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Factores que determinan la comunidad de hongos micorrícicos arbusculares del bosque esclerófilo en Chile central mediterráneo

Factors that determine the arbuscular mycorrhizal fungal community of the sclerophyllous forest in the Mediterranean central Chile

Tesis para optar al grado de Doctor en Ciencias Biológicas, Área Botánica

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"Estúdiese biología porque es "irrelevante" – esto es, estúdiesela por sí misma-, porque al igual que el arte, la música y la literatura, es una aventura para la mente y un alimento para el espíritu" Helena Curtis & N. Sue Barnes, Biología, 2001.

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Resumen

La micorriza es una asociación mutualista que ocurre entre ciertos grupos de hongos del suelo y la mayoría de las plantas. Su función principal es el intercambio de compuestos orgánicos e inorgánicos entre la planta y el hongo, en donde el hongo toma nutrientes y agua desde el suelo y se los entrega a la planta a cambio de carbohidratos y lípidos. Además esta asociación ayuda a las plantas a sobrellevar el estrés abiótico y biótico que pueda generar el ambiente. En consecuencia, la ocurrencia de la micorriza influye positivamente tanto a la planta como a los hongos que la componen. La asociacion micorrícica puede ser de varios tipos dependiendo de los hongos y plantas que la establecen y de las estructuras que forman. Dado esto se clasifican en micorriza arbuscular (MA), ectomicorriza (EcM), micorriza orquideoide (MOr) y micorriza ericoide (MEr). En cuanto a los hongos que forma la MA u hongos micorrícicos arbusculares (HMA) se ha demostrado su influencia positiva y clave en proyectos de restauración de ecosistemas degradados, principalmente del hemisferio norte. En Chile central se encuentra el *matorral chileno*, que corresponde a unos de los solamente cinco ecosistemas de clima mediterráneo a nivel mundial. Es considerado un *hotspot* de biodiversidad debido a la alta proporción de especies de plantas endémicas presentes y que a su vez están amenazadas por factores antropogénicos. En este contexto los HMA se presentan como una propuesta atractiva para diseñar herramientas que permitan la recuperación del *matorral chileno*. No obstante su aplicación aún es restringida ya que en este ecosistema existe muy poca información respecto a patrones ecológicos que puedan finalmente aplicarse en su restauración. Dicho esto, esta tesis tiene como objetivo general identificar los factores que determinan la abundancia, diversidad y composición comunitaria de los HMA de un bosque esclerófilo. Esta formación vegetacional es muy característica y la mas extendida en el *matorral chileno*.

Para esto se realizaron dos análisis previos, correspondientes a (1) compilar información bibliográfica para apoyar el supuesto de una alta frecuencia de micorriza arbuscular asociada al *matorral chileno* y (2) corroborar la presencia de micorriza arbuscular en las plantas dominantes de un bosque esclerófilo representativo del *matorral chileno*. Como resultados principales se obtuvo que la MA es el tipo más frecuente de micorriza presente en el *matorral chileno*. Además todas las especies dominantes del bosque esclerófilo estudiado (*Peumus boldus*, *Lithrea caustica*, *Quillaja saponaria*, *Cryptocarya alba, Kageneckia oblonga* y *Escallonia*

pulverulenta), tienen estructuras de HMA en sus raíces sin embargo en base a la frecuencia e intensidad micorrícica se concluyó que todas salvo *L. caustica,* forman indudablemente MA. Con estos resultados se procedió finalmente a identificar los factores que determinan la abundancia de esporas de los HMA en el suelo y la comunidad molecular de los HMA en suelo y raíz. Se encontró que la abundancia de esporas varía significativamente según las estaciones del año. Además, la diversidad y composición comunitaria de los HMA varía significativamente respecto del hábitat donde se encuentren los hongos (raíz o suelo) y según a la especie de planta hospedera a la que están asociados. Además, tanto la abundancia de esporas, como la diversidad y composición comunitaria de los HMA del bosque esclerófilo estudiado varían significativamente en relación a múltiples factores físicos y químicos del suelo. Se concluye que mientras que la esporulación y germinación de esporas ocurre diferenciadamente a lo largo de las estaciones del año, las distintas especies de plantas se asocian a una proporción pequeña de HMA de forma específica, y en mayor grado a la mayoría del pool de HMA disponibles en el suelo. Además, se destaca la mayor abundancia de hongos del genero *Glomus*, *Claroideoglomus* y *Paraglomus* en este sistema y la ocurrencia de 20 taxones virtuales nuevos a nivel global y exclusivos del bosque esclerófilo estudiado. Al haber una diversidad y composición comunitaria de HMA diferenciada por especies de plantas indica la importancia de estos organismos como simbiontes de esas plantas y por lo tanto su necesidad de ser considerados en planes de restauración futuros para mejorar el desempeño de las plantas y preservar también el reservorio de HMA en los suelos del bosque esclerófilo del *matorral chileno*.

