylogenetic diversity underlay the restoration of essential EFs. Fungal phylogenetic diversity significantly explained the trajectories of C mineralization potential, while bacterial diversity was involved in the restoration of organic C and P hydrolysis. In all cases, the lowest the levels of phylogenetic diversity, the highest the microbial EF rates. We have previously reported high levels of microbial productivity at low levels of phylogenetic diversity (Pérez-Valera et al., 2015). Such a negative relationship might be mediated by the overrepresentation of a few lineages that are highly productive under carbon-enriched conditions, a pattern reported worldwide for soil bacteria (Goberna et al., 2014; Goberna and Verdú, 2018). In these 20-year fire chronosequences, the decrease with time since fire of microbial phylogenetic diversity mediated by the restoration of soil organic carbon (Pérez-Valera et al., 2018) underlies the recovery of C mineralization potential and nutrient cycling.

In short, our results suggest that, in Mediterranean Basin ecosystems, the relationship between soil microbial diversity and ecosystem functions is resilient to fire. While efforts to date have largely addressed short-term effects of fires on soils, further research is needed to better understand their long-term consequences on the complex above-belowground linkages. Careful assessment of whether upcoming changes in the frequency and severity of fires disrupt the resilience of biological communities and the diversity-EF relationship is fundamental to ensure the preservation of diverse and sustainable fire-prone ecosystems.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2020.107948.

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