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Short-Term Response of Soil Bacterial Communities after Prescribed Fires in Semi-Arid Mediterranean Forests

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Abstract: Low-intensity burnings could be an effective silvicultural tool to prevent the occurrence and severity of wildfires. Nevertheless, their use as a forest fuel reduction tool may have a negative impact on soil properties. The aim of this investigation was to study the impact of a low-intensity prescribed fire on the main chemical properties of the soil (pH, electrical conductivity, and total organic carbon), and the diversity and composition of the soil bacterial communities in a semi-arid forest in SE Spain. Two similar stands were treated with a low-intensity prescribed burn in spring and autumn 2018 and were compared to an unburned stand. All soil samples were collected at the same time (autumn 2018). The chemical properties of the soil showed no significant differences between the prescribed burns and the control forest. Shannon and Pielou's diversity indices presented values significantly lower in the burned soils compared to the control. Prescribed burning did not modify soil bacterial community structure at the phylum level, but NMDS analysis did reveal a difference between soil bacterial communities at the genus level. Both prescribed burnings favoured some bacterial taxa over others, suggesting different thermal and bacterial resistance. The presence of *Massilia*, *Pseudomonas* and *Arthrobacter* could suggest a short-term ecosystem recovery. Therefore, prescribed burning in semi-arid forests could be suitable as a preventive tool against wildfires.

Keywords: controlled burn; low-intensity fire; prescribed fire; soil bacteria; soil resilience

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1. Introduction

Wildfires are considered major perturbations that directly and indirectly affect forest ecosystems, resulting in habitat loss or degradation [1,2]. Wildfires occur with greater frequency and intensity in those parts of the world where high temperatures, reduced precipitation, and fuel accumulation create conditions suitable for fire ignition and spread [3]. Moreover, natural wildfire patterns are changing worldwide, due to the combined effect of climate change and land-use changes, and increasing wildfires are expected even in non-fire-prone ecosystems [3,4]. In Europe, approximately 65,000 fire events and more than 500,000 ha of burns take place in Mediterranean forest ecosystems every year, with Spain being the worst affected country in Europe [1]. Nowadays, the greater efficiency of forest fire fighting services is paradoxically increasing the spread of fire, as their rapid response and effectiveness in dealing with forest fires is allowing for increased fuel loads and connectivity between forest areas [4]. In addition, the lack of forest management and prolonged periods of drought in the Mediterranean basin is increasing species mortality in forest ecosystems [5], thus providing a larger quantity and surface area of forest fuel accumulation that can be burned.

Fire directly and indirectly affects both biotic and abiotic forest ecosystem factors, among which soil is considered a key component [6]. According to Certini [7], fires significantly alter the physical, chemical and biological properties of soils, thus affecting

their capacity to supply environmental goods and services [8]. In the wake of a wildfire, the following short-term impacts can be observed: a significant loss of soil organic matter content, the formation of a hydrophobic layer that reduces infiltration, a change in soil pH, and the loss of vegetation cover [6,7,9,10]. Other authors have also reported an increase in soil electrical conductivity [11] and loss of nutrients through erosion and runoff after wildfires, as well as subsequent rainfalls affecting the wildfire affected area [8].

The physical and chemical properties of soil are considered to be driving factors of the microbial communities of soils [12]. The impact of forest fires on soil ecology is crucial, due to the important role soil microorganisms play in biogeochemical soil cycles, as well as in the transformation of soil organic matter into soil nutrients [13–15]. Lucas Borja et al. [14] observed that wildfires favoured *Firmicutes* and *Proteobacteria* soil communities, while *Acidobacteria Gp4* and *Bacteroidetes Ohtaekwangia* decreased their relative abundances after burning in a mesocosm experiment. In addition, Sáenz de Miera et al. [16] showed an increase in the phyla *Proteobacteria* and *Firmicutes* after forest fires, although the same authors also reported that differences between the relative abundances of different soil microorganisms may depend on the type of fire severity, forest ecosystem, and associated vegetation.

Finding adequate forest management tools to reduce forest fuel accumulation and to create landscape heterogeneity may help to reduce the extent and severity of wildfires in the future [17–19], while making forest ecosystems more resilient to global climate change [19]. In this context, there is a growing interest in the use of prescribed burns, and forest managers are increasingly taking them into consideration as a tool for reducing wildfire risk [20,21]. Prescribed burning has been described by Fernandes et al. [22] as the planned use of low-intensity fire, applied in order to eliminate fuel that could generate high-intensity fires. The post-fire physico-chemical and biological processes in soils are very complex, due to the large variability of influential factors (e.g., quantity of ash, level of vegetation removal, site morphology, weather, and post-fire management [23]). This variability can lead to unexpected responses of soil to fire. That is, even low-intensity prescribed fires can significantly change soil properties (e.g., Alcañiz et al. [20]). Conversely, high-severity prescribed fires are more generally understood, so their effects can be better anticipated (e.g., Lucas Borja et al. [14]). Thus, scientific understanding of the effect of prescribed burning on soil biological properties is still limited [20], and its impact on soils in arid and semi-arid Mediterranean areas is especially in need of further study. In addition, soil microbial communities have been considered as early response indicators of any change occurring in soils, as they are particularly sensitive to any anthropogenic or environmental disturbance [14,24].

Analysing the changes that low-intensity prescribed burns could produce on soil microbial communities may provide valuable information to help managers determine whether they could be used as valid management tools in fragile arid and semi-arid ecosystems, and improve planning strategies and actions on vegetation that increase the resilience of the ecosystem to the impact of fire.

The aim of this investigation was to determine the effects of low-intensity prescribed fires on soil chemical properties and soil bacterial communities in semi-arid forests. An unburned forest area was also used as a reference level for comparisons. Our aim was to understand the impact of prescribed burning on the soil of a forest in a semi-arid environment in order to ascertain the resilience of bacterial communities in semi-arid forest soils to prescribed fire. We hypothesized that prescribed burns would not affect soil chemical properties, but that some changes would occur in the bacterial communities of soils just after the application of a prescribed burn, compared to unburned soils. This research could help us to make decisions in preventive forest fire management, as the particularity of each soil and habitat will be affected differently by prescribed fire treatment.

2. Materials and Methods

2.1. Study Site

The study was conducted in the mountain range of Filabres, near to the municipality of Senés (Almería, SE Spain; 37°13′25″ N, 02°20′33″ W) (Figure 1). The climate is semi-arid Mediterranean, with an annual rainfall averaging 335.45 mm and temperatures averaging over 10.47 °C (data recorded between 2001 and 2020 by meteorological station installed by Zapata-Sierra et al. [25]; 37°13′58.85″ N, 02°22′16.94″ W). The experimental area has a steep topography with slopes exceeding 30% and an elevation of 1550 m. a. s. l., where the soil has been classified as eutric Regosols [26]. The vegetation is largely constituted of conifers planted in reforestation plans carried out in 1940. The principal species are Mediterranean maritime pine (*Pinus pinaster* Aiton.) with a tree density of 615 ind. ha^{-1}. The area has been subjected to forest management through silviculture treatments, which has allowed the colonization of scrubland formed mainly by *Retama sphaerocarpa* L., *Artemisia barrelieri* Besser., *Adenocarpus decorticans* Boiss., *Thymus zygis* L., *Festuca granatensis* Boiss., and *Bupleurum* sp.

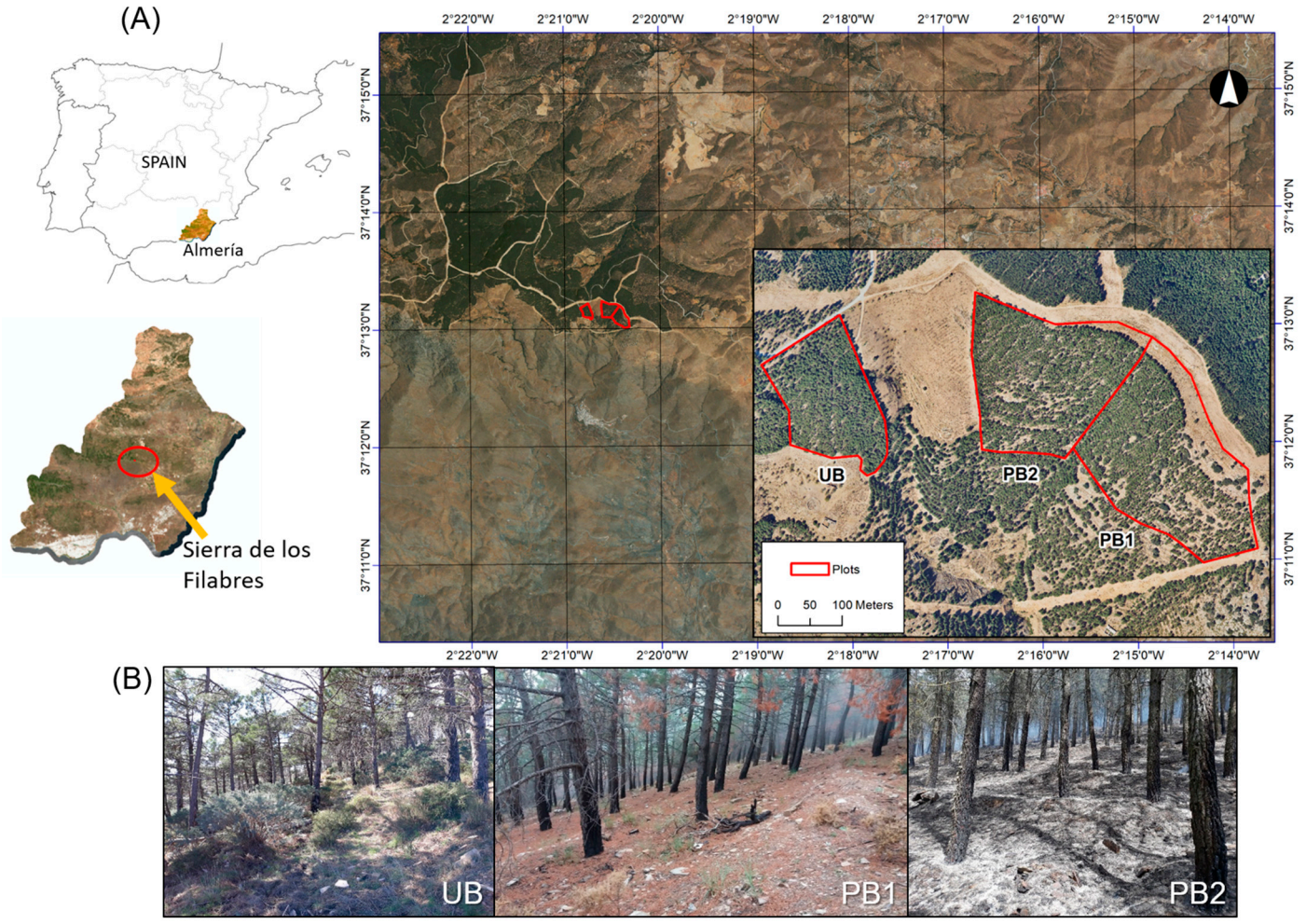

Figure 1. (**A**) Geographical location (southeast Spain, Almería), aerial view of study area with the experimental site. (**B**) Detail view of the different stands selected at the sample collection date. Unburned stand (UB), burned stand seven months after prescribed burning (PB1), burned stand (PB2).

