



## Early responses of soil health indicators to organic amendments and plant establishment at a fire-affected sclerophyllous forest

### Respuestas iniciales de indicadores de salud de suelo a enmiendas orgánicas y establecimiento de vegetación en un bosque esclerófilo incendiado

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#### ABSTRACT

Climate change and inappropriate landscape management have increased the intensity and severity of wildfires in Chilean Mediterranean ecosystems, including sclerophyllous forests. During the 2016-2017 summer, a megafire affected more than 5,000 km<sup>2</sup> in the country, compromising these ecosystems. Despite advances on aboveground recovery following fires, belowground restoration has been less explored in these forests. This study aimed to evaluate early responses of simple and approachable soil health indicators to different organic amendments and vegetation establishment methods. Six months following organic amendment application (compost, swine or poultry manure) and plant establishment (either seeds or seedlings), soil health indicators including heterotrophic microbial counts (colony-forming-units -CFUs) and physicochemical characteristics were determined in a fire-affected sclerophyllous Mediterranean forest of central Chile. The organic amendments, plant establishment, and their interaction, significantly affected soil CFUs, while chemical parameters including pH and electrical conductivity were solely affected by organic amendment. The swine manure-plantation treatment and the unburned reference forest had four- and five-fold more CFUs than the control, respectively. No effect was observed in soil gravimetric water content, organic matter content, or aggregate distribution. Overall, less marked effects were registered in soil physicochemical conditions as compared to microbial counts.

#### RESUMEN

El cambio climático y un manejo inadecuado del paisaje han aumentado la intensidad y severidad de los incendios forestales en ecosistemas Mediterráneos chilenos, incluyendo los bosques de tipo esclerófilo. Durante el verano 2016-2017, un megaincendio afectó más de 5.000 km<sup>2</sup> en el país, comprometiendo estos ecosistemas. A pesar de los avances en la recuperación de la vegetación después de los incendios, la recuperación del suelo ha sido menos explorada en estos bosques. Este estudio evaluó la respuesta temprana de indicadores simples y accesibles de salud del suelo a diferentes enmiendas orgánicas y establecimiento de vegetación. Seis meses después de la aplicación de la enmienda orgánica (compost, estiércol de cerdo o de aves de corral) y del establecimiento de la vegetación (semillas o plántulas), los indicadores de salud del suelo, incluyendo recuentos microbianos heterotróficos (unidades formadoras de colonias -UFCs) y características fisicoquímicas se determinaron en un bosque Mediterráneo esclerófilo incendiado del centro de Chile. Las enmiendas orgánicas, el establecimiento de la vegetación, y su interacción afectaron significativamente las UFCs del suelo, mientras que el pH y la conductividad eléctrica se vieron afectados solamente por la enmienda orgánica. El tratamiento de estiércol porcino- plántulas, y el bosque de referencia sin quemar tuvieron cuatro y cinco veces más UFCs que el control, respectivamente. No se observó ningún efecto en el contenido gravimétrico de agua, en el contenido de materia orgánica, o en la distribución de agregados. En general, se registraron efectos menos marcados en las condiciones fisicoquímicas del suelo en comparación con los recuentos microbianos.

*Palabras clave:* calidad de suelo, restauración de suelo, incendios forestales, mega-sequía.

#### INTRODUCTION

Human activity and climate change are increasing the intensity and severity of fire events worldwide (Santín and Doerr, 2016), especially in Mediterranean regions, which are highly susceptible due to their dry

summers and elevated temperatures (Bodí *et al.*, 2012). Recent human activities have especially affected fire regimes of Mediterranean ecosystems in Chile (29-38°S Latitude) (Fernández *et al.*, 2010). In the Chilean territory, native forests and shrublands, including those within Mediterranean zones, have been historically the

ecosystems most affected by fires (69%), followed by forest plantations (22%), and agricultural lands (about 9%) (Fernández *et al.*, 2010; de la Barrera *et al.*, 2018). Recently, during the 2016–2017 summer, a megafire affected more than 5,000 km<sup>2</sup> in south-central Chile (32–40°S Latitude), an area where about 74% of the Chilean population inhabits (CONAF, 2017; de la Barrera *et al.*, 2018; McWethy *et al.*, 2018). These fire events not only had environmental outcomes by impacting protected areas, but also economic and social consequences, as important components of the country's economy (agriculture and forestry) and highly populated areas were affected (McWethy *et al.*, 2018). In the future, such events are anticipated to be exacerbated by abnormal climatic events, especially in vegetated areas including native sclerophyll forests (González *et al.*, 2018; McWethy *et al.*, 2018). In this context, the megadrought registered in the country since 2010, which is expected to reduce rainfall between 30% and 45% (Garreaud, 2020), represents an additional pressure for these Mediterranean ecosystems.

