

Article

Wildfire Effects on the Soil Respiration and Bacterial Microbiota Composition in Mediterranean-Type Ecosystems

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Abstract: This work provides insights into the effect of fire on soil processes in Mediterranean-type ecosystems in Cyprus. Soil samples from mountainous sites that were subjected to a summer wildfire and adjacent control samples were collected. Incubations were used to estimate basal respiration and isolate soil CO₂ release of heterotrophic microorganisms from autotrophic root respiration and heterotrophic respiration from litter decomposition. Physicochemical property changes, bacteria community changes using DNA extraction and 16S rRNA gene analysis, and the effects of ash and fresh litter addition were studied to reveal the microbial composition and the post-fire soil function. Laboratory incubation showed that burned soils constantly showed higher microbial respiration rates compared with control unburned areas, even six months after a fire. Adding ash to unburned samples increased microbial respiration, suggesting that increased nutrient availability positively correlates with the increased release of CO₂ from fire-affected soil. Elevated temperatures due to the wildfire exerted significant effects on the composition of soil bacterial microbiota. Nevertheless, the wildfire did not affect the alpha-diversity of soil bacteria. New communities of microorganisms are still able to decompose fresh plant material after a fire, but at a slower rate than natural pre-fire populations.

Keywords: wildfire; bacterial microbiota; soil respiration; gene analysis; incubations; ash addition



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1. Introduction

The anthropogenic climate-change-driven amplification of wildfire severity and frequency has been extensively experienced worldwide in recent years, while also being probed in the scientific literature [1]. The ascending global temperatures, extreme heat-waves, and prolonged drought periods due to shifting precipitation dynamics are, consequently, positively correlated with an elevated susceptibility to wildfires, particularly in regions predisposed to arid conditions [2]. Wildfires result in ecological catastrophe, loss of human life, long-term public health impacts due to air pollution, and substantial economic losses [3,4]. It is, therefore, an imperative to understand the causes of wildfires, as well as the effects on burned environments and, most importantly, burned soils in order to facilitate environmental regeneration and the long-term sustainability of burned environments [5,6].

A large number of factors affect the properties and function of soils after a fire. Extensive reviews of the changes in soil physical, chemical, and biological properties caused by fire have been carried out, highlighting the crucial impact of fire intensity on exerted changes [7,8]. Immediate effects concern the destruction or killing of part of the microbial microflora due to elevated temperatures and the loss of soil water [9,10]. Intermediate and long-term effects on burned soils reflect the interaction between the initial degree of change, revegetation, management practices, and weather conditions on the one hand, and soil properties and topography on the other [11,12].

Particular attention has been paid to soil microbiological changes after a fire. These changes may be reasonably explained by soil organic matter (SOM) loss, SOM transfor-

mation, soil structural changes that result in new surfaces for microbes, ash build-up, and pH increase [13]. In turn, modified microbial composition and populations caused by fires may have significant impact on soil ecosystem processes and services, since the microbial biomass and activity act as a source of or sink for labile nutrients and controls, among others, the overall content and chemical fractions of SOM [14,15].

The impacts of wildfire on soil biota diversity and activity have been well elucidated [16]. A wildfire in Spain resulted in significant bacteria population increase, whereas cyanobacteria and fungi populations were diminished [17]. As far as the fungal phyla and fungal functional groups are concerned, basidiomycetes and mycorrhizal taxa displayed particularly low tolerances in relation to fire severity in Alaska [18]. Goberna et al. [19] showed that archaea, bacteria, and fungi responded differently following a Mediterranean shrublands wildfire, with the changes mainly reflected at the community structure rather than the total number of microbial groups. Some microbial groups not only resisted but even benefited from the fire, probably due to the increased abundance in labile forms of C, N, and other macronutrients. While archaea decreased few days after the fire, bacterial populations and microbial biomass increased compared with pre-fire conditions [19].

The study of soil respiration provides a powerful tool for assessing soil function and ecosystem services. Respiration is an index of nutrients contained in organic matter being converted to forms available to crops (e.g., nitrates, phosphates, sulfates). Soil respiration rates indirectly reflect the level of soil fertility and functionality [20]. Therefore, soil respiration rates are constantly evaluated as a key indicator during soil quality and degradation studies [21], and they consequently constitute a key indicator in forest ecological restoration monitoring and research [22].

Soil basal respiration was significantly lower in burned stands compared to unburned ones in a boreal forest ecosystem, with the deterioration being positively correlated with fire severity [18]. Moreover, Castaldi et al. [13] showed that the average daily field CO₂ emissions were significantly lower in burned compared with the control plots one month after a wildfire in a tropical forest. However, the difference between burned and unburned plots did not remain statistically significant eight months after the fire [13]. Prescribed fire did not exert any significant impacts on soil respiration, since a similar evolution between the burned and control plots in both the mixed and pure forest stands was shown [23]. Similarly, burning in a conifer site did not significantly affect soil respiration rates compared with unburned controls [24]. Meanwhile, soil respiration rates were even shown to be increased following wildfires, as increased rates of CO₂ release were measured in the burnt areas compared with those in the unburnt control sites, with the differences being highly dependent on the type of vegetation cover and time since a fire [25,26]. Likewise, Tufekcioglu et al. [27] showed that prescribed fire resulted in increased soil respiration rates in a *Pinus brutia* stand, compared with the control ones.

Nevertheless, studies measuring soil respiration rates in the field cannot easily isolate the contribution of heterotrophic soil microbial respiration from the autotrophic component of the soil respiration (plant roots) and CO₂ release from litter decomposition [28]. Estimates of basal respiration using soil incubations may overcome problems of spatial soil heterogeneity and variability related to the patchy distribution of vegetation (especially in Mediterranean-type ecosystems) and perplexity due to after-fire changes in soil temperature and moisture regimes, which significantly affect soil CO₂ fluxes [29].

This present study was carried out following a short but highly destructive wildfire in a mountainous area in Cyprus, and it was based on laboratory analyses and incubations. Microbial respiration was estimated few days after the fire, aiming to test the hypothesis that the capacity of burned soil samples to decompose native soil organic matter or added incoming fresh litter is equivalent to that of unburned soils. Measurements taken immediately after the fire were also compared with respiration rates six months later. The effect of the fire on microbial characteristics was also investigated by measuring soil respiration after fire ash application, with ash being known to contain macro and micro nutrients needed for microbial growth. DNA analysis was employed to investigate whether eventual soil

respiration differences between burned and unburned samples were related to bacterial assembly changes.

2. Materials and Methods

2.1. Study Sites and Sampling

A wildfire initiated on 3 July 2021 at the mountain areas of Larnaca and Limassol districts of Cyprus, and in the course of a few hours, burned about 55 km² of land due to very strong winds. The fire burned pine forests, shrubland, and cultivated areas.