2.2. Prescribed Burning Operations and Experimental Design

In early 2018, two forest areas were selected for burnings. Two zones were selected inside the burned areas (Figure 1) and an experimental plot of 50 m × 50 m was delimited in stands PB1 and PB2. An unburned area of the same characteristics was chosen as a control and reference ecosystem (UB). Both areas (Figure 1) constituted strategic management points and were considered key areas for forest fire prevention actions that could provide strategic support in secure forest firefighting work, according to the regional forest service. Specifically, the aim of the prescribed burning treatment was to reduce the risk of forest fires by cutting down fuel continuity and reducing the surface fuel load. The first prescribed burning was carried out on 17 April 2018 (hereinafter PB1), and the second burn was carried out seven months later, on 29 November 2018 (PB2), by forest firefighters from the Andalusian Forest Fire Extinction Service (INFOCA). The same ignition technique was used at both experimental sites. In addition, a control line was carried out manually with rows which were 0.5 m wide and deep enough to reach the mineral soil. This control line was made in order to contain the fire within the area designated to be treated and prevent the fire from escaping. The procedure chosen was the ignition pattern by "strip burning", which consisted of burning the fuel manually in lines parallel to the contour lines and 2–3 meters in advance of the control line. The width of the strips in the strips-burning was 2–3 m for PB1 and 1.5 to 2.5 m for PB2 (data provided by INFOCA). The data collection methodology used was described by Molina et al. [27] and were provided by the forest fire laboratory LABIF. Meteorological data during the prescribed burning were measured by meteorological stations specialized in fires (Krestell 5500FW Fire Weather Pro) and these can be seen in Table 1. The temperatures recorded during the burn were measured with thermocouples connected to a data logger. Temperatures reached, at a depth of 3 cm, did not exceed 35 °C in both PB1 and PB2 (data provided by the LABIF forest fire laboratory). Regarding soil surface moisture at the litter layer, both PB1 and PB2 showed 11.40% and 11.46%, respectively. Finally, 2.5 cm of the litter layer was consumed during both PB1 and PB2 (LABIF forest fire laboratory).

On 30 November 2018, three composite soil samples (mixing 10 sub-samples) were randomly collected from each experimental plot (PB1, PB2 and control). First, the organic layer of leaf litter and burnt soil was removed. Samples were taken randomly to guarantee representativeness, and covered the total area of each plot. A total of nine samples were transported in isothermal bags to the laboratory. A portion of soil was air-dried at ambient temperature, then the fine soil fraction was separated and homogenised using a 2 mm sieve and set aside for chemical parameter analysis. Another portion was kept at −20 °C and used for DNA extraction. PB1 and PB2 were compared separately with UB plots in the different analysis carried out in this study.

Table 1. Prescribed burns main characteristics (Data provided by INFOCA and LABIF).

	Air Temperature (°C)	Relative Air Humidity (%)	Wind Velocity (Km h^{-1})	Spread Rate (m/min)	Flame Length (m)	Mean Surface Temperature (°C)	Max Surface Temperature (°C)	Mean Residence Time (s)
PB1	18.95 ± 1.98	35.6 ± 3.5	5.99 ± 4.56	1.6–1.87	0.2–1.0	632.5	759.0	73.5
PB2	20.17 ± 2.95	22.20 ± 3.03	2.75 ± 3.26	0.81–1.71	0.5–0.9	589.9	832.5	61.7

2.3. Chemical Soil Properties

Some of the main chemical properties of the soil were selected as having an early response to fire [7]. The pH values were determined in distilled water solutions with a 1:2.5 *w*/*v* ratio and measured with a pHmeter (LAQUA PH1100, HORIBA, Tokyo, Japan). The same proportion of aqueous suspension (1:2.5 soil/water) was used to determine the electrical conductivity (EC) with a digital conductivity meter (LAQUA EC1100, HORIBA, Tokyo, Japan). Total organic carbon (TOC) was determined using a wet method of oxidation with potassium dichromate, following the Walkey and Black method [27] with a modification proposed by Mingorance et al. [28], resulting in a colorimetric method that was measured with a spectrophotometer Spectronic Helios Gamma UV-Vis (Thermo Fisher Scientific, Waltham, MA, USA).

2.4. Composition and Diversity of Bacterial Communities in Soils Treated and Untreated with Prescribed Fire

To determine the bacterial communities in the soils, deoxyribonucleic acid (DNA) extraction was performed on all collected samples. DNA extraction was performed on 0.25 g of soil using the DNeasy PowerSoil kit (QIAGEN, Hilden, Germany), following the protocol described by the manufacturer. The concentration and quality of DNA contained in each sample was quantified using a Nanodrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

High-throughput sequencing was performed at the Centre for Comparative Genomics and Evolutionary Bioinformatics (CGEB), Dalhousie University, Nova Scotia, Canada. Regions V4-V5 (400–500 bp) of bacterial 16S ribosomal RNA gene were amplified in vitro by polymerase chain reaction (PCR) using primers 515F/806R Walters et al. [29]. Paired-end sequencing was performed using the Illumina MiSeq platform (Reagent Kit v3—2 × 300 cycles) as described in Comeau et al. [30]. The sequences were processed with Quantitative Insights Into Microbial Ecology version 2 (QIIME2 v18.8) software [31] following the protocol of Comeau et al. [30]. Minimal bacterial features were acquired using the Deblur tool for sequence quality control. Amplicon sequence variants (ASVs) were obtained and checked against the SILVA library database (version 132) using the classify-sklearn classification method for taxonomic assignment. To check that no contamination occurred during DNA extraction, a blank control without a sample was added to one of the DNA extraction kit tubes. Negative PCR controls (1 per 96-well plate) were also included and tested for cleanliness by sequencing on the MiSeq to check that they showed no constant reading in this code combination.

Alpha diversity indices were estimated to compare soil bacterial diversity between burned and unburned plots. For this purpose, the number of ASVs observed in each sample was used with QIIME2 software. The phylogenetic diversity indices of Faith, Pielou and Shannon were sampled uniformly at 20,000 reads per sample.

2.5. Statistical Analyses

A permutational analysis of variance (PERMANOVA) was used to determine any changes to the chemical properties of the soil, or the bacterial diversity and abundance of the different burned soil samples (PB1 and PB2), compared with the control (UB) [32]. When the number of permutations was less than 100, the Monte Carlo test was performed. Similarly, PERMANOVA was used to study any differences between the bacterial composition of the samples. This non-parametric analysis uses permutation tests to obtain P-statistic values, and does not rely on the assumptions of traditional parametric ANOVA (normality and homogeneity of sample variances) [32]. Chemical data were represented by a Euclidian distance matrix, which was normalised before analysis. Analyses were performed for all the treatments combined, as well as each individual prescribed burn date. A similarity matrix was carried out on the bacterial abundance data obtained from the different samples using Bray–Curtis distances and Euclidean distances for the chemical parameters. Subsequently, we evaluated which groups these significant differences occurred between for each factor

and variable, using a paired *t*-test. In addition, PERMANOVA and NMDS analyses based on Bray–Curtis dissimilarity were carried out in order to compare the bacterial community compositions in soils treated with prescribed burning (PB1 and PB2), with unburned soils used as a reference (UB). Similarity percentage analysis (SIMPER) of samples collected from the different burn and control stands was performed as previously described [33] to determine which bacterial taxa contributed most to the differences in community structure between the different soils sampled, compared to the control soils stand.

The statistical package PRIMER + PERMANOVA version 7 software (PRIMER-E Ltd., Plymouth Marine Laboratory, Plymouth, UK) for Windows was used for PERMANOVA, NMDS and SIMPER analysis [34]. Graphs of phyla relative abundance and boxplots were performed using RStudio software (version 1.2.5042).

3. Results

3.1. Prescribed Burning Effects on Soil Properties

PERMANOVA analysis showed no significant differences ($p > 0.05$) in the main chemical properties evaluated between the burned and unburned soils (Table 2). Electrical conductivity (EC) was comparatively higher in the recently burned soils (PB2) than in the unburned soils (UB). The pH values obtained from all the plots were close to neutral, around 7.2–7.5, with no significant statistical differences between the burned and unburned plots. The total organic carbon content (TOC) was similar in the burned (PB1 and PB2) and unburned plots (UB) (Table 2).

Table 2. Chemical properties of unburned soils (UB) and after prescribed fire treatment at different dates (PB1 and PB2) (mean ± standard deviation).