Massive plantations of fire-prone exotic conifers, and land clearing by burning for agriculture or forestry, have increased the intensity and severity of fires, significantly decreasing the capacity of the native Chilean vegetation to recover (Úbeda and Sarricolea, 2016; González *et al.*, 2018). Given this scenario, the implementation of active restoration efforts following fire events is of extreme relevance. In Chile, early actions have been proposed to restore plant cover and reduce soil erosion following fires (Arellano *et al.*, 2018). Although such managements are critical to assist proper ecosystem recovery (Úbeda and Sarricolea, 2016; Arellano *et al.*, 2018), improvements of belowground conditions following fires in Mediterranean forests of central Chile have been less explored.

Many post-fire restoration efforts seem to have failed by not including the soil component (including physicochemical and biological parameters) as part of their programs (Thomas *et al.*, 2014). Soil health, which is defined as the capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, and properly provide ecosystem services (FAO, 2008), is an important aspect for post-fire plant recovery and succession (Harris, 2009). Indicators of soil health known to be affected by fires include chemical properties such as pH (Hart *et al.*, 2005), organic matter and organic carbon contents (Certini, 2005), and macronutrient contents (DeBano, 1991; Hart *et al.*, 2005; Bodí *et al.*, 2012), among others. Soil physical properties affected by fire include structure, porosity, and aggregation (DeBano, 1991). It is also well established that soil biological properties show greater sensitivity to fires, compared to abiotic properties (Hart *et al.*, 2005). This is of special concern as soil microbiota has been shown to contribute to the recovery of plant esta-

blishment through the restoration of key belowground metabolic and ecosystem functions (Fierer, 2017). Thus, restoring not solely vegetation, but also soil microbial communities and its physicochemical environment, is fundamental to reach a proper restoration, that takes into account the complex relationships between plants and its belowground surrounding environment.

The present study aimed to identify the early effects of different organic amendments (compost, swine or poultry manure) and different methods of plant establishment (sowing or plantation), on soil microbial counts and physicochemical conditions in a sclerophyllous forest located in the Mediterranean zone of central Chile affected by the 2016–2017 megafire. We asked whether simple and approachable soil properties were capable to detect the effects of organic amendments and plant establishment methods shortly after their implementation under these conditions. Thus, this early assessment can shed light on initial states of soil health and point towards future studies considering more in-depth soil evaluation in forest ecosystems prone to wildfires.

## MATERIALS AND METHODS

### Study area

This study was conducted within the Chilean Mediterranean zone, specifically in the O'Higgins Region, which was one of the three administrative regions most affected by the 2016–2017 megafire (CONAF, 2017). The research site is located at the Region's dryland zone (i.e. 'secano interior'), specifically at the east front of the coastal mountain range in the Pumanque commune (34°36.502'S; 71°42.281'W; 130 m a. s. l.). The mean annual temperature registered for this area is 15.4 °C, with 5.3 °C and 29.9 °C as minimum and maximum temperatures, respectively. The annual rainfall registered at this site was 537.6 mm, 440.6 mm, 578.2 mm, and 451.0 mm for 2015, 2016, 2017, and 2018, respectively. The research site is located in a private land covered by native sclerophyllous forests, with the canopy dominated mainly by *Quillaja saponaria* Molina, *Lithraea caustica* Hook. & Arn., and *Peumus boldus* Molina, followed by *Trevoa trinervis* Miers, *Azara serrata* Ruiz & Pav., and *Colliguaja odorifera* Molina in the understory. According to information provided by local residents, within the last 30 years this site has been mainly used by smallholder for livestock pasture and wood charcoal production and had not experienced fire events until January of 2017. Soils are classified as Aquic Dystrochrepts (Inceptisol) and developed from colluvial and alluvial deposits, which at the study site are rather shallow (~40 cm depth), consists mainly of sandy loam textures (58% sand, 34% silt, and 8% clay), and are located on a 10% slope facing south.

## Experimental design

In early June 2018, it was established a randomized complete design with two factors: 1) organic amendment type (no amendment, compost, poultry or swine manure), and 2) vegetation establishment method (sowing and plantation), plus a control (no amendment and no plant establishment), totalizing nine treatments randomly distributed in duplicate experimental plots (3 x 3 m<sup>2</sup>) within an area of approximately 50 x 50 m (Table 1). In addition to the amended plots and control, there were two plots at a nearby unburned forested area (aprox. 500 m distance) included as reference, which had similar vegetation and use as the affected area before burning. Poultry and swine manure were organic materials easily available at the area while compost was a commercial product originated from agricultural wastes (see Additional Material: Table S1 for chemical characterization of the organic amendments). Organic amendments were manually incorporated over the upper 30 cm of soil, following rototilling at the same depth. All amended plots were covered with a mulch layer consisting of a wheat and oat straw mix. The vegetation sown or planted consisted of the three dominant tree species originally found at the site (*P. boldus*, *Q. saponaria*, and *L. caustica*).