Abstract

A mycorrhiza is a mutualistic association between certain groups of soil fungi and most plants. The main function is the exchange of chemical compounds between the plant and the fungus, in which the mycobiont takes up nutrients and water from soil and delivers to the phytobiont in exchange for carbohydrates and lipids. Moreover, this association helps plants in withstand the biotic and abiotic stress of the environment. In consequence, the occurrence of the mycorrhiza positively influences both the plant and the fungal partner. There are several mycorrhizal types, depending on the groups of plants and fungi that form the mycorrhizal association: Arbuscular mycorrhiza (AM), Ectomycorrhiza (EcM), Orchid mycorrhiza (OrM) and Ericoid mycorrhiza (ErM). Particularly, it has been shown the positive and essential influence of the arbuscular mycorrhizal fungi (AMF) in programs of restoration of degraded ecosystems, mainly in the northern hemisphere. In central Chile, the *Chilean matorral* is one of only five Mediterranean-type ecosystems in the world. It is considered a biodiversity *hotspot*, because of the high proportion of endemic species, many of them threatened due to anthropogenic factors. In this context, AMF are attractive for developing tools for the *Chilean matorral* recovery. Nonetheless, the application is still restricted since there is scarce information regarding AMF ecological patterns of this ecosystem. Therefore, the main aim of this thesis is to identify the factors that determine the abundance, diversity and community composition of the AMF community of the sclerophyllous forest. This plant formation is very characteristic and the most extended in the *Chilean matorral*.

In order to achieve this aim, two previous analysis were performed. First, (1) a bibliographic compilation was done to support the idea of a high frequency of AM in the *Chilean matorral* and (2) corroborate the presence of AM in the dominant plants of a representative sclerophyllous forest of the *Chilean matorral.* As main results, AM were found to be the more frequent mycorrhizal type in the *Chilean matorral*. Moreover, all the dominant plant species of the sclerophyllous forest studied (*Peumus boldus*, *Lithrea caustica*, *Quillaja saponaria*, *Cryptocarya alba, Kageneckia oblonga* y *Escallonia pulverulenta*), showed AMF structures. However, due the frequency and intensity of mycorrhizal structures it was concluded that all, but *L. caustica* form with certainty AM. With these results we continued with (3) determining the factors that influence the AMF spore abundance and (4) the molecular community of AMF

in the soil and roots of associated plants. It was found that spore abundance varies significantly in relation to the seasons. Also, diversity and composition of AMF, varies significantly in relation to the AMF compartment (soil or roots) and to the host plant species. Also, spore abundance, molecular diversity and community composition of AMF varies in regard to several physico-chemical soil factors. It is concluded that while sporulation and germination of spores occur differentially along the year, the different plant species associate in a specialist manner to a small proportion of AMF, while most of the AMF are plant generalists. There is a high abundance of AMF of the genera *Glomus*, *Claroideoglomus* and *Paraglomus* and 20 new virtual taxa were detected and may be exclusive of the sclerophyllous forest studied. Not only mycorrhizal traits but also community structure in relation to plant species should be considered in programs of restoration. This will be important in improving the plant fitness as well as preserve the AMF diversity of the sclerophyllous forest of the *Chilean matorral*.

Capítulo I

1. Introducción general

La micorriza es una asociación mutualista que ocurre entre ciertos grupos de hongos del suelo y raíces de la mayoría de las plantas (Smith and Read, 2008). La evidencia indica que existe desde hace al menos 407 millones de años (Trewin and Rice, 2004). Actualmente se estima que al menos el 92% de las plantas terrestres forman esta asociación (Brundrett and Tedersoo, 2018).

