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Seasonal responses of C and N related soil conditions in a fire affected Mediterranean Chilean forest following organic amendments and plant establishment

Respuestas estacionales de condiciones del suelo relacionadas al C y N en un bosque Mediterráneo Chileno afectado por un incendio tras aplicación de enmiendas orgánicas y establecimiento de plantas

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ABSTRACT

Understanding how soil chemical/physical and biological parameters behave after the increasingly common fires in Mediterranean Chilean ecosystems is critical to boost their recovery. Incorporating organic amendments in soil to support its post-fire recovery is promising; however, there are important gaps regarding the seasonal responses of soil parameters following such additions. This study aimed to identify the effects of different organic amendments (compost, poultry, and swine manure) and methods of plant establishment (sowing or plantation), through the seasons observed in a sclerophyll Mediterranean Chilean forest over one year. Season over organic amendments and plant establishment method had a very strong effect on most of the twelve soil parameters evaluated. Across seasons, soil pH, electrical conductivity, water content, and aggregate stability were less variable than C and N pools, colony forming units (CFUs) of heterotrophic and free-living N_2 fixing microorganisms, and urease and ammonia monooxygenase activities. Manure-based amendments increased NO_3 and heterotrophic CFUs during fall and summer, respectively. The use of compost resulted in greater soil organic matter and carbon, mostly in summer. Different responses of soil abiotic and biotic properties after fire and organic amendments can likely influence differently processes related to ecosystem recovery, particularly those related to C and N cycles.

RESUMEN

Comprender cómo se comportan los parámetros químicos/físicos y biológicos del suelo después de incendios cada vez más comunes en ecosistemas Mediterráneos Chilenos es fundamental para facilitar su recuperación. Incorporar enmiendas orgánicas en el suelo para potenciar su recuperación post-incendio es prometedor; pero existen brechas importantes respecto a las respuestas estacionales del suelo tras dichas adiciones. Este estudio tuvo como objetivo identificar los efectos de diferentes enmiendas orgánicas (compost, estiércol de ave y porcino) y métodos de establecimiento de planta (siembra o plantación), a través de las estaciones observadas en un bosque esclerófilo Mediterráneo Chileno durante un año. La estacionalidad, más que el tipo de enmienda y método de establecimiento, tuvo un fuerte efecto en la mayoría de los doce parámetros edáficos evaluados. A lo largo de las estaciones, el pH, conductividad eléctrica, contenido de agua, y la estabilidad de agregados fueron menos variables que diferentes formas de C y N, unidades formadoras de colonias (UFC) de microorganismos heterótrofos y fijadores de N_2 de vida libre, y que las actividades de ureasa y amonio monooxigenasa. Las enmiendas a base de estiércol aumentaron el NO_3 y las UFC heterótrofas durante otoño y verano, respectivamente. El uso de compost resultó en una mayor cantidad de materia orgánica y C en el suelo, principalmente en verano. Las diferentes respuestas de propiedades abióticas y bióticas del suelo después de incendios y enmiendas orgánicas probablemente pueden influir de manera diferente en procesos relacionados con recuperación ecosistémica, en particular los relacionados con los ciclos de C y N.

Palabras clave: bosque esclerófilo, ciclo del Nitrógeno, megasequía, restauración del suelo, salud del suelo.

INTRODUCTION

Fire occurrence in Mediterranean ecosystems of Chile (29-38 °S Latitude) has drastically raised over the last decades (Úbeda and Sarricolea, 2016). Although most of the fires registered in Chile are of anthropogenic origins (accidental or intentional), changes in local climatic conditions are also related to the increase of these events (Úbeda and Sarricolea, 2016; McWethy *et al.*, 2018). This is the case of increments in fire activity registered in the last decades, which have been related to an extended period of megadrought affecting the country since 2010 (González *et al.*, 2018). Such megadrought represents a multiyear period exhibiting annual rainfall deficits between 25% and 45% (Garreaud *et al.*, 2020), period that also evidences increases in temperature between 0.5 °C and 1 °C relative to 30 years (Garreaud *et al.*, 2017).

In this context, the country's south-central region (32-40 °S Latitude) experienced during the 2016-2017 summer an historical large-magnitude fire, a so-called extreme fire event or megafire, affecting close to 600,000 ha, the largest area recorded to be affected by fires since the early 1960s (De la Barrera *et al.*, 2018; McWethy *et al.*, 2018). This megafire not only affected the most populated regions in the country, but also Mediterranean sclerophyll forests, which are ecosystems especially prone to the occurrence of fire events due to land clearing and conversion to flammable exotic plantations, local climate change effects, and human induced ignition (González *et al.*, 2018; McWethy *et al.*, 2018). Indeed, the combination of changes in climate, and land cover and use have been globally and nationally recognized to increase the occurrence of megafires in Mediterranean ecosystems (McWethy *et al.*, 2018; Moreira *et al.*, 2020).

Projected trends on fire behavior in Chile are expected to endanger the resilience of forest ecosystems (Moreira *et al.*, 2020). Thus, efforts towards restoration of ecosystem functions following fires are crucial under scenarios where natural recovery is hampered (Muñoz-Rojas, 2018). In Mediterranean forests of central Chile, the focus of post-fire restoration has been mostly on the recuperation of aboveground vegetation, but the recovery of belowground conditions, including soil biodiversity has been less explored (Marín and Rojas, 2020; García-Carmona *et al.*, 2021). Such studies are critical, as understanding how soil biological and physicochemical conditions behave after fires is of central importance to elucidate ecosystem restoration (Thomas *et al.*, 2014). Soil properties as pH (Hart *et al.*, 2005), organic matter and carbon contents (Certini *et al.*, 2011), macronutrient contents (DeBano, 1991; Hart *et al.*, 2005; Bodí *et al.*, 2012), oxygen concentration (Certini, 2005), soil structure, porosity, and aggregation (DeBano, 1991), among others, are well known to be severely impacted by fires. However, how these changes affect soil microbial communities, which are usually more affected by fire than abiotic properties (Hart *et al.*, 2005; Guénon and Gros, 2016; Marín and Rojas, 2020), is less understood. This is of especial concern, since these communities are decisive to support plant establishment, nutrient cycling, among other key ecosystem functions (Fierer, 2017; Pérez-Valera *et al.*, 2019).

In addition, the effects of fire on a central soil component as soil organic matter (SOM) are highly variable and depend on fire type, intensity, slope, pre-fire soil conditions, among other factors, and can range from total loss to increases up to 30% (González-Pérez *et al.*, 2004). Moreover, fires have drastic effects on the C and N cycles, and overall cause significant losses of both macronutrients (Raison *et al.*, 2009; Pellegrini *et al.*, 2018). For example, Lavoie *et al.* (2010) found that fire immediately reduced total ecosystem C, in addition to P pools, by 40% and 27%, respectively. Increases in fire frequency have shown to reduce the availability of different N pools (Ojima *et al.*, 1994), and plant uptake (Grogan *et al.*, 2000).