Topsoil (top 0–10 cm) was sampled from inter-canopy areas in burned and unburned control areas immediately after (7 days), shortly after (1 month, but before any autumn rains), and 6 months after the wildfire. Burned soil was sampled inside the burned site, whereas unburned control samples were collected from sites being either in close proximity (few meters) or far away (few hundreds of meters) from the fire's front line. Control unburned samples collected in close proximity to the fire front could have been influenced by fire to a certain degree, while the ones far away provided the presumably unaffected control soil samples. The burned soil and the respective unburned control (close and far away from the fire front) samples were collected from three sites along the fire front: an olive grove, a pine forest, and a shrubland macchia area. Each site included three plots, and each plot provided one composite sample that was used for incubations, physicochemical properties, and DNA analyses. Composite samples were transferred to the laboratory in plastic bags. One small part of the soil without litter was immediately stored at −20 °C for one month before DNA extraction, and the rest was emptied in metal trays and air-dried in a shaded place for a few days. Then, it was passed through a 2 mm sieve and stored for a few more days until the analysis of initial descriptive properties and incubations for quantifying soil respiration rates. Sieved samples collected from the burned sites were clearly darker compared with the unburned ones, despite being sieved.

The abbreviations of the sites included in the study are as follows: OGB: Olive Grove Burned, OGCn: Olive Grove Control near the fire front line, OGCf: Olive Grove Control far away from the fire front line, MVB: Macchia Vegetation Burned, MVCn: Macchia Vegetation Control near the fire front line, MVCf: Macchia Vegetation Control far away from the fire front line, PFB: Pine Forest Burned, PFC: Pine Forest Control (near the fire front).

2.2. Measurements of Soil Physicochemical Properties

The general physico-chemical properties of the soils were measured in the three sets of samples (burned, unburned near, unburned far away). More precisely, soil texture, soil pH, electrical conductivity (EC), and total CaCO₃ and percentage of organic carbon (%C) (using the Walkley–Black oxidation method) contents were measured.

Soil texture was determined by the hydrometer method [30], while soil pH was determined with a pH meter (Mettler Toledo, SevenGo SG2, Switzerland) in a 1:2.5 sample-to-water suspension. The same suspension was used for soil EC measurement using a benchtop conductivity meter (Mettler Toledo, Five Easy F30, Switzerland). The Bernard calcimeter was used for the assay of the percentage of total CaCO₃. Soil inorganic N (NH₄⁺-N and NO₃[−]-N) was extracted with 2M KCl, and nitrate in extracts was determined calorimetrically by the copper–cadmium column method [31]. The NH₄⁺-N estimation was based on the emerald green color formed when ammonia and sodium salicylate react in the presence of sodium hypochlorite at a high pH. The color reaction was catalyzed by the presence of sodium nitroprusside, as previously described [31]. The soil Water Holding Capacity (WHC) was estimated as proposed by Grace et al. [32]. An oven-dried soil of known weight was placed in porous funnel and soaked with water to saturation. The water tension was then standardized by applying a suction of 0.1 MPa. The sample was then reweighed, and WHC was determined as the percentage of oven-dry soil.

2.3. Soil Respiration Rates

Soil respiration rates were determined by trapping the evolved CO₂ from soil samples in NaOH. Soil samples were remoistened by adding water corresponding to 70% of their WHC, and 50 g of subsamples were incubated in 2 L gas-tight jars at 25 °C. A water film was constantly present in the bottom of the jars in order to maintain a vapor saturated atmosphere inside the jars. Evolved CO₂ was captured in a vial containing 40 mL of 0.5 mol L⁻¹ NaOH, and the quantity of CO₂-C absorbed in the alkali was determined by titration with 0.2 mol/L HCl. The rate of respiration was calculated by the method proposed by Alef [33].

The experiment consisted of five incubations of soil samples, which were carried out successively so that the results of each could be taken into account in the design of the following. The first incubation was intended to show the effect of fire of the basal respiration of soil without roots or litter. It was carried out using soil samples taken from the burned site close to the last front line of the wildfire and their three respective non-burned control areas at the other site of the fire front line. The samples concerned all three sites of this study (the olive grove, the pine forest, and the Mediterranean macchia system). Replicates of each site represented different field plots. First, the results of microbial respiration pointed to the need for a second sampling, which was carried out one month after the fire to include a second control area.

The second incubation was intended to show whether differences revealed during the first incubation may be simply due to the increased amount of fire ash in the burned soil. They were carried out using two “unburned” controls: one from sites very close to the front line of the fire, ensuring exactly the same soil type and management practices (for the case olive grove), and one almost 200 m away from the fire front line to ensure conditions that were surely not affected by high temperatures. For the second incubation, soil respiration from samples coming from unburned sites near the fire line and unburned sites at a distance from the fire line was compared with the respiration of these soils mixed with ash coming from burned surface litter. The amount added depended on the soil organic carbon (SOC) of the soil, and it was such as to reach the SOC of the respective burned samples (Table 1).

Table 1. Chemical characteristics and texture of soils coming from burned (B) and control unburned (C) sites. Standard Error of the Mean is shown next to each column of values.

	pH	EC dS/m	C %	Inorganic N (µg g ⁻¹)	CaCO ₃ %	Sand %	Silt %	Clay %
OGB	7.64 ± 0.22	0.64 ± 0.13	1.64 ± 0.11	18.97 ± 18.89	8.27 ± 1.10	66.24	14.77	18.99
OGCn	7.00 ± 0.02	0.13 ± 0.04	1.44 ± 0.05	0.00 ± 0.00	6.42 ± 0.40	66.84	14.47	18.69
OGCf	7.09 ± 0.05	0.15 ± 0.05	1.41 ± 0.04	4.12 ± 4.19	6.37 ± 0.52	67.60	14.65	18.75
PFB	7.82 ± 0.06	0.30 ± 0.02	0.61 ± 0.03	0.00 ± 0.00	9.84 ± 0.91	83.44	5.20	11.36
PFC	7.58 ± 0.05	0.10 ± 0.03	0.45 ± 0.02	0.00 ± 0.00	5.56 ± 0.43	74.24	12.10	13.66
MVB	7.82 ± 0.04	0.80 ± 0.02	1.71 ± 0.08	7.63 ± 4.78	10.91 ± 0.88	63.84	18.65	17.51
MVCn	7.53 ± 0.03	0.14 ± 0.02	1.47 ± 0.05	0.00 ± 0.00	6.20 ± 0.33	71.84	8.80	19.36
MVCf	7.50 ± 0.02	0.17 ± 0.08	1.55 ± 0.05	0.00 ± 0.00	6.35 ± 0.41	68.44	12.54	20.02

The third incubation aimed to complement the second incubation results by revealing the magnitude of contribution of decomposable organic carbon in ash to the overall microbial respiration of burned samples. It refers to the incubation of the ash used in the second incubation trial alone (without soil), and it was intended to estimate the carbon mineralization potential contained in it. A second treatment in this incubation included ash mixed with a very small amount of soil to boost microbial activity. The carbon content of ash was 5.04%

The fourth incubation was intended to provide an indication of the capacity of the burned soils to decompose plant litter inputs. It was carried out using soil from two burned (olive grove and macchia) and their two respective unburned sites (near and far

away from the fire front). Samples were incubated alone or mixed with 0.2 g of lucerne (*Medicago sativa* L.) litter.

“The fifth incubation concerned samples collected six months after fire and intended to show if the observed trends immediately after fire persisted.”

Soil samples from burned, unburned near, and unburned far away from the fire sites were collected from the same areas as the initial samplings (but not exactly the same plots).

Control blank incubations, constituting jars containing the alkali trap but no soil, were run in triplicates in all incubation sets. Soil samples were always prepared in double and one of them was used for the measurement of the initial pH_(H2O) (1:2.5 soil to water ratio) and EC.