La función principal de la micorriza es el intercambio de compuestos quimicos entre la planta y el hongo (Smith and Read, 2008), en donde el hongo realiza la toma de nutrientes y agua desde el suelo y se los entrega a la planta a cambio de carbohidratos (Smith and Read, 2008) y lípidos (Jiang et al., 2017; Keymer et al., 2017; Luginbuehl et al., 2017). Además esta asociación ayuda a las plantas a sobrellevar el estrés abiótico y biótico que pueda generar el ambiente (Pozo et al., 2015). En consecuencia, la ocurrencia de la micorriza beneficia tanto a la planta como a los hongos que la componen. Por una parte hay un efecto directo en el desempeño de la planta e indirecto en cuanto a las interacciones planta-planta, incrementando la diversidad y regulando la composición de las comunidades (van der Heijden et al., 2015). Y por otra parte la identidad y abundancia de la planta afectará la diversidad y la composición de las comunidades de hongos micorrícicos (Davison et al., 2016).

El tipo de asociación micorrícica puede variar entre diferentes plantas y en función de su ambiente y su fase de vida, por lo que una forma de abordar esta variación es utilizar una aproximación basada en los rasgos micorrícicos de las plantas (Moora, 2014). Dependiendo de los taxones de hongos y plantas involucrados en la simbiosis micorrícica se pueden formar distintos tipos de micorriza (Moora, 2014). La mas común es la micorriza arbuscular (MA), la cual es formada entre hongos de la división Glomeromycota (Tedersoo et al., 2018) y aproximadamente el 71% de las plantas terrestres conocidas (Brundrett and Tedersoo, 2018), incluidas las de la superdivisión Bryophyta sensu lato y Pteridophyta. Se caracteriza por formar una estructura intracelular en el tejido radicular llamada arbúsculo, en donde ocurre el intercambio de compuestos químicos entre la planta y el hongo. También pueden formar vesículas, en donde se almacenan reservas. Otro tipo de micorriza es la ectomicorriza (EcM) y se caracteriza por ocupar el espacio intercelular de la raíz formando la llamada red de Hartig y por formar un manto que envuelve a la raíz. Se forma mayoritariamente entre especies de hongos

de las divisiones Ascomycota y Basidiomycota y el 2% de las plantas de la división Spermatophyta (Brundrett and Tedersoo, 2018). Por último, las plantas de la familia Orchidaceae y un grupo de las Ericaceae forman micorriza orquideoide (MOr) y micorriza ericoide (MEr) respectivamente (Brundrett and Tedersoo, 2018). Otro rasgo micorrícico de las plantas es el estatus micorrícico el cual indica la frecuencia de ocurrencia de la simbiosis en las especies de plantas. Puede ser categorizado como plantas siempre colonizadas por hongos micorrícicos o micorriza obligada (MO); algunas veces o micorriza facultativa (MF); o nunca micorrizada (NM) (Moora, 2014; Smith and Read, 2008). La distribución del tipo y del estatus de micorriza en una comunidad vegetal pueden indicar el grado de dependencia de la relación simbiótica entre las plantas y los hongos que forman la micorriza. Por ejemplo, cuando existe una alta proporción de tipos de micorriza con estatus obligado, significa que las plantas dependen totalmente de la micorriza y por lo tanto regulan el desempeño de la planta, influyendo finalmente en la diversidad de esa comunidad.

Dicho todo esto, es evidente el rol clave que la micorriza puede tener en las comunidades vegetales existentes. Sin embargo, gran parte de las conclusiones han emergido de estudios del hemisferio norte en donde, por ejemplo, se conocen los tipos de micorriza presentes en muchas de las distintas plantas además de la diversidad de los hongos que forman la micorriza y de los factores que la determinan (Bueno et al. 2017a). En contraste, en el hemisferio sur, mas específicamente en Sudamérica la proporción de estudios es considerablemente menor y en Chile, son relativamente pocos los estudios que han analizado la diversidad de tipos de micorriza, así como la diversidad de hongos que forman la micorriza y muchos menos son los estudios enfocados en los factores que determinan la estructura de las comunidades de los hongos micorrícicos (Aguilera et al., 1998, 2014; Benedetti et al., 2018; Borie et al., 2010; Carrillo et al., 1992; Casanova-Katny et al., 2011; Castillo et al., 2006, 2016; Dhillion et al., 1995; Garrido, 1988; Godoy et al., 1994; Godoy and Mayr, 1989; Godoy and Palfner, 1997; Herrera et al., 2016; Marín et al., 2017, 2016; Medina et al., 2015; Montero Sommerfeld et al., 2013; Mujica et al., 2016; Palfner, 2001; Torres-Mellado et al., 2012). En consecuencia, muy poco se puede decir a nivel local si la micorriza cumple un rol importante en las comunidades de plantas.