The application of organic amendments following land burning has been widely used as a sustainable soil management practice, since they positively impact soil organic matter, biodiversity, and nutrient cycling (Cellier *et al.*, 2014; Guénon and Gros, 2016; García-Carmona *et al.*, 2021). These amendments can immediately boost soil fertility, microbial growth/activity, and plant establishment, while in the long term they improve soil structure and stability, consequently reducing the risks of erosion and nutrients leaching (Guerrero *et al.*, 2001; Ojeda *et al.*, 2003; Larchevêque *et al.*, 2010; García-Orenes *et al.*, 2013; Rhoades *et al.*, 2017). However, these results greatly depend on the type of organic amendment used, including origin, stability, and maturity, in addition to the doses and the methods of application to soils (Hueso-González *et al.*, 2018). Among organic amendments, fresh materials as animal manures have been shown to boost microbial activity short after application in fire-affected soils, while more stable materials as compost have been shown to potentially accumulate more soil C in the long term not only in burned soils (García-Carmona *et al.*, 2021), but also in soils under organic agricultural management and pastures (Bhowmik *et al.*, 2016).

Although the responses of soil conditions to seasonal variations following the occurrence of fires have been previously studied (Brockway *et al.*, 2002; Nardoto and Bustamante, 2003), much less is known about edaphic responses following the addition of organic amendments in a context of soil health (i.e., the capacity to function as a living system to support ecosystem benefits) recovery following fires. Therefore, the objective of this study was to compare responses of soil abiotic and biotic properties in soils amended with different organic amendments (compost, swine, and poultry manure) and plant establishment methods (sowing or plantation) at a sclerophyll forest affected by the 2016-2017 megafire across the four seasons typically observed in the Mediterranean zone of central Chile. These seasonal effects were considered as both originating from the season itself but also as time elapsed from treatment establishment; thus, different statistical models were performed to incorporate this consideration. In the present study, we asked whether soil abiotic and biotic conditions would respond in a deeper extend to seasonal variation as opposed to organic amendment and plant establishment method over a one-year period. To accomplish this, we determined C and N pools, selected enzyme activities, microbial counts, and specific soil chemical/physical variables.

MATERIALS AND METHODS

Study area

The study was conducted in a previously surveyed site (Marín and Rojas, 2020; García-Carmona *et al.*, 2021), which consist of a Mediterranean sclerophyll forest with the following dominant woody species: *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. This site is located within the Pumanque commune (34° 36.502' S, 71° 42.281' W; 130 m a.s.l.; MAT: 15.4 °C; annual rainfall: 451.0 mm for 2018), specifically at the 'secano interior' biome (dryland to the east front of the coastal mountain range), in the O'Higgins Region, one of the administrative regions most affected by the 2016-2017 megafire. According to local residents, at least for the last 30 years before 2017, there have been no reports of fire events in the specific site. At this location soils are classified as Aquic Dystroxerepts (Inceptisol), exhibiting depths of around ~40 cm, and a sandy loam class texture, with 10% slope facing south (Marín and Rojas, 2020; García-Carmona *et al.*, 2021).

Experimental design and soil sampling

A completely randomized design with two factors was established in early June of 2018 in a fire-affected area (50 m x 50 m) within the research site (Marín and Rojas, 2020). This design consisted of 1) organic amendment type (no amendment, compost, poultry, and swine manure), and 2) vegetation establishment method (sowing or plantation) (Table 1). In addition, a selected unburned native forest area (20 m x 20 m) at approximately 500 m distance was included as reference (Marín and Rojas, 2020). All treatments (10 in total) were randomly distributed in duplicate plots (3 m x 3 m), within the corresponding research areas, with at least 1.5 m of separation among each other. Chemical analyses of organic amendments have been previously reported (Marín and Rojas, 2020). Briefly, poultry manure, swine manure, and compost had pH values of 6.77, 8.30, and 7.41, and electrical conductivity (EC) values of 3.56 mS cm^{-1}, 2.03 mS cm^{-1}, and 1.12 mS cm^{-1}, respectively. Organic carbon and total nitrogen contents were 10.38% and 0.83% in compost, 23.47% and 1.92% in poultry manure, and 12.89% and 0.75% in swine manure. The dry weight values were 65.64%, 94.32%, and 89.41% for compost, poultry manure, and swine manure, respectively. Total P and cations contents were rather low, with total P values of 0.50%, 0.52%, and 0.70%, and Ca values of 4.14%, 0.25%, and 1.41%, for poultry and swine manure, and compost, respectively. Metal concentrations were slightly high, reaching levels up to 14,587 mg kg^{-1} of Fe in compost, 584 mg kg^{-1} of Mn in swine manure, and 208 mg kg^{-1} of Zn and 103 mg kg^{-1} of Cu in poultry manure (Marín and Rojas, 2020). These amendments were manually incorporated over the top 30 cm of soil, following rototilling at the same depth. All amended plots were covered with a mulch layer (1 cm approx.) 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 (*Peumus boldus*, *Quillaja saponaria*, and *Lithraea caustica*) (Marín and Rojas, 2020) (Table 1).

For the present work, plots from a previous study (Marín and Rojas, 2020) were revisited. Such study aimed to evaluate early responses of simple and approachable soil health indicators (i.e., heterotrophic colony forming units (CFUs), pH, electrical conductivity (EC), gravimetric water content (GWC), and aggregates stability (AE)) six months following fire. Main finding from that previous work evidenced the influence of organic amendments and plant establishment in microbial conditions only, while chemical parameters were solely affected by the type of organic amendments used (Marín and Rojas, 2020). For the present study, soil samples were obtained in 2019 and 2020, over the four seasons typically observed in the Mediterranean zone of central Chile (Armesto *et al.*, 2007): Fall (April 2019; 10 months after treatment establishment), Winter (July 2019; 13 months later), Spring (October 2019; 16 months later), and Summer (January 2020; 19 months later) (Table 1). At each plot, five soil subsamples were collected at the corners and center, at a 6 cm depth in the A horizon, following the removal of organic debris. Soil subsamples were thoroughly mixed in the field to obtain a composite sample per plot (1 kg approx.). These samples were transported to the laboratory (under sterile and refrigerated conditions, approx. 4 °C) and then split in two portions to obtain: 1) a fraction for biological analyses and for the determination of gravimetric

Table 1. Treatments and date of soil sampling timing for the present study.

Cuadro 1. Tratamientos y tiempos de muestreo del suelo para el presente estudio.