	EC (mS/cm^{-1})	pH	TOC (%)
UB	0.038 ± 0.00 a	7.246 ± 0.02 a	1.370 ± 0.35 a
PB1	0.030 ± 0.01 a	7.593 ± 0.20 a	1.652 ± 0.16 a
PB2	0.058 ± 0.02 a	7.393 ± 0.41 a	1.595 ± 0.02 a

EC: electrical conductivity, TOC: total organic carbon content. Different letters indicate statistical differences for each treatment ($p \leq 0.05$). UB: unburned control soils; PB1: soils treated with prescribed burning seven months before sample collection; PB2: soils treated with prescribed burning immediately prior to sample collection.

3.2. Bacterial Community Richness and Diversity

In general, the prescribed burning treatments did not produce significant changes to the bacterial properties of the soil. A slight increase in ASVs values was observed in the burned soils (PB1 and PB2) compared to the unburned control soils (UB; Table 3). The Faith phylogenetic index, although presenting very similar values for the burned soils compared to the control, showed a slight increase in PB2 soils compared to UB (Table 3). Shannon and Pielou indices showed significantly ($p \leq 0.05$) higher values in UB soils than in both burned soils (PB1 and PB2). However, this difference was greater for PB2, which showed the lowest values for both indices (Table 3).

Table 3. Diversity index results expressed as mean ± standard deviation for each experimental burned (PB1 and PB2) and unburned plots (UB).

	ASV Observed	Faith	Shannon	Pielou
UB	1044 ± 4.58 a	53.58 ± 0.78 a	8.97 ± 0.03 a	0.89 ± 0.00 a
PB1	1052 ± 17.43 a	53.40 ± 0.65 a	8.74 ± 0.03 b	0.87 ± 0.00 b
PB2	1047 ± 18.87 a	54.43 ± 0.85 a	8.65 ± 0.03 c	0.86 ± 0.00 c

Different letters indicate statistical differences for each treatment ($p < 0.05$). UB: unburned control soils; PB1: soils treated with prescribed burning seven months before sample collection; PB2: soils treated with prescribed burning immediately prior to sample collection.

3.3. Analysis of Bacterial Communities after Prescribed Burning Treatment

The number of detected sequences belonging to the bacterial domain was 872,516. Metagenomic analysis showed nine dominant phyla in all soils sampled (Figure 2). Overall,

Proteobacteria was the most abundant (38.8%), followed by *Acidobacteria* (19.85%), *Actinobacteria* (16.15%) and *Planctomycetes* (13.20%), while phylum *Chloroflexi* (2.35%) and *Verrumicrobia* (1%) were the least abundant (Figure 2). Although comparatively different proportions were observed depending on the time since the prescribed burning treatment, the PERMANOVA analysis ($p \leq 0.05$) for the relative abundance of the main phyla showed significant differences between PB2 soils and UB soils, while there were no statistically significant differences between PB1 and UB. Soils recently treated with prescribed burning (PB2) showed a slight increase in *Proteobacteria*, *Firmicutes* and *Bacteroidetes* phyla, compared to the control soils (UB). However, PB2 showed that the relative abundance values of phyla *Acidobacteria*, *Actinobacteria*, *Gemmatimonadetes* and *Chloroflexi* were lower than UB soils. On the other hand, the prescribed burning seven months earlier showed fewer differences compared to UB soils. *Plantomycetes* and *Firmicutes* showed a comparatively higher relative abundance for PB1 than UB (Figure 2).

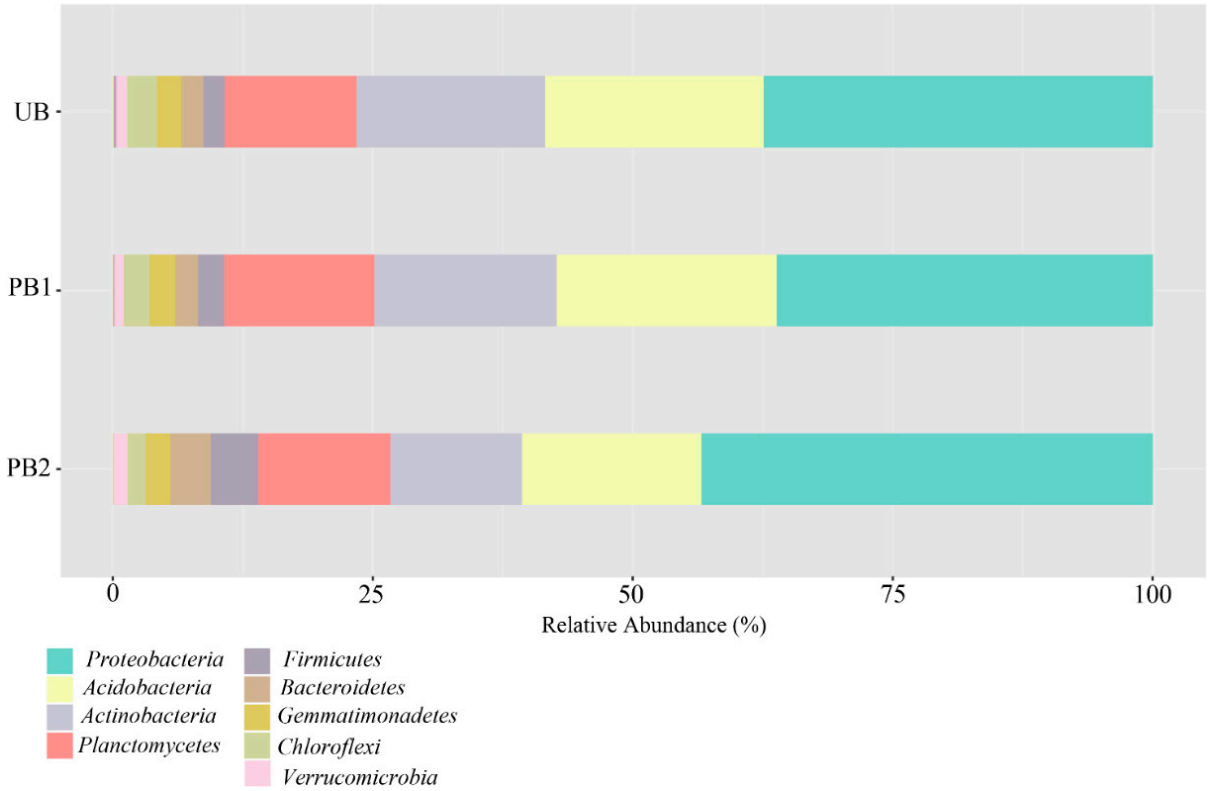

Figure 2. Distribution and relative abundances of bacterial phyla in burned (PB1 and PB2) and unburned control soils (UB). PB1: soils treated with prescribed burning seven months before sample collection; PB2: soils treated with prescribed burning immediately prior to sample collection.

Proteobacteria was the phylum with the highest relative abundance in all plots, both burned (PB1 and PB2) and unburned soils (UB). The most abundant classes in this phylum in PB1 and UB were *Deltaproteobacteria*, followed by *Alphaproteobacteria* and *Betaproteobacteria*, while *Gammaproteobacteria* was the least abundant. Nevertheless, the other two groups, *Betaproteobacteria* and *Alhaproteobacteria*, presented a relative abundance similar to PB1 and UB (Figure 3). On the other hand, *Gammaproteobacteria* was the most abundant class included in Proteobacteria phylum in PB2, while *Deltaproteobacteria* was the least abundant (Figure 3); both bacterial phyla showed a lower percentage of relative abundance than in UB. However, the percentages for *Betaproteobacteria* were 26% for both UB and PB2, and 24% for *Alhaproteobacteria*.

A total of 164 bacterial taxa were identified at the genus or next higher taxonomic level available, with a relative abundance greater than 0.1% in all samples. PERMANOVA

test ($p \leq 0.05$) showed significant differences in bacterial communities at the genus or next higher taxonomic level identified in the PB2 compared to UB, while in PB1 there were no statistically significant differences compared to UB. In general, the NMDS analysis showed three clearly differentiated groups according to the execution of the prescribed burning treatment and the time elapsed since the prescribed burning treatment execution (Figure 4). The right side of the graph shows the cluster grouping the PB2 samples, while the UB samples are grouped on the left side. Interestingly, the PB1 samples are placed in a central position between the PB2 and UB samples.

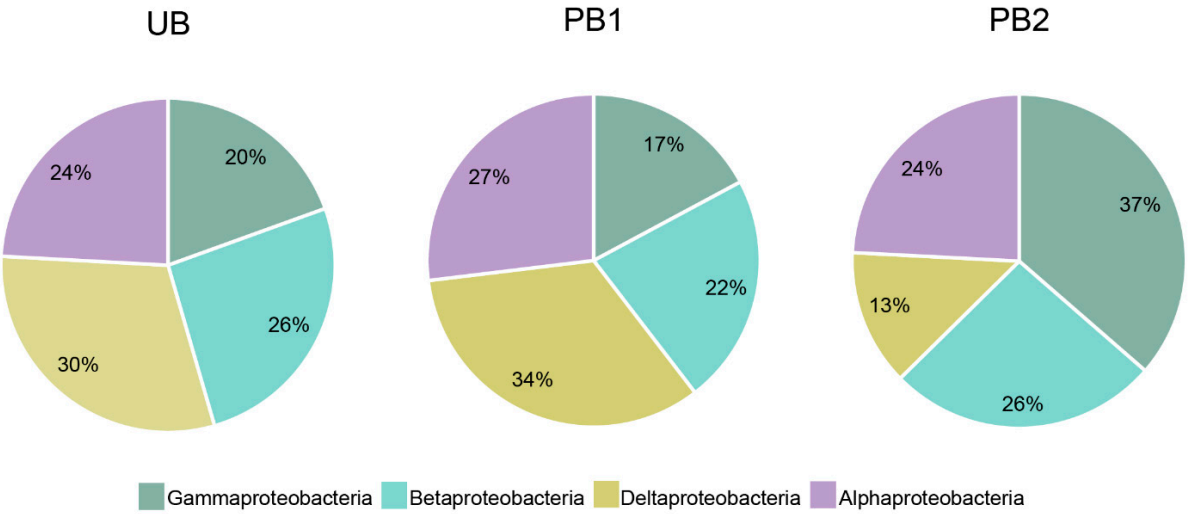

Figure 3. Differences between main classes of the phylum Proteobacteria in different soils treated and not treated with prescribed fire. UB: unburned control soils; PB1: soils treated with prescribed burning seven months before sample collection; PB2: soils treated with prescribed burning immediately prior to sample collection.

