

The Soil Microbiome of the Laurel Forest in Garajonay National Park (La Gomera, Canary Islands): Comparing Unburned and Burned Habitats after a Wildfire

Pablo J. Villadas ¹, Sara Díaz-Díaz ², Antonio Rodríguez-Rodríguez ³, Marcelino del Arco-Aguilar ⁴, Antonio J. Fernández-González ¹, Juan Pérez-Yépez ², Carmen Arbelo ³, Juana M. González-Mancebo ⁴, Manuel Fernández-López ¹ and Milagros León-Barrios ^{2,*}

¹ Grupo de Ecología Genética de la Rizosfera, Departamento de Microbiología del Suelo y Sistemas Simbióticos, Estación Experimental del Zaidín, CSIC, 18008 Granada, Spain; pablo.villadas@eez.csic.es (P.J.V.); antonio.fernandez@eez.csic.es (A.J.F.G.); manuel.fernandez@eez.csic.es (M.F.L.)

² Departamento de Bioquímica, Microbiología, Biología Celular y Genética (área Microbiología), Universidad de La Laguna, 38200 La Laguna, Spain; saradiazdiaz.94@hotmail.com (S.D.D.); juanernestoperezypepez@gmail.com (J.P.Y.)

³ Departamento de Biología Animal, Edafología y Geología, Universidad de La Laguna, 38200 La Laguna, Spain; antoro@ull.es (A.R.R.); carbelo@ull.es (C.A.)

⁴ Departamento de Botánica, Ecología y Fisiología Vegetal, Universidad de La Laguna, 38200 La Laguna, Spain; marco@ull.edu.es (M.A.A.); jglezm@ull.edu.es (J.M.G.M.)

* Correspondence: mileonba@ull.edu.es; Tel.: +34-922318481

Received: 8 October 2019; Accepted: 16 November 2019; Published: 20 November 2019

Abstract: The evergreen laurel forest is a relic of ancient subtropical/tropical forests, of which the best remnant in the Canary Islands is in Garajonay National Park, on La Gomera island. The soil microbiome associated with a mature undisturbed (unburned) laurel forest was characterized at two locations at different topographical positions on the mountain: The slope and the ridge crest. Given the unusual circumstance of an intense wildfire that severely affected part of this forest, the burned soils were also studied. The soil in undisturbed areas was relatively uniform. The bacterial community composition was dominated by bacteria from phyla Proteobacteria, Acidobacteria, and Actinobacteria. The wildfire changed the composition of the bacterial communities. The Acidobacteria, Actinobacteria, and Alphaproteobacteria (dominant class in unburned forests) significantly decreased in burned soils along with a parallel high increase in Betaproteobacteria, Bacteroidetes, and Firmicutes. We further showed the dramatic effect of a wildfire on the soil microbiome of the laurel forest, appearing as a loss of species richness and diversity, species dominance, and changes in the composition of the bacterial communities.

Keywords: laurel forest; wildfire; soil microbiome; Garajonay National Park; pyrosequencing

1. Introduction

The evergreen laurel forest of the Canary Islands is a relic of subtropical/tropical Tethyan forests. Widely developed on all continents around 40° N in the Miocene, they were forced to shift southward, being replaced in Europe by Mediterranean vegetation, as a consequence of Pleistocene glacial periods and the later development of the Mediterranean climate [1]. Part of that arboreal vegetation reached the Canary Islands and found refuge within the cloudy belts on their windward

slopes [2], influenced by condensation from the moist trade-wind regime that mitigates the dryness of the Mediterranean climate elsewhere. In this humid forest, laurifolious trees (Lauraceae) predominate, along with other broadleaf evergreen trees from diverse families. *Laurus novocanariensis*, *Ilex canariensis*, and *Morella faya* are species widely distributed in this forest and constitute the principal matrix in which other typifying species are interspersed [2]. In its Garajonay National Park (NP) (UNESCO World Heritage since 1981), the small island of La Gomera hosts the best remnant of laurel forests in the Canary Islands.

The effects of wildfires are particularly important on oceanic islands like the Canaries. They are small and fragile ecosystems that contain a high percentage of endemic species, which have few opportunities to escape from a sudden threat like habitat destruction. Most fires in the archipelago occur in the pine forest dominated by *Pinus canariensis*, favored by the more arid conditions typical of this higher altitude vegetation belt, with easily combustible material like pine-litter and understorey vegetation. In contrast, in mature laurel forests, the fire spreads with difficulty due to the characteristic high humidity in this formation, and the comparative shortage of easily combustible elements. However, preceded by a winter and spring with scarce rainfall, 2012 was one of the driest summers ever registered in these islands, with temperatures above 30 °C and 20% relative humidity. This set the conditions for a fire that burned more than 10% of La Gomera [3], affecting 750 ha of Garajonay NP.

Wildfires produce great impact in the short, medium, and long term on the whole forest ecosystem. Soil microbial populations are first directly affected by the high temperatures reached during wildfires and later, indirectly, as a consequence of the changes in the physicochemical properties of burned soils. Quantity and quality of organic matter, fluctuations in carbon and nitrogen contents, increase in soil pH, and changes in nutrient availability and water content are common examples [4–6] that alter the dynamics of the microbial communities, causing severe disturbances in biogeochemical cycles [7–10]. Assessment of the changes in the activities of specific microbial groups from conventional culture-based approaches or from DNA fingerprints and sequence analyses of particular genes [8–13] have produced valuable information on the immediate effect of fires. However, this approach provides a limited vision of the true impact of fire on the overall microbial community composition. Furthermore, many conclusions about the impact of fires on soil microorganisms have been inferred from studies monitoring prescribed or controlled fires with moderate effects [8,10,14–16]. The transitory fire release of nutrients easily available to microorganisms causes rapid recolonization by specific groups. Through the increase of microbial biomass and certain activities of the carbon and nitrogen cycles [8–13], these may even lead to a stimulating benefit of fire on surviving soil microorganisms. However, other studies based on evidence accumulated during recent years from data analyses obtained from high-throughput sequence technologies show that wildfires damage the overall microbial biome and the impact of wildfires on the microbial community composition can persist for more than a decade [17–21].

In the Canary Islands, studies of fire impact on soils have mainly focused on changes in physicochemical properties [3,22,23], whereas those regarding soil microorganisms are almost inexistent, being only performed on burned pine forest soils [24,25]. The wildfire affected highly valuable areas from a botanical, zoological, and edaphological point of view. Indeed, recovery of the natural vegetation is predicted to take several decades [3]. However, the impact on soil microbiota was not evaluated by the NP authorities. There is no previous characterization of the microbial diversity in the laurel forest soils, nor a description of the effects of the fire on it beyond the effect on the physicochemical properties. Here, we used 454-pyrosequencing to describe the diversity of the bacterial community composition of Garajonay laurel forest in undisturbed areas and in a severely burned area. One main objective was therefore to describe and compare the soil bacterial diversity associated with undisturbed mature Canarian laurel forest soils in Garajonay NP, which hosts the best remnant of this forest in the islands. For this purpose, we chose two undisturbed (unburned) mature laurel forests differing in their topographical location on the mountain, a crest and a slope forests (UCLF, unburned crest laurel forest and USLF, unburned slope laurel forest). As a second

objective, using this previous knowledge, we assessed the short-term (15–16 weeks after fire) impact of that wildfire on the forest.

2. Materials and Methods

2.1. Sampling Localities

The soils analyzed in this study were collected in a northern area of Garajonay NP. Plots were selected in two localities at 1040 and 1060 m a.s.l. (meters above sea level), where mature laurel forest is well represented (over 100 years old). Two laurel forests with similar orientations were distinguished by their different topographical location on the mountain: The crest forest on the summit and the slope cloud forest growing at mid-altitude on the mountainside. The crest forest is much more subject to fog-laden winds and is more effective in moisture catchment. Nevertheless, the slope forest is a cloud forest that also receives extra water through fog precipitation. Although both forests represent the mature stage of laurel forest, they differ in species composition and abundance, particularly the greater abundance of *Erica arborea* in crest forest, a response to the paradoxical drying effect of wind in the canopy. Thus, two types of bulk soil samples (separated 120 m in distance and 20 m in altitude) were collected in areas not affected by the fire (undisturbed soils). Samples named USLF (unburned slope laurel forest) were taken from a 20 × 20 m unburned plot, and the so-called UCLF (unburned crest laurel forest) samples were taken from a 15 × 15 m unburned plot. The USLF plot had trees up to 19 m tall and a canopy cover of 90%. The tree species composition (number of individuals in the 20 × 20 m plot) was: *Laurus novocanariensis* (16 individuals), *Ilex canariensis* (11 individuals), *Picconia excelsa* (10 individuals), and *Morella faya* (5 individuals). The UCLF plot had trees with maximum heights of 16 m and a canopy cover of 85%. The tree species composition (individuals in the 15 × 15 m plot) was: *L. novocanariensis* (18 individuals), *E. arborea* (14 individuals), *M. faya* (7 individuals), and *I. canariensis* (6 individuals). A third location in the surroundings of these two plots was an area where the 2012 summer wildfire burned a crest laurel forest stand near the UCLF plot, 250 m away. This forest on the top of the ridge, BCLF (burned crest laurel forest), was a severely burned zone (all vegetation was dead) with the characteristics of a crest forest before the wildfire. Soil samples were taken 3 to 4 months after the wildfire from non-rhizospheric soils (bulk soil at 1 to 2 m from the closest trees) and 5 to 10 cm deep. From each location, three replicates were taken, separated at least 3 m from each other. Soil samples were collected from plots UCLF and BCLF on 11 December 2012. In plot USLF, soil samples were taken on 21 December 2012 and kept frozen at −20 °C for no more than seven weeks, before DNA extraction.