Treatments[1]	Date of soil sampling	Month of sampling following amendment establishment
Reference[2]	April 2019 (Fall) July 2019 (Winter) October 2019 (Spring) January 2020 (Summer)	10 13 16 19
Control[3]		
Control-sowing		
Control-plantation		
Compost-sowing		
Compost- plantation		
Poultry manure- sowing		
Poultry manure- plantation		
Swine manure- sowing		
Swine manure- plantation		

[1] Species sown or planted corresponded to *Peumus boldus*; *Quillaja saponaria*; and *Lithraea caustica* established in June 2018.

[2] Unburned sclerophyllous forest included as reference ecosystem, with similar vegetation and use as the burned area before land burning.

[3] Plots for this treatment corresponded to burned soils without the addition of amendments, mulch layer or plant establishment.

water content (GWC), and 2) a second fraction for the remaining soil analyses.

Soil analyses

Soil chemical and physical analyses: Soil pH and electrical conductivity (EC) were determined in a 1:10 soil:water ratio after weighting 10 g of oven-dried and sieved (to a 2 mm diameter) soil in 100 ml of distilled water; GWC was determined by oven-drying 10 g of soil sample at 105 °C to constant weight (Sadzawka *et al.*, 2006). Aggregate stability (AS) was determined by wet sieving (Kemper and Rosenau, 1986) using a wet sieving apparatus (Eijkelkamp Soil & Water. Giesbeek, Netherlands).

Nitrogen and Carbon pools: Soil organic matter (SOM) was determined by calcination at 550 °C (CNA-SCCS, 2007). Total carbon (C) was determined by a CN Elemental Analyzer (LECO corporation, 2014). Contents of NH_4^+ and NO_3^- were determined by the Kjeldahl digestion method using sulfuric and salicylic acids (Mulvaney, 1996; Sadzawka *et al.*, 2006).

Enzyme activity and microbial counts: Urease activity was determined using the indophenol blue technique according to García *et al.* (2003), while ammonia monooxygenase (AMO) activity was determined by the methyl fluoride and dimethyl ether method (Dick *et al.*, 1996; Arp *et al.*, 2002). Microbial counts of culturable aerobic heterotrophic mesophiles were determined by plate count of colony-forming-units (CFUs) (Novo *et al.*, 2015). The 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 three days at 25 °C (Novo *et al.*, 2015). Similarly, CFUs of culturable free-living N_2 fixers were determined by serial dilutions in 0.1% peptone buffer and spread plating on a carbon source (sucrose, lactate, and mannitol) medium (Barraquio *et al.*, 1988; Novo *et al.*, 2015). The CFU counts were adjusted for GWC and reported based on oven-dried soil weight.

Statistical analysis

The homogeneity of variances and normality of the residuals were checked using the Bartlett test and graphical checks, respectively, before ANOVAs. Three-way ANOVAs were performed to evaluate the effects of amendment, vegetation establishment method, season, and their interaction in soil chemical/physical parameters, C and N pools, and related enzyme activity and microbial counts using the R-base functions ‘*lm*’ and ‘*anova*’ in Rstudio (R Core Team, 2020). Two model types were run: in the first one, season was considered as a categorical variable, while in the second one it was considered as a continuous variable (number of months elapsed after treatment establishment). We consider necessary to test the effects of season in this way, due to the relatively short time passed after the fire event, treatment application, and the evaluated seasonal responses. In this way it is possible to check whether the results obtained are mostly explained by the season itself or by the time following amendment establishment. Most post-fire soil recovery studies treat the time as a categorical variable, as they tend to sample across several years, but for seasonal responses to fire, other approaches should be considered (Moura *et al.*, 2022). In addition, two series of Tukey HSD tests were performed to test the nature of the differences among 1) the 10 treatments, and 2) the four seasons by each treatment, using the base function '*Tukey HSD*' in RStudio.

RESULTS

Season had very strong effects on most soil conditions when analyzed as a categorical variable (Table 2a), except for NO_3^- and CFUs of culturable free-living N_2 fixers. The type of organic amendment applied had very strong effects on soil pH, NO_3^-, total C, and SOM contents, and to a lesser degree on GWC, AS, and NH_4^+, while no effect was observed for the biotic parameters evaluated (Table 2a). Among the twelve variables analyzed, NO_3^- was the parameter that responded the most to the interactions tested, with the only exception of that between vegetation and season, while GWC significantly responded to the interaction between amendment and season (Table 2a). Similar trends were observed when season was considered as a continuous variable (number of months after treatment establishment; Table 2b), affecting all soil parameters except for AS, NO_3^-, and CFUs of culturable free-living N_2 fixers. Comparable to model 1, model 2 evidenced the effects of organic amendments in the same soil parameters evaluated except for GWC and NH_4^+. The method of vegetation establishment (sowing nor planting) had no effects on biotic and abiotic parameters with the two models tested (Table 2).

Overall and across seasons, soil chemical/physical parameters (Figure 1) were less variable than C and N pools (Figure 2) and biotic parameters (Figure 3, Figure 4). Moreover, when the four seasons were taken together within the same treatment, most of these variables showed no significant differences among them, with the exception of pH which was significantly lower in reference soils (5.75 – 6.18) than the rest of the samples (6.56 – 7.14) (Figure 1a), and NO_3^- contents, which were significantly greater in soils receiving poultry manure and plants (4.55 – 58.13 mg kg^{-1}) than the other treatments (0 – 13.42 mg kg^{-1}) (Figure 2d).

Regarding chemical/physical parameters (Figure 1), seasonal variation of GWC behaved as expected, as significantly higher values were registered in winter (15.69% – 21.98%), followed by fall (5.27% – 6.51%), spring (1.92% – 3.22%), and summer (0.40% – 1.63%), with all treatments following the same pattern (Figure 1c). Besides GWC, variation across seasons was most notable in EC, usually with higher values in the fall (0.138 – 0.169 dS m^{-1}), followed by winter (0.134 – 0.142 dS m^{-1}), spring (0.116 – 0.142 dS m^{-1}), and summer (0.111 – 0.126 dS m^{-1}) (Figure 1b). pH values were in general slightly higher during winter (6.18 – 7.11) than the rest of the seasons (5.75 – 7.14) (Figure 1a). As per AS, vales did not show a clear trend, which can likely be due to the legacy effect of rototilling shading the influence of treatments; however, in general these were lower during the winter (Figure 1d).

The C and N pools behaved differently according to seasons (Figure 2), which in general showed more notable responses in summer and fall. The highest values for SOM were always observed during summer for all treatments (14.5% – 22.95%), followed by spring (8.32% – 14.965%) and fall (6.44% – 12.765%) in most of the cases (Figure 2a). As expected, total carbon followed a very similar pattern to that observed for SOM (Figure 2b). The NH_4^+ contents showed similar patterns across season for the treatments evaluated, with an average of 2 to 3-fold higher values in fall (19.775 – 61.41 mg kg^{-1}) when compared to the second greatest values observed in winter (13.145 – 21.145 mg kg^{-1}), which drastically decreased in the other two seasons (0 – 11.12 mg kg^{-1}) (Figure 2c). On the contrary, NO_3^- content did not follow a clear seasonal pattern and showed great variation (0 – 58.13 mg kg^{-1}), with the higher value observed in fall in soils receiving poultry manure and plants (Figure 2d).