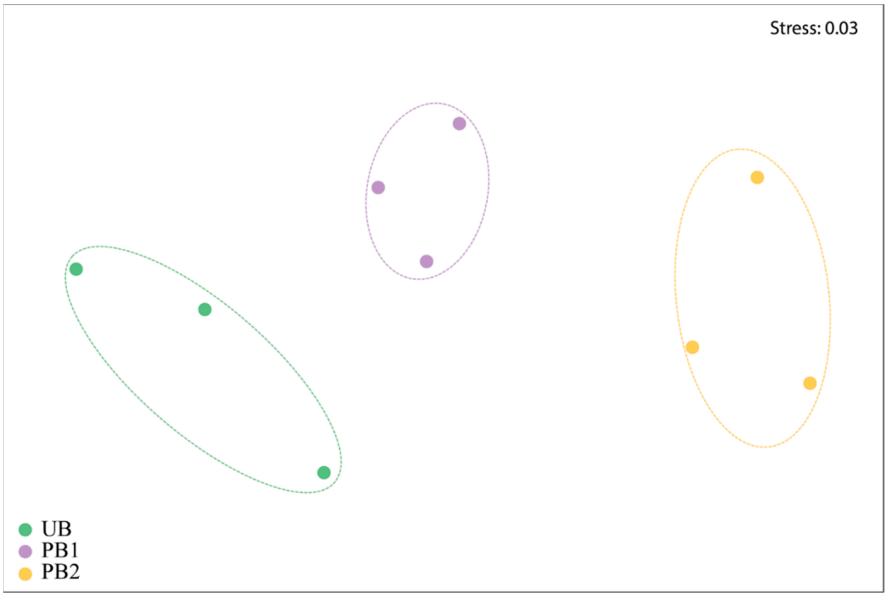

Figure 4. Non-metric multidimensional scaling (nMDS) ordination based on Bray–Curtis similarity, showing the bacterial community structures derived from relative abundance based on genus level across the different samples of burned (PB1 and PB2) and unburned soils (UB). The stress value denotes the goodness of fit. UB: unburned control soils; PB1: soils treated with prescribed burning seven months before sample collection; PB2: soils treated with prescribed burning immediately prior to sample collection.

3.4. Genus-Level Dissimilarity Analysis of Bacterial Communities in Soils Treated with Prescribed Burning and Unburned Soils

The PERMANOVA test, by time factor since the application of the prescribed burn treatment, showed significant differences ($p \leq 0.05$) between the control (UB) and after recent burn (PB2) sample groups, while UB and PB1 showed no differences between them. The bacterial taxa that contributed most to the similarity in the different groups of samples showed similar abundances among UB and PB2 soils, as well as UB and PB1 soils, with an average similarity of 90.75–90.85% and 90.75–92.71%, respectively (Table 4). The cumulative contribution of the PB2 (11.86%) and PB1 (9.91%) groups of samples soils was similar compared to UB (9.37%). The *Proteobacteria* phylum contributed the most to the similarity in the different plots. The family *Xanthobacteraceae* contributed the most to the similarity among all the sample groups. The genus *Mycobacterium* only contributed to similarity in the sample group PB1 (Table 4).

The average dissimilarity in the pairwise comparison of the different prescribed fire treatments on different dates in relation to the UB presented low values. The dissimilarity ratio between the control soil (UB) and the soil just after prescribed burning (PB2) presented values of 13% (Table 5). The dissimilarity ratio between UB and PB1 was 9.99% (Table 5). The cumulative contribution of dissimilarity for the selected group of bacteria between UB and PB1 was 11.59%, while between UB and PB2 plots it was 16.61%. The phylum that contributed most to dissimilarity in the UB–PB2 plots relative to the rest was *Proteobacteria*, with the taxa that contributed most to this relationship being: *Massilia*, *Pseudomonas*, *Luteibacter*, [F] *Archangiaceae*, *Firmicutes Bacillus* [O] *Bacillales*, and *Acidobacteria Subgroup 10*. The phylum that contributed most to dissimilarity in the UB–PB1 plots was *Acidobacteria*, with the bacterial communities that contributed most being: *bacterium clone S111*, [O] *Subgroup 7*, and *RB41 Subgroup 4 uncultivated soil* (Table 4).

Table 4. SIMPER shows the relative abundance of the contribution of bacterial phyla and taxa with the lowest level of classification, the total and cumulative contribution to the average similarity as a percentage. Degree of similarity for each treatment. Contributions of bacterial taxa to mean percent similarity less than 1.5% are not shown.

Phylum	Bacterial Taxa	Abundance (%)	Contribution (%)	Cumulative Contribution (%)
UB average similarity: 90%				
Proteobacteria	[F] *Xanthobacteraceae*	2.73	2.68	2.68
Acidobacteria	[C] *Subgroup_6*	1.78	1.82	4.5
Proteobacteria	*Sphingomonas*	1.72	1.75	6.25
Acidobacteria	*RB41*	1.59	1.57	7.82
Planctomycetes	*Singulisphaera*	1.53	1.55	9.37
PB1 average similarity: 92%				
Proteobacteria	[F] *Xanthobacteraceae*	3.18	3.27	3.27
Acidobacteria	*RB41*	1.95	1.96	5.23
Planctomycetes	*Singulisphaera*	1.8	1.8	7.02
Proteobacteria	*Sphingomonas*	1.68	1.72	8.74
Acidobacteria	[C] *Subgroup_6*	1.56	1.59	10.33
Actinobacteria	*Mycobacterium*	1.56	1.53	11.86
PB2 average similarity: 90%				
Proteobacteria	[O] *Xanthobacteraceae*	3.33	3.49	3.49
Planctomycetes	*Singulisphaer*	1.69	1.73	5.22
Proteobacteria	*Sphingomonas*	1.59	1.61	6.83
Acidobacteria	*RB41*	1.55	1.57	8.4
Acidobacteria	*Subgroup_6*	1.52	1.51	9.91

UB: unburned control soils; PB1: soils treated with prescribed burning seven months before sample collection; PB2: soils treated with prescribed burning immediately prior to sample collection. Capital letters in square brackets indicate the lowest taxonomic level of classification: [O]: order, [C]: class, [F]: family. Bacterial taxa without letters in square brackets represent the genus level.

Table 5. Pairwise SIMPER analysis with comparison of the three treatments in terms of relative abundance of bacterial phyla and genera, or lowest ranked taxonomic level. Percent dissimilarity and percent cumulative total contribution are shown. Contributions less than 1.5% are not shown.

Phylum	Bacterial Taxa	Av. UB	Av. PB1	Contribution (%)	Cumulative Contribution (%)
UB and PB1 dissimilarity = 9%					
Actinobacteria	*Mycobacterium*	1.02	1.56	2.53	2.53
Proteobacteria	[F] *Xanthobacteraceae*	2.73	3.18	2.14	4.68
Acidobacteria	[O] *Subgroup 7*	0.36	0.12	1.88	6.55
Firmicutes	*Cohnella*	0.32	0.69	1.75	8.30
Acidobacteria	*RB41 Subgroup 4 uncult.*	1.59	1.95	1.73	10.03
Acidobacteria	*soil bacterium clone S111*	0.31	0.27	1.56	11.59
UB and PB2 dissimilarity 13%					
Proteobacteria	*Massilia*	0.63	1.25	2.28	2.28
Proteobacteria	[F] *Xanthobacteraceae*	2.73	3.33	2.20	4.48
Proteobacteria	*Pseudomonas*	0.11	0.71	2.19	6.67
Acidobacteria	*Subgroup 10*	1.41	0.85	2.05	8.73
Firmicutes	*Bacillus*	0.89	1.37	1.75	10.47
Firmicutes	[O] *Bacillales*	0.46	0.89	1.57	12.04
Proteobacteria	*Luteibacter*	0.27	0.62	1.54	13.58
Proteobacteria	[F] *Archangiaceae*	0.64	0.23	1.53	15.11
Actinobacteria	*Mycobacterium*	1.02	1.43	1.50	16.61
Proteobacteria	*Massilia*	0.63	1.25	2.28	2.28

UB: unburned control soils; PB1: soils treated with prescribed burning seven months before sample collection; PB2: soils treated with prescribed burning immediately prior to sample collection. Capital letters in square brackets indicate the lowest taxonomic level of classification: [O]: order, [C]: class, [F]: family. Bacterial taxa without letters in square brackets represent the genus level.

The bacterial taxa that contributed most to similarity and dissimilarity in the different study soils are *Proteobacteria* [F] *Xanthobacteraceae* and *Actinobacteria Mycobacterium*, whose contribution to dissimilarity was greatest in the UB–PB1 relationships. Meanwhile, *Proteobacteria Massilia*, [F] *Xanthobacteraceae*, and *Proteobacteria Pseudomonas* had the highest significant contribution to the UB–PB2 dissimilarity relationship.