2.2. Physicochemical Analysis of the Soils

Chemical analyses were performed on air-dried, 2-mm-sieved soil. Three replicas collected at each location were separately analyzed for burned soils. A mixture resulting from three replicates was analyzed for unburned soils, for which a large body of data were available from previous studies (Rodríguez-Rodríguez et al., 2009). Soil organic carbon (SOC) was determined by wet oxidation of the carbon by the 1N dichromate Walkley-Black procedure [26]. Total nitrogen (N) was determined by Kjeldahl digestion, followed by distillation and titration. Mineralization was performed in a mixture of concentrated H₂SO₄ and acetylsalicylic acid using Se as a catalyst, in a TECATOR Mod. 1007 digester. Soil pH was measured in water (1:2.5 soil:liquid ratio). Plant-available phosphorous was extracted [27] and colourimetry determined by the molybdenum blue method [28,29]. Available cations were determined by the ammonium acetate extraction method at pH 7. Sodium and potassium were determined by flame spectrophotometry, and calcium and magnesium by an atomic absorption spectrophotometer (Perkin Elmer Mod. 3100) in the presence of 1% lanthanum. Soil particle size distribution was calculated [30]. Moisture retention capacity was measured in fire-disturbed samples [31]. Water repellence was measured using the WDPT (water drop penetration time) [32]. Five classes of WR persistence were used for WDPT: Wettable (WDPT ≤ 5 s), slightly water repellent (5–60 s), strongly water repellent (60–600 s), severely

water repellent (600–3600 s), and extremely water repellent (>3600 s) [33]. The molarity of the ethanol droplet (MED) test quantifies water repellence as the lowest ethanol concentration permitting droplet penetration within 5 s.

2.3. DNA Extraction, PCR Amplification, and Pyrosequencing of Bacterial Soil DNA

Total bacterial genomic DNA was extracted from soils using the “PowerSoil DNA Isolation Kit” (MoBio Laboratories Inc., Carlsbad, CA, USA). DNA was extracted from collected soil samples (kept at $-20\text{ }^{\circ}\text{C}$) 6 to 7 weeks after collection. Extracted genomic DNA was checked in 1.5% agarose gel electrophoresis Gel Red™ (Biotium, Hayward, CA, USA) and quantified in a NanoDrop 1000 Spectrophotometer. Extracted DNA was kept at $-80\text{ }^{\circ}\text{C}$ until sequencing. 16S rRNA fragments were obtained by amplification of hypervariable V3-V5 using the universal primers U341F and U926R [34]. Primers contained pyrosequencing adaptors (MIDs) and 8-bp barcodes associated with the reverse primer [35]. The PCR mix reaction (25 μL final volume) contained 25 pmoles of each primer, 1.8 mM MgCl_2 , 0.2 mM dNTPs, 1 \times PCR buffer, 5 μL TaqMaster PCR Enhancer, 1U TaqMaster (5 Prime Inc., Gaithersburg, MD, USA), and 10 ng template DNA. The temperature profile consisted of 4 min $94\text{ }^{\circ}\text{C}$, 25 \times (15 s $94\text{ }^{\circ}\text{C}$; 45 s $55\text{ }^{\circ}\text{C}$; 1 min $72\text{ }^{\circ}\text{C}$), and 10 min $72\text{ }^{\circ}\text{C}$. Various replicate PCRs were made for each sample. Amplicons of the same sample were pooled to reduce per-PCR variability and were purified through Filter Units Ultracel-100 K membranes (Amicon, Cork, Ireland). DNA was quantified using the Quant-iT PicoGreen® dsDNA Assay Kit (Life Technologies, Carlsbad, CA, USA). Equimolecular amounts of DNA samples (3 replicates per each soil type) were prepared and pyrosequenced in Roche 454 FLX Titanium Plus (LifeSequencing S.L., Valencia, Spain).

2.4. Pyrosequencing Data Analysis

Raw sequences were processed with MOTHUR version 1.40.2 [36]. Trimming followed the criteria defined [37], leaving out those sequences (1) with homopolymers longer than 8 bp, allowing two mismatches to the barcode and three to the primer sequences; (2) sequences with unidentified nucleotides (Ns); (3) sequences with a quality index less than 20; and (4) those identified as chimeras, virtual operational taxonomic units (OTUs). Only fragments of sizes ≥ 390 nt were considered for the analysis. Sequence alignment was performed [36], and the bacterial sequences were clustered into OTUs with a similarity of at least 97%. OTU distribution among samples was used to calculate rarefaction curves, Chao 1 richness estimator index, Simpson index and its inverse, Shannon diversity index, equity, and coverage. Alpha diversity was calculated from normalized rarefaction curves; for other analyses, we used non-rarefied reads. To compare alpha diversity indices, a one-way ANOVA was performed for each index after confirming that these data were parametric (Levene and Shapiro tests) and Tukey HSD as a post-hoc test to perform pairwise comparisons using R version 3.5.2. Statistics of the taxonomic profiles found in the samples were done with STAMP v2.0.9, (<http://kiwi.cs.dal.ca/Software/STAMP>) using a parametric *t*-test at a 95% confidence interval [38]. Ginkgo [39] was applied in principal component analysis (PCA) and principal coordinate analysis (PCoA), using relative abundance of sequence at genus level. The Bray–Curtis dissimilarity was used to generate the distance matrix in PCoA. CANOCO v4.5 [40] was used for canonical correspondence analysis (CCA) after transformation of some physicochemical parameters as recommended [41]. InteractiVenn [42] was used for Venn graphics, modified with CorelDraw X3 (Corel Corporation, Ottawa, Canada). Alpha diversity was calculated from normalized rarefaction curves; for other analyses, we used non-rarefied reads.

3. Results

3.1. Chemical Characteristics of Soils from Laurel Forest in Garajonay NP

Data shown in Table 1 correspond to the three locations studied in the present study. The undisturbed laurel forest soils in the NP are acidic, have very high soil organic carbon contents (SOC) and total nitrogen (N), and a high C/N ratio, while showing a deficit for available P, K, Ca, and Mg (except Mg in UCLF). Particle size and texture analyses indicated the predominant texture

was silty-loam and loamy. As is usual for andic soils in Garajonay, water retention and available water was high or very high, indicating high water retention at wilting point as well as low water repellence, coinciding with earlier results [22]. These results agree with the values obtained in a previous large study of the soils in the National Park [43], which included the locations sampled here. In the burned soils, losses of 47% in SOC and 17% in total N decreased the C/N ratio by 36%, the soil pH increased by 9%, probably due to the alkalizing effect of dissolved ash [4], P and Ca contents increased slightly, and K doubled, while Mg decreased to half. Texture was altered in the silt fraction (37% increase) and a moderate increase (7%) was observed in the clay fraction. A slight increase was detected in water repellence (probably due to ash). There was a 54% decrease in water retention capacity at field capacity and this decrease was about 32% at wilting point, meaning a 68% drop in the plant-available water retention capacity, which indicates that the fire reached a high level of severity [44].

Table 1. Physicochemical characteristics of soils from Garajonay National Park in unburned and burned areas of the laurel forest.

