The effect of prescribed fires on abiotic and biotic factors in the southern region of Puerto Rico[1,2]

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ABSTRACT

Field fires can modify soil nutrient cycling and alter soil microbial communities (SMC), although the latter is not well understood. In the southern region of Puerto Rico, field fires have become a significant problem during the dry season. To mimic the effects of a field fire, we performed prescribed fires on a hillside at the Juana Díaz Agricultural Experiment Substation in October 2015 and March 2017. A complete randomized block design was established in Yauco soil (Typic Calciustolls) that included the following treatments: negative control (unburned), positive control (burned plots, no remediation), mulching treatment (burned plots remediated with *Leucaena* spp. mulch), and surfactant treatment (burned plots remediated with a surfactant). In the first burning (2015), soil samples were collected before burning and at 30, 180, and 420 days after burning (DAB). In the second burning (2017), soil samples were collected at 30, 90, and 270 DAB. Soil physicochemical properties and microbial community structure were assessed using phospholipid fatty acid (PLFA) analysis. Overall, burning increased soil exchangeable Ca^{2+} (except after 30 DAB in the second burning) and decreased exchangeable K^+ when compared to unburned soils. Compared to unburned plots, total fungal PLFA was significantly lower in burned plots with or without mulch and surfactant treatments, and total bacterial PLFA did not differ between burned and unburned plots after 30 days. Total microbial biomass was significantly ($P<0.05$) higher in mulch and surfactant treated burned soil compared to unburned and burned plots without treatment after 90 DAB (2017) and 420 (2015) DAB. The use of mulch and surfactant treatments in prescribed burning fields increased microbial communities 90 DAB. This study emphasizes short-term changes in microbial communities and suggests they are highly resilient to disturbances after prescribed fires.

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Key words: mulch, prescribed fires, soil microbial communities, surfactant, PLFA

RESUMEN

El efecto de quemas intencionales en factores abióticos y bióticos en la región sur de Puerto Rico

Los incendios de campos (IC) pueden modificar el ciclo de nutrientes en el suelo y alterar las comunidades microbianas, sin embargo, estas últimas no son bien entendidas. En la región sur de Puerto Rico, los IC son un problema en la época seca. En este estudio se realizaron quemas intencionales en una zona con ladera en la Subestación Experimental Agrícola de Juana Díaz en octubre 2015 y marzo 2017. El estudio se estableció en un suelo Calciustolls Típico, serie Yauco. Se utilizó un diseño en bloques completamente aleatorizado con los siguientes tratamientos: control negativo (sin quemar), control positivo (quemado, sin remediar), mantillo (quemado y remediado con mantillo de *Leucaena* spp.) y surfactante (quemado y remediado con surfactante). En 2015 (primera quema), las muestras de suelo se recolectaron antes de la quema y a 30, 180 y 420 días después de la quema (DDQ). En la segunda quema en 2017, las muestras de suelo se recolectaron a los 30, 90 y 270 DDQ. Se evaluaron las propiedades fisicoquímicas del suelo y la estructura de la comunidad microbiana se determinó mediante el análisis de los ácidos grasos de los fosfolípidos (AGF). En general, la quema aumentó el contenido de Ca^{2+} intercambiable (excepto en la segunda quema luego de los 30 días) y disminuyó el contenido de K^+ al compararse a suelos no quemados. La concentración de hongos totales (AGF) fue significativamente menor en predios quemados con y sin remediación con mantilla o surfactante comparado con predios no quemados, y las bacterias totales (AGF) no difirieron entre predios quemados y no quemados a los 30 DDQ. La biomasa microbiana total (AGF) fue significativamente mayor ($P<0.05$) en predios quemados y tratados con mantilla y surfactante que en predios no quemados o quemados sin tratamiento luego de 90 (2017) y 420 (2015) DDQ. El uso de los tratamientos mantilla y surfactante en predios con quema aumenta las comunidades microbianas luego de 90 días. Este estudio muestra cambios a corto plazo en las comunidades microbianas, sugiriendo que estas son altamente resilientes a disturbios luego de una quema.

Palabras claves: mantillo, quema prescrita, surfactantes, comunidades microbianas del suelo, AGF

INTRODUCTION

The southern region of Puerto Rico is characterized by a dry climate, and ustic and aridic soil moisture regimes (Muñoz et al., 2018). This geographic zone is on the leeward side of an orographic effect that produces high rainfall in the windward north mountains of the central region of the island and drier southern slopes and coastal south (U.S. Geological Service, 2016). Recently, field fires have become a significant problem, and their frequency has increased due to low precipitation. Most of the field fires are anthropogenic, resulting from accidental or intentional ignition for agricultural purposes or other reasons (Glogiewicz and Baez, 2001; Monmany et al., 2017; Van Beusekom et al., 2017). In 2015, the Department of Natural and Environmental Resources (DNER) and the Fire Department of Puerto Rico (FDPR) reported 4,243 fires affecting more than 5,666 hectares on the island (Figueroa, 2016). Most of these fires occurred on hillsides near the ocean and during the dry season between the months of January and April (González-Toro, 2008).

Field fires are more common in the southern area of the island where vegetation consists of grasses and invasive plant species such as Guinea grass (*Megathyrsus maximus*), white leadtree (also known as Leucaena trees) (*Leucaena leucocephala*), and lebbek tree (*Albizia lebbeck*). This type of vegetation makes a suitable environment for higher fuel load production, which in combination with high temperatures and a source of ignition, is responsible for spreading fires. These fires may have an effect on soil properties, especially soil biology. A study by Dangi et al. (2010) emphasized the importance of fire frequency and intensity on plants, soil microbial communities (SMC), and the overall ecosystem function. Severe fires (e.g., exceeding 250° C) can destroy above- and belowground biomass, and SMC, and alter abiotic environmental conditions. Fires can affect soil structure and porosity with apparent alterations in biomass and SMC (Dangi et al., 2010). Also, depending on the intensity of the fire, it can induce soil water repellency, which decreases water infiltration by moving and concentrating hydrophobic compounds produced in plants. Soil water repellency is also produced by fungal and microbial activity, affecting soil particles up to three feet below the surface (DeBano et al., 1998; Fidanza et al., 2005; Keizer et al., 2005). The heat and ash produced during a fire can modify and affect nutrient cycling and the bio-physicochemical properties of the soils (Díaz-Raviña et al., 1992; Santín et al., 2016; Santín and Doerr, 2016); thus, alterations can also impact the microbial communities present in the soil (Vázquez et al., 1993). Significant loss of organic matter (OM), nitrogen (N), and phosphorous (P) can occur depending on the intensity of the fire (Neary, 2004; Certini, 2005). Ash produced during the fires can be a source of nutrients, especially cations such as calcium (Ca) and magnesium (Mg) that were stored in plants and litter (Khanna and Raison, 1986; Andreu et al., 1996; Pereira et al., 2013). This increase in cations is accompanied by a temporary increase (up to three units) in pH (Badía and Martí, 2003a). However, this increase in pH is reduced over time as levels of cations are reduced with time.

