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Evaluation of Density and Viability of Arbuscular Mycorrhizal Spores in *Austrocedrus chilensis* Forests Affected by Wildland Fires in Patagonia, Argentina

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Simple Summary

Wildfires represent a recurrent disturbance in the Patagonia Andean region, with increasing frequency in recent decades. This study analyzed how fires could affect the density of arbuscular mycorrhizal spores in *Austrocedrus chilensis* forests, an endemic arbuscular-mycorrhizal conifer vulnerable to fire. Our study area was settled in a widespread area affected by wildfire, with varying severities, in February 2015. Soil samples were collected from three sites with different precipitation ranges and three different fire severities each, evaluated 10 months and 5 years after the fire. Our key findings were a) post-fire, spores were significantly more abundant in sites affected by moderate severity fires than unburned or high fire severity; b) after 5 years, burned sites showed no differences between severities, but the driest sites showed no changes since fire occurred with the same pattern and spore density; c) seedlings from the bioassay showed less than 25% mycorrhizal colonization in burned sites; d) wildfires reduce arbuscular mycorrhizal spores and alter soil properties; e) drier sites have less resilience, requiring active restoration. Post fire management must consider that wildfires reduce mycorrhizal presence and alter soil properties, especially in dry conditions where natural recovery is limited.

Abstract

Background: Wildfires represent a recurrent disturbance in the Patagonia Andean region with increasing frequency in recent decades. *Austrocedrus chilensis* is an arbuscular-mycorrhizal (AM) endemic conifer particularly vulnerable to fire, a situation that will worsen with climate change. In February 2015, a wildfire affected 5700ha of *Austrocedrus chilensis* forests with varying severities (WFS). The aim of this study was to determine and compare the density of AM spores (AMS) in soil affected by different WFS and non-affected sites, considering site features. **Methods:** Ninety soil samples were collected from three sites 10 months and 5 years after fire. AMS' density was determined, a bioassay was set, and soil physicochemical features were evaluated. **Results:** after the wildfire, spores were significantly more abundant in sites affected by moderate severity fires. After 5 years, burned sites showed no differences between severities, but the driest sites showed no changes since fire occurred. Seedlings from the bioassay showed less than 25% mycorrhizal colonization growing in soil from burned sites, regardless fire severity compared with unburned soils. **Conclusions:** For restoration strategies, it must be considered that wildfires reduce mycorrhizal spores and mycelium, alter soil properties, and that drier conditions have less resilience, requiring active restoration.

Keywords: AM inocula; restoration; native forests; forests fire

1. Introduction

Wildfire has been a recurrent disturbance in the Patagonia Andean region, causing significant environmental, social and economic impacts similar to those in other countries around the world [1,2]. In these forests, the natural fire regime varies according to the precipitation regime. In general, fire frequency increases from the humid sites in the west, towards the xeric sites in the East [3]. In the past, fire frequency increased in the majority of *A. chilensis* stands after 1850, coincidentally with an increase in number of indigenous people that inhabited the region and the beginning of European settlement. This frequency peaked at the end of the 19th century, and started to decline from then on because indigenous population decreased, and the creation of several National Parks in the region brought about the policy of fire suppression [1]., while during the last three decades, the number of wildfire ignitions has risen in the whole central Patagonia Andean region [4,5]. As rains are markedly limited to autumn-winter in the region, summer provides favorable conditions for wildfires occurrence, combining the greatest water deficit and the highest temperatures [6]. Moreover, in a climate change scenario, with predictions of 1°C to 3°C temperature increase, and a 10% to 30% precipitation decrease for West Patagonia [7,8], wildfire frequency is expected to rise [9,10]

Austrocedrus chilensis (D. Don) Pic. Sern et Bizarri is an endemic and emblematic conifer of northern Patagonia forests, one of the most affected by wildfires [11]. It grows in pure or in mixed stands interspersed with *Nothofagus dombeyi* Mirb. Oerst. and other tree species between 37°07′and 43°44′ S latitude [12]. *Austrocedrus chilensis* is an obligate seeder [13,14] that does not resprout after fires [15], highly vulnerable to fire given the thin bark and persistent, dry, branches that favor fire access to the crown [16], and the high resin foliage contents that burns explosively [17]. It forms extensive stands valuable for forestry and tourism, and of significant ecological importance, covering an area of 100.000 ha [18]. However, fire and diseases [19–21], invasions by other plant species [22]as well as herbivory on saplings after fires [23] have generated severe degradation, resulting in great economic and environmental losses [24]. *It* has been declared as a ‘near threatened’ species by The IUCN (International Union for the Conservation of Nature) since 2013.