Parameter	Soil Sample		
	USLF	UCLF	BCLF ^a
SOC (g kg ⁻¹)	105	155	83 (±13.26)
Total N (g kg ⁻¹)	6.6	10.5	8.7 (±0.09)
C/N	15.9	14.8	9.5 (±3.02)
pH (H ₂ O)	5.8	5.8	6.3 (±0.75)
Available P (mg kg ⁻¹)	13.7	15.0	16.0 (±3.25)
Available K (cmol.kg ⁻¹)	0.5	0.8	1.5 (±0.4)
Available Ca (cmol.kg ⁻¹)	5.9	9.4	10.6 (±6.48)
Available Mg (cmol.kg ⁻¹)	2.0	4.4	2.2 (±0.22)
Clay (%)	15.8	10.4	11.1 (±3.35)
Silt (%)	50.1	47.8	65.7 (±5.74)
Sand (%)	34.1	41.8	23.2 (±6.19)
Coarse fragments (g kg ⁻¹)	150	150	233 (±32.3)
Texture	Silty loam	Loamy	Silty loam
Water retention ^b			
33 kPa	626	867	403(±56.0)
1500 kPa	262	345	236 (±38.0)
Available water	364	522	167 (±23.8)
Water repellency ^c			
MED	Wettable	Slightly w.r.	Slightly w.r.
WDPT	Slightly w.r.	Wettable	Wettable

USLF = Unburned Slope Laurel Forest; UCLF = Unburned Crest Laurel Forest; BCLF: Burned Crest Laurel forest. ^a Mean values of three replicates and standard deviations. ^b Water retention, 33 kPa = Water retention at field capacity, 1500 kPa = Water retention at wilting point = Water available for plants. ^c Water repellency (w.r.), MED = Molarity of Ethanol Droplet test, WDPT = Water Drop Penetration Time).

3.2. Alpha Diversity of the Microbial Communities

We obtained 163,286 raw reads, which, after the trimming step, resulted in 106,864 high-quality sequences. Sequence numbers ranged from 4370 to 56,914 per sample. Rarefaction curves tended towards asymptote (Figure S1), and coverage values (Table 2) ranged from 89.31% to 98.68%, indicating that the sequencing effort was sufficient and the sequences obtained were representative of the bacterial communities in these soils. Alpha diversity (Table 2) was calculated from normalized data using 4370 sequences per sample (in total, 13,110 sequences per type of forest soil). The number of operational taxonomic units (OTUs) was significantly higher in unburned soils than in BCLF (average of about 50% lower). The total species richness and diversity was similar in unburned soils and significantly lower in BCLF. In comparison with the unburned soils, the higher equity indices in the BCLF soils suggest species dominance.

Table 2. Alpha diversity. Diversity indices for the three replicates from laurel forest soils on the unburned slope (USLF) and crest (UCLF) and the burned crest (BCLF) forests. Results correspond to normalized 4370 sequences. The average is shown with the standard deviation between brackets. Bold letters after the standard deviation indicate statistically significant differences in the averages along columns according to Turkey HSD test results (p value < 0.05).

Group	OTUs	Chao	InvSimpson	Simpson	Shannon	Equity	Coverage
USLF A	624.63	963.82	86.61	0.01	5.27	0.8180	0.9388
USLF B	853.64	1482.89	152.87	0.01	5.77	0.8551	0.9048
USLF C	958.09	1566.26	152.51	0.01	5.90	0.8599	0.8931
average	812.12	1337.66	130.66	0.01	5.65	0.8443	0.9122
	(170.56) a	(326.43) a	(38.15) a	(0.00) a	(0.33) a	(0.0229) a	(0.0237) a
UCLF A	660.23	1006.26	84.99	0.01	5.31	0.8181	0.9335
UCLF B	704.29	1026.83	59.37	0.02	5.29	0.8061	0.9313
UCLF C	761.70	1076.88	96.81	0.01	5.54	0.8356	0.9274
average	708.74	1036.66	80.39	0.01	5.38	0.8199	0.9307
	(50.88) a	(36.32) ab	(19.14) a	(0.01) a	(0.14) a	(0.0148) a	(0.0031) ab
BCLF A	182.84	241.45	13.39	0.07	3.36	0.6442	0.9868
BCLF B	514.64	820.26	12.14	0.08	4.17	0.6676	0.9464
BCLF C	258.00	313.12	26.98	0.04	4.02	0.7244	0.9831
average	318.49	458.28	17.50	0.06	3.85	0.6787	0.9721
	(173.98) b	(315.53) b	(8.23) b	(0.02) b	(0.43) b	(0.0412) b	(0.0223) b

3.3. Bacterial Community Composition of the Soil Microbiome in Undisturbed Laurel Forests

The soil microbiomes from unburned laurel forests, UCLF and USLF, were very alike to each other (Figure 1, Figure 2). Both soils were characterized by the presence of bacteria from three main phyla: Acidobacteria (38.55%–26.83%), Proteobacteria (23.66%–37.13%), and Actinobacteria (17.78%–17.35%) (Figure 1a). Bacteroidetes, Firmicutes, Gemmatimonadetes, Planctomycetes, Chloroflexi, Verrucomicrobia, and candidate division WPS2 were less abundant phyla (<2%). Unclassified phyla represented 7.54% to 8.12%. Statistically significant differences (p value < 0.05) at the phylum level (Figure S2a) were only found for the relative abundance of Proteobacteria, Acidobacteria, and Latescibacteria. This latter phylum was not detected in crest samples, and in USLF, it only accounted for less than 0.08% of the sequences. Significant differences were obtained for the two most represented classes in unburned soils (Figure S2b): The Alphaproteobacteria, more abundant in USLF (19.88%) than in UCLF (11.77%), and the Acidobacteria-Gp1 with an opposite trend (9.41% USLF, 16.59% UCLF). Three other underrepresented classes, Acidobacteria-Gp25, Armatimonadetes-Gp4, and Latescibacteria, were also significantly more abundant on USLF (Figure S2b). *Acidobacteria Gp1* and *Gp2* were the most abundant genera in both forests (12.66% and 14.14% in UCLF and 7.31% and 7.47% in USLF, Figure S3a). Other abundant genera (>1.0% relative abundance) in both undisturbed forest soils were *Actinoallomurus*, *Gemmatimonadetes*, *Gaiella*, *Gp3*, *Gp6*, *Solibacter*, *Bradyrhizobium*, *Mycobacterium*, *Steroidobacter*, *Phenylobacterium*, *WPS-2*, *Rhizomicrobium*, and *Conexibacter*. Statistically significant differences (p values < 0.05) (Figure S3b) were only found for less abundant genera (<1% relative abundance, except *Mycobacterium*). The large percentage of sequences showing no close similarity with any previously described genera is noteworthy; these made up 44.40% to 53.84% of the sequences from the undisturbed laurel forest soils.

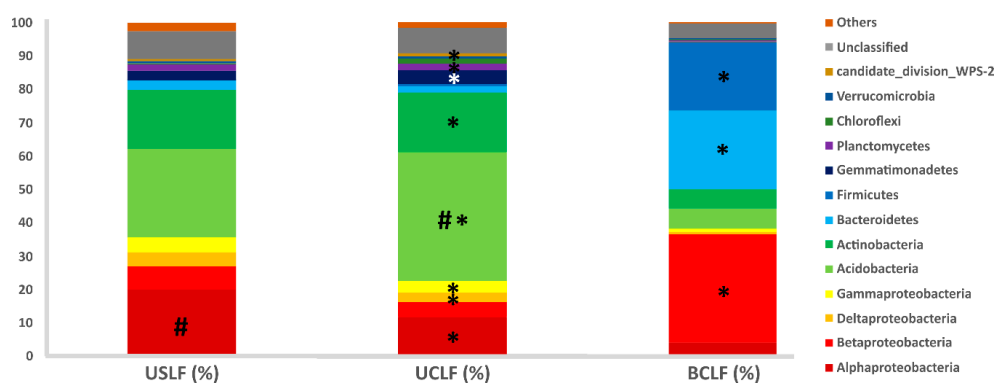


Figure 1. Relative abundance of the dominant bacterial phyla in undisturbed and burnt soils of the laurel forest at the Garajonay National Park. Comparison of two undisturbed forests, the slope and crest laurel forests (USLF and UCLF) and burnt crest forest (BCLF) areas. For phylum Proteobacteria, classes alpha, beta, and gamma are also shown. Phyla accounting for less than 0.7% relative abundance are included under ‘Others’. # and * refer to taxa with a significantly increased relative abundance in two to two comparisons. Statistically significant differences in the relative abundance are shown in Supplementary Figure S2.