## Soil sampling and analyses

Soil was sampled in November 2018, six months after treatment establishment. At each plot, five sub-samples were collected at the corners and center of the plot, at a 6 cm depth in the A horizon following the removal of organic debris (1 kg each sample approx.). The sub-samples were thoroughly mixed in the field to obtain a composite sample per plot, which was split in two portions to obtain: 1) a fraction for biological analysis and for the determination of gravimetric water content, and 2) a second fraction for physicochemical analyses. The former portion was transported to the laboratory under sterile and refrigerated conditions.

The soil biological parameter measured consisted of standard aerobic heterotrophic plate count by determination of colony-forming-units (CFUs) (Maier *et al.*, 2009). CFUs were determined by serial dilutions in 0.1% peptone buffer and spread plating on 1.8% R2A medium (Difco, Detroit, MI), placed in an incubation chamber for 3 days at 25 °C. CFU counts were adjusted for gravimetric water content and reported on the basis of oven-dried soil.

Soil physicochemical parameters included some known to be affected by fires: gravimetric water content, determined by oven-drying samples at 70 °C for

**Table 1.** Treatments used in the study. All treatments, except T0 and T1, were covered with a mulch layer consisting of a wheat and oat straw mix.

**Cuadro 1.** Tratamientos usados en este estudio. Todos los tratamientos, excepto T0 y T1, fueron cubiertos con una capa de mulch consistente en una mezcla de paja de trigo y avena.

Treatment	Amendment (m <sup>3</sup> ha <sup>-1</sup> )	Amendment (T ha <sup>-1</sup> )	Sowing <i>Pb</i> ; <i>Q.s</i> ; <i>L.c</i> <sup>b</sup> (kg ha <sup>-1</sup> )	Plantation <i>Pb</i> ; <i>Q.s</i> ; <i>L.c</i> (plants ha <sup>-1</sup> ) <sup>c</sup>
(T0) Reference <sup>a</sup>	--	--	--	--
(T1) Control	0	0	0	0
(T2) Control-sowing	0	0	17; 33; 39	
(T3) Control-plantation	0	0		2.220; 2.220; 2.220
(T4) Compost-sowing	200	120	17; 33; 39	
(T5) Compost-plantation	200	120		2.220; 2.220; 2.220
(T6) Poultry manure-sowing	200	160	17; 33; 39	
(T7) Poultry manure-plantation	200	160		2.220; 2.220; 2.220
(T8) Swine manure-sowing	200	107	17; 33; 39	
(T9) Swine manure-plantation	200	107		2.220; 2.220; 2.220

<sup>a</sup>Corresponds to unburned sclerophyllous forest included as reference ecosystem, which had similar vegetation and use as the burned area previous to fire occurrence. <sup>b</sup>Planted species: *Pb*, *Peumus boldus*; *Q.s* *Quillaja saponaria*; *L.c* *Lithraea caustica*. <sup>c</sup>This plantation density (six plants per plot—two of each species) was for experimental purposes only.

48 h; soil pH and electrical conductivity (EC), determined in a 1:10 ratio after weighting 10 g of oven-dried and sieved (to a 2 mm diameter) soil in 100 ml of distilled water (Sadzawka *et al.*, 2006). Soil aggregate distribution was determined by passing 5 g of refreshed, oven-dried and sieved soil through 2 mm, 250 µm, and 53 µm mesh diameter soil sieves (Sadzawka *et al.*, 2006). Soil organic matter content was determined in oven-dried and sieved samples by acid dichromate oxidation, followed by colorimetric determination of reduced chromate (Sadzawka *et al.*, 2006).

**Statistical analysis**

Analyses of variance (ANOVA) were performed after testing the homogeneity of variances and normality of the residuals using the Bartlett test and graphical checks, respectively. Two-way ANOVA was performed to evaluate the effects of amendment, vegetation establishment method, and their interaction on soil health indicators using the base functions ‘*lm*’ and ‘*anova*’ of the software R Studio v.1.1.463 (RStudio Team, 2019). Significant effects were determined by the Tukey HSD test, using the base function ‘*TukeyHSD*’ in RStudio. Additionally, a principal component analyses (PCA) was implemented using the base function ‘*prcomp*’ and the package ‘*ggfortify*’ (Tang *et al.*, 2016) in RStudio to establish the relationship between the different treatments and variables measured.

**RESULTS**

In late November 2018, six months after treatment establishment, all amended plots evidenced plant growth, consisting mainly of wheat and oat introduced with the mulch layer used. However, some herbaceous species consisting of *Brassica campestris*, *Conium maculatum*, and *Loasa triloba* were present more fre-

quently in manure amended plots (see Additional Material: Table S2 for more details). Germination of sown species was still not evident by that time, while the percentage of seedlings standing varied from 50% for *P. boldus* and *L. caustica* in compost amended plots to 100% for *Q. saponaria* and *L. caustica*, in swine manure amended plots, and 100% for *L. caustica*, in poultry manure amended plots (see Additional Material: Table S2 for more details).

Soil biological and physicochemical conditions evidenced different responses to the treatments established (Table 2). The type of organic amendment and vegetation establishment method (sowing and plantation), and their interaction, significantly affected soil CFUs, while pH and EC were solely affected by the former. No effect of either factor was observed on gravimetric water content, soil organic matter, and soil aggregate distribution.