Existen propuestas teóricas que indican que la micorriza es una relación que se da en casi todos los ecosistemas del planeta (Read, 1991; Read et al., 2004; Read and Perez-Moreno,

2003) y con un 92% de las plantas terrestres (Brundrett and Tedersoo, 2018), no obstante, en Chile no existe mucha evidencia que afirme las propuestas mencionadas previamente. La mayor parte del conocimiento existente proviene de los bosques templados, específicamente de la zona desde los 37º hasta los 52º de latitud sur aproximadamente y que es casi nulo el conocimiento de esta simbiosis en Chile central y norte (Aguilera et al., 1998, 2014; Benedetti et al., 2018; Borie et al., 2010; Carrillo et al., 1992; Casanova-Katny et al., 2011; Castillo et al., 2006, 2016; Dhillion et al., 1995; Garrido, 1988; Godoy et al., 1994; Godoy and Mayr, 1989; Godoy and Palfner, 1997; Herrera et al., 2016; Marín et al., 2017, 2016; Medina et al., 2015; Montero Sommerfeld et al., 2013; Mujica et al., 2016; Oehl et al., 2012; Palfner, 2001; Torres-Mellado et al., 2012).

En Chile central se encuentra el *matorral chileno* (Armesto et al., 2007), el cual corresponde a uno de los cinco ecosistemas de clima mediterráneo del planeta caracterizado por tener veranos secos y cálidos con inviernos húmedos y fríos (Rundel and Cowling, 2013). Además, es considerado un hotspot de biodiversidad debido a la alta proporción de especies de plantas endémicas presentes y que a su vez están amenazadas por factores antropogénicos (Myers et al., 2000), lo que implica que es un ecosistema de alta prioridad de conservación. Para poder conservar un ecosistema es clave conocer sus características y sus procesos, y si bien se sabe bastante respecto a la diversidad de plantas, animales, características físicas y climáticas, entre otros (Armesto et al., 2007), existen pocos estudios enfocados en los hongos micorrícicos, cuyo rol es clave tanto en las comunidades de plantas (van der Heijden et al., 2008), como en modular el funcionamiento ecosistémico (Bardgett and van der Putten, 2014). Respecto al conocimiento asociado a hongos micorrícicos en el *matorral chileno*, se conoce el tipo de micorriza de aproximadamente el 17% de las plantas de este ecosistema (Silva-Flores et al., in review). Por otra parte, algunas comunidades de hongos micorrícicos han sido descritas (Benedetti et al., 2018; Marín et al., 2017), mientras que los estudios que describen comunidades de hongos micorrícicos junto con los factores que las regulan son escasos y restringidos a OrM (Herrera et al., 2016; Mujica et al., 2016) o MA en bosques de montaña de *Nothofagus spp.* (Marín et al., 2017). El *matorral chileno*, está compuesto por varias formaciones vegetacionales, muchas de las cuales se encuentran amenazadas (Armesto et al., 2007). La formación vegetal de mayor extensión en la zona mediterránea de Chile central es el bosque esclerófilo (Luebert and Pliscoff, 2017) del cual se desconocen los factores determinan la diversidad y composición

de las comunidades de HMA. No obstante, una aproximación para realizar una predicción al menos respecto al tipo de micorriza que debiese estar en esta formación vegetacional, es buscar el tipo de micorriza para la especie de planta de interés, en otro ecosistema o buscar el tipo de micorriza conocida de una especie filogenéticamente relacionada con la especie de planta de interés (Bueno et al. 2018). Ninguna de las dos aproximaciones es completamente precisa en entregar el tipo micorrícico (Bueno et al. 2018), no obstante, proveen un punto de partida para su futura investigación. Entonces, en base a evidencia en otros ecosistemas (Carrillo et al., 1992; Fontenla et al., 2001, 1998) y otras especies de plantas filogenéticamente relacionadas (Wang and Qiu, 2006) se puede esperar que las plantas del bosque esclerófilo debiesen ser del tipo AM.