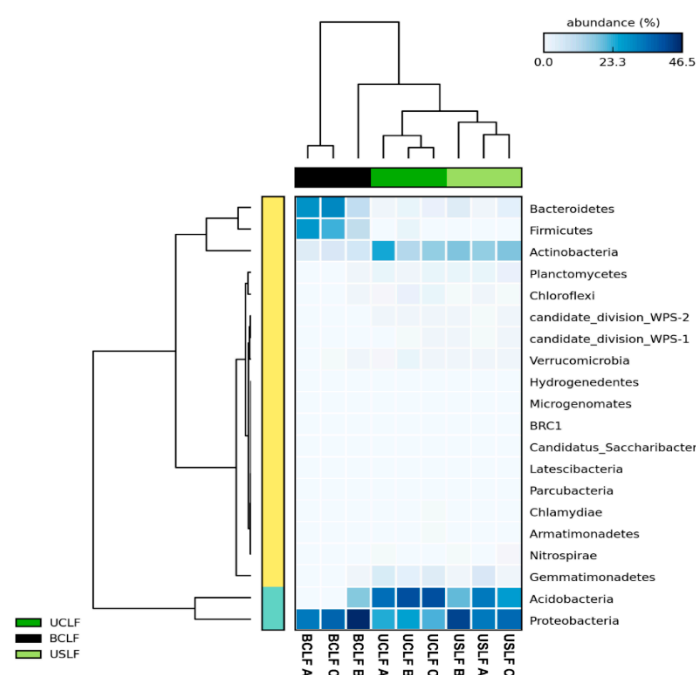


Figure 2. Heatmap plot of dominant phyla in undisturbed (USLF and UCLF) and burnt (BCLF) laurel forest soils.

3.4. Effect of a Wildfire on the Soil Microbiome in the Crest Laurel Forest

A heatmap plot of taxonomic groups at the phylum level (Figure 2) shows the relationships among replica samples, confirming the likeness of the microbiome in the undisturbed areas (USLF and UCLF) and further distinguishing them from burned soils, BCLF. One BCLF replicate occupied an intermediate position between undisturbed and burned soils, which could be due to a soil patch being somehow partially protected from fire, probably due to the heterogeneous effects of fire on particular soil characteristics at the specific sample point or to changes in wind direction during the fire [45]. Differences in the bacterial structure communities, the beta diversity, between unburned and burned soils were assessed by principal coordinate analysis (PCA) (Supplementary Figure S4).

Comparing the bacterial community composition of UCLF versus BCLF (Figures 1 and 3 and Supplementary Figures S5–S8), fire caused profound changes in the soil microbiome. Although all phyla present in UCLF were still detected in BCLF, their relative abundance significantly varied

(Figure 1, Figure S5). Acidobacteria and Actinobacteria, two phyla prevailing in UCLF soils (38.5% and 17.9% relative abundance, respectively), dropped to almost 6% after fire exposure. Proteobacteria continued to be a dominant phylum in burned soils, and was even slightly increased (Figure 1), although at the class level (Figure S6), the decrease in Alphaproteobacteria was statistically significant, dropping from 11.78% to 4.21%. Delta- and Gammaproteobacteria also underwent a drop from 2.98% and 3.42% to 0.79% and 0.88%, respectively. The parallel high increase in Betaproteobacteria from 4.45% to 32.36% was also significant (Figure 1, Supplementary Figure S6). Minor phyla (<2% relative abundance in UCLF), such as Gemmatimonadetes, Planctomycetes, and candidate WPS2, became almost undetectable in BCLF, while unclassified phyla dropped from 7.54% to 4.26% in BCLF samples. Quite the opposite, phyla Bacteroidetes and Firmicutes, scarcely represented in UCLF (1.75% and 0.70% relative abundance), became predominant in BCLF (23.23% and 20.48% relative abundance, respectively) (Supplementary Figure S5), and Sphingobacteria (Bacteroidetes) and Bacilli (Firmicutes) were the most significantly abundant classes (Figure S6). Striking changes were also observed in the genus composition (Figure 3, Supplementary Figure S7) and distribution (Supplementary Figure S8). A decrease in *Acidobacteria* Gp1 and Gp2 stands out (more than 80% in BCLF compared to UCLF). The opposite effect was found in genera *Paenibacillus* (Firmicutes), *Pedobacter* (Bacteroidetes), and *Massilia* (Betaproteobacteria), which became dominant in BCLF (from almost undetectable percentages to 14.16%, 12.72%, and 9.1%, respectively). *Dyadobacter* (Bacteroidetes), *Cohnella* (Firmicutes), and *Arthrobacter* (Actinobacteria) were also statistically more abundant in BCLF (p -value < 0.04) (Figure 3).

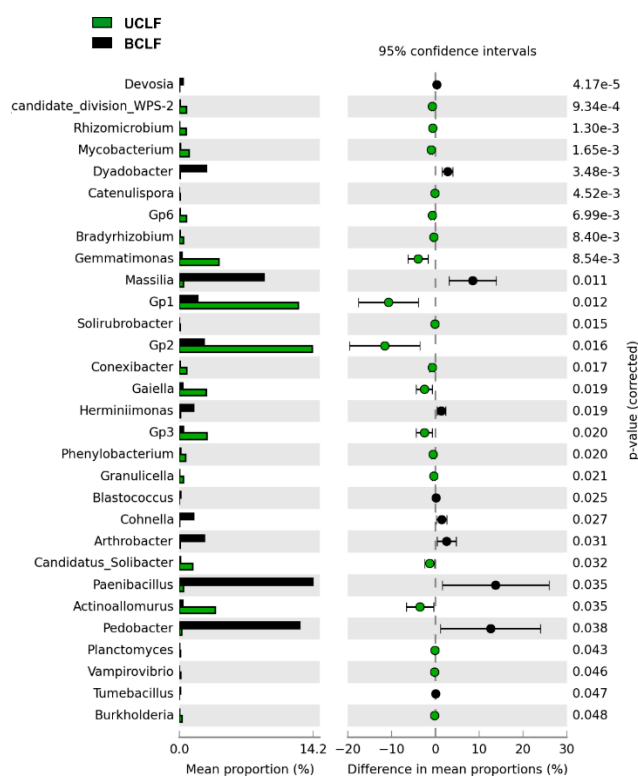


Figure 3. Relative abundance of genera exhibiting significant differences in undisturbed (UCLF) and burnt (BCLF) crest laurel forest soils. t -test at the 95% confidence interval.

3.5. Influence of Environmental Variables on Soil Communities

Canonical correspondence analysis (CCA) (Supplementary Figure S9) was performed to study the influence of soil organic carbon (SOC), total nitrogen, C/N ratio, pH, cations (Mg, K, Ca, and P), soil texture, and available water in genus distribution, in terms of relative abundance. The Monte Carlo test was statistically significant (999 permutations, p -value = 0.005), indicating a strong positive correlation between pH (p -value = 0.003), and was marginally significant for the C/N ratio

(p -value = 0.077) and genus distribution in burned and unburned soils. Other variables not influencing the bacteria distribution are not shown in the figure.

3.6. The Core Microbiome

The OTU numbers and sequence percentages characteristic of each soil and in the core microbiome are shown in a Venn diagram (Figure 4). USLF soil had the largest number of exclusive OTUs, 983 (6.4% of sequences, relative abundance), while the crest forest had 723 (4.23% of sequences) and 302 (5.46% of sequences) OTUs in unburned and burned soils, respectively. However, the core microbiome with only 249 OTUs constituted 62% of the total sequences. In the unburned soils, the large number of OTUs belonging to the genus *Gaiella* (Actinobacteria) and the *Acidobacteria Gp6* in USLF (Table S1) and to *Acidobacteria Gp1* and *Gp2* in the UCLF (Table S2) was remarkable. OTUs in BCLF (Table S3) belonged to genera *Paenibacillus* (Firmicutes), *Flavobacterium*, *Pedobacter*, *Dyadobacter* (Bacteroidetes), *Amycolatopsis* (Actinobacteria), and *Delftia* (Betaproteobacteria). It is worth mentioning that in USLF and UCLF soils, genera with a large percentage of sequences also had a large number of OTUs (Tables S1 and S2), whereas in BCLF soils, the genera contained a large percentage of the sequences with a low number of OTUs (Table S3). This suggests a dominance of particular species, supporting data from α -diversity. The core microbiome was characterized by OTUs belonging to: (i) The *Acidobacteria Gp1*, *Gp2*, *Gp3*, *Gp6*, *Gemmatinomas* and the candidate *Solibacter*; (ii) Actinobacteria from genera *Gaiella*, *Actinoallomurus*, *Arthrobacter*, and *Mycobacterium*; (iii) Proteobacteria of genus *Massilia* and *Bradyrhizobium*; and (iv) the *Bacteroidetes Flavobacterium* and *Pedobacter* (Table S4). In this core microbiome (Table S4), the genera *Massilia* (Betaproteobacteria) and *Pedobacter* (Bacteroidetes) stood out for their high percentage of sequences in the core microbiome (above 3% each of total sequences) belonging to one single OTU. Also represented by one OTU were genera *Arthrobacter*, *Bradyrhizobium*, and *Mycobacterium*, with a relatively large percentage of sequences: 0.66% to 0.895% of the total core genome sequences. A large percentage of sequences in the core microbiome (28.54%) were unclassified.