Regarding its mycorrhizal status, *A. chilensis* is an obligated AM species [25,26]. This mutualism is of great ecological importance, involved in seedlings establishment and tree growth, with major roles in absorption, transport and translocation of nutrients (P, N, C, K and Ca) [27], increased water availability and tolerance to drought [28,29], and resistance to pathogen attack [30]. On the other hand, AM Fungi (AMF) improves soil physicochemical properties [31] and plays a role in nutrient cycling [32], serving as a key element of ecosystem health and supporting vegetation recovery following degradation [33]. Nevertheless, AMF are highly susceptible to fire [34–36], especially mycelial structures and colonized AM root tips. Although, AM spores (resistance structures) have been reported as not drastically affected by fire [37–39]; fire heat could even break spore dormancy [40]. Anyway, fire can reduce AMF diversity compared with unburned areas [34,41,42] but, if edaphic, weather and vegetation conditions are favorable, AM community could recover in a few decades [43,44], contributing to a quick ecosystem recovery after fire [45,46]. Although mycorrhizas are crucial for maintaining biodiversity and ecosystem function, there is surprisingly limited knowledge regarding the landscape-scale biogeographical patterns of AMF species and the environmental factors that affect their distributions, including their response to fire severity. Empirical work has linked certain edaphic properties (e.g., soil texture, pH, nitrogen, phosphorus) to AMF distributions [47–49]. Dispersal capabilities of AMF are likely species-specific and environmentally dependent [50], although the mechanisms are poorly understood and have not been addressed for *A. chilensis* forest.

During February of 2015, near to Cholila (Chubut, Argentina), an extreme behavior wildfire, the most severe in the past century, occurred. From the total 27,101 ha burned, approximately 5700 ha corresponded to pure and mixed *A. chilensis* forests [5]. Therefore, it constituted a suitable scenario

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to assess the AM spore bank´s post fire condition, understand its evolution over time to properly manage restoration actions. In this sense, the aims of this study were 1) determine and compare over time the abundance of AMS in *A. chilensis* forest areas affected by different WFS, in relation to non-affected patches, and considering sites features and 2) analyze the behavior of the AM spore bank through its infectivity on *A. chilensis* seedlings analyze the behavior of the AM spore bank of those burned forest areas on *A. chilensis* seedlings.

2. Materials and Methods

2.1. Study Area

This study was carried out in three pure stands of *A. chilensis* forest affected by 2015 wildfire, located near "Las Horquetas" in Villegas Valley (A), Tigre River (B) and Cholila lake (C) in Chubut province (Figure 1). Three different fire severities were selected as treatments in each location. Fire severity was categorized considering the damage in trees, following [51]: unburned forests (green areas of forest), moderately affected forests (areas with partial tree burned), and severely affected forests (areas with total trees burned) (Figure 1). The slope ranged between 10 % and 35 % in all sites, while the aspect was mostly southeast, except for unburned site A and severely affected site B (northeast) and unburned site B (southwest). Site A Annual precipitation ≅ 2000 mm; Site B annual precipitation ≅ 1500 mm; Site C Annual precipitation ≅ 800 mm [52].