Soil microorganisms carry out essential processes that support plant productivity and maintain soil health and ecosystem function (Pérez-Guzmán et al., 2020). Microorganisms are responsible for driving essential ecosystem processes such as nutrient cycling, OM decomposition, plant nutrient uptake, and maintenance of soil structure (Dangi et al., 2020; Pérez-Guzmán et al., 2020; Dangi et al., 2013; Dangi et al., 2012) and are particularly sensitive to changes in soil quality due to wildfire or prescribed fire disturbances (Barreiro and Díaz-Raviña, 2021). Soil health and quality depend on maintaining diverse and vigorous biological communities that are responsible for these processes (Lehman et al., 2015). Fires alter SMC activity and composition directly through heat-induced microbial mortality (DeBano et al., 1998; Hart et al., 2005), and the post-fire soil recovery is determined to great extent by its impact on soil microorganisms (Barreiro and Díaz-Raviña, 2021). Plant and vegetative community structures are imperative elements of SMC structure and the recovery from a fire will depend on the development of plant communities (Grayston et al., 2001). Some ecosystem studies have demonstrated a direct relationship between microbial diversity and plant productivity, especially after a disturbance (Tilman, 1999; Hooper et al., 2005). It has been observed that SMC increases with increasing plant productivity (Liao et al., 2018), although Bai et al. (2007) detected an inverse relationship between microbial diversity and plant productivity.

Previous reviews have described decreased microbial biomass after a fire (Certini, 2005; Syaufma and Ainuddin, 2011). Hart et al. (2005) emphasized that bacteria biomass tends to be more resistant to fire heat than fungi biomass during moderate-intensity fires, and its recovery may take months or even years (Barreiro and Díaz-Raviña, 2021). Changes in vegetation can reduce microbial biomass with the succession of greater aboveground diversity than homogeneous plant cover (Fioretto et al., 2009). These strong links between plant species and SMC suggest that years after a fire, variations in plant structure can have a greater influence on SMC dynamics than the direct impact of the fire disturbance itself (Hart et al., 2005).

Soil-applied surfactant has proven effective in reducing soil water repellency and improving ecosystem restoration after a fire (DeBano and Conrad, 1974; Madsen et al., 2012). Water repellency is a naturally occurring phenomenon (most common in forested areas) that reduces water infiltration, soil-water retention, and unsaturated hydraulic conductivity in various soil types and soil textures (DeBano and Rice, 1973; DeBano, 2000). In Puerto Rico, soil water repellency has been observed in the soil surface of secondary forests and grasslands in different soil textures from sandy clay loam to clay (Nieves-Rivera, 2003). It originates from naturally occurring water-repellent compounds in plants (e.g., waxes), and fungal and microbial activity, covering soil particles (DeBano, 2000; Fidanza et al., 2005; Keizer et al., 2005).

Since the 1960s, soil-applied surfactant has been studied as a remediation strategy in burned forests, grassland, and chaparral vegetation. However, the use of surfactants later gained interest as a vegetation restoration strategy to restore soil health after wildfires by improving soil hydrological conditions (DeBano and Conrad, 1974; DeBano, 2000) and agricultural conditions to promote plant growth by increasing soil water storage (Cooley and Lowery, 2000), thus reducing the time that the soil is without surface cover after burning.

Soil management practices such as the application of different types of soil covers like mulch can have a considerable effect on soil temperature (Wang et al., 2011; Li et al., 2013), organic matter content (Zhou et al., 2013) and other measures of soil health after a fire (Robichaud et al., 2013; Henry and Bergeron, 2005). Soil microorganisms respond quickly to these changes in soil conditions. Thus, the objective of this study was to determine the effects of prescribed fires and the use of soil surfactants and mulch on soil physicochemical properties and microbial communities

MATERIALS AND METHODS

Study area and soil sampling

The research site was located at Juana Díaz – Agricultural Experiment Substation (AES) (18° 01’ 47.17” N and 66° 31’ 13.19” W) with an elevation of 36 m above sea level. Annual precipitation fluctuates between 508 and 1,016 mm, and mean annual temperature fluctuates between 26.1 and 27.2° C with the months of December, January, and February as the driest of the year (Muñoz et al., 2018). Figure 1 shows Juana Díaz-AES average monthly precipitation for 2015-2017. The soil at the site is a Yauco silty clay loam (Fine-silty, carbonatic, isohyperthermic Typic Calciustolls) (Muñoz et al., 2018) with 45% sand, 20% silt, and 35% clay. This soil series is formed from calcareous sediments located at the base slope of mountains with 2 to 5% slope. Before burning, the predominant vegetation at the experimental site was Buffel grass (*Cenchrus ciliaris*), Guinea grass (*Megathyrsus maximus*), and Leucaena trees (*Leucaena* spp.).