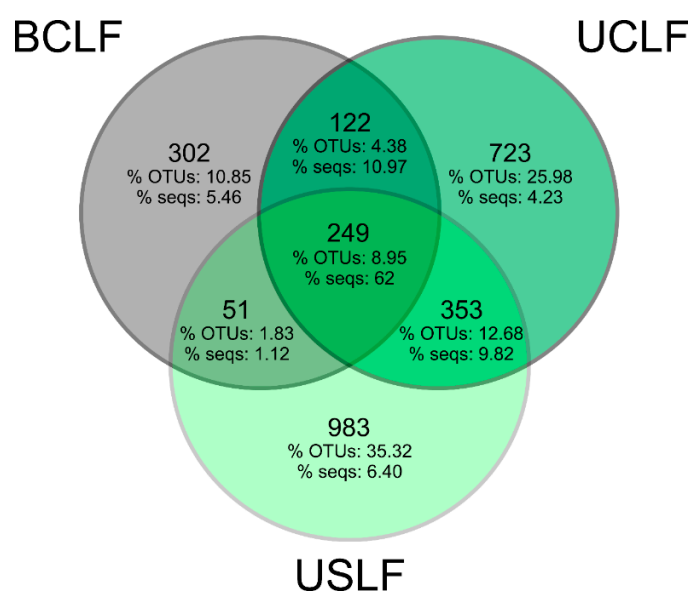


Figure 4. Venn diagram showing the OTU numbers and percentage of sequences in undisturbed laurel forest (USLF and UCLF) and burnt forest (BCLF) soils. The diagram also shows OTUs and percentages of sequences in the core microbiome.

4. Discussion

Here, we studied the soil microbiome of a laurel forest and showed that this relic forest hosted a large diversity of novel bacteria, with 44.40% to 53.84% of sequences corresponding to potentially novel undescribed genera. The use of a high-throughput sequencing methodology revealed the

overall composition of the soil bacterial communities of this forest, and evaluated the short-term impact (15–16 weeks after fire) of the wildfire. The two selected locations differed in their topographic situation, tree composition, plant species abundance, and soil characteristics. However, these differences appeared to not have had a great impact on the bacterial communities, since both unburned forests shared similar soil microbiomes dominated by bacterial communities belonging to Proteobacteria, Acidobacteria, and Actinobacteria. Only a few taxa showed significant differences in relative abundance. The wildfire drastically affected the soil microbiome of the laurel forest. Burned soil had lower species richness and diversity. Besides this, the composition of the bacterial communities was also strongly modified: A soil microbiome dominated by Acidobacteria, Alphaproteobacteria, and Actinobacteria turned into a community of Betaproteobacteria, Bacteroidetes, and Firmicutes. The severe fire completely burned the vegetation at the sampled sites, and quite likely had an immediate direct effect on the soil microbial biomass. Additional post-fire alterations observed in the physicochemical characteristics of the soil should have altered the composition of the bacterial communities, especially due to the loss of the upper organic layer that was completely burned. The laurel forest burned soils showed losses in SOC and N content and a decrease in the C/N ratio and a pH increase that seems to have had a high influence on the composition of the microbial communities (Figure S9), similar to other severe fires [6,46,47]. These effects all are the most common post-fire disturbances influencing normal bacterial activities, eventually modifying bacterial communities [8–10,18,48]. Furthermore, as a probable consequence of the losses in organic carbon [49,50], the water retention capacity and available water decreased. Nevertheless, the increase in silt fraction may have partially compensated some losses in water retention [51].

Acidobacteria is a large phylum of ubiquitous bacteria and one of the most abundant prokaryote phyla in soils [52,53]. Likewise, these were dominant bacteria in the undisturbed laurel forest soils. In UCLF soils, Acidobacteria were the most abundant group (38.5% relative abundance) and the one that suffered the highest drop (84.4% decrease) in BCLF soils. The direct effect of fire on this non-sporulating group of gram-negative bacteria is likely to be the main reason for it. The ecological role that this phylum plays in soils remains broadly unknown due to their unculturability. Sequence-based approaches show they are highly diverse [53] and that different subgroups show inconsistent correlations regarding Ca, Mg, K, P, carbon content, C/N ratio, and soil pH [54–58], making it difficult to evaluate how soil changes affect Acidobacteria. However, most studies agree that soil pH is the main driver behind the abundance and composition of Acidobacteria, and several subgroups show a positive correlation with low pH conditions [53,55,59,60], with subgroup *Gp1* being especially sensitive to an increase in pH [61]. Nevertheless, other subgroups, such as *Gp2*, negatively correlate with pH [53]. The two most abundant subgroups in UCLF were *Gp1* and *Gp2*. Both underwent a drastic drop in abundance in burnt soils, suggesting that the slight increase in soil pH of the BCLF did not affect them. Furthermore, Acidobacteria are supposedly oligotrophic and more used to complex polymers of C and N [53], which is more characteristic of undisturbed forests. A higher availability in soluble C, due to mineralization being accelerated by fire, negatively correlates with Acidobacteria abundance [54].

Bacteria from other phyla thrived quickly in burned soils. The huge increase observed in the relative abundance of some bacterial groups in BCLF soils could be due to the opportunistic overgrowth of fast-growing bacteria that take advantage of less acidic pH and more copiotrophic conditions or can tolerate or are able to utilize chemical forms of carbon present in burned soils. Contrary to Acidobacteria, higher C mineralization rates positively correlate with the abundance of Betaproteobacteria and Bacteroidetes [54]. Consistent with previous reports that showed a predominance of Proteobacteria in many types of soils, [52,55,62,63], they were an abundant phylum in the soils of the Garajonay laurel forest. Moreover, coinciding with other frequent post-fire effects described in other forests, the previous dominance of Alphaproteobacteria was greatly diminished [13,18,21] in favor of Betaproteobacteria [13,18,20,54,64] in burned soil. Especially predominant in BCLF soils were the Betaproteobacteria of genus *Massilia*. Polycyclic aromatic hydrocarbons are released after a forest fire [65] and as they are able to metabolize them [66,67], members of *Massilia*

can flourish in burned soils. Moreover, *Massilia* is a major group of rhizospheric bacteria colonizing the roots of many plants at early successional stages of the root microbiome [68], suggesting that these bacteria could play a role in plant fitness. The fact that its sequences were highly represented in the core microbiome but strikingly all belonged to one OTU is very suggestive that this specific taxon is important for the laurel forest soil ecosystem and it might play a positive role in the post-fire forest ecosystem. The high increase in Bacteroidetes detected in BCLF soils is another common observation in many burned soils [18,20,64]. It has been related to a shifted balance in the carbon cycle and carbon mineralization rates [52,54]. In our study, the increase in genera *Pedobacter* and *Dyadobacter* was particularly high. *Dyadobacter* has been isolated from diverse environments worldwide and the genus includes several species that can degrade heterocyclic aromatic hydrocarbons. The abundance of *Dyadobacter* in the BCLF might correlate with an opportunistic advantage to the chemical conditions prevailing in burned soils. Its potential use in bioremediation [69] could be important for detoxifying burned soils. *Pedobacter* species have been described in very diverse environments, including plant rhizosphere and forest soils [70,71], but to our knowledge, this is the first time that *Pedobacter* has been related to burned soils and we cannot yet explain its striking increase in BCLF soils. It is worth noting that similarly to *Massilia*, genus *Pedobacter* also has a high percentage of sequences in the core microbiome, all belonging to one single OTU, which suggests a relevant role of this particular species in the laurel forest ecosystem.

The Firmicutes-producing endospores take advantage of fires by withstanding the high temperatures reached during a wildfire, so their predominance is frequent in the soils of many burned habitats [12,13,18–21,64]. In the present study, we indeed detected a huge increase in the endosporulates *Paenibacillus* and *Cohnella*. These two genera contain many free-living N₂-fixing species [70–74], in concordance with the fact that N cycle-related sequences found in burned soils showed a dominance of such free-living spore-forming N₂ fixers [11,12]. Furthermore, many species in these two genera are frequent colonizers of the plant rhizosphere [70,73,74]. As N₂ fixers, they may play a role in recovering the N losses observed in BCLF.