Biological soil conditions assessed in this study evidenced high variation among data as reflected by large standard deviations (Figure 3, Figure 4). Regardless, slight tendencies were observed across seasons. Regarding microbial counts, CFUs of culturable aerobic heterotrophic mesophiles where higher during the summer (0.3 – 30.0 x 10^7 CFUs g^{-1}) compared to the other seasons (0.002 – 4.1 x 10^7 CFUs g^{-1}), with the greatest values observed in soils receiving swine manure, followed by those receiving poultry manure (Figure 3a). As opposed to CFUs of aerobic heterotrophic mesophiles, CFUs of free-living N_2 fixers showed no clear pattern; however, higher values were observed in fall for soils receiving poultry manure and seeds (40.6 x 10^4 CFUs g^{-1}), and in spring for soils with compost and plants (37.1 x 10^4 CFUs g^{-1}) (Figure 3b). Enzyme activity showed clear responses according to season (Figure 4). Urease activity was generally greater during spring (8.9 – 69.4 µg of NH_4^+ g^{-1} h^{-1}) and fall (35.3 – 64.0 µg of NH_4^+ g^{-1} h^{-1}), followed by winter (9.6 – 34.6 µg of NH_4^+ g^{-1} h^{-1}), while in summer its activity was almost negligible (0 – 0.6 µg of NH_4^+ g^{-1} h^{-1}) (Figure 4a). The AMO activity was generally greater in the summer (0.7 – 1.9 µg of NO_2^- g^{-1} h^{-1}), except for the compost amended soils receiving seeds and plants, where higher activities were registered in fall (1.4 µg of NO_2^- g^{-1} h^{-1}) and winter (1.5 µg of NO_2^- g^{-1} h^{-1}), respectively (Figure 4b).

Table 2. Three-way ANOVAs showing the effects of organic amendment type (amendment t.), vegetation establishment method (vegetation m.), and time (season) on the soil parameters evaluated. Model 1: time as categorical variable (seasons). Model 2: time as continuous variable (months elapsed since treatments establishment).

Cuadro 2. ANOVAs de tres factores mostrando los efectos del tipo de enmienda orgánica (*amendment t.*), El método de establecimiento de la vegetación (*vegetation m.*), y el tiempo (*season*) sobre los parámetros del suelo evaluados. Modelo 1: tiempo como variable categórica (estaciones). Modelo 2: tiempo como variable continua (meses transcurridos desde el establecimiento de los tratamientos).

Factor	pH	EC	GWC	AS	Urease	AMO	NH_4+	NO_3-	TC	SOM	Mes	Nfx
a. Model 1: time as categorical variable												
Amendment t.	32.270^{***}	0.211^{ns}	3.537^{*}	3.681^{*}	0.751^{ns}	2.193^{ns}	3.214^{*}	10.849^{***}	14.659^{***}	11.597^{***}	1.133^{ns}	2.098^{ns}
Vegetation m.	0.369^{ns}	1.014^{ns}	1.007^{ns}	1.098^{ns}	0.003^{ns}	0.080^{ns}	0.287^{ns}	1.495^{ns}	0.075^{ns}	0.263^{ns}	0.464^{ns}	0.234^{ns}
Season	13.906^{***}	61.445^{***}	1053.158^{***}	8.652^{***}	24.407^{***}	15.440^{***}	72.519^{***}	2.117^{ns}	111.423^{***}	47.208^{***}	5.032^{**}	1.195^{ns}
Amend t.: Veget m.	1.970^{ns}	0.966^{ns}	0.441^{ns}	1.168^{ns}	1.930^{ns}	0.583^{ns}	0.514^{ns}	5.612^{**}	1.546^{ns}	1.152^{ns}	0.430^{ns}	0.924^{ns}
Amendment t.: Season	0.587^{ns}	1.071^{ns}	3.896^{***}	0.826^{ns}	0.182^{ns}	1.463^{ns}	1.308^{ns}	2.405^{*}	1.207^{ns}	1.370^{ns}	1.160^{ns}	0.608^{ns}
Vegetation m.: Season	1.020^{ns}	1.464^{ns}	0.546^{ns}	1.348^{ns}	0.684^{ns}	0.955^{ns}	0.357^{ns}	1.100^{ns}	0.407^{ns}	0.230^{ns}	0.324^{ns}	1.203^{ns}
Amend t.: Veget m.: Season	1.008^{ns}	1.517^{ns}	0.639^{ns}	0.326^{ns}	1.559^{ns}	1.835^{ns}	0.596^{ns}	3.486^{**}	0.733^{ns}	0.452^{ns}	0.499^{ns}	1.280^{ns}
R^2	0.851	0.849	0.988	0.627	0.719	0.708	0.865	0.773	0.913	0.842	0.513	0.508
b. Model 2: time as a continuous variable												
Amendment t.	31.684^{***}	0.185^{ns}	0.102^{ns}	2.960^{*}	0.579^{ns}	1.375^{ns}	2.059^{ns}	6.297^{***}	5.919^{***}	6.665^{***}	1.201^{ns}	2.122^{ns}
Vegetation m.	0.298^{ns}	0.888^{ns}	0.029^{ns}	0.883^{ns}	0.003^{ns}	0.050^{ns}	0.184^{ns}	0.868^{ns}	0.030^{ns}	0.151^{ns}	0.492^{ns}	0.237^{ns}
Season	16.641^{***}	157.736^{***}	22.306^{***}	0.677^{ns}	35.299^{***}	8.191^{**}	108.880^{***}	3.457^{ns}	99.501^{***}	46.943^{***}	9.382^{**}	1.147^{ns}
R^2	0.667	0.690	0.240	0.166	0.343	0.161	0.620	0.297	0.613	0.507	0.174	0.124

Mes=culturable aerobic heterotrophic mesophiles. Nfx=culturable free-living N_2 fixer bacteria. F values are presented with p-values as asterisks. *p*-value: *<0.05, **<0.01, ***<0.001, ^{ns}=non-significant.