Two prescribed burnings were performed with the collaboration of the Fire Department of Puerto Rico (FDPR) in October 2015 and March 2017. A complete randomized experimental design with four treatments and four replicates (16 plots) was established. Each plot consisted of an area of 6.0 m by 12.2 m, with the longest section parallel to the slope. All plots had the same aspect, soil type, and slope percentage. The treatments were: positive control (burned, no remediation), nega-

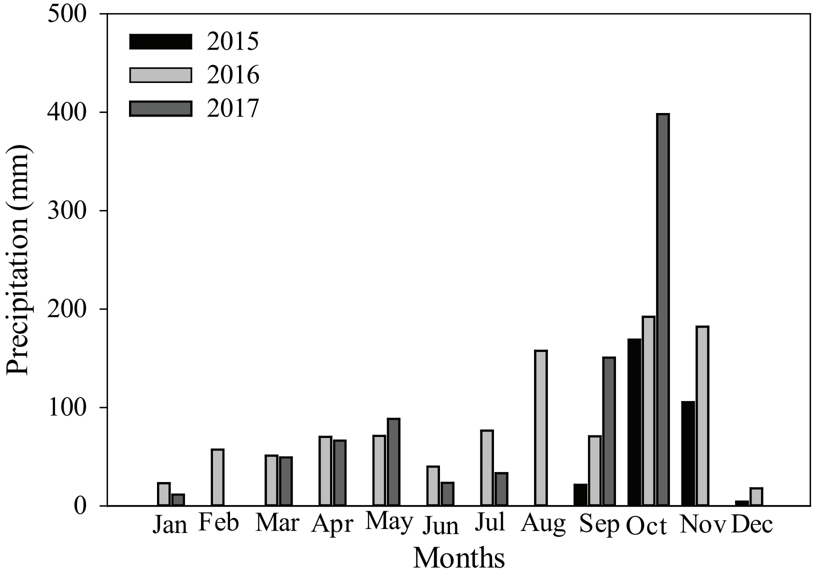

FIGURE 1. Monthly mean precipitation values (2015-2017) in mm for AES- Juana Díaz site.

tive control (non-burned sites), mulching (burned and covered with 1.27 cm of *Leucaena* spp. mulch after burning), and surfactant (burned and covered with surfactant after burning). Both mulching and surfactant were applied no later than one day after each prescribed burning. The surfactant used was IrrigAid® Gold (Aquatrols® New Jersey, USA)[6] which contains the active ingredients alkoxylated polyols and glucoethers at a 10% and 5% ratio, respectively. This product was hand sprayed using a 15 L diaphragm pump backpack sprayer at a ratio of 5 ml/m^2.

Composite soil samples were collected at a depth of 0 to 15 cm from each plot before and after the prescribed fires. For the burning performed in 2015, soil samples were collected before burning, and at 30, 180, and 420 days after burning (DAB). In the second burning, performed in 2017, samples were collected at 30, 90, 180, and 270 DAB. However, the samples collected at 180 DAB (for chemical and biological analysis) and 270 DAB (for chemical analysis) were lost due to an

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[6] Company or trade names in this publication are used only to provide specific information. Mention of a company or trade name does not constitute an endorsement by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

electrical outage caused by Hurricane María. Soil samples for chemical analysis were oven-dried at 65° C for 48 h, ground, and sieved through a 2-mm screen. Samples for phospholipid fatty acid (PLFA) analysis were placed in sealed plastic bags, stored on dry ice immediately after collection, and taken to the laboratory where they were placed in a -20° C freezer until analyzed.

Soil physicochemical analysis

Soil chemical analyses were performed at the Central Analytical Laboratory, Agricultural Experiment Station, University of Puerto Rico. Soil pH and electrical conductivity (EC) were measured in a 1:2 (v:v) soil/water mixture with Orion Star A215 pH and EC meter (Thomas, 1996). Exchangeable calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+), and sodium (Na^+) were extracted using 1 M NH_4OAc (Sumner and Miller, 1996) buffered at pH 7.0 and determined with an AA spectrometer (Thermo Electron Corporation S-Series AA S-2 Spectrometer). Nitrate (NO_3-N) was extracted using 2N potassium chloride and determined colorimetrically using a Quick Chem Analyzer. Soil OM was determined using humid digestion and colorimetry of Walkley and Black as described by Nelson and Sommers (1996) and available phosphorus (P) using the Olsen method (Kuo, 1996).

Soil microbial community structure analysis

The microbial community was assessed using Phospholipid Fatty Acid (PLFA) analysis. This was performed at Wards Laboratory, Inc. at Kearney, NE (Clapperton et al., 2005). Total soil lipids were extracted using dichloromethane (DMC): methanol (MeOH): citrate buffer (1:2:0.8 v/v). A lipid-class separation was conducted in silica gel columns, and the neutral, glycol and phospholipids fractions were eluted by sequential leaching. The fatty acids were converted to fatty acid methyl esters by transesterification and were analyzed using an Agilent 7890 A gas chromatograph equipped with a 7693 autosampler and a flame ionization detector; peaks were identified using the Microbial Identification Inc. (MIDI) Sherlock System.

The abundance of individual PLFA was expressed as micrograms (µg) of PLFA per gram of dry soil. The quantification was performed using the relative area under specific peaks, as compared to the 19:0 peak value, which was calibrated according to a standard curve made from a range of concentrations of the 19:0 FAME (fatty acid methyl ester) standard dissolved in hexane. Individual fatty acids have been used as signatures for various functional groups of microorganisms (Bossio et al., 1998; Pankhurst et al., 2002). Selected terminal-branched saturated PLFAs (i15:0, a15:0, i16:0, a16:0, i17:0, and a17:0) were used as markers for Gram-positive (Gram+) bacteria (Federle, 1986; Zelles, 1997). Selected monounsaturated and cyclopropyl-saturated PLFAs 16:1ω5, 16:1ω9, 17:1ω9, cy17:0, 18:1ω11, and cy19:0 were used to represent Gram-negative (Gram–) bacteria, and the PLFA 14:0, 15:0, and 17:0 for unspecific bacteria (Federle, 1986; Frostegård et al., 1993; Zelles, 1997). The polyenoic, unsaturated PLFA 18:2ω6c was used as an indicator of fungal biomass (Federle, 1986; Frostegård and Bååth, 1996; Huang et al., 2011). The PLFA 16:1ω11 or 20:0 was used to represent arbuscular mycorrhizal fungi (Olsson, 1999; Huang et al., 2011). The biomarkers for PLFA 20:3 at 6 and 20:4 at 6 were used as indicators for protozoa biomass (Cavigelli et al., 1995). The rhizobia PLFA biomarkers contained 16:0, 17:0, 18:0, and 19cycloω9C fatty acids (Jarvis and Tighe, 1994). Total bacteria were calculated as the sum of Gram+, Gram–, and unspecific bacteria. The total PLFA biomass was calculated as the sum of all the extracted PLFAs and reported as total µg PLFA biomass/g.

The PLFA 16:1ω5 cis, a structural component of arbuscular mycorrhizal fungi (AMF) (Olsson, 1999), has been used as a biomarker for viable AMF hyphal density (Buyer et al., 2010; Olsson, 1999), although it is also found in Gram-negative bacteria (Zelles, 1997).