2.4. DNA Extraction and High-Throughput Sequencing

Soil DNA from burned and unburned samples was extracted using the DNeasy PowerSoil Kit (QIAGEN N.V., Venlo, The Netherlands). Following the extraction process, the DNA samples were quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA) and subsequently stored at -20°C for further analysis. To delineate the bacterial community composition, the V3–V4 variable region of the 16S rRNA gene was amplified using PCR primers 515/806. Pair-end (2×300 bp) sequencing using a MiSeq (Illumina, San Diego, CA, USA) platform was performed at the Genome Sequencing facilities of the Environmental Microbiology and Biotechnology Center (Agricultural Research Institute, Nicosia, Cyprus). DADA2 pipeline was used to quality-filter, de-noise, de-replicate, merge, and chimera-check the raw sequencing data. Taxonomic assignment was performed with QIIME2 sklearn-based classifier against SILVA rRNA database Release 132 at 99% identity [34]. The process was performed using a Jupyter Notebook [35] web-based platform of the Bioinformatic Facility of the Environmental Microbiology and Biotechnology Center (Agricultural Research Institute, Nicosia, Cyprus). The data were uploaded to ENA (European Nucleotide Archive), supported by EMBL-EBI, with primary Accession PRJEB41189.

2.5. Data Analysis

C-CO₂ release data were analyzed to indicate changes in daily respiration rate and cumulative respiration over time. Fitting of exponential association functions ($Y = Y_{\max} \times (1 - e^{-kx})$) to cumulative CO₂ data was initially attempted to designate rate constants (k) that could be used for the comparison between treatments by using GraphPad Prism (GraphPad Software, version 9.5.1., Boston, MA, USA). However, as k co-varies with $Y_{\max}$ in a free fitting procedure, rate constant utilization would not be appropriate for such comparisons. Instead, average daily respiration estimates were used. These were calculated as the weighted mean of the daily respirations obtained after each incubation interval measurement. The comparison of the results of CO₂-C evolution for each incubation interval was subjected to analysis of variance (ANOVA) using GraphPad Prism (GraphPad Software, version 9.5.1., Boston, MA, USA).

All statistical computations regarding microbiome analysis were conducted using RStudio (Version 2022.07.2). Prior to analysis, taxa lacking sequence hits and ASVs present in less than 5% of samples were excluded. The α -diversity metrics, including the Fisher, Gini-Simpson, Inverse Simpson, and Shannon indices, were computed using the microbiome package. A two-way ANOVA was employed to investigate the effects of fire and ecosystem types on bacterial α -diversity. In this analysis, Fire (categorized as Yes or No) and Ecosystem (categorized as Pine forest, Olive grove, or Macchia vegetation) served as fixed factors, with their interaction also considered. The α -diversity metrics were treated as dependent variables. Data assumptions of normality and homoscedasticity were validated using the Shapiro and Levene's tests, respectively, facilitated by the *rstatix* package.

Bacterial β -diversity was ascertained using the Bray-Curtis distance metrics, and the resulting data were visualized using Nonmetric Multidimensional Scaling (NMDS). This was performed after standardizing the data to the minimum number of reads observed

across libraries (1250 reads); every sample that was below this was excluded from the analysis. To compare the dissimilarity between various sample groups, PERMANOVA was applied using the “adonis2” function from the vegan package [36]. An exploration of bacterial community composition distinctions between burned and unburned soil samples was executed and visually presented using the ggplot and ggvenn packages. The Indicator Species Analysis was conducted using the “indicspecies” package. This method involves identifying species that are significantly associated with particular groups of sites based on their occurrence and abundance patterns [37]. The *multipatt()* function was used to perform the analysis, with groups defined based on soil exposure to wildfire. The significance of associations between taxa and soil conditions was tested using a permutation test with 999 permutations, ensuring robust statistical inference. The results were interpreted based on point biserial correlation coefficients and associated *p*-values, identifying taxa distinctly associated with each habitat.

3. Results

3.1. Results of Soil Physico-Chemical Characteristics as Affected by Wildfire

The analysis of the basic physico-chemical characteristics of the sampled soils showed that the burned soils had greater pH and EC values compared with the control samples (Table 1). This result was also confirmed by sample analyses of additional soils that were not used in incubations. The increase was in the range of 0.24 to 0.64 units for pH and 0.20 to 0.66 dS m⁻¹ for EC. The content of CaCO₃ was always found to be greater in burned than unburned areas, a result probably attributed to the way that CaCO₃ concentration was measured (Bernard method) rather than to the effect of fire. An excess CO₂ release after hydrochloric acid application coming from the organic material of the burned soil samples could have resulted to an overestimation of CaCO₃. The average values of %C content were greater in the burned than in the unburned soils, but the differences were not statistically significant.

The variance of the data of inorganic N concentrations extracted seven days after the fire was great, thus hindering differences and making comparisons between burned and unburned sites non-statistically significant (Table 1). Mean values, though, showed increased mineral N in the burned areas of both olive grove and macchia sites in relation to their respective unburned ones. Mineral N consisted mainly of ammonium while nitrates appeared only in traces. No effect of fire and no elevated soil ammonium concentrations were shown six months after the wildfire.

3.2. Soil Respiration

Results of the first incubation experiment (*burned* vs. *unburned*) revealed that the average rate of CO₂ release ranged between 0.0076 and 0.1035 mg CO₂ g⁻¹ dry soil. The microbial basal respiration from samples coming from burned sites was constantly greater than that from their respective unburned sites, and differences were always statistically significant. This was true for all measurement time intervals and cumulative respiration results that are shown in Figure 1. The greatest respiration was shown in macchia burned soils followed by olive grove burned soils and then by pine forest burned soils. The magnitude of difference of the average daily respiration between burned and unburned soils also depended on the type of vegetation in the studied sites, being greatest in the macchia site and followed by the pine forest and the olive grove.

The addition of ash in the control unburned soils (second incubation experiments) resulted in increased microbial respiration. The percentage increase associated with ash addition, though, was much smaller in relation to the percentage difference in respiration of burned and unburned samples obtained at the previous incubation (Table 2). Burned olive grove soil, for example, showed 178% increase in average daily respiration in relation to the respective unburned soil, whereas when this soil was mixed with fire ash, it showed an increase of only 41% in average daily respiration. Mixing with ash also resulted in increased soil pH: 7.21 for OGCn + Ash, 7.33 for OGCf + Ash, 7.74 MVCf + Ash, and 7.62 for

MVCn + Ash. Ash by itself, with or without a soil inoculum showed no measurable CO₂ release at all but one internal incubation, which was even very small and lasted for only two days (third incubation). The respiration of ash was considered, therefore, as negligible, and the results are not presented.