3.5. Relative Abundance of Bacterial Communities as a Function of Time since Prescribed Burning Treatment

In general, the behaviour of bacteria at the genus level was different depending on the time elapsed since the application of the prescribed fire treatment, compared to the control plots. On the one hand, it was observed that a particular group of bacteria clearly decreased their relative abundance with respect to UB, regardless of the time elapsed after treatment with prescribed burning, both for PB1 and PB2 (Figure 5). Bacteria in this group included *Actinobacteria* (*Pseudonocardia* and *Arthrobacter)* or *Acidobacteria* [G] *Acidobacteria_bacterium_WX27* and *Acidobacteria* [C] *Subgroup 6*. However, seven months after the application of the prescribed burn (PB1), the relative abundance values of other bacterial communities remained similar to UB. These included: *Proteobacteria* [F] *Archangiaceae*, *Proteobacteria* [G] *Sphingomonas*, *Planctomycetes* [F] *Isosphaeraceae* and *Acidobacteria* [G] *Subgroup_10*. The same bacterial taxa were clearly more affected by the recent prescribed burn (PB2) compared to UB (Figure 6), showing a decrease in their relative abundance. In contrast, *Proteobacteria* [F] *Xanthobacteraceae*, *Planctomycetes* [G] *Singulisphaera*, *Actinobacteria* [G] *Mycobacterium*, and some *Firmicutes* ([G] *Sporosarcina* and [G] *Cohnella*) were favoured by prescribed burning treatments regardless of the time since application, showing abundance values in both cases higher than UB (Figure 7). Moreover, *Proteobacteria* [G] *Luteibacter*, *Proteobacteria* [G] *Pseudomonas*, *Proteobacteria* [G] *Massilia*, and others such as *Firmicutes* ([G] *Bacillus* and [O] *Bacillales*) showed relative abundance values in PB1 similar to UB (Figure 8). However, the same bacterial taxa presented an increase in their relative abundance and were favoured by recent burning (PB2) in comparison with UB. In addition, other bacterial groups such as *Firmicutes* [G] *Paenibacillus*, *Bacteroidetes* [G] *Chitinophaga*, *Acidobacteria* [G] *uncultured_microorganism*, or *Acidobacteria* [G] *uncultivated soil bacterium Clone111* showed resistance to the prescribed burning treatment, and their relative abundance was not af-

fected regardless of the time elapsed since burning, presenting similar relative abundance with respect to the relative abundances shown by UB (Figure 9).

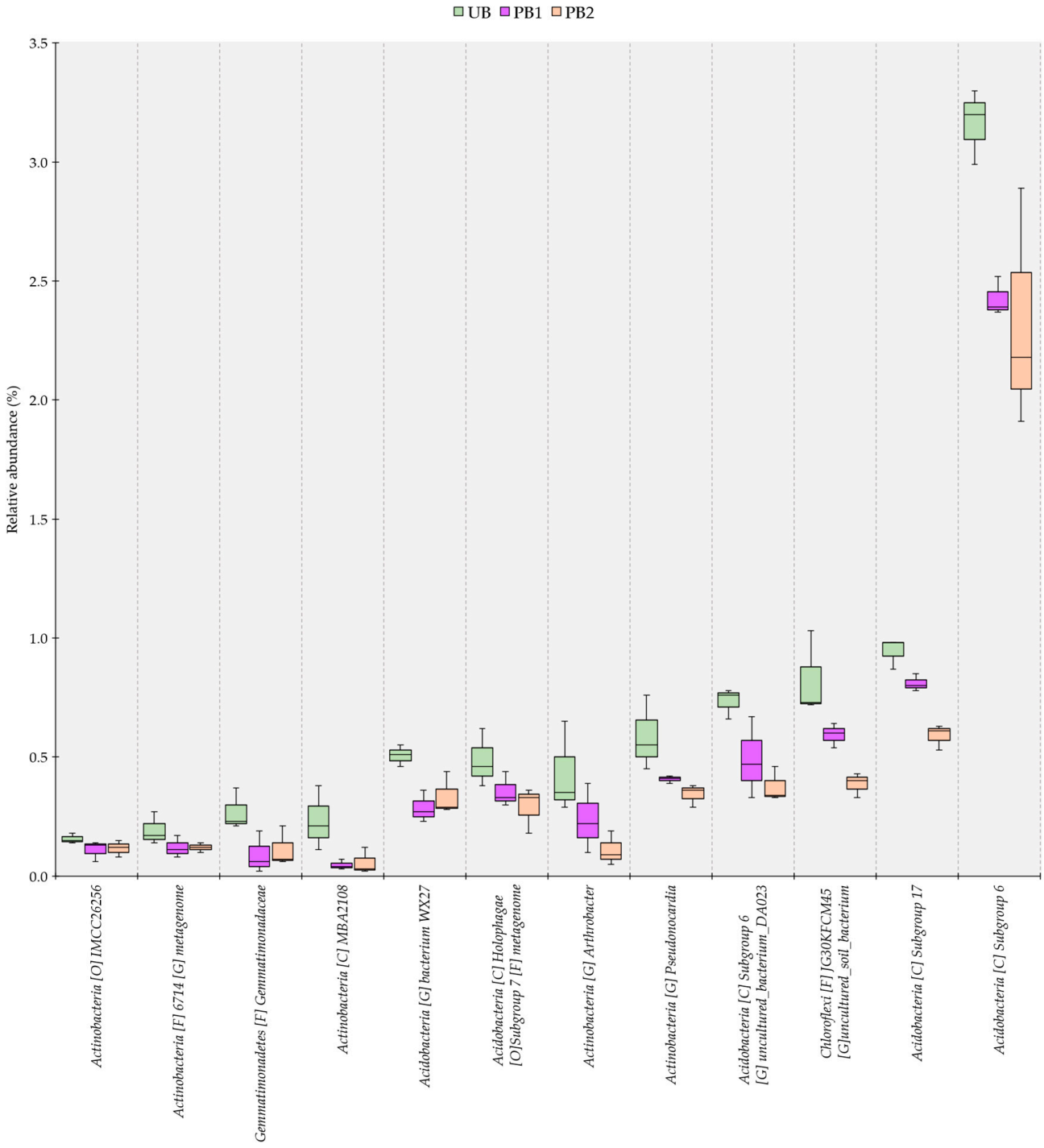

Figure 5. Bacterial groups decreasing in relative abundance (%) after prescribed burn treatment regardless of time since burn application compared to UB. UB: control; PB1: soils treated with prescribed burning seven months before sample collection; PB2: soils treated with prescribed burning immediately prior to sample collection. Boxes include 50% of the data between the first and third quartiles (interquartile range) and the central line, the median. The whiskers include those values that deviate from the first and third quartiles up to a maximum distance of 1.5 times the interquartile range. Capital letters in square brackets indicate the lowest taxonomic level of classification: [O]: order, [C]: class, [F]: family, [G] genus.

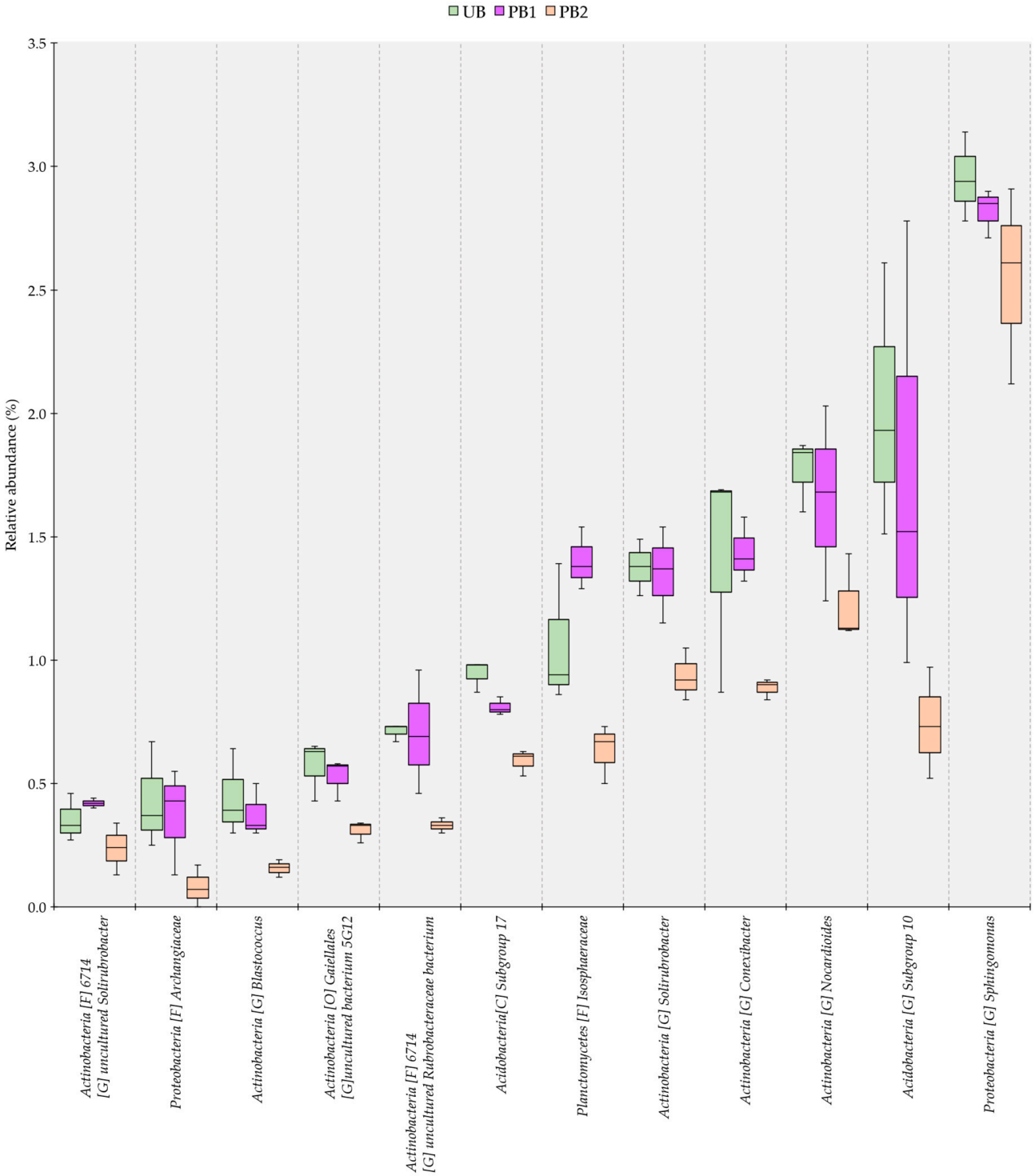

Figure 6. Bacterial groups showing a relative abundance (%) similar to UB seven months after application and bacterial group decreasing in relative abundance (%) after a recent prescribed burning treatment (PB2) respect to UB. UB: control; PB1: soils treated with prescribed burning seven months before sample collection; PB2: soils treated with prescribed burning immediately prior to sample collection. Boxes include 50% of the data between the first and third quartiles (interquartile range) and the central line, the median. The whiskers include those values that deviate from the first and third quartiles up to a maximum distance of 1.5 times the interquartile range. Capital letters in square brackets indicate the lowest taxonomic level of classification: [O]: order, [C]: class, [F]: family, [G] genus.