Data Analysis

Two-way analysis of variance (ANOVA) followed by means separation using Tukey's Honestly Significant Difference test were utilized to examine differences among microbial community composition and soil chemical properties within the sampling date. Statistical analysis was done using SAS 9.1 (SAS Institute, 2003). A canonical discriminant analysis was used to compare soil microbial communities from the different treatments to determine the similarity among microbial communities. In this multivariate analysis of variance (MANOVA), the absolute area of each biomarker was used to identify the linear combination of variables that best-separated soil microbial community structure. The canonical variates were graphed to summarize group differences (Buyer et al., 2002). All statistical analyses were performed at the $P<0.05$ significance level.

RESULTS

Impact of prescribed fire on soil physicochemical properties

Soil exchangeable Ca^{2+} and K^{+} varied between treatments and for each DAB (Table 1). For example, in 2015, significantly higher Ca^{2+} and significantly lower K^{+} concentrations were observed in the positive con-

TABLE 1.—*Soil pH, electrical conductivity (EC), organic matter (OM) and macronutrient content in Yauco soil after prescribed burnings at Juana Díaz Agricultural Experiment Substation sites.*

		pH	EC*	OM	P	NO_3^-	Ca	K	Mg	Na
Days after burning	Treatment		mmho/cm	--%--	---------mg/kg---------		------------------cmol$_c$/kg------------------			
1st burning										
30	Negative Control	8.12	0.458	4.92	16.7	161.0	17.7 b[+]	2.0 a	2.5	0.1
	Positive Control	8.08	0.467	4.26	12.8	207.0	18.5 a	1.2 b	2.4	0.1
	Mulch	8.08	0.462	4.56	13.8	163.0	18.5 a	1.3 b	2.8	0.1
	Surfactant	8.10	0.466	4.53	11.4	205.0	18.7 a	1.1 b	2.5	0.1
180	Negative Control	8.29	0.346	4.85	12.6	95.4	17.5 b	1.7 a	2.3	0.1
	Positive Control	8.39	0.263	4.89	9.35	63.7	18.3 a	1.0 b	2.3	0.1
	Mulch	8.38	0.259	4.58	8.90	46.0	18.3 a	1.0 b	2.6	0.1
	Surfactant	8.41	0.241	4.90	7.85	43.0	18.4 a	0.8 b	2.4	0.1
420	Negative Control	8.20	0.514	5.13	10.8	141.0	35.1 b	1.9 a	3.5	ND
	Positive Control	8.16	0.328	5.22	9.19	61.5	36.4 a	1.0 b	3.4	ND
	Mulch	8.16	0.349	5.27	8.68	55.0	36.8 a	1.2 b	3.9	ND
	Surfactant	8.17	0.374	5.32	8.39	127.0	36.6 a	0.8 b	3.5	ND
2nd burning										
30	Negative Control	8.18	0.346	4.96	7.18	57.2	34.5 a	1.2 a	4.4	0.2
	Positive Control	8.10	0.462	5.06	1.68	64.6	33.6 b	1.1 ab	4.2	0.2
	Mulch	8.09	0.465	4.99	2.41	67.0	33.6 b	1.1 ab	4.4	0.2
	Surfactant	8.08	0.467	5.16	1.61	79.8	32.5 c	1.0 b	5.8	0.3
90	Negative Control	7.85	0.683	5.98	10.3	80.4	48.3 b	1.3 a	3.6	0.1
	Positive Control	7.93	0.718	5.58	7.78	33.6	49.7 b	0.8 b	3.9	0.1
	Mulch	7.83	0.890	6.22	9.05	65.4	51.2 a	0.9 b	4.7	0.1
	Surfactant	7.95	0.673	5.83	7.05	30.6	48.8 b	0.7 b	4.0	0.1

[+] Means followed by different letters in a column for each “day after burning” are significantly different by Tukey’s test at $P< 0.05$.

*EC-electrical conductivity, OM-organic matter; NO_3^--nitrate, P-phosphorus, Ca-calcium, K-potassium, Mg-magnesium, Na-sodium, and ND-Not detectable.

trol (burned), mulch, and surfactant treatments compared to the negative control (unburned). However, in the second burning at 30 DAB, Ca^{2+} concentration was significantly higher in the negative control compared to the other treatments, while K^+ concentration in the negative control was only significantly higher than that in the surfactant treatment. In the second burning, at 90 DAB, a significantly higher concentration of Ca^{2+} was measured in the mulch treatment when compared to the other treatments. Significantly lower K^+ concentrations were observed in the positive control (burned), mulch, and surfactant compared with the negative control (unburned) treatment. No significant differences ($P>0.05$) were observed for pH, EC, OM, P, Mg, Na, and NO_3^-. The ash from burning organic material contributes to the higher concentration of Ca^{2+} observed. This cation prevails in the soil exchangeable system, occupying exchangeable sites preferentially over Mg^{2+}, K^+, and Na^+.

Impact of prescribed fire on microbial biomass and communities

First prescribed burning

The treatments did not have a consistent effect on total microbial biomass, total fungi, total bacteria, and total protozoa on the three sampling dates after the first prescribed burning in October 2015 (Figure 2). However, treatment response was observed after the first 30 DAB. Total microbial, fungal, and protozoan PLFAs were significantly lower in the positive control (burned), mulch, and surfactant treatments when compared to the negative control (unburned) at 30 DAB. After 420 days, higher microbial PLFAs were found in mulch and surfactant treatments compared to both controls (Figure 2). Also, at 420 DAB higher protozoa PLFA mean values were found under mulch treatment followed by surfactant, negative control (unburned), and positive control (burned) (Figure 2). Total bacterial PLFA did not show significant differences ($P>0.05$) between treatments at any sampling date after burning.