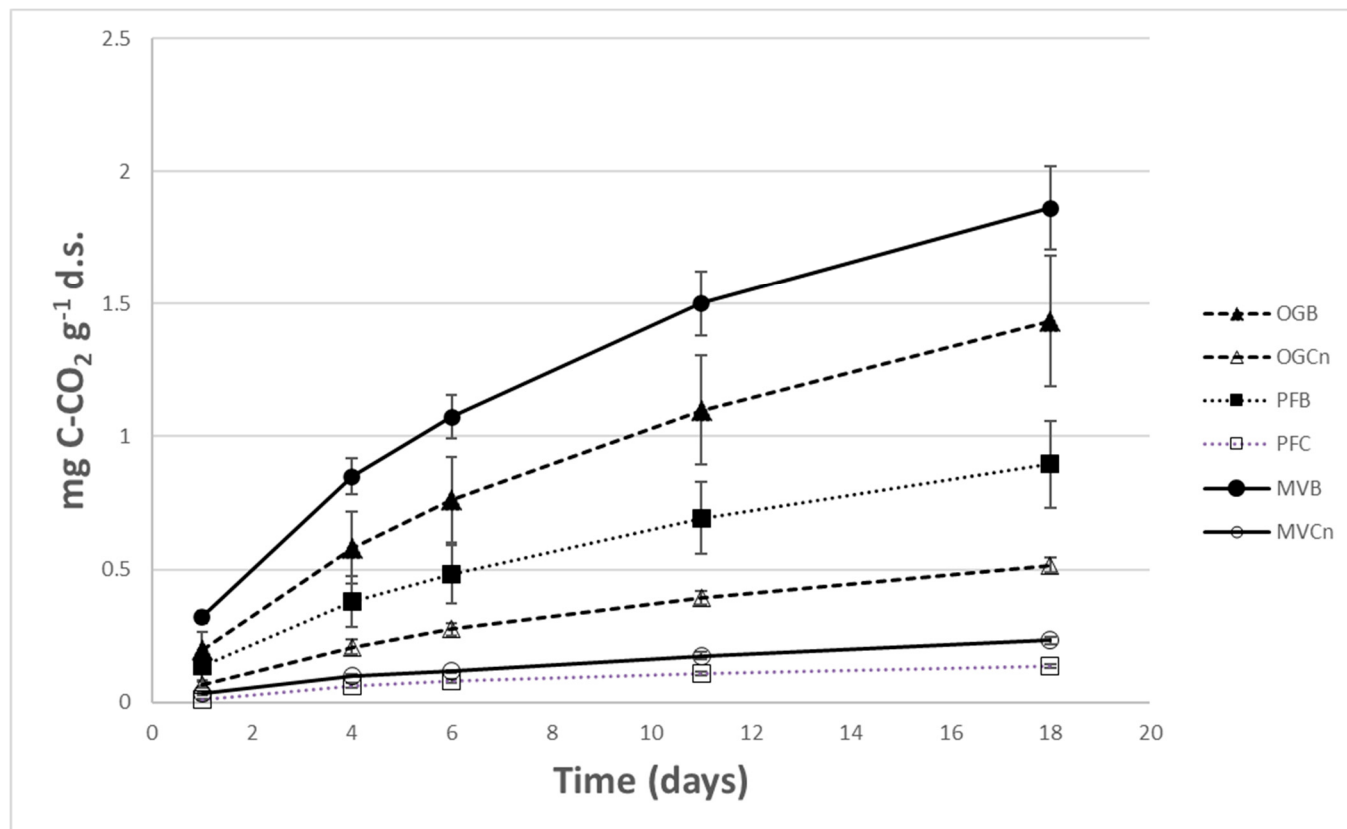


Figure 1. Cumulative microbial respiration in mg of C-CO₂ release per g of dry soil during incubation (first incubation) of soil samples coming from burned (B) and unburned (control-C) sites on either side of the fire front line. OG: Olive Grove, PF: Pine Forest, MV: Macchia Vegetation.

The addition of dry plant material of lucerne in soil samples significantly boosted microbial respiration in all burned and unburned soils. The microbial respiration rate of soil samples amended with plant litter was always higher in burned soils compared with their respective unburned control samples. However, by subtracting CO₂ due to soil-only emissions, respiration data indicated that the decomposition of *Medicago sativa* at the same temperature and moisture conditions and for the same period of time was greatest in the unburned soils. Indicatively, the total amount of released CO₂ was 89% higher in the burned olive grove soil samples receiving alfalfa litter and incubated for 21 days, but 219 and 989% higher when the litter was incorporated in the respective unburned control samples (Figure 2).

The fifth incubation experiment, concerning samples collected six months after the wildfire, revealed that the absolute values of CO₂ release rates were constantly lower compared with the respective ones measured in samples collected just few days after the fire. The comparison of respiration between burned and unburned areas six months after the fire showed essentially the same result patterns as those immediately after the fire. Samples coming from burned areas respired with a greater rate than those from unburned areas. For the olive grove in particular, even the percentage difference between burned and unburned samples seemed to have not changed after six months. For the macchia vegetation, though, this difference was much restricted (Table 3).

Table 2. Average daily respiration in mg of C-CO₂ released per g of dry soil during incubation (second incubation) of soil samples coming from unburned (control-C) sites either near (Cn) or far away (Cf) from the fire front line. The +Ash samples were those of which the soil was mixed with a certain amount of burned litter ash, and the last column indicates the percentage increase in relation to the control after ash addition. The average daily respiration results of the first incubation are also shown for comparison.

Soil (Treatment)/Average Daily Respiration		% Increase in Relation to Control	
OGCn	OGCn + Ash		
0.0401	0.0567	41	Results of 2nd incubation
OGCf	OGCf + Ash		
0.0144	0.0286	98	
MVCn	MVCn + Ash		
0.0209	0.0314	50	
MVCf	MVCf + Ash		
0.0340	0.0463	36	
OGCn	OGB		
0.0287	0.0797	178	Results of 1st incubation
MVCn	MVB		
0.0129	0.1035	702	
PFC	PFB		
0.0076	0.0497	554	

Table 3. Average daily respiration in mg of C-CO₂ released per g of dry soil during incubation (fifth incubation) of soil samples taken six months after the fire event and coming from burned (B) and unburned (control-C) sites either near (Cn) or far away (Cf) from the fire front line. The last two columns indicate the percentage increase in relation to the controls.

Soil	Average Daily Respiration	% Increase in Relation to Control Cn	% Increase in Relation to Control Cf
OGB	0.0273	174	270
OGCn	0.0099		
OGCf	0.0074		
MVB	0.0264	180	9
MVCn	0.0094		
MVCf	0.0242		

3.3. Influence of Fire on Bacterial Phyla and Their Correlation with Vegetation

From the data collected, we obtained a total of 3,317,501 quality sequences, with each sample ranging from 1816 to 8247 sequences (average 5045). The dominant phylum observed was Proteobacteria (~36%), followed by Actinobacteriota (~24%), Acidobacteriota (~12%), Bacteroidota (~11%), Planctomycetota (~5%), Chloroflexi (~4.5%), Verrucomicrobiota (~3.5%), and Firmicutes (~1.5%) (Figure 3A). Both fire and vegetation type significantly influenced the abundance of bacterial phyla (Figure 3A). For instance, the macchia ecosystem witnessed a notable increase in the abundance of Acidobacteriota and Firmicutes, while the olive grove saw an uptick in soil Cyanobacteria. In contrast, burned soils from the macchia ecosystem exhibited a marked reduction in the relative abundance of Bacteroidota (Figure 3B).