1.1 Hongos micorrícicos arbusculares

Los hongos micorrícicos arbusculares (HMA) se ubican dentro de la raíz de las plantas y en el suelo formando variadas estructuras características (Figura 1). Salvo una excepción, no se reconocen estructuras reproductivas sexuales (Pawlowska, 2005), por lo que el concepto biológico de especie en el sentido estricto no es aplicable para ellos (Smith and Read, 2008).

Las primeras descripciones de los HMA, se realizaron en base a características morfológicas y anatómicas desde esporas asexuales (Koide and Mosse, 2004). Como los HMA no parecen reproducirse sexualmente y solo parece haber reproducción clonal y parasexual (Pawlowska, 2005), es que se han descrito "especies" en base a características morfológicas, emergiendo así el concepto de morfoespecie. Por otro lado, para que los HMA sean reconocidos como morfoespecies, los hongos deben estar aislados y depositados en alguna colección reconocida (*e.g.* INVAM) (Koide and Mosse, 2004), un proceso complejo considerando la naturaleza simbiñotica obligada de estos hongos que requiere co-cultivo con raíces vegetales. Complementariamente se han realizado estudios moleculares que han permitido identificar con mayor detalle la diversidad de los HMA, permitiendo conocer un aislado junto con su identidad molecular y las relaciones filogenéticas con otras morfoespecies (Schüβler et al., 2001). Con todo esto, actualmente se ha llegado a describir alrededor de 300 morfoespecies, lo que en comparación a las 300.000 especies de plantas descritas (Hart and Klironomos, 2002), parecen representar un grupo poco diverso a nivel global.

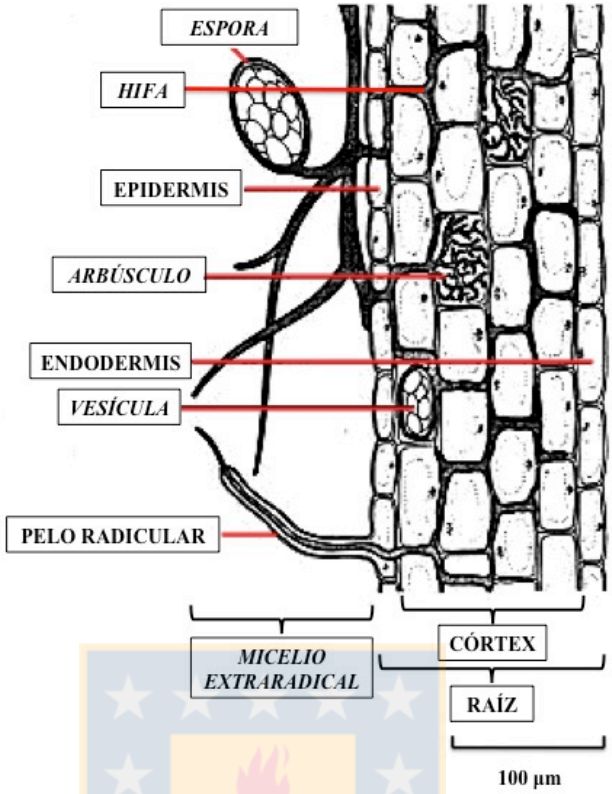

Figura 1: Esquema de una micorriza arbuscular, mostrando las estructuras de los HMA presentes en el suelo (esporas y micelio extraradical) y estructuras dentro de la raíz (arbúsculos, vesículas e hifas). Imagen modificada desde http://www.science.kennesaw.edu/~jdirnber/Bio2108/Lecture/LecBiodiversity/BioDivFungi.html