The effect of fire on bacteria in the phylum Actinobacteria is more variable. An increase has been reported [21,75] or apparently not been greatly affected [17,18]. Thus, the severe decrease detected in members of Actinobacteria in burned soils from Garajonay does not appear to be common in other research. In our study, the decrease in Actinobacteria could be due to the severity of the wildfire in the sampled area. One main concern is how this decrease could negatively affect the N cycle in the mature laurel forest ecosystem, where legumes are rare components usually limited to the forest margins. Biological N₂ fixation linked to the symbiosis between *Morella faya* (a dominant or common tree in laurel forest) and *Frankia* is likely the key entry channel of N into the forest ecosystem. *Frankia* was not detected among the most abundant genera in this study. This may be explained by the sampled soil being non-rhizospheric. However, other free-living N₂-fixing actinobacteria could have a prominent role in the N cycle. In our work, while the decrease in Actinobacteria was reflected in most genera, *Arthrobacter* greatly increased in BCLF during the next few months. By fixing N₂, many members of genus *Arthrobacter* [76] may contribute to N entering the ecosystem. This agrees with previous studies that describe an abundance of *Arthrobacter* as a post-fire effect in other forests [21,76]. *Arthrobacter* has been related to the degradation of aromatic compounds that appear in burned soils [77]. More interesting, is its proposed role as an essential element in forest recovery, by acting as plant growth-promoting bacteria [21]. Interestingly, in the core genome, *Arthrobacter* presented a large percentage of sequences, all belonging to one OTU. This again points to this particular species being important in laurel forest soils. Plant inoculation with culture-isolated bacteria of strains well-adapted to burned soil conditions and showing plant growth promotion could be a useful strategy in re-afforestation after wildfires.

5. Conclusions

This study revealed that about 50% of sequences correspond to undescribed genera, suggesting that a wide unknown bacterial diversity may be specific to laurel forest soils. Our results also revealed the dramatic effect of a wildfire on their bacterial community composition, despite the soil

science perspective having been that mature laurel forest soils are largely protected from fires by their high humidity levels. The severity of the 2012 fire completely burned some areas of a forest over 100 years old and practically eliminated the most abundant bacterial communities, substituting them with other probably opportunistic bacteria. The recovery of the characteristic vegetation of this forest is thought to require many decades. Microorganisms can be good indicators of the ‘health’ status of a forest ecosystem and thus used to monitor soil recovery. Our results indicate that the bacterial groups likely to play key functional roles in these soils were lost. Recovering them from unaltered soils for use as inoculants aiding plants to be sown in reforestation campaigns could help accelerate forest recovery.

Supplementary Materials: The following are available online at www.mdpi.com/1999-4907/10/12/1051/s1, Figure S1: Rarefaction normalized curve calculated at OTU level, Figure S2: Significant differences at phylum level (a) and class level (b) between the soil microbiomes of the unburned laurel forests on the crest and slope, Figure S3: The soil microbiome in the unburned laurel forest soils. (a) Relative abundance at the genus level in the soil microbiome from undisturbed laurel forest on the crest and slope of the mountain. (b) Genera with statistically significant differences in soils from unburned crest and slope laurel forests obtained by a *t*-test, Figure S4: Principal Coordinate Analysis (PCoA) based on 16S rRNA gene sequences classified at genus level from unburned and burned laurel forest soils, Figure S5: Phyla with statistically significant differences in soils from unburned crest forest and burned crest forest, Figure S6: Classes showing statistically significant differences in soils from burned crest forest and unburned crest forest, Figure S7: Relative abundance of genera in the bacterial communities from soils in unburned crest laurel forest and in a crest forest burned after a wildfire, Figure S8: Principal component analysis (PCA) plot of the genera distribution in unburned and burned crest laurel forest soils, Figure S9. Canonical correspondence analysis (CCA) of 16S rRNA gene sequences at genus level in the three types of soil samples, Table S1: Genera with percentage of exclusive sequences above 0.1% and detected OTUs in soils from an undisturbed laurel forest growing on the mountain slope, Table S2: Genera with percentage of exclusive sequence above 0.1% and detected OTUs in soil samples from the undisturbed crest laurel forest, Table S3: Genera with percentage of exclusive sequences above 0.1% and detected OTUs in soil samples from the burned crest laurel forest, Table S4: Genera with percentage of exclusive sequences above 0.6% and detected OTUs in the core microbiome.

Author Contributions: M.L.-B. and M.F.-L. designed the research. M.A.A. and J.M.G.M. performed the soil samples collection, the botanical study and wrote some parts of the introduction, A.R.R. and C.A. the soil chemical analyses, J.P.Y. the DNA soil extractions, S.D.D., A.J.F.G. and P.J.V. the sequencing data analysis. M.L.B. wrote the manuscript. All authors contributed to the data interpretation and critical revision. P.J.V. edited figures and M.L.B. and M.F.L. prepared the final version.

Funding: This work was partially funded by Ministerio de Medio Ambiente y Medio Rural y Marino (grant ref: 111/2010); M.L.B. and M.F.L. laboratories supported part of the research.

Acknowledgments: We thank Angel Fernández, Director of Garajonay N P, for his help in working in Garajonay NP.

Conflicts of Interest: The authors declare no conflict of interest.

Data Availability: The datasets generated or analyzed during the current study are available in the NCBI Sequence Read Archive (SRA) repository (www.ncbi.nlm.nih.gov/sra) under the BioProject accession number PRJNA541921.

References

1. Fernández-Palacios, J.M.; de Nascimento, L.; Otto, R.; Delgado, J.D.; García-del-Rey, E.; Arévalo, J.R.; Whittaker, R.J. A reconstruction of Palaeo-Macaronesia with particular reference to the long-term biogeography of the Atlantic island laurel forest. *J. Biogeogr.* **2011**, *38*, 226–246.
2. Del Arco, M.J.; Wilpret, T.W.; Pérez de Paz, P.L.; Rodríguez-Delgado, O.; Acebes-Ginovés, J.R.; García-Gallo, A.; Martín-Osorio, V.E.; Reyes-Bentancort, A.; Cabrera-Lacalzada, M.V.; García-Avila, S. *Mapa de Vegetación de Canarias*; GRAFCAN: Tenerife, Spain, 2006.