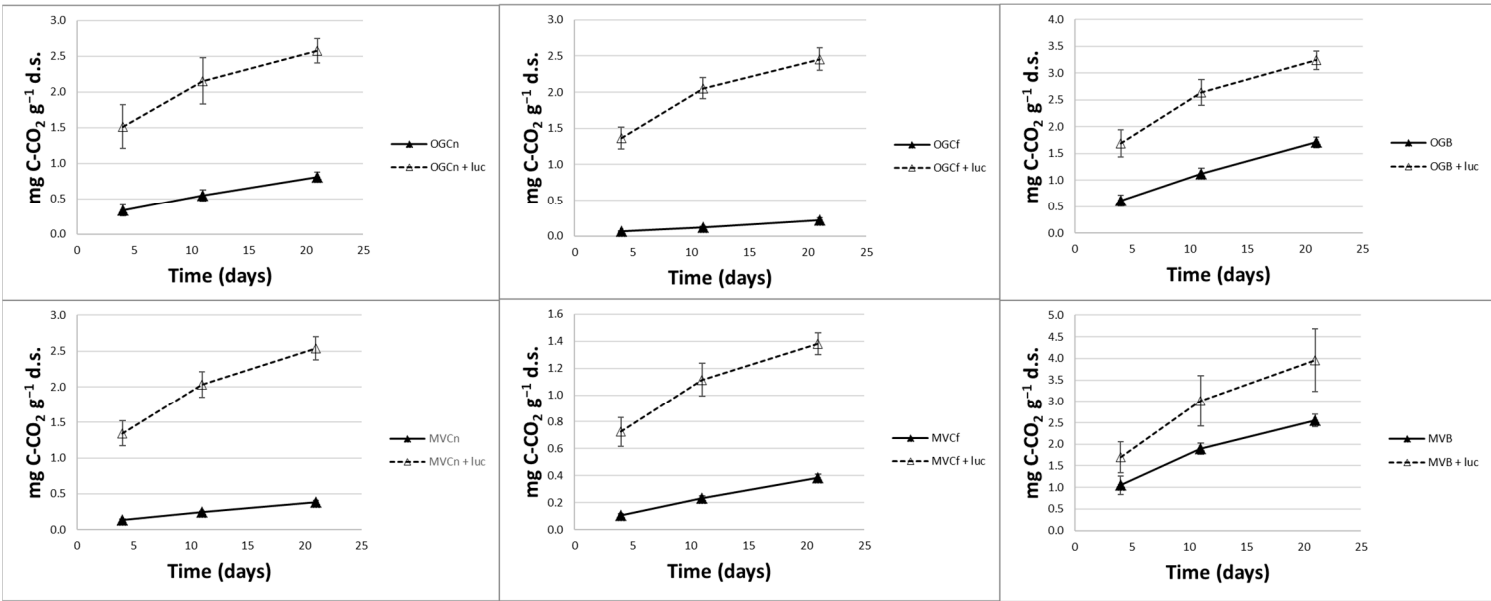


Figure 2. Cumulative microbial respiration in mg of C-CO₂ release per g of dry soil during incubation (fourth incubation) of soil samples coming from burned (B) and unburned (control-C) sites. The results in each graph illustrate differences in respiration of a soil after mixing with lucerne (luc) litter.

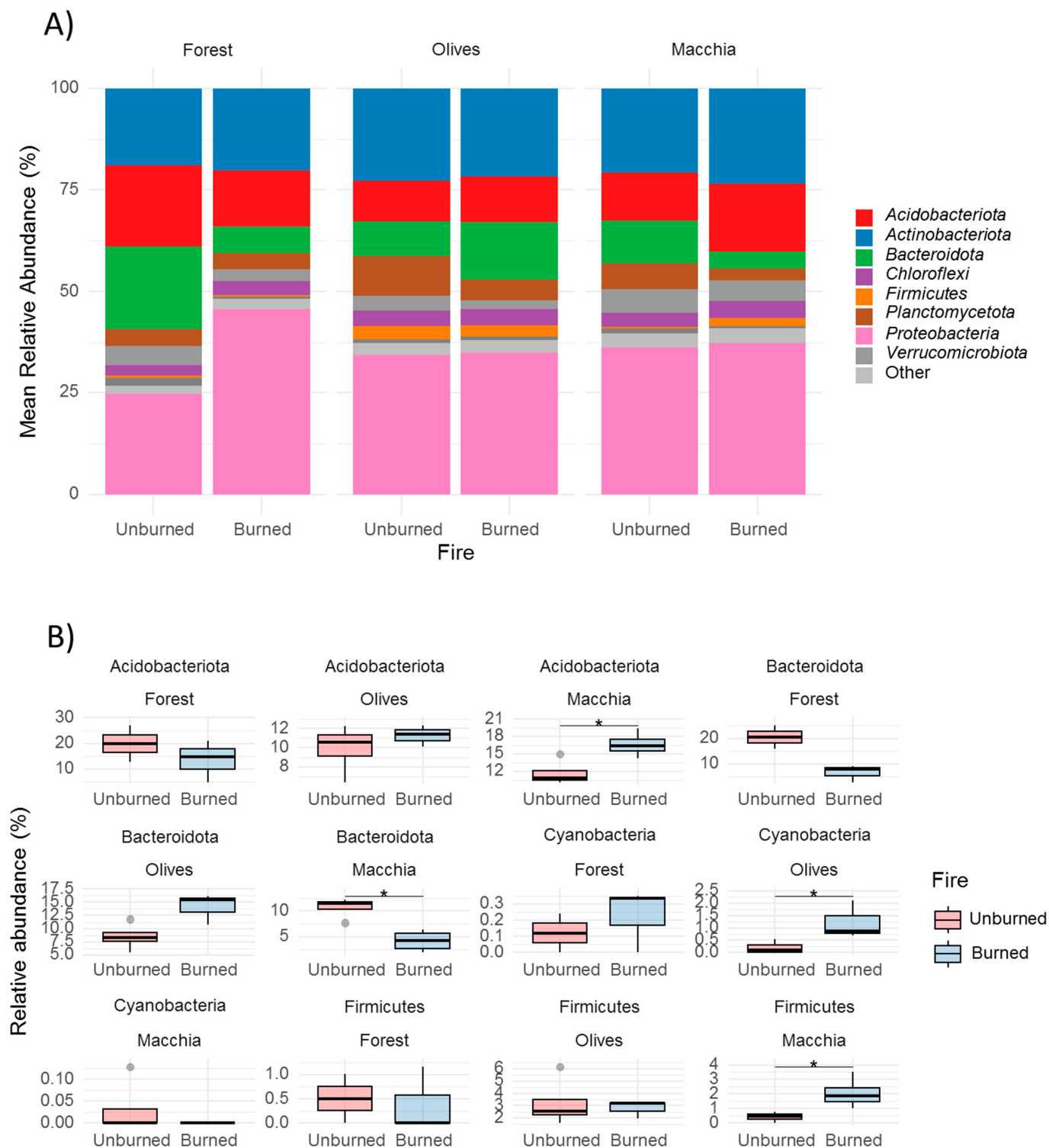


Figure 3. (A) Relative abundance of predominant bacterial phyla (>1%) in the soils sampled from burned and unburned sites in three different ecosystems, namely, Pine forest, Olive grove, and Macchia, as obtained by 16S rRNA gene metabarcoding. (B) Dominant bacteria phyla of burned and unburned soils in different ecosystems were screened out by the Kruskal–Wallis test corrected with the Benjamini–Hochberg algorithm. No sign was considered insignificant; * $p < 0.05$ was considered as significant difference.