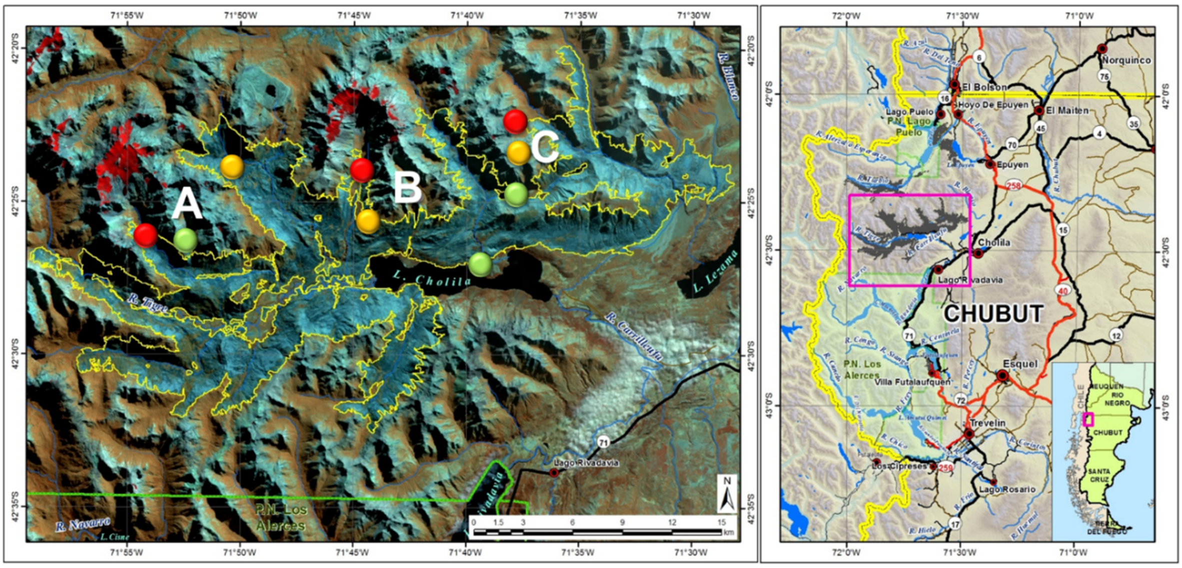

Figure 1. Study area. Sampling sites: Villegas valley (A), Tigre River (B) and Cholila lake (C) (Map from [5]). In the zoomed-in map, the yellow line indicates the wildfire perimeter, green dots unburned sites, yellow dots moderately affected sites and red dots severely affected sites.

2.2. Sampling Design

During January and February 2016, a sampling plot of ≅ 100 m^2 was set and sampled for each treatment / site and revisited and sampled in February 2021. In each plot five soil subsamples of approximately 500 g, from the first 15 cm of soil, were collected and placed in new plastic bags and stored in a refrigerator at 4 ° C until the AMS' evaluation. A total of 90 soil samples were obtained (2016 and 2021) which were processed in duplicate (180 subsamples in total).

In addition, three composite soil samples (~630 cm3 each) of the upper 5 cm of mineral soil were collected at each sampling site in 2016 (27 soil samples) to determine physicochemical parameters.

2.3. Sample Processing; Spore and Soil Analyses

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For the extraction of soil AMS, the wet sieving and sucrose gradient method (adapted from [53]) was used. Soil subsamples of 50 to 75 g per treatment/site were placed in a 20% sodium pyrophosphate solution for 60 minutes, sieved with distilled water using a 25 μ sieve; the obtained fraction was centrifuged at 2000 rpm for 5 minutes to precipitate the AMS. After discarding the supernatant, 50 ml of 50% sucrose solution was added, homogenized and centrifuged at 2000 rpm for 5 minutes. The supernatant was filtered on filter paper (103 slow, XinXing®), placed in a Petri dish and counted under stereomicroscope. Density of AMS was expressed as the amount of AMS per 100 g of dry soil by treatment [54].

Soil analyses were carried out at CIEFAP Soil Laboratory with dried 2-mm sieved fraction of soil. Physicochemical parameters analyzed were: (1) current pH 1:2.5 soil/distilled water ratio [55]; (2) electrical conductivity (EC, ds/m) [56]; (3) organic matter (OM%) [57]; soil organic carbon (C) was estimated from the ratio between organic matter and the van Bemmelen factor of 1.724 [58]; (4) total nitrogen (N, %) by obtained by Kjeldahl method [59]; (5) available phosphorus (P, mg/kg) estimated by the Olsen method [60], recommended for slightly acidic soils such as Andisols [61]; (6) cation exchange capacity (CEC) [62]; and (7) exchangeable bases, to determine calcium (Ca, meq/100g) and magnesium (Mg, meq/100g), sodium (Na, meq/100g) and potassium (K, meq/100g) [63].