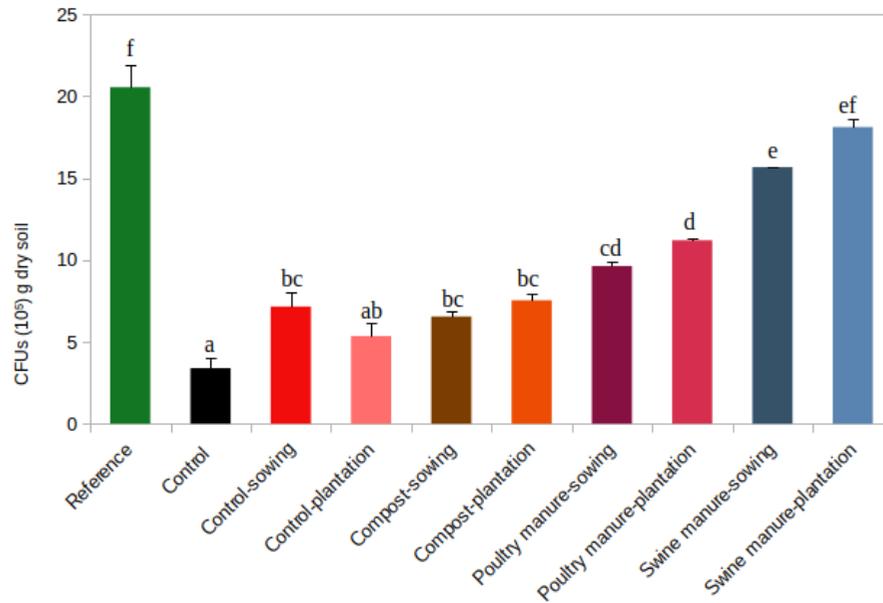
Biological conditions assessed by heterotrophic plate count varied among treatments (Figure 1). Significantly higher CFUs were obtained in the reference plots under the unburned forest, followed by plots amended with fresh materials, i.e. swine and poultry manure. Among compost and manure amended plots, those where seedlings were established evidenced greater microbial counts than sown plots. Soil pH was slightly acidic in all treatments (Figure 2a), fluctuating from an average of 5.90 in reference plots to 6.86 in compost amended plots which were sown. Electrical conductivity in all soils receiving organic amendments was below toxic levels, and in most cases slightly higher than reference plots, varying from 0.07 dS/m in control sown plots to 0.2 dS/m in swine manure amended plots with seedlings (Figure 2b). As compared to reference plots, there were no significant differences registered among treatments for soil gravimetric water (Figure 2c) and organic matter (Figure 2d) contents, where gravimetric water content was in general low

**Table 2.** ANOVAs showing the effects of organic amendment type, plant establishment method, and interactions on the soil parameters evaluated.

**Cuadro 2.** ANOVAs mostrando los efectos del tipo de enmienda orgánica, método de establecimiento vegetal, e interacciones, en los parámetros del suelo evaluados.