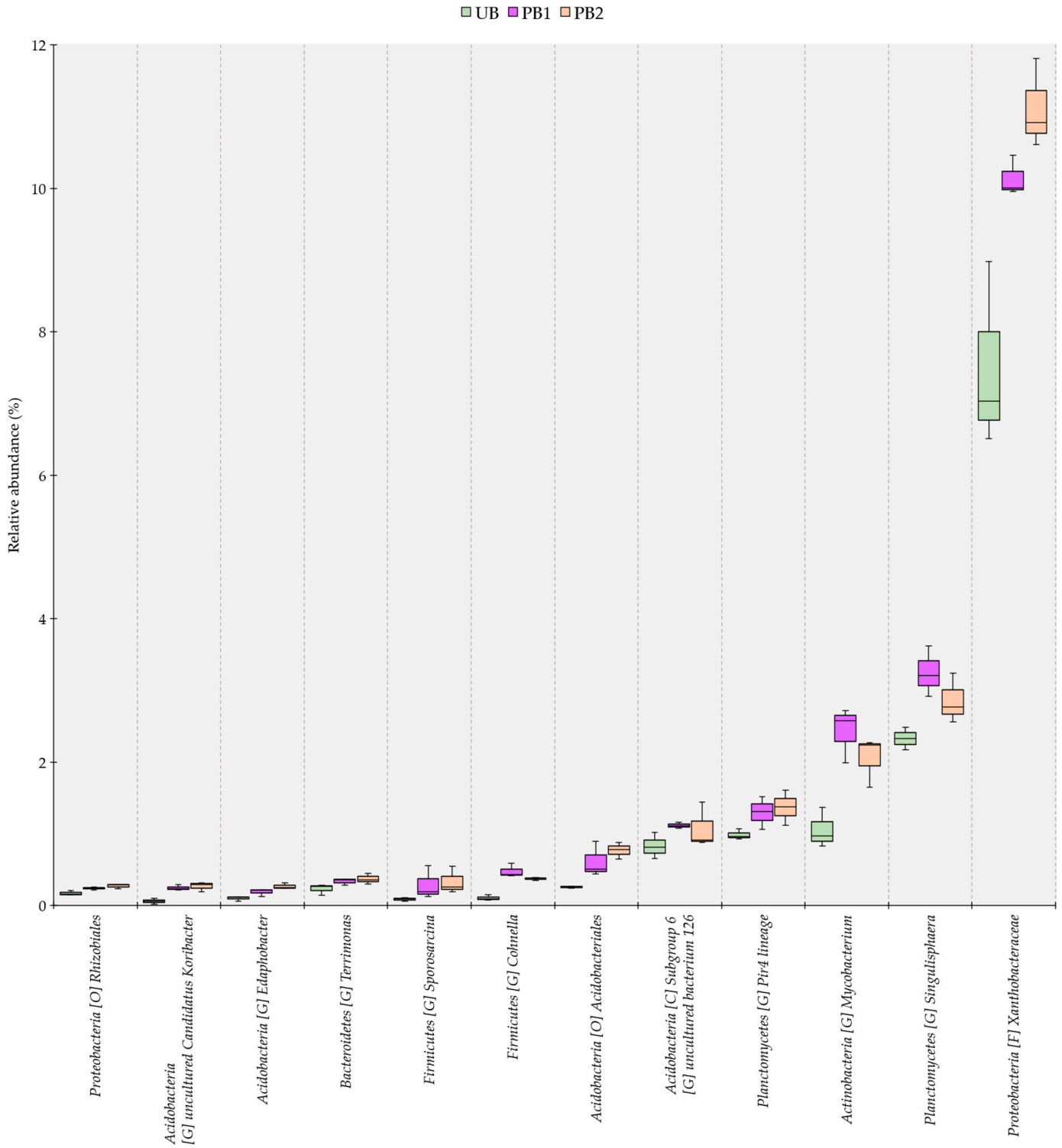

Figure 7. Bacterial taxa increasing in relative abundance (%) after application of prescribed burning regardless of the time elapsed since treatment. UB: control; PB1: soils treated with prescribed burning seven months before sample collection; PB2: soils treated with prescribed burning immediately prior to sample collection. Boxes include 50% of the data between the first and third quartiles (interquartile range) and the central line, the median. The whiskers include those values that deviate from the first and third quartiles up to a maximum distance of 1.5 times the interquartile range. Capital letters in square brackets indicate the lowest taxonomic level of classification: [O]: order, [C]: class, [F]: family, [G] genus.

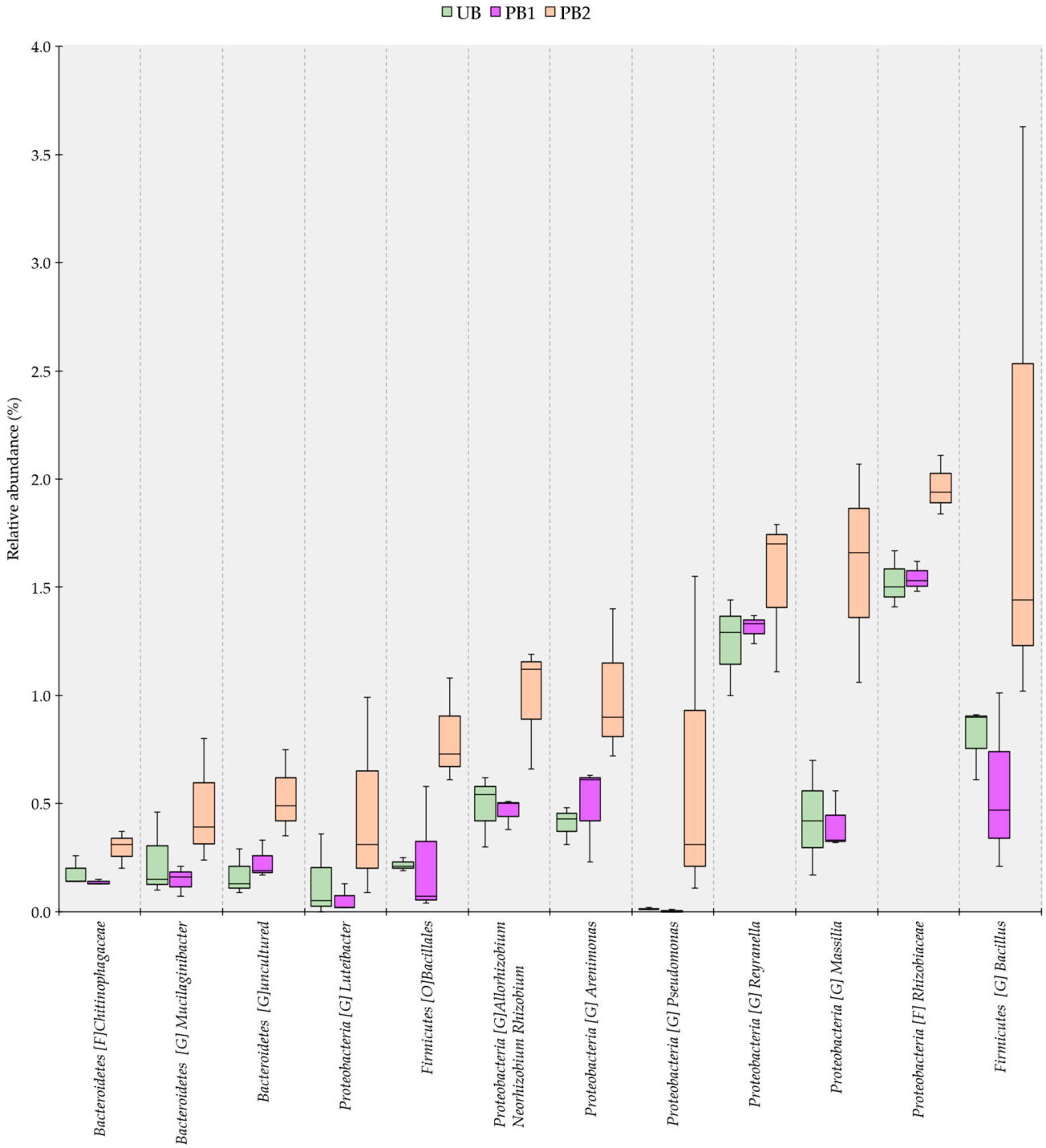

Figure 8. Bacterial taxa showing no difference with respect to UB at seven months after application of a prescribed burn (PB1). Increase in relative abundance (%) of bacteria after application of recent prescribed burning treatment (PB2) compared to UB. UB: control; PB1: soils treated with prescribed burning seven months before sample collection; PB2: soils treated with prescribed burning immediately prior to sample collection. Boxes include 50% of the data between the first and third quartiles (interquartile range) and the central line the median. The whiskers include those values that deviate from the first and third quartiles up to a maximum distance of 1.5 times the interquartile range. Values with a deviation greater than 1.5 times were represented as circles. Capital letters in square brackets indicate the lowest taxonomic level of classification: [O]: order, [C]: class, [F]: family, [G] genus.