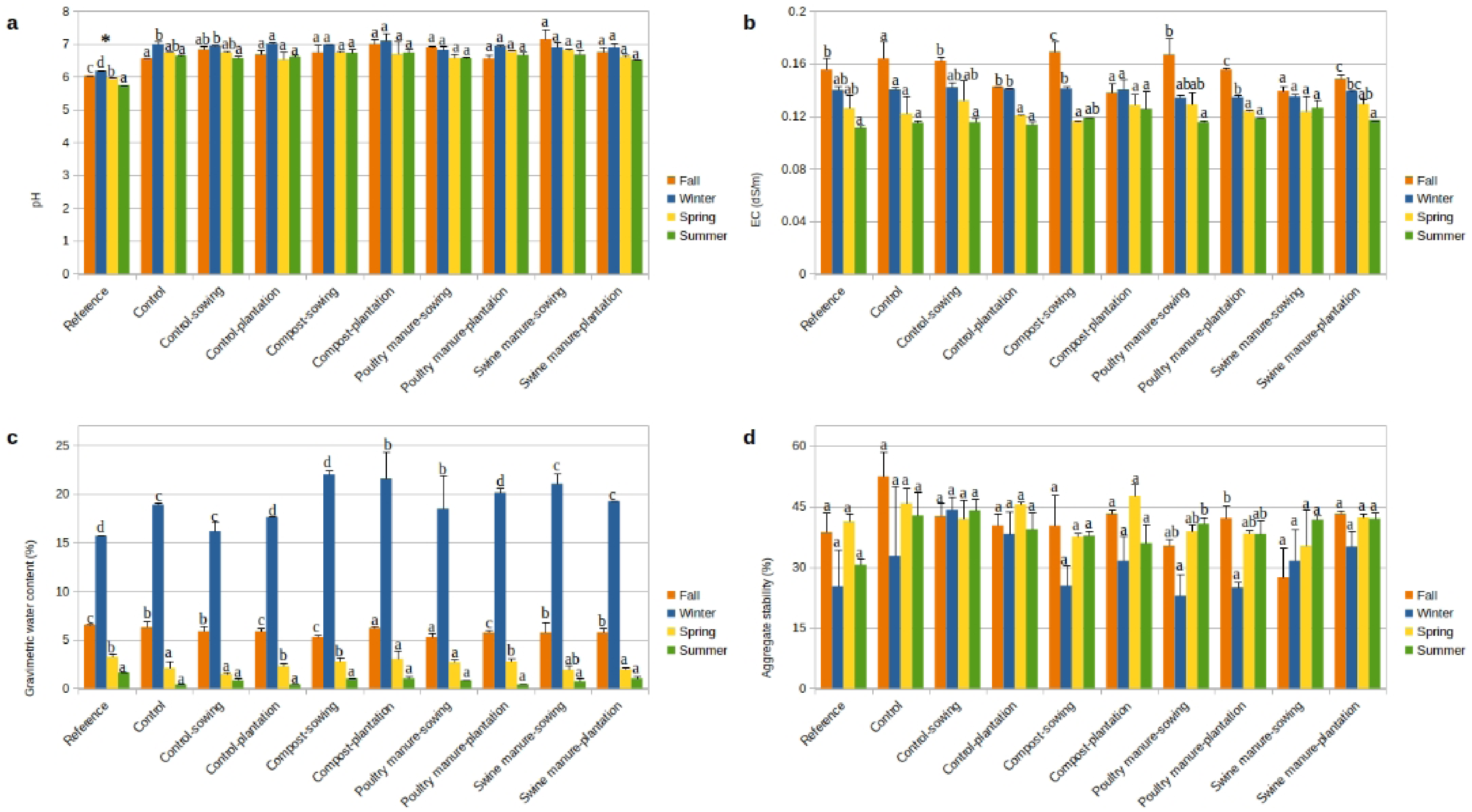

Figure 1. Effects on soil physical-chemical properties. **a.** pH. **b.** Electrical conductivity (EC). **c.** Gravimetric water content (%). **d.** Aggregate stability (%). Small letters indicate significant ($p < 0.05$) differences among seasons, while asterisks indicate significant ($p < 0.05$) differences among treatments, according to the Tukey HSD test.

Figura 1. Efectos sobre las propiedades físico-químicas del suelo. **a.** pH. **b.** Conductividad eléctrica (EC). **c.** Contenido gravimétrico de agua (%). **d.** Estabilidad de agregados (%). Las letras minúsculas indican diferencias significativas ($p < 0,05$) entre estaciones, mientras que asteriscos indican diferencias significativas ($p < 0,05$) entre tratamientos, según la prueba Tukey HSD.