2.4. Bioassay Setup

A soil bioassay, to know AMS real colonization capacity, was conducted with soil sampled 10 months after fire. *Austrocedrus chilensis* seeds were surface sterilized in 10 % sodium hypochlorite for 10 min, sown in sterile distilled water [64], and then hydrated for 24 h in sterile distilled water before storing them at 4 C in a refrigerator for 45 days to stratify (adapted from [65]). Non-AM seedlings were obtained in a growth chamber at 17-19°C, 48–55% relative air humidity, and 16 h photoperiod with 1400 lux radiation with sterilized pumicite (autoclave 3 times at 120 C for 30 min; adapted from [66]) for 30 d. Three weeks later, 2 seedlings were transplanted to a clean, 250 cm3 pot filled with a 1:1 (v/v) mix of soil obtained from each sampling unit and sterilized pumicite (as mentioned above). As a control, 10 pots were filled with mixed (1:1, v/v) sterilized soil (mixing soil from each treatment from all sites) and sterilized pumicite, both autoclaved as previously mentioned. Seedlings were randomly arranged and grown for 18 months in a growing chamber, under the same set up described for germination.

The root system of each seedling was cut into 10 mm lengths portions (approx. 600 mg per seedling) to fit in Tissue-Tek plastic capsules (Fisher Scientific Co., Pittsburgh, PA), cleared in 10% KOH for 30 min at 100°C under water bath and 15% H_2O_2 overnight at room temperature. Cleared samples were immersed 60 min at 4 C in a staining solution of 0.05% trypan-blue in lactoglycerol, rinsed with tap water and stored in lactoglycerol at 4 C until microscopic examination [67]. Arbuscular mycorrhizal colonization percentage for each seedling root system (AM%) was estimated following [68], using the complete root system, as:

AM%= (number mycorrhizal intersects/total intersects)*100

All AM structures (arbuscules, coils, vesicles and hyphae) were count separately and considered as mycorrhizal intersects for AM%.

2.5. Statistical Analysis

Spore density and AM% were analyzed using a two-way generalized linear mixed model (2W-GLMM) with the restricted maximum likelihood estimation method (RELM) with posterior comparisons with the test DGC (test of exclusionary groups). Sites (A, B, C) were treated as blocks (incorporated as a random effect) and different treatments (HIGH, MODERATE and UNBURNED wildfire severity) as fixed-effects [69] with R-DCOM in Infostat [69]. Arbuscular mycorrhizal structures (arbuscules, coils, vesicles and hyphae) were analyzed with a hierarchical clustering analysis using pheatmap, ggplot2 packages for RStudio. Soil physicochemical differences between sites and treatments were analyzed using two-way ANOVA, with treatments nested within each site, and site x treatment interactions. When variables showed significant interaction, treatments were analyzed within each site. Prior to the analysis, the assumptions of normality and homogeneity of variance were verified. To further analyze the relationships between seedlings mycorrhizal status and soil features, Pearson correlation tests and PCA were conducted, including AM%, AM structures, pH, OM%, electrical conductivity, content of N, Mg, P, K, Ca, Na.

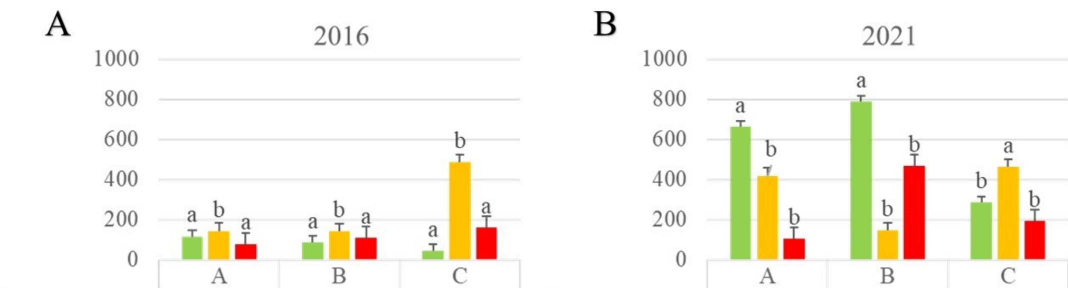
All analyses were performed at a 0.05 significance level with the statistical package InfoStat for Windows version 2020 [70] and the RStudio version 2022.2.0.443 [71].