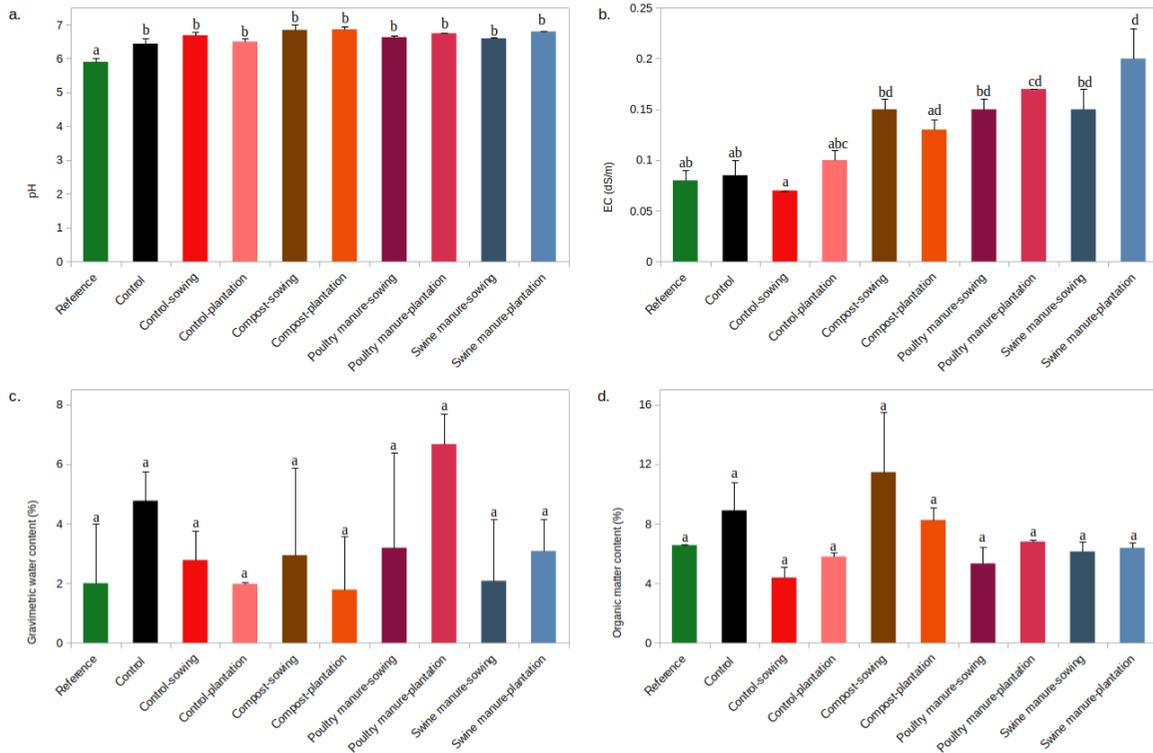
Source of variation	CFUs (10 <sup>5</sup> ) g dry soil	pH	EC (dS/m)	GWC (%)	OM (%)	<53 µm	250µm-53µm	2mm-250µm	>2mm
Amendment	235.965***	18.819***	18.028***	0.216 <sup>ns</sup>	2.240 <sup>ns</sup>	2.842 <sup>ns</sup>	0.817 <sup>ns</sup>	2.231 <sup>ns</sup>	1.377 <sup>ns</sup>
Plant establishment	11.044**	1.090 <sup>ns</sup>	1.975 <sup>ns</sup>	0.888 <sup>ns</sup>	2.100 <sup>ns</sup>	1.246 <sup>ns</sup>	0.427 <sup>ns</sup>	1.274 <sup>ns</sup>	0.601 <sup>ns</sup>
Amendment: Establishment	5.525*	1.563 <sup>ns</sup>	2.140 <sup>ns</sup>	0.041 <sup>ns</sup>	1.052 <sup>ns</sup>	1.458 <sup>ns</sup>	1.495 <sup>ns</sup>	1.170 <sup>ns</sup>	0.464 <sup>ns</sup>

EC=electrical conductivity; GWC=gravimetric water content; OM=organic matter; CFU=colony-forming units. The F<sub>p</sub> values are shown. P values: <sup>ns</sup>, non-significant; \*, <0.05; \*\*, <0.01; \*\*\*, <0.001.



**Figure 1.** Heterotrophic plate count, per treatment, determined as colony-forming units (CFUs) on a factor of 10<sup>5</sup> by gram of dry soil. Different letters represent significant differences at  $p < 0.05$  probability level according to the TukeyHSD test.

**Figura 1.** Recuento heterotrófico en placa, por tratamiento, determinado como unidades formadoras de colonias (UFCs) en un factor 10<sup>5</sup> por gramo de suelo seco. Letras diferentes representan diferencias significativas con un nivel de probabilidad  $p < 0,05$  de acuerdo al test TukeyHSD.



**Figure 2.** Average values by treatment for soil (a) pH, (b) EC (dS/m), (c) gravimetric water content (%), and (d) organic matter (%). Different letters represent significant differences at  $p < 0.05$  probability level according to the TukeyHSD test.

**Figura 2.** Valores promedio por tratamiento para las variables del suelo (a) pH, (b) EC (dS/m), (c) contenido gravimétrico de agua (%), y (d) materia orgánica (%). Letras diferentes representan diferencias significativas con un nivel de probabilidad  $p < 0,05$  de acuerdo al test Tukey HSD.

(1.79% to 6.68%) and organic matter content rather high (5.33% to 11.46%) for all plots.