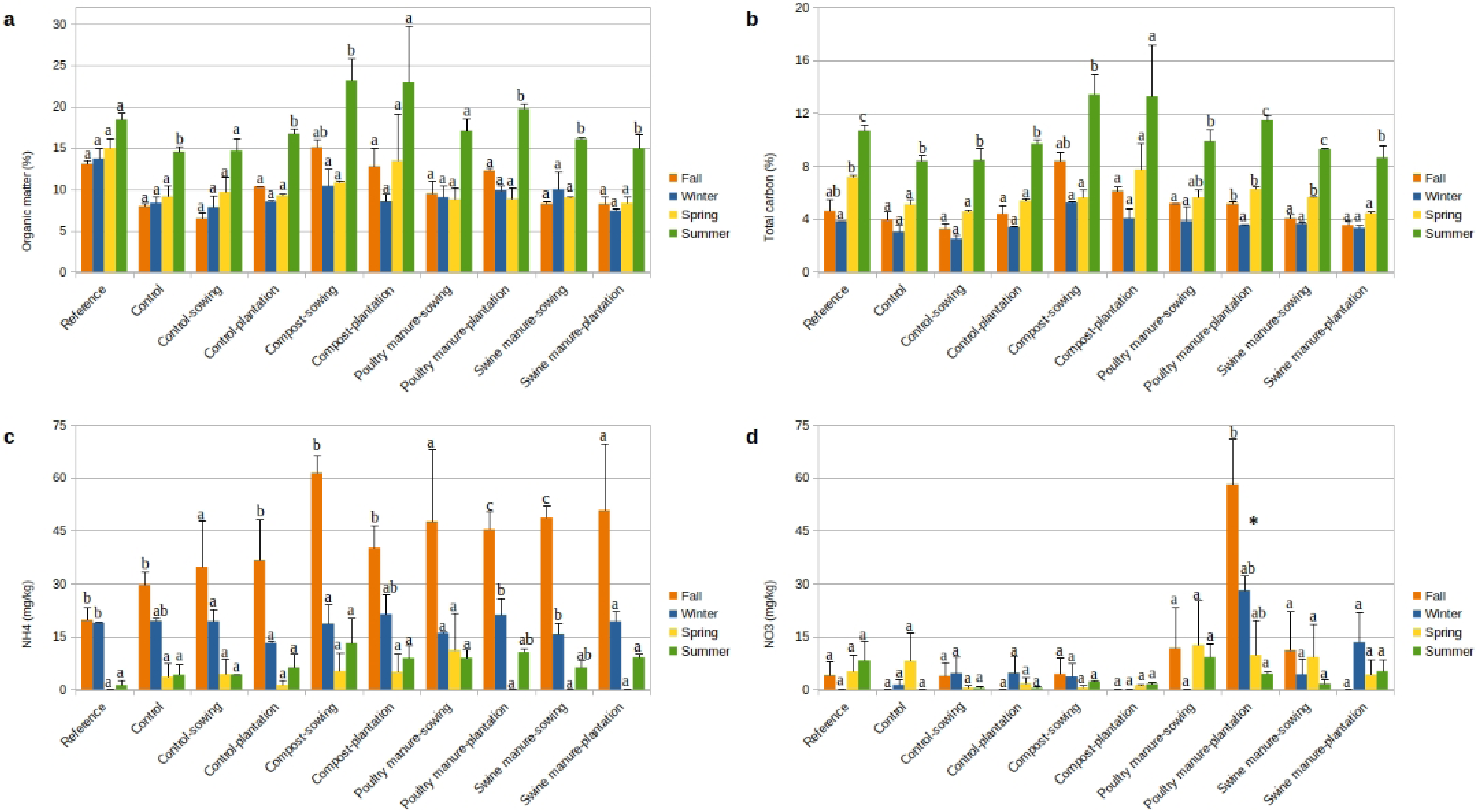

Figure 2. Effects on soil macro-nutrients. **a.** Organic matter. **b.** Total carbon. **c.** NH_4^+. **d.** NO_3^-. Small letters indicate significant ($p < 0.05$) differences among seasons, while asterisk indicate significant ($p < 0.05$) differences among treatments, according to the Tukey HSD test.

Figura 2. Efectos sobre los macronutrientes del suelo. **a.** Materia orgánica. **b.** Carbono total. **c.** NH_4^+. **d.** NO_3^-. Las letras minúsculas indican diferencias significativas ($p < 0,05$) entre estaciones, mientras que asteriscos indican diferencias significativas ($p < 0,05$) entre tratamientos, según la prueba Tukey HSD.

DISCUSSION

Seasonal effects over vegetation establishment and organic amendment treatments

Results indicated that soil biotic and abiotic conditions responded to a greater extent to the seasonal patterns typically observed in the Mediterranean zone of central Chile, than to the organic amendments and plant establishment methods tested in this study. This was particularly the case for most of biotic parameters followed by C and N pools. In forest ecosystems, potential long-term impact of fires on physical, chemical, and biological soil properties have been reported (Xue *et al.*, 2014). In addition, seasonal shifts in soil conditions have been observed after the occurrence of land burning (Brockway *et al.*, 2002; Nardoto and Bustamante, 2003); however, these responses coupled with the application of organic amendments following land burning are less understood. One long-

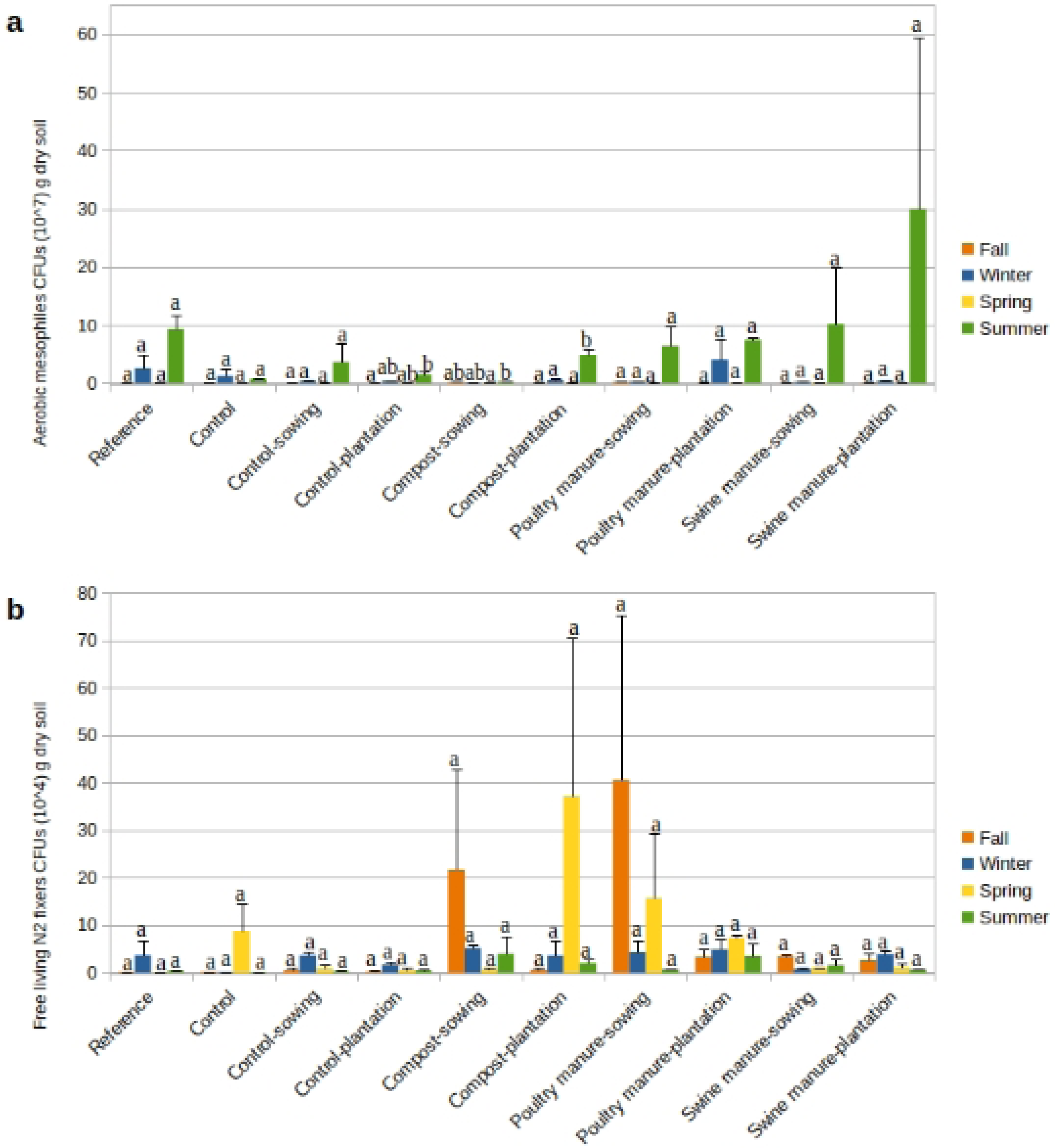

Figure 3. Effects on soil microbial diversity. **a.** Aerobic mesophiles. **b.** Free-living N_2 fixers. Small letters indicate significant ($p < 0.05$) differences among seasons; there were no significant ($p < 0.05$) differences among treatments, according to the Tukey HSD test.