3. Results

3.1. Analyses of Soil AMS Densities and Soil Variables

In total, 24.434 spores belonging to AMF were recovered from 180 soil samples (Table S1). Ten months after wildfire, AM spores' density was significantly and consistently more abundant in moderately affected sites, compared both with the unburned or high affected sites (Figure 2A, 2W-GLMM, F= 5.14; p=0.0115). Also, the driest site (C) tripled the AMS density compared with the other sites for the moderately affected condition (Figure 2A).

After 5 years (2021), burned sites showed no differences in AMS densities between wildfire severities for sites A and B (Figure 2B). Unburned sites presented higher densities from the initial situation (Table S1), and significantly higher than burned treatments, except in the driest site (C) (2W-GLMM, F= 7.39; p=0.0022). Also, the AMS density patterns between treatment differed considering the site (Figure 2A, B). Interestingly, the driest site (C) did not show changes in AMS' density after 5 years in burned sites.



2016	A	B	C
Unburned	118	89	48
Moderately affected	144	143	487
High affected	81	110	162

2021	A	B	C
Unburned	663	788	288
Moderately affected	418	148	463
High affected	108	468	194

Figure 2. AMS' density for site (A, B, C), year (2016, 2021), and WFS treatment (unburned, moderate, high) (Spores/ 100 g of soil). Sites: A= Villegas valley, B= Tigre River and C= Cholila lake. Bars represent SE. Different letters indicate significant differences between treatments (P<0.05, 2W-GLMM).

Regarding soil physicochemical properties, pH was lower in Site A compared to B and C; high severity treatment presented significantly higher pH than the moderate, and both significantly higher than the unburned. Nitrogen was only significantly lower in site B compared to C, while both did not differ with site A; burned treatments from all sites consistently presented lower N than the unburned ones. The EC was significantly higher for the high severity compared to the unburned treatment, while those of moderate severity showed intermediate values. The OM, Na and C/N were consistently higher in the unburned treatment compared to both burned ones. Potassium and Mg evidenced differences only in site B, where the unburned treatments had a significantly higher concentration than the burned ones. Calcium concentration was significantly higher in unburned treatment, compared to both severities in sites B y C, while only to moderate severity in site A (Table 1).

Table 1. Physicochemical properties by sites and wildfire severity. Different letters indicate significant differences (p< 0.05). Capital letters between sites (*S) and lower-case letters between treatments or severity of fire (*T). Where interaction (*SxT) was detected, lower case letters show differences between treatment within each site [Villegas valley (A), Tigre River (B) and Cholila lake (C)]. Variables without letters showed no significant differences.

Sampling sites	A			B			C		
Fire Severity	Unb*	Mod	High	Unb	Mod	High	Unb	Mod	High
pH	6.05	6.71	7.15	6.85	7.31	7.18	6.76	6.8	7.63
*S*T	Aa	Ab	Ac	Ba	Bb	Bc	Ba	Bb	Bc
OM	24.38	11.04	11.02	28.72	8.95	7.46	34.76	12.95	8.51
*T	b	a	a	b	a	a	b	a	a
N	0.72	0.41	0.46	0.66	0.39	0.29	0.91	0.64	0.39
*S*T	Ab	ABa	ABa	Ab	Aa	Aa	Bb	Ba	Ba
P available	40.66	21.65	55.23	24.01	47.29	30.45	54.03	44.48	39.68
Na	0.88	0.88	1.06	1.12	0.76	0.52	1.07	0.71	0.85
*T	b	a	a	b	a	a	b	a	a
K	0.96	1.37	0.94	1.71	0.81	0.55	1.69	1.21	1.68
S*T*				b	a	a			
Ca	16.91	7.08	17.42	38.16	18.42	14.83	43.33	20	32.5
*S*T	b	a	b	b	a	a	b	a	a
Mg	4.08	2.08	5.58	9.33	1.83	1.33	7.5	5.67	4.5
*S*T				b	a	a			
EC	0.13	0.13	0.21	0.08	0.09	0.13	0.12	0.13	0.22
*T	a	ab	b	a	ab	b	a	ab	b
C/N	16.7	13.1	11.8	24.4	11.5	12.5	19.2	9.9	10.8
*T	b	a	a	b	a	a	b	a	a

*Unb= Unburned, Mod= Moderate. OM=%, N= %, Available P= mg/kg, Na= meq/100g, K= meq/100g, Ca= meq/100g, Mg= meq/100g, EC= ds/m, C/N= %.