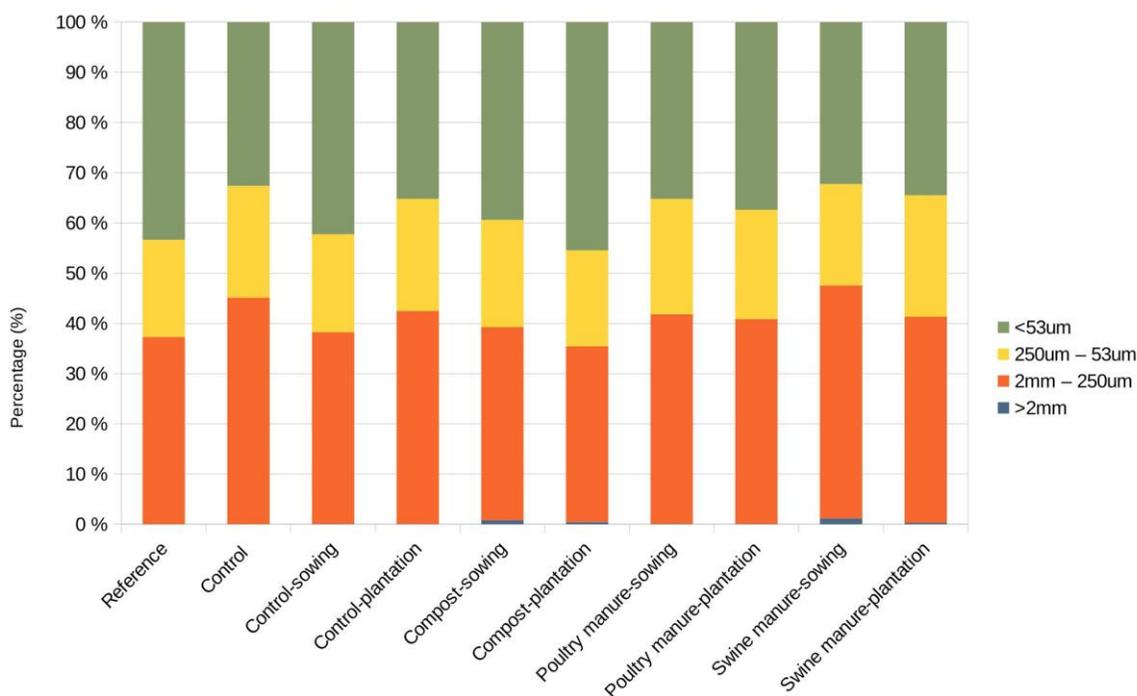
Soil aggregate distribution was very similar among treatments (Figure 3), possibly due to the still present effect of rototilling. Large macro aggregates (> 2 mm) were less common in reference unburned forest plots (0.005%) and most common in plots receiving swine manure and seeds (1.17%); small macroaggregates (2–0.25 mm) were less frequent in compost amended plots with seedlings (35%) and more abundant in plots with swine manure and seeds (46.39%). Microaggregates (250–53 μm) evidenced less variation among treatments, with the lowest proportion found in compost amended plots with seedlings (19.13%) and the highest proportion for swine manure amended plots with seedlings (24.18%). Silt and clay size particles (<53 μm) were less abundant in plots with swine manure and seeds (32.23%) and more abundant in compost amended, sown plots (45.45%).

Overall, variables like soil pH, OM, large macroaggregates, and EC were highly correlated to each other and to swine and compost with plantation and compost with sowing treatments; the control and other poultry and swine (plantation and sowing) treatments were more related to gravimetric water content and microaggregates (see Additional Material: Figure S1 for more details).

## DISCUSSION

Fires have historically played an important role in the configuration of terrestrial landscapes; however, they can also negatively affect them. Soil health can be drastically affected by these disturbances, for example by increasing soil leaching and erosion, and/or by highly reducing soil microbial biodiversity and ecosystem functions (Hart *et al.*, 2005). Among soil health indicators, biological parameters are the most informative regarding soil functioning, especially as soil biota responds rapidly to soil management and disturbances. Given that soil fungi and bacteria are heavily involved in biogeochemical cycles and plant-nutrient supply of carbon and nitrogen, respectively, it is expected that the measurement of their biodiversity and functioning directly reflects soil health. After an environmental disruption, microbial functions (i.e. regulation of net primary production, nutrient cycling, carbon sequestration, water purification; Guerra *et al.*, 2020) seem to recover faster than microbial biodiversity (Paulino *et al.*, 2009; Rivas *et al.*, 2016; Pérez-Valera *et al.*, 2019; Fuentes-Ramirez *et al.*, 2018), and both of these biotic parameters recover earlier than soil physicochemical parameters, which take longer to stabilize.

Although soil microbial biodiversity and functions are recognized as the main drivers of post-fire ecosys-



**Figure 3.** Soil aggregate distribution by particle size per treatment. For the four particle sizes, there were no significant differences between the treatments according to the Tukey HSD test ( $p < 0.05$  probability level).

**Figura 3.** Distribución de agregados del suelo por tamaño de partícula, por tratamiento. Para los cuatro tamaños de partícula, no hubo diferencias significativas entre los tratamientos de acuerdo al test Tukey HSD (nivel de probabilidad  $p < 0,05$ ).

tem recovery, the effects of fire events on soil microorganisms are less understood than those on soil physicochemical properties (Hart *et al.*, 2005). Overall, in our study we found a greater effect of organic amendments on microbial counts compared to physicochemical characteristics. The use of organic amendments in small areas within a larger damaged zone has proven not only to ameliorate soil conditions, but also to promote herbaceous plant colonization and succession within the treated area and its edges (Lupton *et al.*, 2013). Thus, this approach was used as opposed to amending larger areas, which is more costly and less effective (Lupton *et al.*, 2013). Organic amendments including compost, as well as swine and poultry manure (both representing sources of greater labile organic fractions), notoriously increased CFU counts when compared to control plots. In particular, soils amended with swine manure reached values closer to the reference unburned ecosystem, only after about six months, suggesting a greater benefit of the use of this material in soil recovery under the conditions of this study. In addition, CFUs were the only parameter evaluated in this study affected not only by organic amendment application, but also by the method of plant establishment and the interaction of these two factors, with greater counts observed in manure amended plots receiving seedlings. Regarding physicochemical characteristics, although organic amendments significantly increased soil pH and EC when compared to reference plots, these values were still within adequate range for most plants.