The PLFA biomarkers for actinomycetes, AMF, and saprophytic fungi were affected by treatments after 30 days of the first prescribed burning in October 2015 (Figure 3), but gram-negative and gram-positive bacterial and rhizobial PLFA did not show statistical differences ($P>0.05$) between treatments at this stage. The actinomycetes population at 180 DAB was significantly lower in the burned plot (positive control) and burned plots treated with mulch when compared to the unburned and burned with surfactant treatment. However, after 420 days, the plot with surfactant treatment contained a significantly ($P<0.05$) higher number of actinomycetes compared to unburned and burned with mulch treatments and without treatment. Gram-negative and rhizobial PLFA did not show any significant difference due to sam-

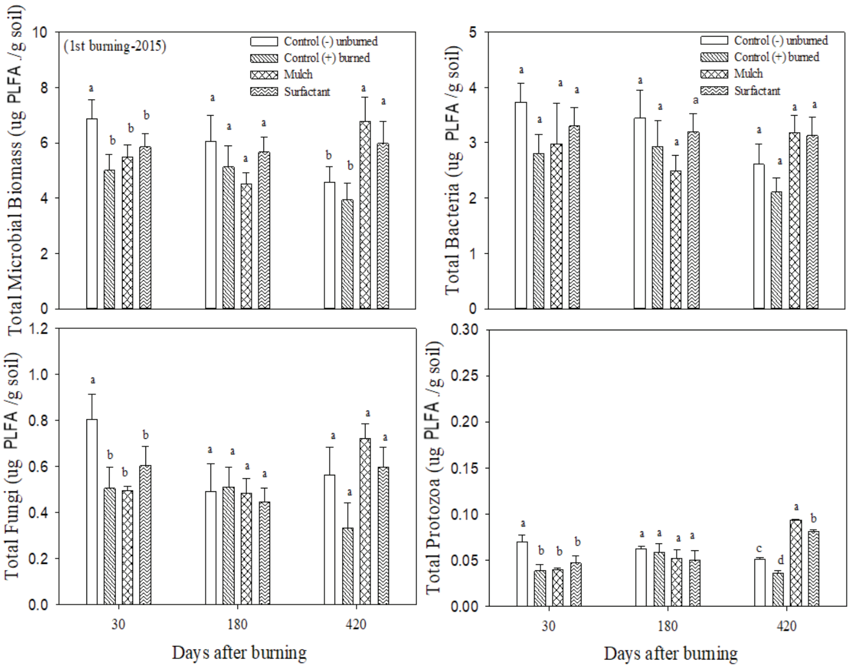

FIGURE 2. Total microbial biomass, total bacteria, total fungi, and total protozoa PLFA for the selected treatments at 30, 180, and 420 days after the first burning in October 2015 in Juana Díaz-AES experimental plot. The error bar indicates standard error. Treatments with the same letter within the same sampling date are not statistically different (P<0.05).

pling stage or treatment. However, Gram-positive bacterial PLFA was significantly lower in mulch treated burned plots after 180 days. While at 420 DAB, Gram-positive bacteria population in unburned control, mulch, and surfactant treated plots was similar, but significantly higher than in the burned control plot. Saprophytic and AM fungi showed the same tendency at 30 DAB, where higher mean values were found in the negative control compared with the other three treatments (Figure 3). While no statistical difference was observed at 180 DAB, at 420 DAB higher mean values for AMF were observed in mulch and surfactant treatments compared with control treatments, and amounts of saprophytic fungal PLFA were significantly lower in burned plots compared to unburned, mulch, and surfactant treated plots.

Second prescribed burning

At 30 DAB, total microbial, bacterial, and fungal PLFAs, were significantly higher in negative control unburned plots compared to

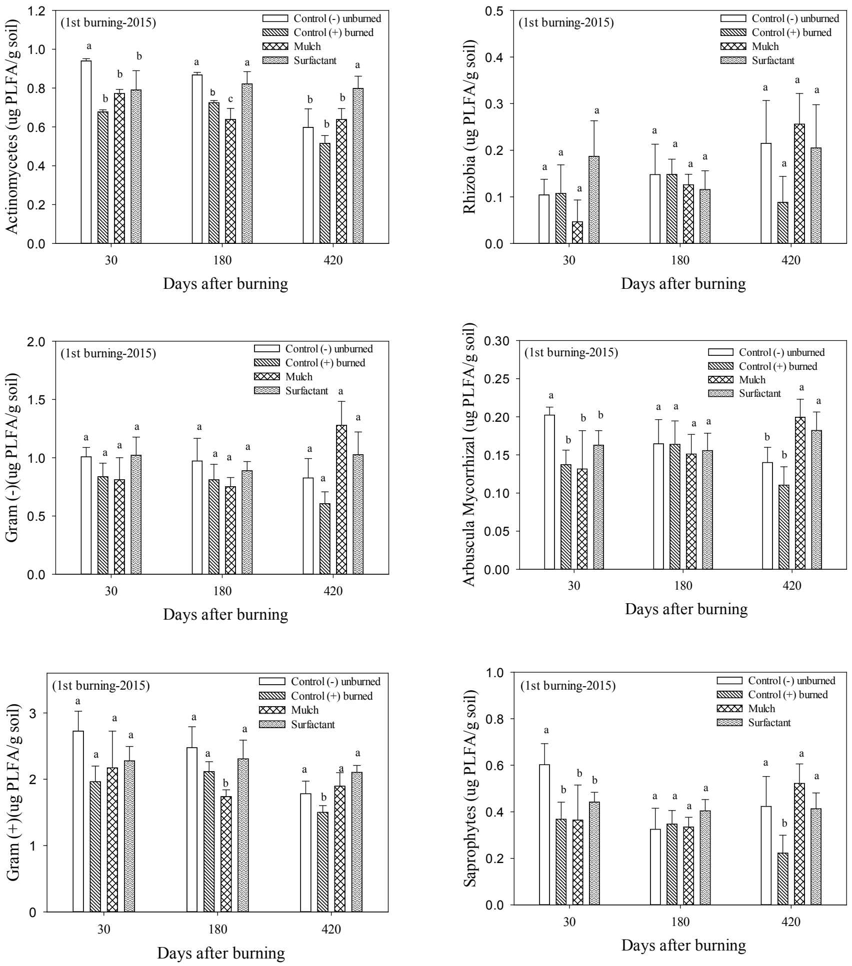

FIGURE 3. Actinomycetes, Gram (+), Gram (-), Rhizobia, Arbuscular mycorrhizal, and Saprophytes PLFA for the selected treatments at 30, 180, and 420 days after the first burning in October 2015 in Juana Díaz-AES experimental plot. The error bar indicates standard error. Treatments with the same letter within the same sampling date are not statistically different (P<0.05).

burned plots with or without treatment. However, at 90 DAB microbial biomass was significantly higher in burned plots with mulch and surfactant treatments compared to unburned and burned plots without any treatment. After 90 days, total bacteria significantly increased with an addition of mulch treatment in burned plots compared to unburned and burned plots with or without surfactant. However, for total fungi, lower values were observed at 90 DAB compared with the other three treatments. While for total bacteria at 90 DAB, higher mean values were observed in mulch treatment compared with the other treatments. No statistical differences between treatments were found at 270 DAB (Figure 4). For total protozoa, no statistical differences (P>0.05) were found between treatments at each sampling date after burning.