3.2. Seedlings AM Colonization from Soil Bioassay

The AM % of 100 *A. chilensis* seedling from the bioassay showed significant differences between wildfire severities treatments, and both significantly lower values compared to the unburned treatment (Figure 3B, Table S2). The AM% for the moderate severity treatment represents 33,8% of the unburned treatment value, while the high severity treatment only 12 %. Control seedlings showed no AM colonization (Figure 3, Table S2), and lead to non-significant differences with the high severity treatment. Consequently, seedlings from the unburned treatment showed the greatest amounts of AM nutrient and photosynthate exchange structures (arbuscules, coils), while seedlings grown in soil affected by wildfire showed mostly explorations and storage of nutrient and photosynthate structures (hyphae and vesicles) but in small amounts especially in wet sites (A= Villegas valley) (Figure 3A, Table S2).

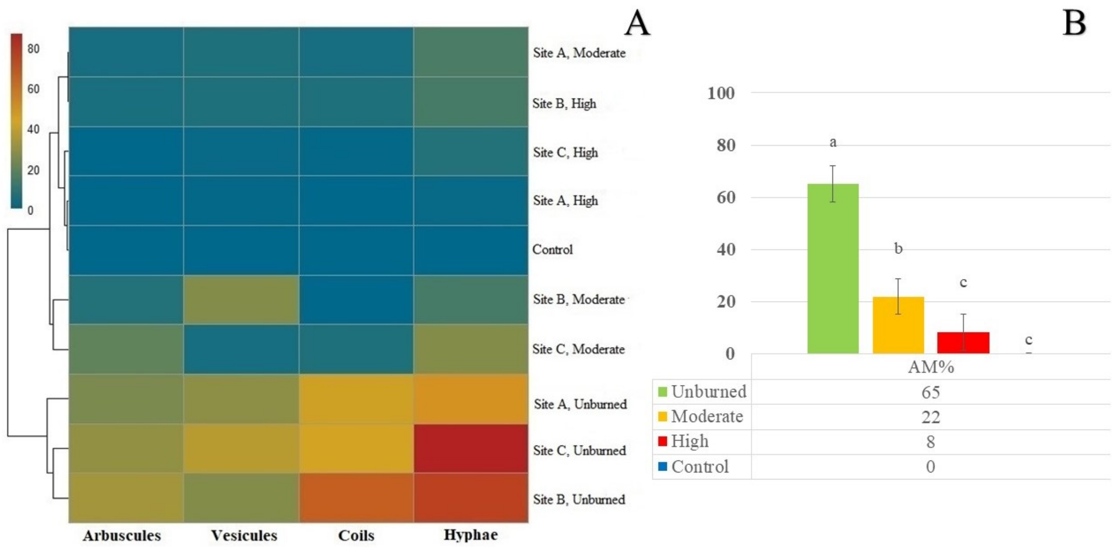


Figure 3. Bioassay results. A) Heatmap showing AM structures per seedling per WFS treatment (unburned, moderate, high) and sites (A= Villegas Valley, B= Tigre River and C= Cholila lake). Different colors refer to different quantity of AM structures per seedling according to color bar (dark red=80 structures/seedling to Blue=0 structures/ seedling). B) Seedling AM colonization per treatment. Bars represent SE. Different letters indicate significant differences between treatments (P<0.05, 2W-GLMM).

3.3. Relationships Between Seedlings AM Colonization, Spore Density and Soil Features

Arbuscular mycorrhizal colonization in *A. chilensis* seedlings was negatively correlated with soil pH and EC and positively with OM% and C/N (Pearson correlation coefficient, r= -0.7, p= 0.05; r= -0.7, p= 0.05; r= 0,8, p= 0,02; r= 0.8, p=0.02; respectively). Arbuscles and hyphae abundance were positively correlated with OM% and C/N and negatively correlated with soil pH (Pearson correlation coefficient, r= 0.9, p=0.003; r= 0.8, p=0.01; r= 0.8, p= 0.01; r= 0.8, p= 0.01; r= -0.7, p= 0.03; r= -0.8, p= 0.02, respectively). Coils' abundance was positively correlated with OM%, Mg content and C/N (Pearson correlation coefficient, r=0.92, p=0,0005; r= 0.7, p= 0.03; r= 0.95, p= 0.0001, respectively). Finally, the vesicles' abundance was negatively correlated with soil electrical conductivity and positively with OM% and C/N (Pearson correlation coefficient, r= 0,7, p=0,03; r= -0.7, p=0.05; r= 0.7, p=0.05, respectively). On the other hand, spore density (2016) was not correlated with any soil feature (Table S3).