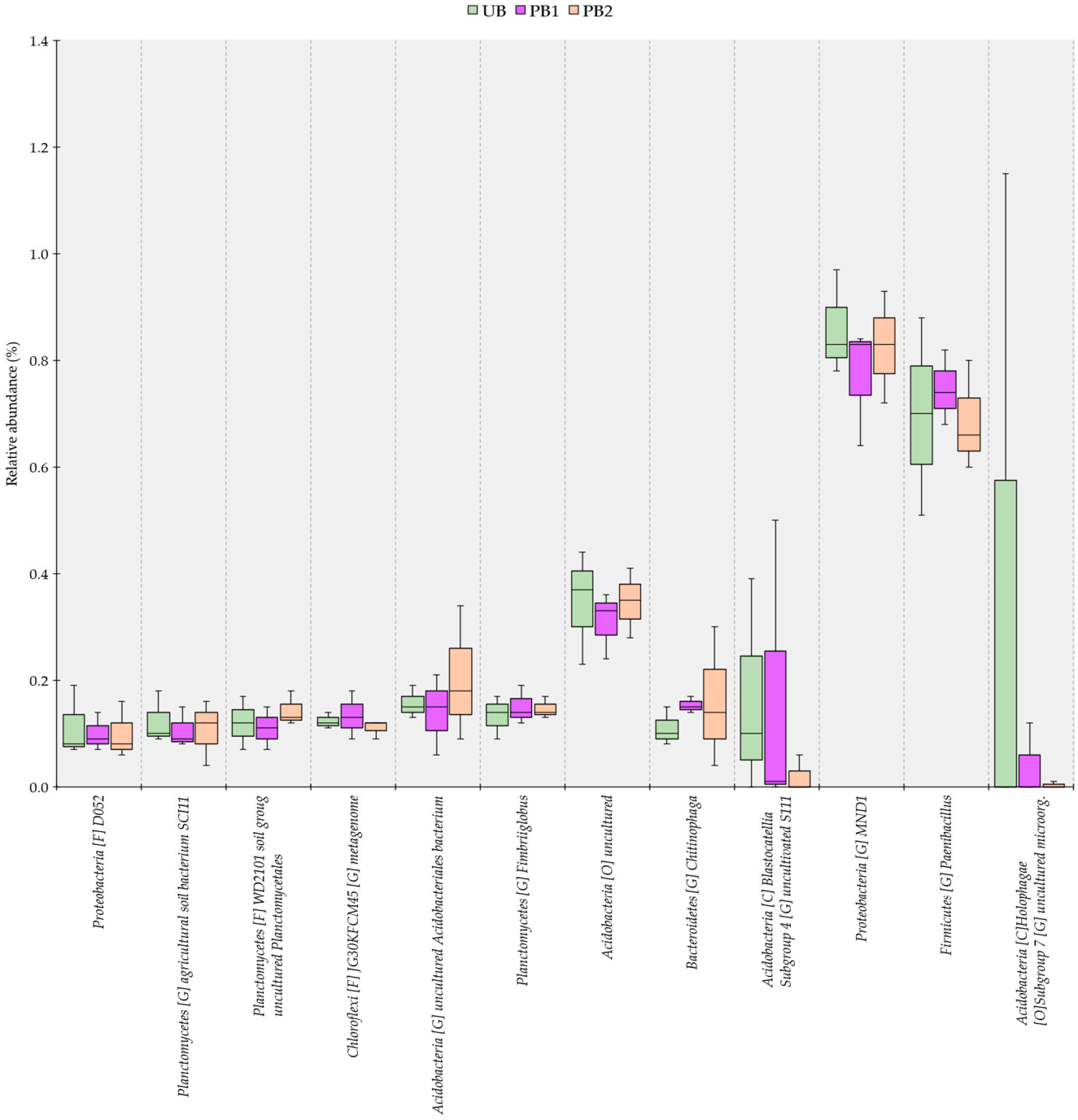

Figure 9. Bacterial taxa showing no change in relative abundance (%) after a prescribed burning treatment, regardless of the time since the application of the burn. UB: control; PB1: soils treated with prescribed burning seven months before sample collection; PB2: soils treated with prescribed burning immediately prior to sample collection Boxes include 50% of the data between the first and third quartiles (interquartile range) and the central line, the median. The whiskers include those values that deviate from the first and third quartiles up to a maximum distance of 1.5 times the interquartile range. Capital letters in square brackets indicate the lowest taxonomic level of classification: [O]: order, [C]: class, [F]: family, [G] genus.

4. Discussion

Prescribed burnings are a tool used in Mediterranean forest ecosystems to reduce forest fuel and, therefore, wildfires [35,36]. In this investigation, we studied the effects

of two low-intensity experimental prescribed burns of similar characteristics on the main chemical properties and bacterial communities of the soil in a semi-arid Mediterranean forest. Taking into account that the plant communities, parent materials, climate, topographies, and environmental conditions in both burns were similar in all stands, the differences in soil chemical properties and bacterial community structure between the different types of burned soils (PB1 and PB2) could be attributed to the time elapsed since each prescribed burning, compared to the reference ecosystem and the prescribed burn seasonality. Our results showed that low-intensity prescribed burning did not compromise the main chemical properties (pH, EC, and TOC; Table 1), nor the bacterial community richness (ASV number and Faith index; Table 2) of the burned soils compared to the control soils. However, it did cause significant changes in the Shannon and Pielou diversity indices (Table 2), as well as in the relative abundance of some soil bacterial taxa (Figure 2) and distribution of soil bacterial community structure (Figure 4), when comparing burned plots with control plots.

Both PB1 and PB2 burned soils showed no significant differences in pH compared to the unburned soils (UB), possibly because the alkalinity of the soils buffered the disturbances caused by the fire [37]. In contrast, PB2 soils showed a slight increase in electrical conductivity with respect to UB soils, as has been reported in other studies in Mediterranean pine forests [37,38], mainly due to the contribution of inorganic ions produced by the combustion of organic matter [7]. The burned soils (PB1 and PB2) showed no statistical differences in TOC content with respect to the UB soils. It is usually held that TOC varies when comparing burned and unburned sites after prescribed fires (e.g., [39]). However, mainly due to the low severity of the PB, our analyses did not show any statistical differences between TOC in burned and unburned sites, in line with data reported by different authors from Mediterranean forest stands affected by prescribed burns. More specifically, Lucas-Borja et al. [14] found no substantial alterations in the chemical properties of soils studied with monoliths extracted from coniferous forests to which low-intensity fire was applied with temperatures higher than those obtained in our research (see temperature reached in PB1 and PB2 material and methods section).

The richness of bacterial taxa and Faith's phylogenetic index showed no significant differences between the control soils (UB) and those burned and collected seven months afterwards (PB1) or those recently burned (PB2), consistent with other previous studies in which no significant changes in alpha diversity were observed [14,40]. This result could be because the temperatures reached in the prescribed burns did not negatively affect the bacterial community. Interestingly, there was a slight increase in the richness of ASVs, both immediately after the prescribed burn (PB2) and seven months later (PB1), compared to the unburned soils (UB), indicating that fire has an immediate effect on the bacterial community after the prescribed burn, and its effects may last up to seven months after the burn (PB1). These changes in the diversity and richness of soil bacterial communities have been described as more pronounced as fire severity increases [14]. Likewise, in PB2 soils, the Faith index increased slightly compared to UB soils. Fontúrbel et al. [13] also observed a progressive increase after prescribed burning, differing significantly from unburned soils. Shannon and Pielou indices presented significant differences between treatments (Table 2). These results suggest a decrease in the diversity indices immediately after prescribed burning, and that, a short time after burning, the abundance of bacterial taxa could recover slightly, although without reaching the species richness values of unburned soils (UB).

Some studies [13,14,41] have found that with the temperatures reached in a low-intensity burn, microbial biomass and its activity are initially reduced, while microbial diversity increases. Despite the decline in the activity of soil microbial communities and changes in soil structure, soil functional diversity also tended to recover in other soils colonized by Mediterranean thermophilic shrubs within a few months after burning [42], possibly due to the recovery of soil microbial communities any time after prescribed burning [43]. This implies that the soil bacterial structure at any time after prescribed burns appears to be similar to the communities of unburned soils, as corroborated by our results (Figure 4).

The analysis of the bacterial community also showed that the relative abundances at phylum level presented different trends depending on the time elapsed from the prescribed burning treatment application (Figure 2). Our results indicated an initial increase in *Proteobacteria* and *Firmicutes* in burned soils, and a decrease in *Acidobacteria* compared to UB. A similar pattern was also observed by Lucas-Borja et al. [14] and Rodríguez et al. [44] in soils affected by Mediterranean pine wildfires. An increase in the relative abundance of *Firmicutes* after the passage of fire has also been described on numerous occasions [14,16,44]. This circumstance could be due to the fact that this bacterial phylum contains species capable of generating spores that germinate at high temperatures [45,46], taking advantage of post-fire conditions to colonize the soil, even if its conditions have changed [47]. In contrast, *Planctomycetes* shows sensitivity to fire [48], although in this study it did not show a large variation after burning, suggesting that the effect of prescribed burning, at least under the conditions of our study, could be very small, even in some fire-sensitive bacterial communities. However, our results differ in the trend found with *Bacteroidetes*, since their relative abundance increased in PB2 soils compared to UB soils. In a study conducted by Rodríguez et al. [44] on soils subjected to high severity forest fires in *Quercus suber* and *Pinus pinea* forests in Sierra de Aznalcollar (Seville), they observed different trends of increase or decrease, depending on the recurrence of wildfires, for the phylum *Bacteroidetes*. Therefore, these results would highlight the different effect of high severity or variable severity wildfires versus low severity fires, such as prescribed burns, on some soil bacterial communities, since changes in soil microbial communities are very extreme after severe wildfires [14,20]. Conversely, prescribed burns produce small changes in the abundance of some communities that usually return to their initial levels soon after burning [43]. Likewise, the fire recurrence study by Rodríguez et al. [44] found an increase in the abundance of *Actinobacteria* in soils burned twice (1997 and 2004) compared to control soils and those burned only once (2004). However, our study showed different results, such as an initial decrease in their relative abundance in recently burned soils (PB2) compared to UB soils, and similar values to UB seven months after a prescribed burn (PB1).