3. Notario, J.; Rodríguez, A.R.; Arbelo, A.D.; Rodríguez, A.R.; Fernández, A.; Gómez, L. Burned soils at La Gomera wildfire: A preliminary GIS analysis. *Flamma* **2015**, *6*, 95–97.
4. Certini, G. Effects of fire on properties of forest soils: A review. *Oecologia* **2005**, *143*, 1–10.
5. Thomaz, E.L.; Antoneli, V.; Doerr, S.H. Effects of fire on the physicochemical properties of soil in a slash-and burn agriculture. *Catena* **2014**, *122*, 209–215.
6. Hobley, E.U.; Le Gay-Bereton, A.J.; Wilson, B. Forest burning affects quality and quantity of soil organic matter. *Sci. Total Environ.* **2017**, *575*, 41–49.
7. Aznar, J.M.; González, J.A.; Badía, D.; Martí, C. At what depth are the properties of gypseous forest soil affected by fire? *Land Degrad. Dev.* **2013**, *27*, 1344–1353.
8. Fritze, H.; Pennanen, T.; Pietikainen, J. Recovery of soil microbial biomass & activity from prescribed burning. *Can. J. Res.* **1993**, *23*, 1286–1290.
9. Acea, M.J.; Carballas, T. Changes in physiological groups of microorganisms in soil following wildfire. *FEMS Microbiol. Ecol.* **1996**, *20*, 33–39.
10. Hernández, T.; García, C.; Reinhardt, I. Short-term effect of wildfire on the chemical, biochemical & microbiological properties of Mediterranean pine forest soils. *Biol. Fertil. Soils* **1997**, *25*, 109–116.
11. Yeager, C.M.; Kornosky, J.L.; Housman, D.C.; Grote, E.E.; Belnap, J.; Kusk, C.R. Diazotrophic Community structure & function in two successional stages of biological soil crusts from the Colorado Plateau & Chihuahuan desert. *Appl. Environ. Microbiol.* **2004**, *70*, 973–983.
12. Yeager, C.M.; Northup, D.E.; Grow, C.C.; Barns, S.M.; Kuske, C.R. Changes in nitrogen-fixing and ammonia-oxidizing bacterial communities in soil of a mixed conifer forest after wildfire. *Appl. Environ. Microbiol.* **2005**, *71*, 2713–2722.
13. Smith, N.R.; Kishchuk, B.E.; Mohn, W. Effects of wildfire and harvest disturbances on forest soil bacterial communities. *Appl. Environ. Microbiol.* **2008**, *74*, 216–224.
14. Covington, W.W.; Sackett, S.S. Soil mineral nitrogen changes following prescribed burning in ponderosa pine. *Ecol. Manag.* **1992**, *54*, 175–191.
15. Kovacic, D.A.; Swift, D.M.; Ellis, J.E.; Hakonson, T.E. Immediate effects of prescribed burning on mineral soil nitrogen in ponderosa pine of New Mexico. *Soil Sci.* **1986**, *141*, 71–76.
16. Hobbs, N.T.; Schimel, D.S. Fire Effects on Nitrogen Mineralization and Fixation in Mountain Shrub and Grassland Communities. *J. Range Manag.* **1984**, *37*, 402–406.
17. Isobe, K.; Otsuka, S.; Sudiana, I.; Nurkanto, A.; Senoo, K. Community composition of soil bacteria nearly a decade after a fire in a tropical rainforest in East Kalimantan, Indonesia. *J. Gen. Appl. Microbiol.* **2009**, *55*, 329–337.
18. Xiang, X.; Shi, Y.; Yang, J.; Kong, J.; Lin, X.; Zhang, H.; Zeng, J.; Chu, H. Rapid recovery of soil bacterial communities after wildfire in a Chinese boreal forest. *Sci. Rep.* **2014**, *4*, 3829.
19. Cobo-Díaz, J.F.; Fernández-González, A.J.; Villadas, P.J.; Robles, A.B.; Toro, N.; Fernández-López, M. Metagenomic Assessment of the potential microbial nitrogen pathways of a Mediterranean forest after a wildfire. *Microb. Ecol.* **2015**, *69*, 895–904.
20. Shen, J.; Chen, C.; Lewis, T. Long term repeated fire disturbance alters soil bacterial diversity but not abundance in an Australian wet sclerophyll forest. *Sci. Rep.* **2016**, *6*, 19639.
21. Fernández-González, A.J.; Martínez-Hidalgo, P.; Cobo-Díaz, J.F.; Villadas, P.J.; Martínez-Molina, E.; Toro, N.; Tringe, S.G.; Fernández-López, M. The rhizosphere microbiome of burned holm-oak: Potential role of genus *Arthrobacter* in the recovery of burned soils. *Sci. Rep.* **2017**, *7*, 6008.
22. Neris, J.; Tejedor, M.; Rodríguez, M.; Fuentes, J.; Jiménez, C. Effects of forest floor characteristics on water repellency, infiltration, runoff and soil loss in Andisols of Tenerife (Canary Islands, Spain). *Catena* **2013**, *108*, 50–57.
23. Dorta-Almenar, I.; Navarro, F.; Arbelo, C.; Rodríguez, A.; Notario, J. The temporal distribution of water-soluble nutrients from high mountain soils following a wildfire within legume scrubland of Tenerife, Canary Islands, Spain. *Catena* **2015**, *135*, 393–400.
24. Durán, J.; Gallardo-Cano, A.; Fernández-Palacios, J.M. Efecto del fuego sobre la biomasa microbiana del suelo de ecosistemas de Pino canario (*Pinus canariensis*). *Cuad. Soc. Esp. Sci.* **2004**, *20*, 181–186.
25. Rodríguez, J.; Turmero, A.; Blánquez, A.; González-Pérez, J.A.; González-Vila, F.J.; Rodríguez Rodríguez, A.; Pérez Leblic, M. I.; Arias Fernández, M. E. Effect of wildfires on the genetic microbial diversity in forest soils from Canary Islands (Spain). *Flamma* **2014**, *5*, 42–44.

26. Nelson, D.W.; Sommers, L.E. Total carbon, organic carbon, and organic matter. In *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*; Page, A.L., Ed.; SSSA, ASA: Madison, WI, USA; 1982; pp. 539–597.
27. Olsen, S.; Cole, C.; Watanabe, F.; Dean, L. Estimation of available Phosphorus. In *Soils by Extraction with Sodium Bicarbonate*; USDA Washington, DC, USA; 1954; pp. 1–19.
28. Murphy, J.; Riley, J.P. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* **1962**, *27*, 31–36.
29. Watanabe, F.S.; Olsen, S.R. Test of an ascorbic method for determining phosphorus in water & NaHCO₃ extracts from soils. *Soil Sci. Soc. Am. J.* **1965**, *29*, 677–678.
30. Gee, G.W.; Bauder, J.W. Particle-size analysis. In *Methods of Soil Analysis. Part 1. Physical & Mineralogical Properties*; SSSA, ASA: Madison, WI, USA; 1986; pp. 383–409.
31. Klute, A. Water Retention: Laboratory Methods. In *Methods of Soil Analysis. Part 1. Physical and Mineralogical Properties*; SSSA, ASA: Madison, WI, USA; 1986; pp. 635–660.
32. Doerr, S.H. On standardizing the “water drop penetration time” and the “molarity of an ethanol droplet” techniques to classify soil hydrophobicity: A case study using medium textured soils. *Earth Surf. Proc. Land* **1998**, *23*, 663–668.
33. Bisdom, E.B.; Dekker, L.W.; Schoute, J.F. Water repellency of sieve fractions from sandy soils and relationships with organic material and structure. *Geoderma* **1993**, *56*, 105–118.
34. Baker, G.C.; Smith, J.J.; Cowan, D.A. Review and re-analysis of domain-specific 16S primers. *J. Microbiol. Meth.* **2003**, *55*, 541–555.
35. Binladen, J.M.; Bollback, T.P.G.P.; Panitz, F.; Bendixen, C.; Nielsen, R.; Willerslev, E. The use of coded PCR primers enables high-throughput sequencing of multiple homolog amplification products by 454 parallel sequencing. *PLoS ONE* **2007**, *2*, e197.
36. Schloss, P.D.; Westcott, S.L.; Ryabin, T.; Hall, J.R.; Hartmann, M.; Hollister, E.B.; Lesniewski, R.A.; Oakley, B.B.; Parks, D.H.; Robinson, C.J.; Sahl, J.W.; et al. Introducing mothur: Open-source, platform-independent, community-supported software for describing & comparing microbial communities. *App. Environ. Microbiol.* **2009**, *75*, 7537–7541.
37. Calleja-Cervantes, M.E.; Aparicio-Tejo, P.M.; Villadas, P.J.; Irigoyen, I.; Irañeta, J.J.B.; Fernández-González, A.J.; Fernández-López, M.; Menéndez, S. Rational application of treated sewage sludge with urea increases GHG mitigation opportunities in Mediterranean soils. *Agric. Ecosyst. Environ.* **2017**, *238*, 114–127.
38. Parks, D.H.; Beiko, R.G. Identifying biologically relevant differences between metagenomic communities. *Bioinformatics* **2010**, *26*, 715–721.
39. DeCáceres, M.; Font, X.; Oliva, F.; Vives, S.; Ginkgo, A. Program for Non-Standard Multivariate Fuzzy Analysis. *Adv. Fuzzy Sets Syst.* **2007**, *2*, 41–56.
40. Ter-Braak, C.J.F.; Smilauer, P. *Canoco for Windows Version 4.5*; Biometris-Plant Research International: Wageningen, The Netherlands, 2002.
41. Clarke, K.R.; Ainsworth, M. A method of linking multivariate community structure to environmental variables. *Mar. Ecol. Prog. Ser.* **1993**, *92*, 205–219.
42. Heberle, H.; Meirelles, G.V.; Da Silva, F.R.; Telles, G.P.; Minghim, R. InteractiVenn: A web-based tool for the analysis of sets through Venn diagrams. *BMC Bioinform.* **2015**, *16*, 169.
43. Rodríguez Rodríguez, A.; Arbelo, C.D.; Notario, J.S.; Mora, J.L.; Guerra, J.A.; Armas, C.M. Los suelos del Parque Nacional de Garajonay. In *Parque Nacional de Garajonay. Patrimonio Mundial*; Fernández, A.B., Turquesa, S.L., Eds.; O.A.P.N.: Madrid, Spain; 2009; pp. 63–91.
44. Stoof, C.R.; Wesseling, J.G.; Ritsema, C.J. Effects of fire and ash on soil water retention. *Geoderma* **2010**, *159*, 276–285.
45. Li, W.; Niu, S.; Liu, X.; Wang, J. Short-term response of the soil bacterial community to differing wildfire severity in *Pinus tabulaeformis* stands. *Sci. Rep.* **2019**, *9*, 1148.
46. Giovannini, G.; Lucchesi, S.; Giachetti, M. Effects of heating on some physical and chemical parameters related to soil fertility and plant growth. *Soil Sci.* **1990**, *149*, 344–350.
47. Knicker, H. How Does Fire Affect the Nature and Stability of Soil Organic Nitrogen and Carbon? A Review. *Biogeochemistry* **2007**, *85*, 91–118.
48. Ferrenberg, S.; O'Neill, S.P.; Knelman, J.E.; Todd, B.; Duggan, S.; Bradley, D.; Robinson, T.; Schmidt, S.K.; Townsend, A.R.; Williams, M.W.; Cleveland, C.C.; Melbourne, B.A.; Jiang, L.; Nemergut, D.R. Changes in