Principal component analysis (PCA) showed that the combination of AM structures AM% and soil features (Figure 4) could be explained by two components, including 69.6% of variance (Table S4, S5); PC1 and PC2 explained 43.1% and 26.5% of the variance, respectively. PC1 discriminates between burned treatments from the unburned, while PC2 discriminates between sites (A= Villegas valley, B= Tigre River and C= Cholila lake).

The N, K, Ca, Mg content (EV=0.715; -0.701; -0.818; -0.680, respectively), arbuscles, vesicles, coils, hyphae abundance (EV= -0.732, -0.788, -0.798, -0.881) AM% (EV=-0.816) were spatial variables that contributed to PC1, grouping variables affected by fire. On the other hand, pH (EV=-0.657) OM % (EV=0.751) and Na content (EV=0.736) contribute to PC2, grouping site dependent variables.

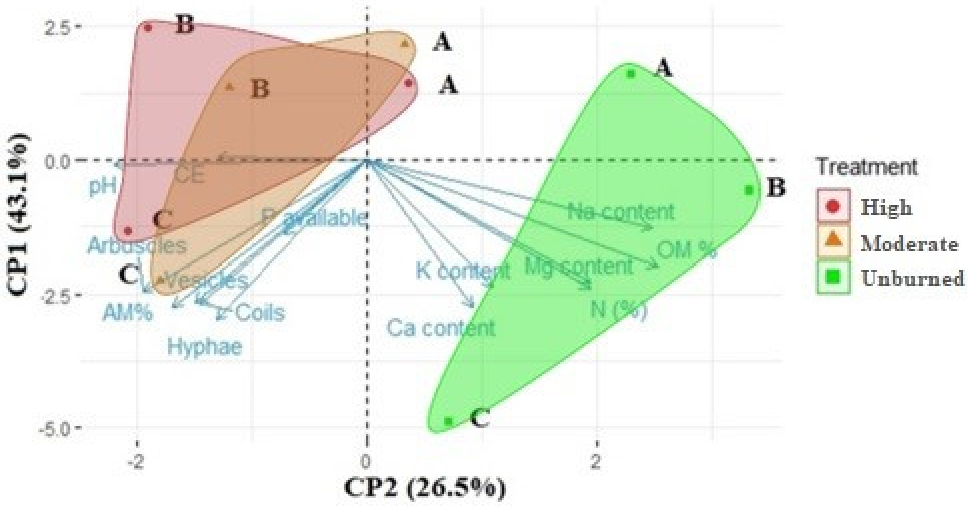


Figure 4. PCA for AM structures and seedling colonization (AM%) and soil features considering sites and WFS (unburned, moderate, high). Sites: A= Villegas Valley, B= Tigre River and C= Cholila lake.

4. Discussion

Arbuscular mycorrhizal colonization and spore-based studies are a gap of knowledge for South American *Austrocedrus chilensis* forests, except for few works about plant mycorrhizal status from last century [25,26,72]. For this reason, this study represents the first analysis regarding bank evolution and infectivity after wildfires for these native ecosystems. Our results show that wildfire events had direct effects on the AMS communities associated with *A. chilensis* forests, and that these effects are affected and vary according to fire severity and site conditions. It has been already stated that fire reduce root AM colonization [73,74] but increase AMS density in the soil [75]. In our study, immediately after wildfire occurs, AMS density was significantly more abundant in the moderately affected treatment in the dry site (C), compared both with unburned to highly affected treatment at the same site and with all treatments from the other wetter sites. On the other hand, seedlings growing on soils affected by wildfires showed low or null AM colonization regardless of WFS. After a wildfire, the surviving soil microecosystem and their ability to recover are essential to forest succession and restoration [76–78]. For those microorganisms beneath the surface, soil is a good insulator, especially when it is dry, and the temperature at 2.5 cm depth may be 50°C when the surface is 100°C [79]. In this sense, the results of this work showed that soon after fire, moderate severity wildfire were associated with higher AMS densities, and that this effect was more pronounced in dryer site conditions. In those xeric sites, spore abundance does not recover even 5 years after the event. [37] found that AM communities are resilient to wildfire on decadal timescales; this resilience appears to depend on the post-fire regrowth of understory vegetation and the subsequent recovery of soil chemical properties. However, regarding the direct effects of wildfire on AM fungi, results have been contradictory, reporting either negative e.g., [80–85], neutral e.g., [34,45,86–88], or positives [89].