Figura 3. Efectos sobre la diversidad microbiana del suelo. **a.** Mesófilos aeróbicos. **b.** Fijadores de N_2 de vida libre. Las letras minúsculas indican diferencias significativas ($p < 0,05$) entre estaciones; no hubo diferencias significativas ($p < 0,05$) entre tratamientos, según la prueba Tukey HSD.

term experiment though, which included prescribed fires and subsequent fertilization in native tallgrass prairies, has shown greater responses of both bacterial and fungi population size, but very few changes in individual taxonomic groups, to seasonal variation rather than to management type (Carson and Zeglin, 2018). The biological responses observed in our study can likely influence soil processes across seasons at the studied ecosystem, as temporal responses of biotic properties, including microbial community structure and activity, have been related to functionality in other forest ecosystems (Vargas and Allen, 2008; Koranda *et al.*, 2013; Luo *et al.*, 2020). Although seasonality was the factor with stronger influences in soil parameters in our study, future work should test if such responses are consistent over longer periods, especially in a scenario of increased drought in Chilean Mediterranean ecosystems (Garreaud *et al.*, 2020), where drier summers or organic amendment legacy effects could alter the patterns observed here.

Variations of soil biological properties across seasons have been attributed to fluctuations in precipitation, soil water content, air and soil temperature,

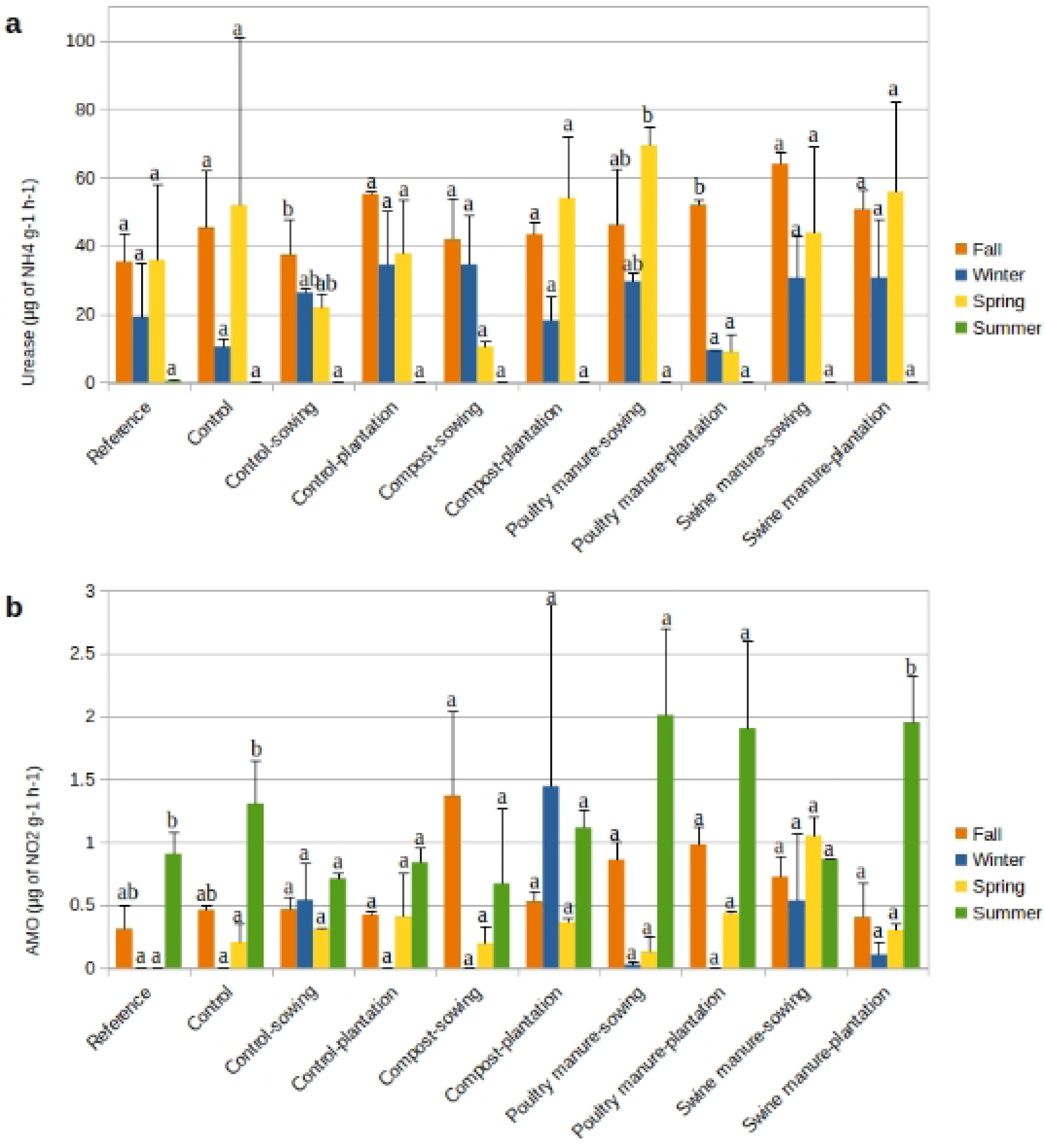

Figure 4. Effects on soil microbial activity. **a.** Urease. **b.** AMO. Small letters indicate significant ($p < 0.05$) differences among seasons; there were no significant ($p < 0.05$) differences among treatments, according to the Tukey HSD test.

Figura 4. Efectos sobre la actividad microbiana del suelo. **a.** Ureasa. **b.** AMO. Las letras minúsculas indican diferencias significativas ($p < 0,05$) entre estaciones; no hubo diferencias significativas ($p < 0,05$) entre tratamientos, según la prueba Tukey HSD.

and labile organic inputs from vegetation (Boerner *et al.*, 2005; Koranda *et al.*, 2013; Carson and Zeglin, 2018; Luo *et al.*, 2020), factors that can likely explain responses according to season registered in our study. In the case of heterotrophic CFUs, higher counts in summer coincided with greater SOM, total C, and with very low GWC values registered for this season. This dry period also coincided with the highest AMO activity observed under the studied conditions, while the greatest values for urease activity were in general higher in spring and fall periods. Increases in soil enzyme activity have been argued to occur under low water contents due to the microbial need to produce enzymes under these circumstances, where access to soil resources is impeded (Alster *et al.*, 2013; Luo *et al.*, 2020). Moreover, decreases in enzyme activity during warm periods as summer have been related to increases in plant transpiration, which ultimately reduce soil water content (Allison and Treseder, 2008; Luo *et al.*, 2020).