Although these results shed light on early stages of soil responses following land burning and organic amendment application, future studies need to include greater number of experimental units (replications) in order to strengthen statistical power and reduce large variability, as it was the case for GMC and OM in our study. Other studies in Chile have looked at the effect of fire on soil microorganisms, especially in temperate rainforests -a contrasting ecosystem to the less studied Mediterranean sclerophyllous forest in this study. In southern Chile, arbuscular mycorrhizal fungi have been shown to be crucial for the recovery of *Araucaria araucana* (Molina) K. Koch populations after severe fires (Paulino *et al.*, 2009; Rivas *et al.*, 2016; Fuentes-Ramirez *et al.*, 2018). The increase in soil microbial diversity occurred at this ecosystem despite a rapid loss of plant diversity, but a short-term response to new nutritional conditions after fires (Fuentes-Ramirez *et al.*, 2018). Similarly, the increase in microbial counts in amended plots reported in the present study could likely be due to the soil nutritional conditions achieved after the use of organic amendments and early plant colonization.

On any terrestrial ecological succession, soil biota, especially microorganisms, are the first ones to arrive

and to engineer the ecosystem so others can follow. Furthermore, the activity of these microorganisms is crucial for the stabilization of soil physicochemical parameters, as the current biodiversity and ecosystem functioning scientific literature suggests (Guerra *et al.*, 2020). Thus, in the context of restoration efforts, particularly after the occurrence of fires, quick and simple but very significant measurement of soil health status, including early microbial biodiversity determinations, should be considered. In accordance with the literature, our work suggests that in a sclerophyllous forest with Mediterranean climate, soil microbial conditions are precisely the first soil health indicators responding to a restoration effort based on the use of different organic amendments and plant establishment methods.

Although this study used a simple soil biological measurement, based on culturable microorganisms, which is known to account for a small portion of the complete microbial community (approximately 1% to 10%), insights from our results encourage further soil biological studies of greater resolution, as they have recently contributed to a better understanding of fire effects on soil microbial biodiversity/structure and their functions (Pérez-Valera *et al.*, 2018, 2019). Recently, studies based on techniques independent of cultivation have shown the impact of fire in soil microbiota with changes in their phylogenetic structure (Pérez-Valera *et al.*, 2019). Such studies have revealed that positive (i.e. species co-occurrence or environmental filtering) and negative ecological (i.e. competitive exclusion) interactions among distinctive groups respond differently to fire occurrences (Pérez-Valera *et al.*, 2017, 2019). Moreover, the dominance of certain groups of microorganisms after fires also seems to explain changes in biodiversity, resulting in the recovery of key microbial-species interactions through the succession after fire (Pérez-Valera *et al.*, 2018, 2019). Thus, future directions should include such techniques to better elucidate the impact of soil restoration approaches based on the use of different organic amendments and plant establishment methods, looking at their effect on soil microbial communities and its physicochemical environment.

## CONCLUSIONS

After six months of treatments application, less marked effects were registered in soil physicochemical conditions as compared to microbial counts. Swine manure had the greatest increase in culturable microorganism counts, as compared to the rest of the treatments evaluated, and the closest values compared to the reference unburned ecosystem. Among amended plots, microbial counts were always higher in those with plants than those receiving seeds.

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## ADDITIONAL MATERIAL

**Table S1.** Chemical properties of the organic amendments used in the study.

**Cuadro S1.** Propiedades químicas de las enmiendas orgánicas usadas en este estudio.

Property	Compost	Poultry Manure	Swine Manure
pH	7.41	6.77	8.3
EC (mS cm <sup>-1</sup> )	1.12	3.56	2.03
OM (%)	18.68	42.24	23.21
OC (%)	10.38	23.47	12.89
Total N (%)	0.83	1.92	0.75
CO:N	12.5	12.22	17.19
Total P (%)	0.7	0.5	0.52
P <sub>2</sub> O <sub>5</sub> (%)	1.6	1.15	1.19
K (%)	0.51	0.42	0.66
K <sub>2</sub> O (%)	0.61	0.51	0.79
Ca (%)	1.41	4.14	0.25
Mg (%)	0.29	0.37	0.28
Cu (mg kg <sup>-1</sup> )	74	103	39
Zn (mg kg <sup>-1</sup> )	201	208	98
Mn (mg kg <sup>-1</sup> )	518	404	584
Fe (mg kg <sup>-1</sup> )	14587	13408	9978