3.4. Wildfire Influence on Bacterial Community Composition and Diversity

Across all tested vegetation types, fire did not significantly affect the richness or evenness of the bacterial community. Across indicators, both unburned and burned soil samples exhibited comparable values (Figure 4). In contrast, when analyzing β -diversity based on the Bray–Curtis distance for taxa with a prevalence of 40% of the samples, fire was found to induce significant compositional shifts in bacterial communities across all ecosystems (PERMANOVA: $R^2 = 12.34\%$, $p = 0.023$) (Figure 5). Further analysis of the distance metrics revealed that these observed differences in the soil bacterial community were more attributable to the fire itself rather than random distribution patterns or specific ecosystems. Upon examining taxa present in 20% of the samples with a relative abundance greater than 0.01%, we observed that regardless of vegetation type, both burned and unburned soils shared 60% of the ASVs. Notably, burned soils had fewer unique ASVs (15%) compared to the 25% unique taxa identified in unburned samples (Figure 6).

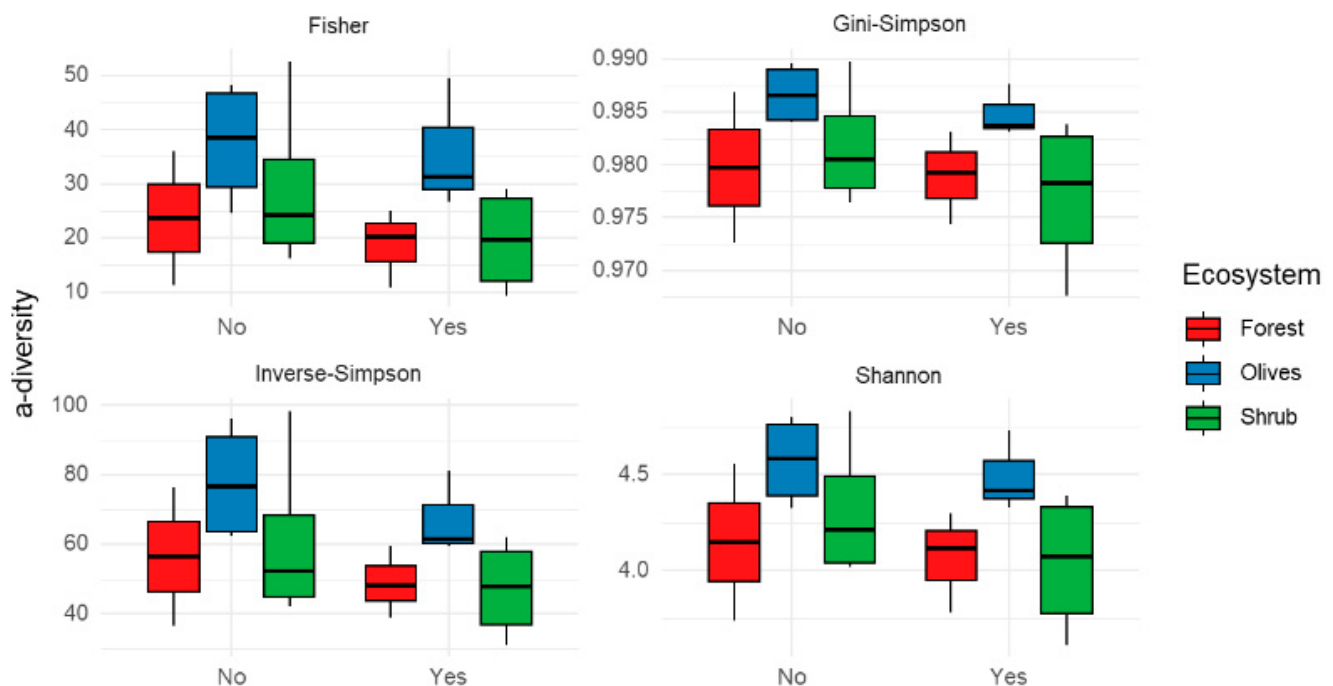


Figure 4. Comparison (Kruskal–Wallis test) of alpha-diversity metrics (Fisher, Gini-Gimpson, Inverse-Simpson, and Shannon) between the bacterial communities of burned and unburned soil samples from three different vegetation type systems. No letters indicate no statistically significant differences.

Indicator species analysis in taxa with prevalence higher than 55% revealed that nine ASVs were significantly associated with soil samples affected by wildfires. These indicators were assigned within the phyla Proteobacteria, Bacteroidota, Acidobacteriota, and Actinobacteriota. The taxa identified include ASV12 (Proteobacteria: *Psychroglaciacola*), ASV30 and ASV580 (uncultured Proteobacteria), ASV195 (Bacteroidota: *Mucilaginibacter*), ASV199 (Bacteroidota: *Pedobacter*), ASV337 (Acidobacteriota: *Paludibaculum*), ASV389 (Actinobacteriota: *Kribbella*), ASV503 (Proteobacteria: *Noviherbaspirillum*), and ASV540 (Proteobacteria: *Novosphingobium*). The associations were statistically significant, with p -values ranging from 0.005 to 0.043 (Table 4). Statistical strength for these associations varied, with values of either approximately 0.756 or 0.845, underscoring a robust link between these taxa and wildfire-impacted soils. On the contrary, soils that did not experience a wildfire were associated with twelve ASVs spanning several microbial phyla: Proteobacteria, Verrucomicrobiota, Bacteroidota, and Actinobacteriota. The taxa identified include ASV22 (Proteobacteria: *Rhodoplanes*), ASV66 (Verrucomicrobiota: *Candidatus Udaeobacter*), ASV72 and ASV75 (Verrucomicrobiota: uncultured and *Ellin517*, respectively), ASV250 (Bacteroidota: *Flavisolibacter*), ASV353 and ASV404 (Actinobacteriota: *Arthrobacter* and

MB-A2-108), and ASV498, ASV513, ASV522, ASV533, and ASV589 (Proteobacteria: *Mas-silia*, *MND1*, *TRA3-20*, *Acidibacter*, and *Reyranella*). These taxa demonstrated statistically significant associations with unburned soils, with p -values ranging from 0.001 to 0.028 and association strengths between 0.791 and 1.00 (Table 4).