The type of organic amendment applied to the fire-affected soils under the sclerophyll forest seemed to influence the size of the culturable microbial heterotrophic CFUs and AMO activity, as higher values during summer were in general obtained from manure amended soils. These results follow previous findings at the same study site, where after six month of treatment establishment greater responses to swine and poultry manures, compared to compost, were observed for heterotrophic CFUs (Marín and Rojas, 2020), and after eight months greater values of basal respiration and microbial biomass were registered (García-Carmona *et al.*, 2021). The stabilization degree of organic amendments is known to affect microbial responses once they are incorporated to soils (Jorge-Mardomingo *et al.*, 2013; García-Carmona *et al.*, 2021): while fresh materials represent a source of greater labile organic fractions with short turnover times (Haynes, 2005), stabilized materials, such as compost, provide a high proportion of stable organic substances contributing to soil nutritional conditions in the long term (García-Carmona *et al.*, 2021). Regarding C and N pools, in the form of SOM, total C, and NH_4^+ contents, these were highly affected by seasons, with the summer period evidencing the greatest values for SOM and C, and the fall season the highest values for NH_4^+. The method of plant establishment (sowing or plantation) had no effects on any of the soil variables measured in this study, even though in a previous study assessing the same treatment, the effect of it was observed in microbial conditions (Marín and Rojas, 2020). Thus, further studies should include the growth plant responses of soil conditions, as it is known that vegetation dynamics following fires influence soil microbial conditions over time (Hart *et al.*, 2005).

Implications for soil restoration and ecosystem functions

The reestablishment of a key soil component as organic matter in restoration processes is an effective strategy to induce positive cascading effects on related soil properties, and consequently on soil health and ecosystem functions recovery (Heneghan *et al.*, 2008; Larney and Angers, 2012; Hueso-González *et al.*, 2018). In a context in which the recovery of ecosystem functionality could be hampered, the correct application of organic amendments would help to regain C and N pools, and thus essential soil microbial functions (Cellier *et al.*, 2014; Luna *et al.*, 2018). In our study, the use of compost as organic amendment resulted in greater soil organic matter and C over time and across seasons, which could ensure greater long-term positive impacts on edaphic conditions due to lower turnover times (Haynes, 2005), for example, strongly influencing nutrient cycling and storage over time, soil structure stability, and water holding capacity, thus improving soil health and productivity.

Although C and N pools were greatly influenced by season, they also responded to the type of organic amendment used (particularly NO_3^-), reflecting the legacy effect of these materials on C and N soil constituents. This phenomenon was reflected by the lasting effect of compost application in total C across seasons, since soils treated with this material showed the highest total C contents after 10-, 13-, 16-, and 19-months following amendment application. In a previous study at this site following eight months of amendment application, soils treated with compost accumulated the most organic carbon and total nitrogen, ensuring long-term nutrient release and thus long-term soil function recovery, as opposed to manure amended soils which evidenced higher mineralization rates, and thus presumably shorter periods of C source consumption (García-Carmona *et al.*, 2021). As opposed to greater total nitrogen contents reported previously for soils amended with compost, inorganic N sources in the present study are comparable among treatments, except for NO_3^- in soils receiving poultry manure and plants, particularly during fall. This increase is consistent with previous studies which have long reported that this type of amendment highly increases soil NO_3^- contents (Ruiz Diaz *et al.*, 2008), and even potentially soil nutrients leaching (Liebhardt *et al.*, 1979; Adams *et al.*, 1994).

Soil microbial functioning is a key component of successful restoration and rehabilitation processes; thus, monitoring soil microbial parameters to describe soil health during these processes is of great importance (Hart *et al.*, 2020). In our study, we confirmed the greater sensibility of biological conditions over chemical/physical parameters following land burning and organic amendment application. Among biological soil indicators, several microbial parameters are used as sensitive markers of ecological stresses suffered by soils, providing immediate and precise information on their recovery, e.g., after a wildfire (Hart *et al.*, 2005; Muñoz-Rojas *et al.*, 2016). In this sense, the activities of soil enzymes involved in the C and N cycles are good predictors of post-fire ecosystem recovery, as they are usually more affected by fire than these nutrients pools (Eivazi and Bayan, 1996; Raison *et al.*, 2009). Similarly, accounting for the microbial diversity of organisms directly involved in certain nutrient cycles (i.e., some bacterial groups involved in the N cycle) represents a direct way to test the effects of fire on such turnover processes (Raison *et al.*, 2009). Direct counts of culturable microorganisms, although not accounting for the majority of the soil microbial community (Martiny, 2019), is a simple and quick indicator of soil microbial diversity that can be used to monitor soil restoration processes, particularly when used for specific taxa (Hart *et al.*, 2020). Indeed, soil biotic status, although known more sensitive to the effects of fires than abiotic conditions (Mataix-Solera *et al.*, 2009), shows variable responses to fires according to the method used to test it (Pérez-Valera *et al.*, 2019). Thus, incorporating more in-depth analyses to better decipher the effect of land burning on soil microbial structure and related functioning is of particular interest, particularly those based on DNA approaches as they have proven to pick to a greater extent the effect of forest fires (Pérez-Valera *et al.*, 2019).

In the case of enzymes involved in the degradation of organic matter, they usually decrease following land burning, while soil enzymes related to the organic N decomposition have been shown to differ in activity after these events (Docherty *et al.*, 2012). Among enzymes related to the N cycle, urease activity is a good indicator to capture the effects of land burning on soil biological conditions (Cetin *et al.*, 2009), while AMO activity, which catalyzes the first and limiting step of nitrification, has been related to change according to fire-induced changes in the vegetative community (Hart *et al.*, 2005). In our study, urease and AMO activities did not show differences across treatments, which could be indicative of the progress in edaphic processes following restoration, as amended soils are comparable to control soils. However, this observation should be taken with caution due to the great variation observed among data. Regardless, their responses to seasons, particularly to spring and fall in the case of urease and summer in the case of AMO, allows to register temporal effects on N-cycle related processes and potential soil functioning across seasons under the studied conditions.

CONCLUSIONS

In a global context where forest fires are predicted to increase, particularly in Mediterranean ecosystems, a better understanding of soil conditions following land burning and post-fire management practices is crucial to support the recovery of aboveground vegetation and functioning. In this study, we observed greater responses of abiotic and biotic soil parameters to the seasonal variation typically observed in central Chile rather than to the type of organic amendment applied or the method used for plant establishment. Among the soil conditions tested, chemical/physical variables and C and N pools were less variable across seasons than most of the biological parameters evaluated, confirming the greater sensitivity of the latter to temporal shifts. Soils treated with manure-based amendments showed greater NO_3^- pools and heterotrophic CFUs, particularly in fall and summer, respectively, while compost-amended soils had greater organic matter and carbon, most notably in summer. Such soil responses likely influence at a different extent ecological process taking place over ecosystem recovery. Thus, further insights related to the implications of these variations in the recovery of soil functions are central to sustaining the resilience of a fire-prone ecosystem such as sclerophyll forests in Central Chile.

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