Thirty days after burning, biomarker PLFA for actinomycetes, Gram-positive and negative bacteria, AMF, and saprophytic fungi were significantly lower in all the burned plots with and without treatments after the second prescribed burning in 2017 (Figure 5). Rhizobia biomarker was not affected by burning with or without treatments, compared to unburned plots. In general, all the PLFA biomarker concentrations were lower after the second burning. After 90 days, actinomycetes, Gram-negative bacteria, rhizobia, and saprophytic fungi remained significantly lower in burned control plots compared to the

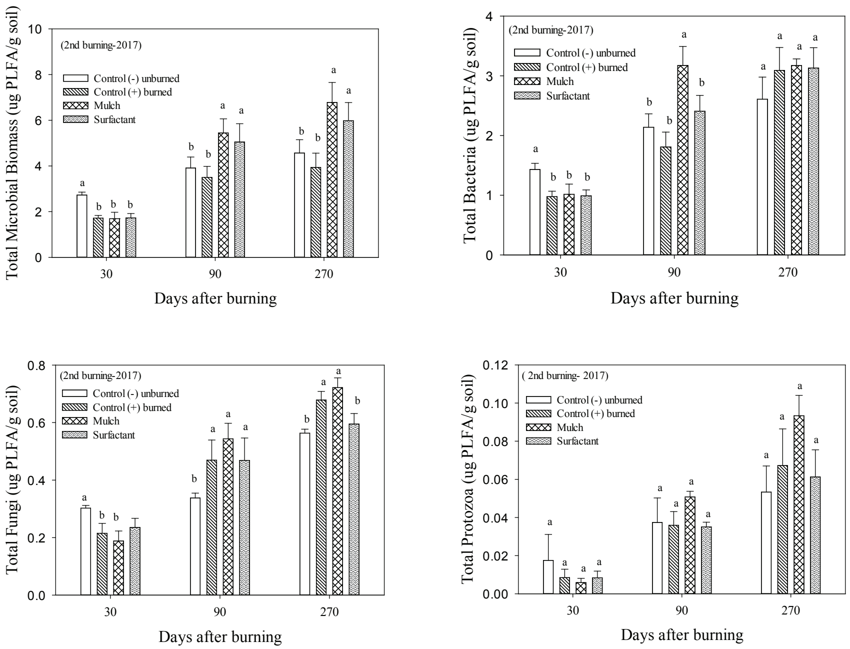

FIGURE 4. Total microbial biomass, total bacteria, total fungi, total protozoa, and actinomycetes PLFA for the selected treatments at 30, 90, and 270 days after the second burning in March 2017 in Juana Díaz-AES experimental plot. The error bar indicates standard error. Treatments with the same letter within the same sampling date are not statistically different (P<0.05).

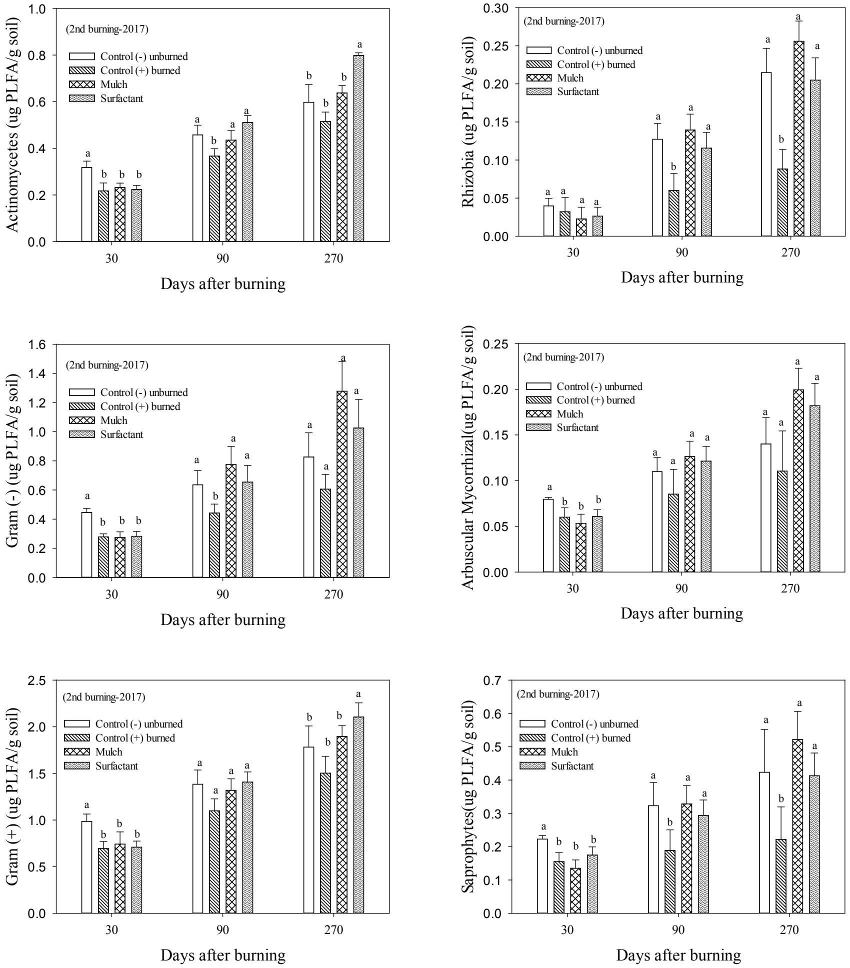

FIGURE 5. Actinomycetes, Gram (+), Gram (-), Rhizobia, Arbuscular mycorrhizal, and Saprophytes PLFA for the selected treatments at 30, 90, and 270 days after the second burning in March 2017 in Juana Díaz-AES experimental plot. The error bar indicates standard error. Treatments with the same letter within the same sampling date are not statistically different (P<0.05).

other treatments. Gram-positive bacteria and AMF PLFA did not show significant differences among unburned and burned plots with and without treatments. After 270 days, actinomycetes and Gram-positive bacterial PLFAs remained significantly higher in burned plots with surfactant treatment; however, Gram-negative and AMF biomarkers did not show any significant difference between unburned and burned plots with and without treatments. Moreover, rhizobia and saprophytic fungal PLFAs were significantly higher in unburned and burned plots with treatments compared to burned plots without any treatments.