Furthermore, the copiotrophic phylum *Proteobacteria* experienced an increase after recent burning, possibly due to organic carbon input (Table 1), since this phylum is involved in pyrogenic C degradation [14]. In addition, it frequents soils with high resource availability, suggesting that part of the bacterial community could be involved in the degradation of organic compounds [49]. The percentage distribution of the phylum *Proteobacteria* follows the same pattern as in other coniferous forest soils in Andalusian Mediterranean forests after wildfires [37,44]. Its higher representation is mainly due to the contribution of *Gammaproteobacteria,* which increased its relative abundance immediately after prescribed burning (PB2) by approximately 30% (Figure 3), being favoured by fire [50]; and, to a lesser extent, *Betaproteobacteria* and *Alphaproteobacteria,* whose increases were milder (around 10%; Figure 3). The effects of fire on these bacterial taxa have been described by other authors, in which *Betaproteobacteria* have been favoured by fire [40,48], while *Alphaproteobacteria* are more sensitive to fire severity [40]. Therefore, the increase in *Alphaproteobacteria* could also suggest that prescribed burning did not affect these fire-sensitive microbial communities, and instead favoured the proliferation of these communities, possibly by nutrient input [40], immediately after burning (comparative increase in TOC in PB2; Table 1). However, we observed a marked decrease immediately after burning (PB2) of *Deltaproteobacteria,* suggesting a higher degree of vulnerability of these communities to warming [48]. Therefore, the degradation of organic compounds by the phylum *Proteobacteria*, *Firmicutes* and *Bacteroidetes* just after burning could lead to a release of substrates that, in turn, favoured a greater proliferation of the phyla *Acidobacteria* and *Actinobacteria* seven months after burning (PB1). Thus, the changes produced in the microbial community structure after prescribed burning point to the fact that these bacterial phyla could be affected differently by fire [51], as previously concluded by Lucas-Borja et al. [14]. In addition, the results showed that prescribed burning produced marked changes in bacterial communities at different post-fire response times at the genus level (Tables 3 and 4; Figures 5–9). The

different trends presented from the relative abundance of the bacterial community at the phylum level show no relationship with the bacterial taxa that compose them, as reported in the studies of Cerda and Robichaud [43] and Rodríguez et al. [37]. However, other authors claim that fire intensity can predict bacterial structure, being a driver of microbial structure change at higher severities [52]. The boxplots revealed different trends in several taxa depending on the prescribed burning and the time elapsed after the burns (Figures 5–9). For example, bacterial taxa such as *Pseudonocardia* and *Arthrobacter*, *DA023* and *WX27* were affected by the prescribed burns (PB1 and PB2; Figure 7) compared with UB, regardless of the time elapsed since its application, possibly due to their greater sensitivity to the heat exposure caused by the prescribed burning. In addition, these bacterial taxa did not recover a relative abundance similar to control soils (UB) months after a prescribed burn (PB1). Although numerous authors have highlighted a rapid recovery of edaphic microbiota after low severity prescribed burns [6,7,20,43], the abovementioned bacterial taxa might need more time to reach values similar to UB. Interestingly, contrary to the findings of the present study after different prescribed burns, *Arthrobacter* genus increased in abundance after a Quercus ilex L. fire in Sierra de Cabrera (NE Spain; Sáenz de Miera et al. [16]). However, their relative abundance was higher in the short term after a prescribed burning (PB1) than immediately after (PB2). Some authors have reported resistance to drying by *Arthrobacter* [53], as well as an important role in nitrogen cycling after wildfire [54] and potential as a rhizosphere bacterium and plant growth promoter, which has led it to be known as a post-fire ecosystem recovery bacterium [55]. In addition, other authors have described their physiology as resistant to starvation, dehydration, and oxidative stress [53,56,57], so it could have increased its relative abundance as environmental conditions after the fire improved.

Another group of bacterial taxa, including *Sphingomonas*, *Solirubrobacter*, *Nocardioides*, and *Blastococcus*, were affected just after the prescribed burn (PB2). However, their relative abundance values were similar to UB soils in soil samples taken seven months after the burn (PB1) (Figure 6). Among them, *Sphingomonas* decreased in abundance after recent prescribed burning (PB2) compared to UB soils, while presenting very similar values to UB soils seven months after the prescribed burning (PB1) (Table 3; Figure 6). These results coincide with other previous studies, in which it decreased its abundance post-burn, suggesting that it could be easily affected by fire [14,44]. *Sphingomonas* has been previously described in other limestone soils as a genus involved in nitrogen fixation [58]. Therefore, a similar relative abundance between PB1 and UB could support the hypothesis that low-intensity prescribed burning could be a good strategy for fuel removal without seriously affecting the functionality of the natural ecosystem in the short term.

Interestingly, another group of bacterial taxa that could have benefited from prescribed burning, both PB2 and PB1, was [F] *Xanthobacteraceae* (Figure 7), which was also the taxon that contributed most to the dissimilarity between samples from burned plots (PB1 and PB2) compared to unburned plots (Table 4). The increase in [F] *Xanthobacteraceae* in burned soils could suggest (in soils affected by prescribed burns in the study area) that the stimulation of biogeochemical cycles may have been favoured, as it has been previously observed that these bacteria play an important role in carbon cycling [59]. Other bacteria, such as *Sporosarcina* (Figure 7), could have been favoured in soils affected by prescribed burns due to their ability to form resistance structures (endospores) (Dworkin, 2006), protecting them from heat and keeping them in a dormant state while the burn treatment occurs, but allowing them to proliferate later. Likewise, another group of bacterial taxa (e.g., *Massilia* and *Pseudomonas*) was favoured immediately after burning (PB2; Figure 8), suggesting interspecific competition with other soil bacterial taxa that would not allow them to proliferate under low-intensity fire conditions [10,20,52]. Lucas-Borja et al. [14], Rodríguez et al. [44], and Whitman et al. [52] obtained similar results regarding *Massilia* genus. *Massilia* is considered an opportunist that takes advantage of post-fire ecosystem conditions, acting as a pioneer of microbial succession [16], as well as being related to vegetation development [46]. This bacterium is probably an opportunistic colonizer that

came from unburned areas or areas that were not affected by prescribed burning. Although its resistance to fire has not been previously described [16], it could have proliferated in the face of reduced competition with other bacterial groups that were more affected.

Finally, the results suggest that the most important factors in understanding trends in the increase or decrease in the abundance of bacterial taxa are their resistance to temperature, their strategies for exploiting fire-generated resources, and the opportunity to occupy (succession) a space relinquished by a bacterial community that has been diminished by disturbance [52]. In addition, these results highlighted the fact that the effective use of technical fire requires a great knowledge of all the factors influencing its impact and the dynamics of each of them over time, as well as the need for medium to long-term sampling after the execution of prescribed burns in order to understand the evolution of bacterial communities. Some studies suggest that the carbon accumulated in the soil from the incomplete combustion of woody materials participates in increasing soil fertility [9], so that prescribed burning could contribute to the recovery of ecosystem functionality. However, studies on how fire affects organic matter cycling, microbial processes, and nutrient loss [6] are insufficient or contradictory, increasing this deficit in Mediterranean environments where bacterial communities are adapted to this type of disturbance [14]. In addition, thermal acclimation of bacterial communities to high temperatures has been found in ecosystems of semi-arid zones; these communities continue to carry out their metabolic processes even at temperatures above 40°C in the presence of humidity [60]. Therefore, new complementary studies are needed to clarify the information gaps that currently exist on the effect of prescribed burning on the biological properties of soils.

5. Conclusions

In order to achieve better management and conservation of semi-arid Mediterranean forests, which are highly vulnerable to wildfires, characteristics related to adaptation to wildfire should be considered. The low-intensity prescribed burns performed in this investigation did not affect the chemical properties studied, so performing burns under similar conditions should not be a problem if care is taken to ensure that the flame residence and the intensity (severity) of the burn do not exceed a level that could affect the organic horizon during its execution. In this study, a slight modifying effect of low-intensity prescribed burning on the structure of soil bacterial communities was detected, indicating that fire has an immediate effect on the microbial community after prescribed burning, and its effects can extend up to seven months after the burns are carried out. However, the relative abundance of some bacterial taxa was favoured, while others decreased immediately after burning. In soils seven-months post-fire treatment, some bacterial taxa were observed to have relative abundances similar to unburned reference soils. The different patterns observed for the bacterial taxa determined in these soils indicate that there could be a rapid recovery of the bacterial community, reaching relative abundance levels shown prior to treatment with prescribed burns. Therefore, our results would suggest that in response to this low-intensity fire disturbance, the resilience of the soils and their bacterial communities could favour the recovery of the ecosystem returning it to its initial state in the short term, as well as being able to withstand the impact of a prescribed burn carried out under adequate conditions. In light of these findings, the silvicultural treatment of prescribed burning could be a viable management tool for the prevention of forest fires, given the effect on the soil properties of Mediterranean semi-arid ecosystems. However, these preliminary results should be handled with caution, and further studies on the impact of prescribed burning on these fragile ecosystems, severely threatened by climate change, are needed.

Author Contributions: Conceptualization: I.M., R.S. and A.T.; methodology, I.M., R.S. and A.T.; validation, I.M., R.O. and M.E.L.-B.; formal analysis, R.S., A.T. and R.O.; investigation I.M., R.S. and A.T.; resources, I.M.; data curation, R.O. and N.R.-B.; writing—original draft preparation, R.S., A.T., I.M. and N.R.-B.; writing—review and editing, R.S. and M.E.L.-B.; visualization, R.S. and N.R.-B.; supervision, I.M., M.E.L.-B. and R.O.; project administration, I.M.; funding acquisition, I.M., R.O. and M.E.L.-B. All authors have read and agreed to the published version of the manuscript.

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