[90] found that natural *Austrocedrus chilensis* regeneration could only be expected to occur in patches that have been unaffected by fire, or in areas affected by low severity wildfires because of low seeds availability. The potential for natural regeneration of this species following large-scale disturbances that eliminate or drastically reduce the forest canopy is limited [11]. The emergence of *Austrocedrus chilensis* seedling pattern varied with site conditions and forest management practices [91]. Additionally, *A. chilensis* seedling survival improves if early successional herbaceous species and the burned canopy are not removed [11]. In this sense, herbaceous and shrubs species from *A. chilensis* understory tended to increase their presence with wildfire [17,92]; as most of them harbor AMF [26,93]they could improve AM inoculum availability and colonization of new *A. chilensis*

seedling, although the specificity of *A. chilensis*-associated AM fungi in relation to understory AM fungal species has not yet been studied. We also found low seedling colonization, mostly with explorations and storage structures (hyphae and vesicles), in treatments with soils of the severely burned forests. Despite the fact that very little is known in this regard, considering that AM symbiosis is crucial to new plant establishment [94], our findings indicate that inoculation with AM fungi should be recommended for the ecological restoration of forests affected by high WFS. Further research should examine its effects on seedling establishment in post-fire plantations.

Post-fire impacts include significant alteration of soil properties, such as pH, OM%, EC, N, C/N ratio, and CEC [51]. It was stated that soil pH increases after wildfires [92] due to the combustion of organic matter [95]. The direct impact of lower pHs on AMF is the limited sporulation and germination of spores in the rhizosphere; soils with neutral pH revealed greater amounts of AMF spores compared with acidic, pH 5.5 soils [96]. When pH rises, as found in burned soils in this work, then a stimulation of AMF sporulation can occur, increasing spore densities as found in sites moderated affected by fire. Arbuscular mycorrhizal fungi can also be stimulated by organic matter contents [27,97]. In this sense, our seedlings revealed AM structures colonization consistently associated with OM soil content, even when wildfire, regardless of WFS, showed significantly lower percentage of OM.

Arbuscular mycorrhizal spore density in natural soils/ environments is highly variable [27] and experimental studies have shown that several factors could be involved in AMS sporulating, such as climatic, geological and/or edaphic features, plant associated phenology, human activities, soil legacy [27,98–100]. In this sense, unburned sites in this study showed a noticeable increase in AMS density after 5 years. Although our sampling design does not allow us to establish causalities, considering sampling plots and methodology were not changed, it could be hypothesized that these differences could be related to previous climatic conditions to each sampling period (January-February 2016 and 2021). In average, the four autumns prior to the first sampling were warmer and drier, with less days with frost in 2016 than in 2021 (Table S6). Other studies have found that warming [101,102] and drought conditions [103] significantly decrease AMS abundance. More local studies are needed to unravel this issue, understand its dynamic and predict evapotranspiration effects on the AMS bank.

Thinking of restoration strategies, it must be considered that wildfire would also cause extramatrical mycelium loss, although some stimulus occurs in moderately affected soil that increased spores' abundance. Moderately burned areas have better chances of natural restoration considering the abundant AM spores bank, while artificial inoculation must be considered for severely affected areas or in burned dry sites.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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Abbreviations

The following abbreviations are used in this manuscript:

AMF	Arbuscular mycorrhizal fungi
AMS	Arbuscular mycorrhizal spores
AM%	Arbuscular mycorrhizal colonization percentaje
WFS	Wildfire severity
AM	Arbuscular mycorrhiza
OM	Soil organic matter
N	Soil Nitrogen content
EC	Soil Electrical conductivity
C/N	Soil carbon nitrogen ratio
2W-GLMM	Two-way general linear mix model
PCA	Principal component analysis

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