Microbial community structure

Canonical multivariate analysis showed significant differences in the soil microbial community structure before and after prescribed fires with surfactant and mulch applications (Figure 6). In 2015, differences in the microbial communities in the positive control and mulch treatments were similar at 30 and 180 DAB. At 420 DAB, microbial communities in mulch treatment were significantly different when compared to the other three treatments. In 2017, microbial communities in the positive and negative controls were significantly different than mulch and control negative treatment 30 days after burning. After 90 days, microbial communities under the mulch treatment were similar to the negative control, which were significantly different under positive control and surfactant treatments.

The ability of the discriminant function to differentiate before and after prescribed fires based on the amounts and types of PLFAs was found to be significant. In 2015, 180 DAB, canonical variate (CV)1 was able to distinguish between a positive control (burned) and surfactant vs. mulch and negative control (unburned). In 2017, 90 DAB, both CV1 and CV2 differentiated the negative control and mulch from the positive control and surfactant. In 2017, 270 DAB CV1 distinguished negative control vs. control positive, mulch, and surfactant, and CV2 discerned negative control and mulch vs. positive control and surfactant (Table 2).

DISCUSSION

This study captured changes in abiotic and biotic factors in positive control (burned), negative control (unburned), mulch, and surfactant treatments after two prescribed burnings in the southern region of Puerto Rico. After two prescribed burnings, exchangeable Ca^{2+} increased and exchangeable K^{+} decreased when compared to unburned soils. It is well known that fires can alter soil properties (Tng et al., 2014; Santín and Doerr, 2016) and sometimes have a fertilizing effect by increasing levels of exchangeable cations (Pyne, 2001; Thomaz et al., 2014; Heydari et al., 2015) as a result of the dissolution of ashes and mineralization of charcoal (Badía et al., 2014). Each nutrient reacts differently to fire, depending on its individual volatilization threshold (DeBano, 2000; Hough, 1981). The combustion of nutrients bound to vegetation and SOM adds inorganic forms of K, Ca, Mg, P, and N to the

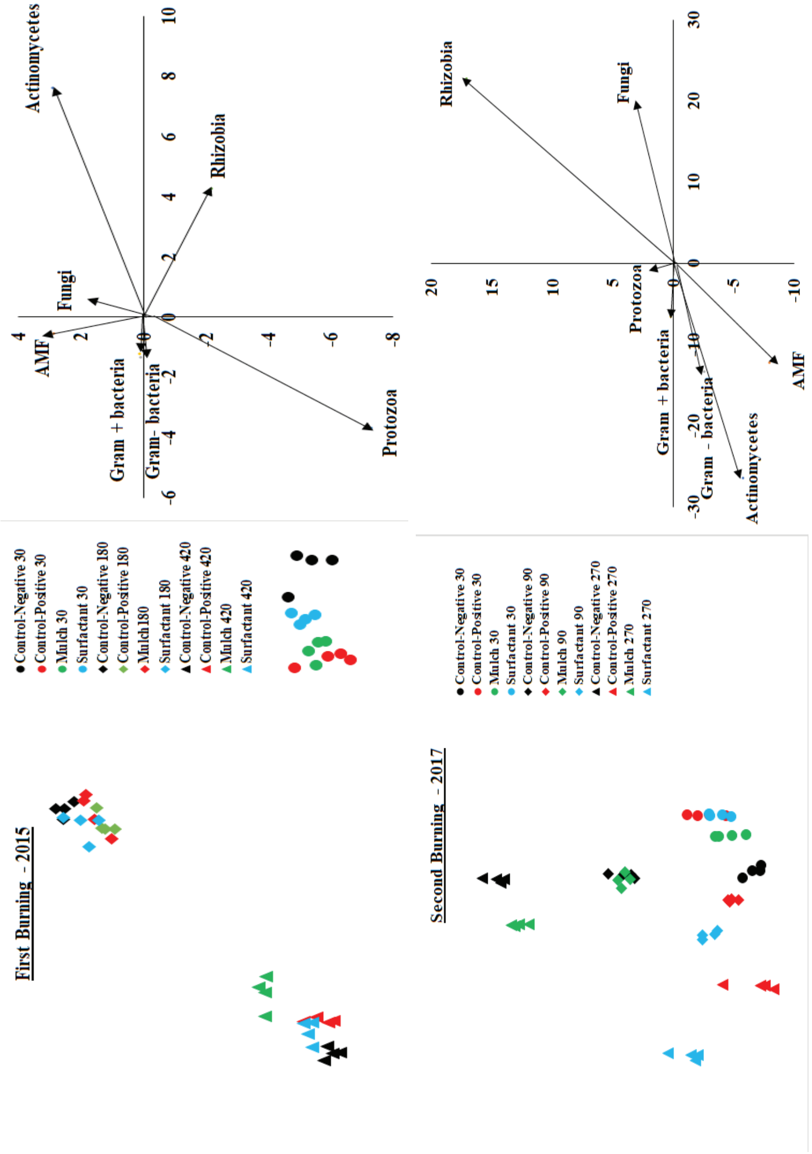

FIGURE 6. Canonical multivariate analysis of variance of PLFA biomarkers. Vectors represent standardized canonical coefficients and indicate the relative contribution of each biomarker group to each canonical variate.

