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

Marín, C.*, Rojas, C.h.c*

* Centro de Investigación e Innovación para el Cambio Climático (GiIC), Universidad Santo Tomás, Santiago, Chile.
* Laboratorio de Ecología Microbiana y Biogeoquímica de Suelos (LEMiBiS), Instituto de Ciencias Agroalimentarias, Animales y Ambientales (ICA3), Universidad de O’Higgins, 3070000 San Fernando, Chile.
* Center of Applied Ecology and Sustainability (CAPES), 8331150 Santiago, Chile.

ARTICLE INFO

Article history:
Received 23.12.2021
Accepted 19.05.2022

Keywords:
N cycle
Sclerophyll forest
Soil health
Soil restoration
N cycle
Megadrought

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, 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, 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, megadequía, 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 multyear 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; Bodi 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 (Pierce, 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 trimervis 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 Dystroxepts (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.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Date of soil sampling</th>
<th>Month of sampling following amendment establishment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control-sowing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control-plantation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compost-sowing</td>
<td>April 2019 (Fall)</td>
<td>10</td>
</tr>
<tr>
<td>Compost-plantation</td>
<td>July 2019 (Winter)</td>
<td>13</td>
</tr>
<tr>
<td>Compost-plantation</td>
<td>October 2019 (Spring)</td>
<td>16</td>
</tr>
<tr>
<td>Poultry manure- sowing</td>
<td>January 2020 (Summer)</td>
<td>19</td>
</tr>
<tr>
<td>Poultry manure- plantation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swine manure- sowing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swine manure- plantation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.

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 monoxygenase (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 NO₃ and CFUs of culturable free-living N₃ fixers. The type of organic amendment applied had very strong effects on soil pH, NO₃, total C, and SOM contents, and to a lesser degree on GWC, AS, and NH₄⁺, while no effect was observed for the biotic parameters evaluated (Table 2a). Among the twelve variables analyzed, NO₃ 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, NO₃, and CFUs of culturable free-living N₃ fixers. Comparable to model 1, model 2 evidenced the effects of organic amendments in the same soil parameters evaluated except for GWC and NH₄⁺. 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 NO₃ 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, values 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 NH₄⁺ 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, NO₃ 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 10⁶ CFUs g⁻¹) compared to the other seasons (0.002 – 4.1 x 10⁶ 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 N₃ fixers showed no clear pattern; however, higher values were observed in fall for soils receiving poultry manure and seeds (40.6 x 10⁴ CFUs g⁻¹), and in spring for soils with compost and plants (37.1 x 10⁴ 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 NH₄ g⁻¹ h⁻¹) and fall (35.3 – 64.0 μg of NH₄ g⁻¹ h⁻¹), followed by winter (9.6 – 34.6 μg of NH₄ g⁻¹ h⁻¹), while in summer its activity was almost negligible (0 – 0.6 μg of NH₄ g⁻¹ h⁻¹) (Figure 4a). The AMO activity was generally greater in the summer (0.7 – 1.9 μg of NO₂ 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 NO₂ g⁻¹ h⁻¹) and winter (1.5 μg of NO₂ g⁻¹ h⁻¹), respectively (Figure 4b).
Cuadro 2. Three-way ANOVAs showing the effects of organic amendment type (amendment t.), vegetation establishment method (vegetation m.), and time (season) on the soils. 

<table>
<thead>
<tr>
<th>Variable</th>
<th>Amendment t.</th>
<th>Vegetation m.</th>
<th>Season</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC</td>
<td><strong>0.01</strong></td>
<td><strong>0.01</strong></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td><strong>0.01</strong></td>
<td><strong>0.01</strong></td>
<td></td>
</tr>
<tr>
<td>Redox</td>
<td><strong>0.01</strong></td>
<td><strong>0.01</strong></td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td><strong>0.01</strong></td>
<td><strong>0.01</strong></td>
<td></td>
</tr>
<tr>
<td>Organic N</td>
<td><strong>0.01</strong></td>
<td><strong>0.01</strong></td>
<td></td>
</tr>
<tr>
<td>Humic N</td>
<td><strong>0.01</strong></td>
<td><strong>0.01</strong></td>
<td></td>
</tr>
<tr>
<td>SOM</td>
<td><strong>0.01</strong></td>
<td><strong>0.01</strong></td>
<td></td>
</tr>
<tr>
<td>C:NC</td>
<td><strong>0.01</strong></td>
<td><strong>0.01</strong></td>
<td></td>
</tr>
<tr>
<td>C:OM</td>
<td><strong>0.01</strong></td>
<td><strong>0.01</strong></td>
<td></td>
</tr>
<tr>
<td>C:O:OM</td>
<td><strong>0.01</strong></td>
<td><strong>0.01</strong></td>
<td></td>
</tr>
</tbody>
</table>

Nota: Las cifras en cursiva indican significación a nivel de 0.05 (**) y 0.01 (**).
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. $\text{NH}_4^+$. 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. $\text{NH}_4^+$. 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](image_url)

**Figure 3.** Effects on soil microbial diversity. **a.** Aerobic mesophiles. **b.** Free-living N\textsubscript{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\textsubscript{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 (Garreau 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,
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 cultivable 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 NH₄⁺ 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 NH₄⁺. 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 NO₃⁻), 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 NO₃⁻ 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 NO₃⁻ 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 NO3− 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.

ACKNOWLEDGMENTS

The authors acknowledge the Galvez family, who permitted site access and provided historical information. This research was supported by the Fondo de Protección Ambiental (FPA) [Project No. 6-RE-002-2018 of the Ministry of Environment], the Agencia Nacional de Investigación y Desarrollo de Chile [ANID PIA/BASAL FB0002] and the University of O’Higgins [postdoctoral fellowship program 2018 for C.M.]. C.M. also thanks the Convocatoria Nacional Subvención a Instalación Academia + Convocatoria Año 2021 + Folio SA77210019 (ANID, Chile).

REFERENCES