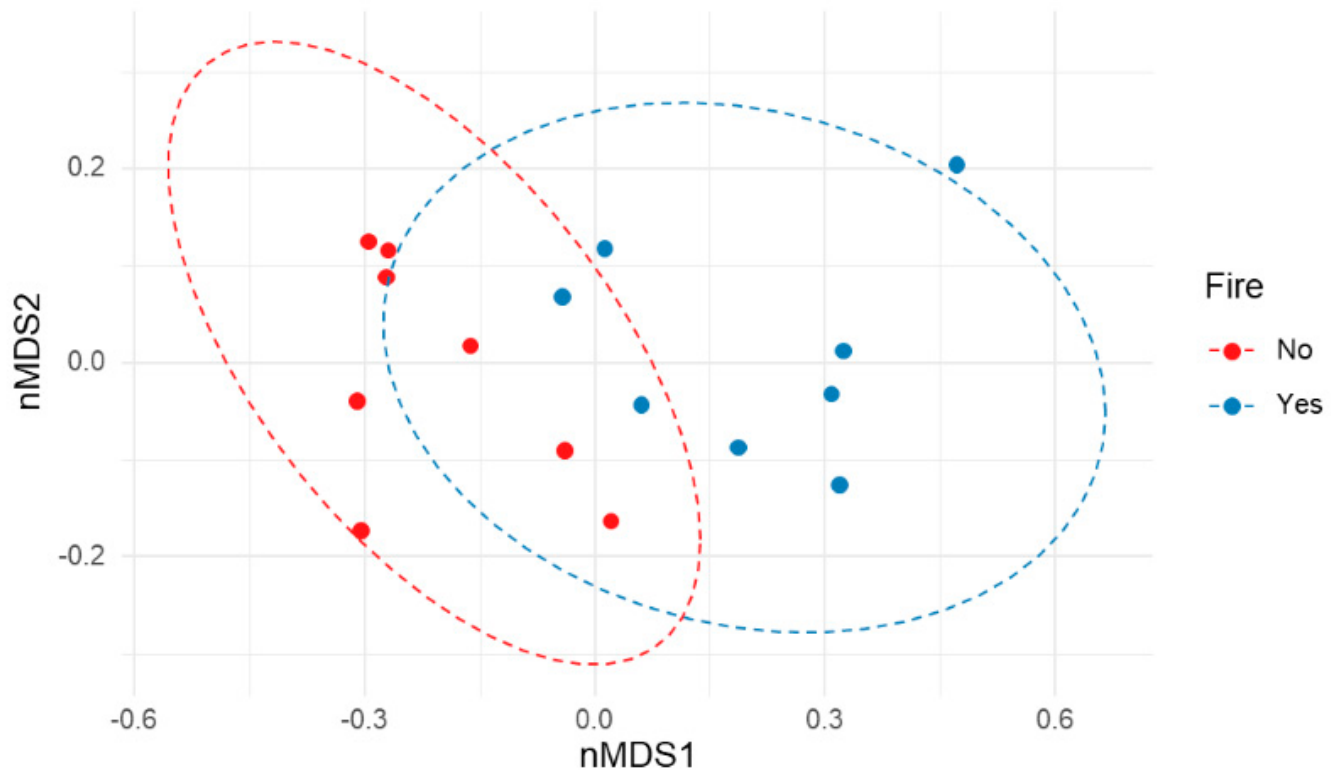


Figure 5. Non-metric Multidimensional Scaling (NMDS) Plot visualization of beta diversity (PERMANOVA: $R^2 = 12.34$, $p = 0.023$), using the Bray–Curtis distances, separating soil samples from burned and unburned sampling sites. Plot ellipses represent the 95% confidence regions for the grouped sampling sites (nMDS stress = 0.17).

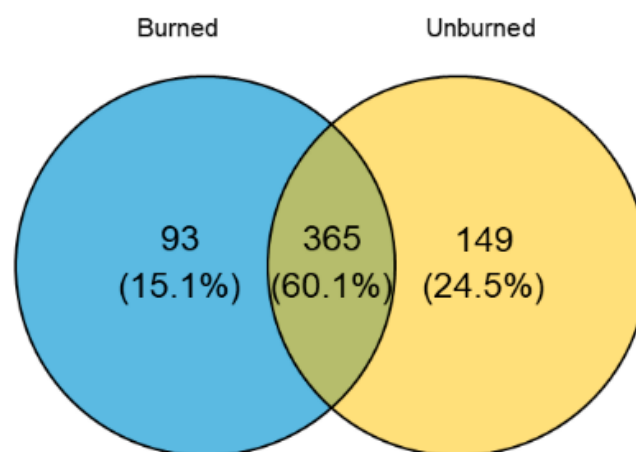


Figure 6. Venn diagrams showing the number of bacterial ASVs shared between burned and unburned soils.

Table 4. Association between Amplicon Sequence Variants (ASVs) and soil samples from unburned and burned locations. The table lists ASVs, their taxonomic classification (Phylum and Genus level), the strength of this association, and the *p*-value indicating the statistical significance of the association.

ASV	Taxon	Association with Samples	Association Strength	<i>p</i> -Value
ASV22	ASV22 Proteobacteria:Rhodoplanes	Unburned	0.791	0.0220
ASV66	ASV66 Verrucomicrobiota:Candidatus_Udaeobacter	Unburned	0.866	0.0090
ASV72	ASV72 Verrucomicrobiota:uncultured	Unburned	0.866	0.0050
ASV75	ASV75 Verrucomicrobiota:Ellin517	Unburned	0.791	0.0260
ASV250	ASV250 Bacteroidota:Flavisolibacter	Unburned	1.000	0.0010
ASV353	ASV353 Actinobacteriota:Arthrobacter	Unburned	0.866	0.0060
ASV404	ASV404 Actinobacteriota:MB-A2-108	Unburned	0.791	0.0260
ASV498	ASV498 Proteobacteria:Massilia	Unburned	0.866	0.0130
ASV513	ASV513 Proteobacteria:MND1	Unburned	0.791	0.0280
ASV522	ASV522 Proteobacteria:TRA3-20	Unburned	0.935	0.0010
ASV533	ASV533 Proteobacteria:Acidibacter	Unburned	0.866	0.0080
ASV589	ASV589 Proteobacteria:Reyranella	Unburned	0.866	0.0050
ASV12	ASV12 Proteobacteria:Psychroglaciecola	Burned	0.845	0.0100
ASV30	ASV30 Proteobacteria:uncultured	Burned	0.756	0.0430
ASV195	ASV195 Bacteroidota:Mucilaginibacter	Burned	0.845	0.0080
ASV199	ASV199 Bacteroidota:Pedobacter	Burned	0.756	0.0230
ASV337	ASV337 Acidobacteriota:Paludibaculum	Burned	0.756	0.0310
ASV389	ASV389 Actinobacteriota:Kribbella	Burned	0.845	0.0060
ASV503	ASV503 Proteobacteria:Noviherbaspirillum	Burned	0.845	0.0050
ASV540	ASV540 Proteobacteria:Novosphingobium	Burned	0.845	0.0070
ASV580	ASV580 Proteobacteria:uncultured	Burned	0.756	0.0220

4. Discussion

The effect of fire on soil properties has quite extensively been studied, especially in Mediterranean-type of ecosystems where wildfires occur very often during the summer period [9,11]. Studies have been undertaken aiming at evaluating the impact of fire on physical properties, such as soil water repellency, infiltration, and aggregate stability, and chemical properties, such as pH, electrical conductivity, and concentrations of C, N, and P. The former can largely indicate after-fire erosion risks and the latter revegetation potential [6,16].

Soil respiration studies are less frequent. Variables affecting soil respiration are associated with either positive or negative microbial responses due to fire. The thermal shock could kill microorganisms and reduce soil respiration, whereas increased nutrient availability in deposited ash would favor microbial activity and CO₂ release [38,39]. Increases in pH and dissolved organic carbon, especially in low-severity fires may enhance microbial respiration, whereas the formation of material characterized by high aromaticity and recalcitrance would result in a drop in microbial activity [40].

Accordingly, contrasting results on basal microbial respiration have been reported in the literature. De Marco et al. [41] attributed greater respiration rates of soils in the months following fire to increased availability of nutrients. Similarly, Guerrero et al. [42] found that the solubilization of carbon due to heating and increased nutrients results in increased colonization by bacteria and increased microbial respiration. However, the exhaustion of easily mineralizable compounds eventually resulted in a decrease in respiration rates with increasing time post fire. On the contrary, in situ measurements of soil C emissions have often been found to be lower after a fire in forest stands [43]. Francos Quijorna [12] found that basal soil respiration and basal soil respiration/microbial C values at the fire-affected sites of a study were significantly lower than those recorded at the control, thus attributing the reduction to the highly recalcitrant charcoal produced during a fire, which is more resistant to microbial decomposition, or the release of organic pollutants and heavy

metals. Lower basal respiration and microbial biomass-C have also been reported by Hernandez et al. [44] for a Mediterranean pine forest, 9 months after a fire.