TABLE 2.—*Structure matrix (pooled with Canonical Structure) and function at Group Centroid.*

1st Burning-2015			2nd Burning-2017		
Variable	CV1	CV2	Variable	CV1	CV2
Actinomycetes	0.41	-0.01	Actinomycetes	-0.19	0.36
AMF	-0.33	0.23	AMF	-0.06	0.16
Gram - bacteria	-0.16	-0.10	Gram - bacteria	-0.09	0.31
Gram + bacteria	-0.04	0.34	Gram + bacteria	-0.15	0.33
Protozoa	-0.17	-0.58	Protozoa	-0.06	0.12
Rhizobia	0.31	-0.31	Rhizobia	-0.07	0.36
Fungi	-0.37	-0.05	Fungi	-0.04	0.24
Group Centroids					
30 DAB			**30 DAB**		
Control (-) unburned	20.92	-5.78	Control (-) unburned	8.81	-6.88
Control (+) burned	13.43	-7.47	Control (+) burned	26.98	-2.82
Mulch	14.21	-6.57	Mulch	20.38	-4.75
Surfactant	16.84	-5.43	Surfactant	26.99	-3.94
180 DAB			**90 DAB**		
Control (-) unburned	1.03	13.72	Control (-) unburned	7.28	3.89
Control (+) burned	-0.12	10.48	Control (+) burned	-0.82	-5.03
Mulch	0.70	11.35	Mulch	5.76	3.96
Surfactant	-0.34	12.26	Surfactant	-12.08	-3.15
420 DAB			**270 DAB**		
Control (-) unburned	-19.01	-7.65	Control (-) unburned	5.80	14.50
Control (+) burned	-16.34	-6.79	Control (+) burned	-28.77	-7.00
Mulch	-14.07	-2.24	Mulch	-8.81	12.65
Surfactant	-17.25	-5.87	Surfactant	-51.53	-1.40

soil (Alcañiz et al., 2016; Schlesinger et al., 2016). Studies by Tomkins et al. (1991) and Santín et al. (2018) reported an increase in soil exchangeable Ca one month after a fire on a Eucalyptus plantation. However, the increase of soil exchangeable Ca was almost gone six months later. In our study, the increase in exchangeable Ca^{2+} was more lasting and almost double its concentration at 420 days after burning. Yauco soil is a Mollisol of carbonatic mineralogy, and under fire $CaCO_3$ can be converted to CaO, a calcium compound of greater solubility, thus contributing to the prolonged effect of Ca^{2+} increase. Higher concentrations of Ca were found under burned soils (positive control, mulch, and surfactant) compared with unburned soil (negative control treatment). Chungu et al. (2020) found results similar to those of our study, and the increase in Ca concentration persisted for one to two years after a fire event. Tomkins et al. (1991), Santín et al. (2018) and Chungu et al. (2020) also found an increase in soil exchangeable K, differing from our results. However, Bridges et al. (2019) found a reduction in K concentrations under burned soils when compared to unburned soils. A probable reason is that the available K was immobilized within mineral structures driven by thermal (burning) (Bridges et al. (2019) and remedial (mulching and surfactant) treatments. Probably, once the clay mineral dehydrates as a result of the heating process, K^+ is trapped in the interlayer space of montmorillonite and vermiculite present in Yauco soil. Also, the increase in exchangeable Ca^{2+} may cause displacement of K^+ adsorbed to the exchange sites, facilitating the loss of Ca^{2+} by lixiviation and erosion. In addition, it has been found that high soil moisture content, erosion, and leaching processes can significantly decrease the availability of K (Fonseca et al., 2017; Kuchenbuch et al., 1986), although, none of them can be attributed to the results obtained at our experimental site.

At our experimental site, the highest soil surface mean temperature recorded was 538° C, which is categorized as medium to high severity fire. Wolfe and Van Bloem (2011) found that in grass dominated dry areas in Puerto Rico, ground level peak fire temperature was approximately 540° C. Based on the recorded temperature a significant decline in total microbial biomass was expected following a fire, as suggested by Dooley and Treseder (2012). However, a remarkable reduction (around 70%) was observed 30 DAB only after performing the second burning but none in the first year. Studies have shown a decrease in microbial biomass after a fire and a recovery that may take months or even years (Certini, 2005; Barreiro and Díaz-Raviña, 2021). Other studies have shown that prescribed fires do not change total microbial biomass in the long term (Dooley and Treseder, 2012).

The addition of mulch or a surfactant to the soil increased total microbial biomass in both years after the prescribed fires. Soil management practices such as mulching can have considerable effects on soil temperature, evaporation (Wang et al., 2011; Li et al., 2013), organic matter content (Zhou et al., 2013), and increased soil water retention which in turn can stimulate soil microbial activity and biomass (Shen et al., 2016). Also, soil surfactant application can increase soil water content which in turn increases plant activity and increases soil microbial biomass and activity (Ahmadi et al., 2018).

Total microbial biomass in both years was significantly higher in mulch and surfactant-treated burned soils, compared to unburned and burned plots without any treatment after 90 and 420 DAB. No significant differences were observed between unburned and burned plots with and without treatments in the first year. Our results agree with previously reported data where higher bacterial concentrations were found in burned soils as compared to unburned soils (Grasso et al., 1996; Badía and Martí, 2003b). Also, similar to our results, Grasso et al. (1996) and Mataix-Solera et al. (2002) showed that bacterial populations returned to pre-fire levels (around 3 to 4 ug PLFA/g soil).

Soil fungi populations were more sensitive to prescribed burning than bacteria populations. Similar results were observed by Dooley and Treseder (2012). Total fungal PLFA was significantly lower in burned positive sites compared to unburned sites whereas total bacterial PLFA did not differ between burned and unburned sites 30 DAB. A meta-analysis study by Dooley and Treseder (2012) found that soil fungi abundance declines by an average of 47.6% following fires, and fungal responses to fire may have the same response mechanisms as SMC as a whole.

CONCLUSIONS

An increase in exchangeable Ca^{2+} and a decrease in exchangeable K^+ were observed on burned plots with and without mulch and surfactant treatments compared to unburned soils. The increase in Ca^{2+} can be attributed to the ash from organic matter and from the solubility of $CaCO_3$ present in Yauco soil, a Mollisol with a carbonate mineralogy. Under fire, $CaCO_3$ can be converted to CaO, a more soluble calcium compound. The solubility of $CaCO_3$ in water is approximately 15 mg/L at 25° C, whereas the solubility of CaO is 1 g/840 ml. The displacement of exchangeable K^+ by exchangeable Ca^{2+} from the exchange sites and subsequent loss by leaching should be a factor contributing to the decrease in K^+. Another factor contributing to lower levels of K^+ can be entrapment in 2:1 clay minerals as hydration decreased by the fire. Prescribed fire also decreased microbial biomass 30 days after burning. However, most PLFA biomarkers returned to similar or higher values in a short period of time after fire. The use of mulch and surfactant seemed to help the recovery of microbial communities in both years. Results from this short-term study suggest that soil microbial communities are highly resilient to disturbance after prescribed fires.

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