- assembly processes in soil bacterial communities following a wildfire disturbance. *ISME J.* **2013**, *7*, 1102–1111.
49. Emerson, W.W. Water retention, organic carbon and soil texture. *Aust. J. Soil Res.* **1995**, *33*, 241–251.
 50. Rawls, W.J.; Pachepsky, Y.A.; Ritchie, J.C.; Sobecki, T.M.; Bloodworth, H. Effect of soil organic carbon on soil water retention. *Geoderma* **2003**, *116*, 61–76.
 51. Reichert, J.M.; Albuquerque, J.A.; Kaiser, D.R.; Reinert, D.J.; Urach, F.L.; Carlesso, R. Estimation of water retention and availability for Rio Grande do Sul soils. *R. Bras. Ci. Solo* **2009**, *33*, 1547–1560.
 52. Janssen, P.H. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. *Appl. Environ. Microbiol.* **2006**, *72*, 1719–1728.
 53. Kielak, A.; Barreto, C.; Kowalchuk, G.A.; van Veen, J.A.; Kuramae, E. The Ecology of Acidobacteria: Moving beyond Genes and Genomes. *Front. Microbiol.* **2016**, *7*, 744.
 54. Fierer, N.; Bradford, M.A.; Jackson, R.B. Toward an ecological classification of soil bacteria. *Ecology* **2007**, *88*, 1354–1364.
 55. Jones, R.T.; Robeson, M.S.; Lauber, C.L.; Hamady, M.; Knight, R.; Fierer, N. A comprehensive survey of soil acidobacterial diversity using pyrosequencing & clone library analyses. *ISME J.* **2009**, *3*, 442–453.
 56. Faoro, H.; Alves, A.C.; Souza, E.M.; Rigo, L.U.; Cruz, L.M.; Al-Janabi, S.M.; Monteiro, R.A.; Baura, V.A.; Pedrosa, F.O. Influence of soil characteristics on the diversity of Bacteria in the southern Brazilian Atlantic forest. *Appl. Environ. Microbiol.* **2010**, *76*, 4744–4749.
 57. Navarrete, A.; Kuramae, E.E.; de Hollander, M.; Pijl, A.S.; van Veen, J.A.; Tsai, S.M. Acidobacterial community responses to agricultural management of soybean in Amazon forest soils. *FEMS Microbiol. Ecol.* **2013**, *83*, 607–621.
 58. Sile, J.A.; Margesin, R. Abundance and Diversity of Bacterial, Archaeal, and Fungal communities Along an Altitudinal Gradient in Alpine Forest Soils: What Are the Driving Factors? *Microb. Ecol.* **2016**, *72*, 207–220.
 59. Lauber, C.L.; Hamady, M.; Knight, R.; Fierer, N. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl. Environ. Microbiol.* **2009**, *75*, 5111–5120.
 60. Männistö, M.K.; Tirola, M.; Haggblom, M.M. Bacterial communities in Arctic fields of Finnish Lapland are stable but highly pH-dependent. *FEMS Microbiol. Ecol.* **2007**, *59*, 452–465.
 61. Sait, M.; Davis, K.E.; Janssen, P.H. Effect of pH on isolation and distribution of members of subdivision 1 of the phylum Acidobacteria occurring in soil. *Appl. Environ. Microbiol.* **2006**, *72*, 1852–1857.
 62. Nacke, H.; Thürmer, A.; Wollherr, A.; Will, C.; Hodac, L.; Herold, N.; Schöning, I.; Schruppf, M.; Daniel, R. Pyrosequencing-Based assessment of bacterial community structure along different management types in German forest and grassland soils. *PLoS ONE* **2011**, *6*, e17000.
 63. Yun, Y.; Wang, H.; Man, B.; Xiang, X.; Zhou, J.; Qiu, X.; Duan, Y.; Engel, A.S. The Relationship between pH and bacterial communities in a single Karst ecosystem & its implication for soil acidification. *Front. Microbiol.* **2016**, *7*, 1955.
 64. Weber, C.F.; Lockhart, J.S.; Charaska, E.; Aho, K.; Lose, K.A. Bacterial composition of soils in ponderosa pine and mixed conifer forests exposed to different wildfire burn severity. *Soil Biol. Biochem.* **2014**, *69*, 242–250.
 65. Vila-Escalé, M.; Vegas-Vilarrúbia, T.; Prat, N. Release of polycyclic aromatic compounds into a Mediterranean creek (Catalonia, NE Spain) after a forest fire. *Water Res.* **2007**, *41*, 2171–2179.
 66. Bodour, A.A.; Wang, J.M.; Brusseau, M.L.; Maier, R.M. Temporal change in culturable phenanthrene degraders in response to long-term exposure to phenanthrene in a soil column system. *Environ. Microbiol.* **2003**, *5*, 888–895.
 67. Wang, H.; Lou, J.; Gu, H.; Luo, X.; Yang, L.; Wu, L.; Liu, Y.; Wu, J.; Xu, J. Efficient biodegradation of phenanthrene by a novel strain *Massilia* sp. WF1 isolated from a PAH-contaminated soil. *Environ. Sci. Pollut. Res. Int.* **2016**, *23*, 1378–1388.
 68. Ofek, M.; Hadar, Y.; Minz, D. Ecology of Root Colonizing *Massilia* (Oxalobacteraceae). *PLoS ONE* **2012**, *7*, e40117.
 69. Willumsen, P.A.; Johansen, J.E.; Karlson, U.; Hansen, B.M. Isolation & taxonomic affiliation of N-heterocyclic aromatic hydrocarbon-transforming bacteria. *Appl. Microbiol. Biotechnol.* **2005**, *67*, 420–428.
 70. Kwon, S.W.; Son, J.A.; Kim, S.J.; Kim, Y.S.; Park, I.C.; Bok, J.I.; Weon, H.Y. *Pedobacter rhizosphaerae* sp. nov. and *Pedobacter soli* sp. nov., isolated from rhizosphere soil of Chinese cabbage (*Brassica campestris*). *Int. J. Syst. Evol. Microbiol.* **2011**, *61*, 2874–2879.

71. Yang, D.J.; Hong, J.K. *Pedobacter solisilvae* sp. nov., isolated from forest soil. *Int. J. Syst. Evol. Microbiol.* **2017**, *67*, 4814–4819.
72. Seldin, L.; *Paenibacillus*, Nitrogen Fixation and Soil Fertility. In *Endospore-Forming Soil Bacteria*; Logan, N., Vos, P., Eds.; Springer: Berlin/Heidelberg, Germany, 2011; pp. 287–307.
73. Wang, L.Y.; Chen, S.F.; Wang, L.; Zhou, Y.G.; Liu, H.C. *Cohnella plantaginis* sp. nov., a novel nitrogen-fixing species isolated from plantain rhizosphere soil. *Anton. Leeuw.* **2012**, *102*, 83–89.
74. Wang, L.Y.; Wang, T.S.; Chen, S.F. *Cohnella capsici* sp. nov., a novel nitrogen-fixing species isolated from *Capsicum annuum* rhizosphere soil, and emended description of *Cohnella plantaginis*. *Anton. Leeuw.* **2015**, *107*, 133–139.
75. Pizarro-Tobías, P.; Fernández, M.; Niqui, J.L.; Solano, J.; Duque, E.; Ramos, J.L.; Roca, A. Restoration of Mediterranean forest after a fire: Bioremediation and rhizoremediation field-scale trial. *Microb. Biotechnol.* **2014**, *8*, 77–92.
76. Sellstedt, A.; Richau, K.H. Aspects of nitrogen-fixing Actinobacteria, in particular free-living and symbiotic Frankia. *FEMS Microbiol. Lett.* **2013**, *342*, 179–186.
77. Westerberg, K.; Elvang, A.M.; Stackebrandt, E.; Jansson, J.K. *Arthrobacter chlorophenolicus* sp. nov., a new species capable of degrading high concentrations of 4-chlorophenol. *Int. J. Syst. Evol. Microbiol.* **2000**, *50*, 2083–2092.



© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).