In this study, differences in soil respiration between burned and unburned sites were, however, clear and statistically significant. In laboratory incubation conditions, burned soils respired more than unburned ones. The positive effects of fire on CO₂ soil fluxes were documented in all three ecosystem types that were selected along the front line of the wildfire. All soils in the studied area were young and poorly developed with low organic matter contents. Olive grove soils respired more than macchia soils, which respired more than Pine forest soils. Differences in respiration rates between sites, however, should be attributed to a combination of effects by topography/erosion variability and by vegetation cover. Fire effects, though, as they were expressed by the difference in CO₂ release between burned and unburned samples, were ranked as follows: Macchia > Pine forest > Olive grove. Respiration decreased six months after the fire, but the difference between burned and unburned soil samples persisted. Apparently, the soil six months after the fire that was used in incubations was more exhausted in decomposable organic matter in relation to the soil immediately after the fire, as fresh above-ground or root litter had not been incorporated yet. The percentage increase due to fire in macchia decreased and approached that of olive grove.

Ash addition to unburned soil samples provoked an increase in CO₂ release, indicating organic C supply or enhanced nutrient provision to soil microbes. However, when incubated alone, ash did not provide any evidence of decomposition. Hence, the contribution to basal respiration of burned soils of eventual, not fully stable forms of C contained in ash cannot be considerable. A better nutrient status is often used as an explanation for the proliferation of some microbial groups and the increase in saprophytic and decrease in autotrophic microorganisms after a fire [17]. The important role of ash in post-fire soil respiration was also emphasized by Sánchez-García et al. [38] who worked in a savanna environment. Adding wildland fire ash enhanced CO₂ emissions by up to three times compared with pre- and post-fire soils without ash, suggesting that this was the result of the high content of readily available nutrients in the ash.

Burned soils did not lose their capacity to decompose plant residues. Hence, it can be reasonably alleged that services provided by soil microbial activity are not fully disrupted by fire. The addition of alfalfa multiplied CO₂ release in incubated soils. The overall respiration of burned soils continued to be greater than that of the unburned ones even after the addition of litter. However, the subtraction of CO₂ corresponding solely to soil samples (not amended with organic matter) showed a smaller decomposition potential at the burned soils. If greater basal respiration of burned soils was simply a result of soil nutrient availability contained in ash, the addition of the same (qualitative and quantitatively) litter in burned soils would result in similar or even higher CO₂ release compared with that of unburned soils. The results of the incubation with alfalfa, therefore, provide a strong indication of either a shift in microbial community composition after a fire or a decrease in enzymatic activity due to elevated pH values. The exoenzymes of the otherwise similar-in-capacity microbes may operate less effectively at the post-fire conditions of elevated pH [45,46]. Alternatively, heat-resistant microorganisms dominating soil microbiome after a fire may well be capable of decomposing exogenous organic material, but not at the rate of the undisturbed unburned microbial community.

DNA analysis has illuminated the intricate bacterial dynamics in response to environmental variables such as fire events. Previous studies on Mediterranean ecosystems after wildfires have documented an upsurge in the abundance of Proteobacteria, Actinobacteria, and Firmicutes, alongside a decline in Bacteroidetes, echoing our results [47,48]. In the current study, Proteobacteria were dominant in post-fire environments, followed by Actinobacteriota and Acidobacteriota. Notably, these bacterial groups possess characteristics that may confer advantages in burned soils. For instance, Nelson et al. [49] observed a pronounced increase in the abundance of Firmicutes and Actinobacteria in soils affected by wildfires. These findings further highlight the enhanced expression of thermal resistance

genes, such as those for sporulation and heat shock proteins, suggesting these as adaptive strategies against wildfire heat. This upregulated function was notably associated with taxa within the Actinobacteria and Firmicutes phyla. Beyond the impact of fire, vegetation type can significantly influence bacterial communities, stemming from variations in soil characteristics and pre-existing bacterial assemblies before the fire event [50]. Our observation of increased Acidobacteriota and Firmicutes abundance in the shrub ecosystem post-fire only suggests that specific bacterial groups thrive under certain ecosystem conditions. Indeed, the implementation of indicator analysis revealed that specific bacterial taxa are associated with soil samples derived from burned and un-burned sites. The overall reduction of taxa abundance in burnt soils is expected since the immediate heat and combustion during wildfires can act as a pulse disturbance for bacterial assemblies. For example, the taxa associated with soils sampled from unburned sites were assigned in the genus of *Arthrobacter* and *Massilia*, which is in contrast with previous findings that suggest that these genera could be a good indicator to fire events [51]. The differences observed between our study and the previous could be attributed to the sampling time of the soil samples. In the current study, samples were collected seven days after the fire event while in the studies reporting an increase in *Arthrobacter* and *Massilia* abundance, the soil samples were collected two months after the fire. It is, therefore, possible that at early stages after a fire incidence, the abundance of both genera decreases and gradually increases due to its copiotrophic characteristics [52]. On the contrary, members of *Mucilaginibacter*, *Paludibaculum*, *Noviherbaspirillum*, and *Noviherbaspirillum* have been reported to be among the most dominant taxa during the early-month recovery after a fire, and this is in line with the indicator analysis performed in this study [53,54]. The presence of pyrogenic organic matter in burnt soils and habitat heterogeneity have been reported to affect the abundance of bacterial taxa that are proliferated in burnt soils [55].

Current results indicate that while fire does not significantly alter the richness or evenness of bacterial communities across different ecosystems, it does induce compositional shifts in these communities. This observation aligns with prior research that found that bacterial α -diversity remains largely unchanged in soils subjected to either controlled or natural wildfires [48,56]. The short duration of the wildfire under investigation, combined with soil temperature thresholds, likely preserved bacterial richness and evenness across the examined ecosystems. The observation that burned soils had fewer unique ASVs compared with the unburned samples is particularly intriguing (Figure 6). The observed response might be due to the potential reduction in the abundance of specific bacterial taxa sensitive to high temperatures by fire, resulting in fewer unique ASVs in burned soils. Such shifts, as highlighted by [47], can be attributed to the direct and indirect effects of fire, including alterations in soil properties, nutrient availability, and the physical structure of the habitat.

5. Conclusions

The basal respiration of burned soils was higher than of the unburned ones. Part of the difference should be attributed to ash, which apparently provides nutrients for microbial growth. When litter ash was added to the soils, the CO₂ release during incubations significantly increased in all types of soil used. Additionally, soil DNA extraction and 16S rRNA gene analysis revealed changes in bacterial community composition and a shift in the dominance of bacterial species, which should also be considered as being responsible for the increase in soil respiration after a fire. Soils after a fire do not lose their ability to decompose fresh litter despite these changes, although the rate of this decomposition is smaller than at the unburned samples. More extended research is needed in order to reveal whether these decomposition process changes are the result of ecosystem adaptation to fire so that SOM content is more rapidly replenished.

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draft, A.C. and P.D.; Writing—review & editing, A.C.; Supervision, A.C. and E.H.; Project administration, A.C. and E.H. All authors have read and agreed to the published version of the manuscript.

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