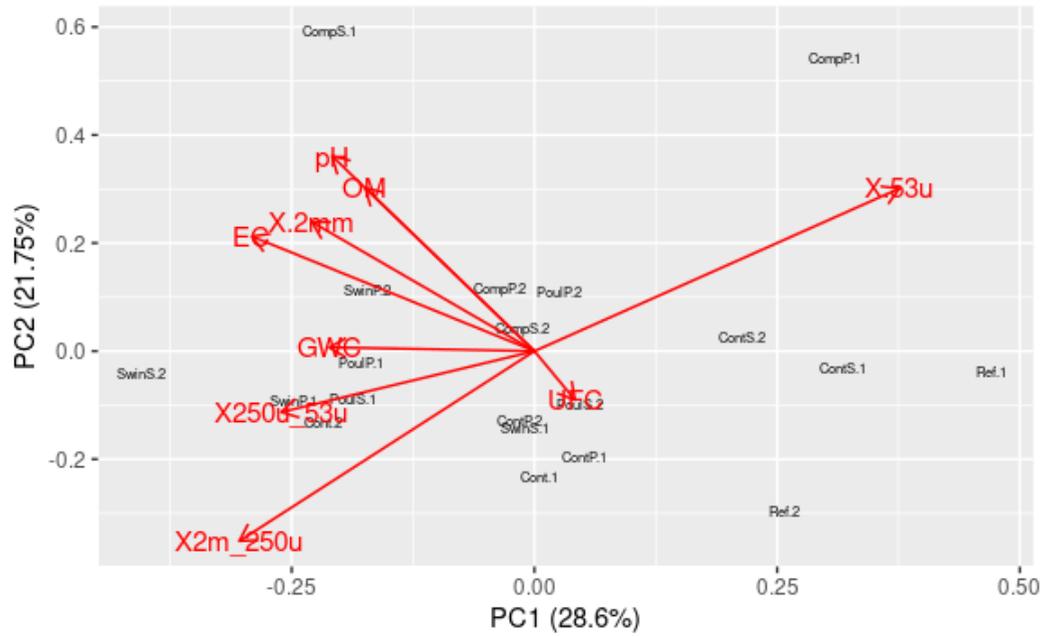
Chemical methods based on the test method for the examination of composting and compost (TMECC), US Composting Council and the Chilean norm of compost, classification and requirements (NCh 2880, 2015). Briefly, total N, P, K and micro and macronutrients obtained by acid digestion using HCl followed by atomic absorption spectrophotometry.

**Table S2.** State of vegetation six months after treatments establishment.

**Cuadro S2.** Estado de la vegetación seis meses después del establecimiento de los tratamientos.

Treatment	Plant cover (%) <sup>a</sup>	Height (cm)	Dominant vegetation <sup>b</sup>	Survival (%) of <i>P.b</i> ; <i>Q.s</i> ; <i>L.c</i> <sup>c</sup>
(T0) Reference <sup>d</sup>	--	--	--	--
(T1) Control	--	--	--	--
(T2) Control-sowing	35	50	Wheat, oat, <i>Brassica campestris</i>	
(T3) Control-plantation	60	60	Wheat, oat, <i>B. campestris</i>	75; 75 <sup>e</sup> ; 75 <sup>f</sup>
(T4) Compost-sowing	90	135	Wheat, oat, <i>B. campestris</i>	
(T5) Compost-plantation	73	110	Wheat, oat, <i>B. campestris</i> , <i>Conium maculatum</i> , <i>Raphanus raphanistrum</i>	75 <sup>f</sup> ; 50 <sup>e</sup> ; 50
(T6) Poultry manure-sowing	85	110	Wheat, oat, <i>B. campestris</i> , <i>C. maculatum</i> , <i>Loasa triloba</i>	
(T7) Poultry manure-plantation	80	115	Wheat, oat, <i>B. campestris</i> , <i>C. maculatum</i> , <i>L. triloba</i> , <i>R. raphanistrum</i> , <i>Malva sylvestris</i>	75; 75 <sup>e</sup> ; 100
(T8) Swine manure-sowing	85	135	Wheat, oat, <i>B. campestris</i> , <i>C. maculatum</i> , <i>L. triloba</i>	
(T9) Swine manure-plantation	93	85	Wheat, oat, <i>B. campestris</i>	100 <sup>f</sup> ; 75 <sup>e</sup> ; 100

<sup>a</sup>Plant cover of voluntary herbaceous species and species germinated from seeds introduced in mulch. <sup>b</sup>Dominant vegetation of voluntary herbaceous species and species germinated from seeds introduced in mulch. <sup>c</sup>Planted species: *P.b*, *Peumus boldus*; *Q.s* *Quillaja saponaria*; *L.c* *Lithraea caustica*. <sup>d</sup>Corresponds to unburned sclerophyllous forest included as reference ecosystem, which had similar vegetation and use as the burned area previous to fire occurrence. <sup>e</sup>Leaves with some brown stains. <sup>f</sup>Yellow leaves.



**Figure S1.** Principal component analysis (PCA) among the different edaphic variables measured and treatments.

**Figura S1.** Análisis de componentes principales (PCA) entre las diferentes variables edáficas medidas y los tratamientos.

For variables: EC=electrical conductivity; GWC=gravimetric water content; OM=organic matter; CFU=colony-forming units. For treatments: Cont=control; Ref=unburned reference forest; Comp=compost; Swin=swine manure; poul=poultry manure; P=plantation; S=sowing.