Las aproximadamente 300 morfoespecies actualmente reconocidas, han sido descritas principalmente en base a diferencias morfológicas y anatómicas de las esporas, sin embargo una gran parte de las estructuras de los HMA (arbúsculos, vesículas e hifas) se encuentran dentro de la raíz de la planta y estas estructuras no permiten la identificación del hongo a nivel de morfoespecie (Merryweather and Fitter, 1998; Sanders, 2004). Además, existe evidencia de que no todas las especies forman esporas, lo que también genera una dificultad para la descripción de especies. Favorablemente, el avance de las técnicas moleculares ha permitido avanzar en la determinación y delimitación de especies. Actualmente se utilizan métodos moleculares que utilizan genes nucleares de ARNr para la identificación de los HMA presentes en las raíces de las plantas y en el suelo (Helgason et al., 1998; Kohout et al., 2014; Öpik et al., 2014). Con esta aproximación se ha encontrado que existe una mayor diversidad de HMA de la que se pensaba analizando solo morfoespecies a partir de solamente esporas del suelo. Gracias a las técnicas

moleculares hoy se cuenta con datos de diversidad molecular de HMA o filogrupos. Para el caso de los HMA se ha propuesto que a estos filogrupos se les denomine "taxones virtuales" (TV), que permite generar una taxonomía estándar de la diversidad molecular de HMA conocidos hasta la fecha (Öpik et al., 2014, 2010). Dado esto, hoy se reconocen aproximadamente 425 TV de HMA (MaarjAM database, estado Octubre 2018), de los cuales aproximadamente 60 pueden ser asignados al concepto de morfoespecie (Öpik et al., 2014). Todo esto indica que existe una diversidad mayor a lo pensado previamente. Por último, al momento de describir los HMA que componen una comunidad determinada, es necesario considerar tanto las raíces como el suelo. Los HMA habitan ambos compartimentos y cuando se ha comparado suelo con raíz para la descripción de comunidades de HMA, se ha encontrado que los hongos presentes en el suelo no son necesariamente los mismos presentes en las raíces y viceversa (Clapp et al., 2002).

1.2 Comunidades de hongos micorrícicos arbusculares

La abundancia, diversidad y composición comunitaria de los HMA pueden depender de múltiples factores, los que pueden variar entre ecosistemas. Esto sugiere que la abundancia, diversidad, y composición comunitaria es sitio-dependiente, por lo que es recomendable investigar los posibles factores que afectan las comunidades de los HMA siempre en el sitio de interés.

La especie del fitobionte se conoce como un factor que afecta a las comunidades de HMA, inclusive en otros ecosistemas Mediterráneos (Gollotte et al., 2004; Helgason et al., 2002; Pivato et al., 2007; Sánchez-Castro et al., 2012; Santos-González et al., 2007; E Torrecillas et al., 2012; Torrecillas et al., 2012; Wang and Wang, 2014). También se ha reportado reiteradamente la relevancia de las características físico-químicas del suelo (Alguacil et al., 2008; Baoming et al., 2013; Lin et al., 2012; Schechter and Bruns, 2008; Wirsel, 2004), las estaciones del año (Helgason et al., 1999; Daniell et al., 2001; Husband et al., 2002a,b; Dumbrell et al., 2011; Dumbrell, 2013) e inclusive el compartimento examinado de los HMA (suelo vs raíz de la planta hospedera) (Varela-Cervero et al., 2016, 2015).

Dicho todo lo anterior, esta tesis tiene la siguiente pregunta de investigación, hipótesis de trabajo y objetivos.

2. Pregunta de investigación

¿Qué factores determinan la estructura comunitaria de hongos micorrícicos arbusculares del bosque esclerófilo del *matorral chileno*?

3. Hipótesis

La especie de planta hospedera, las características físico-químicas del suelo y el ciclo anual determinan la abundancia de los hongos micorrícicos arbusculares del bosque esclerófilo, además los mismos factores junto con el compartimento examinado de los hongos micorrícicos arbusculares (suelo o raíz) determinan también el patrón detectado de la diversidad y composición comunitaria.

4. Objetivo general

Identificar los factores que determinan la estructura comunitaria de los hongos micorrícicos arbusculares del bosque esclerófilo en el *matorral chileno.*

5. Objetivos específicos

Objetivo específico 1

Compilar información bibliográfica que permita apoyar el supuesto de una alta frecuencia de micorriza arbuscular asociada al *matorral chileno*.

Objetivo específico 2

Corroborar la presencia de micorriza arbuscular en las plantas dominantes de un bosque esclerófilo representativo del *matorral chileno.*

Objetivo específico 3

Describir correlaciones entre la especie de planta hospedera, los factores físico-químicos del suelo, el ciclo anual y la abundancia de los hongos micorrícicos arbusculares en un bosque esclerófilo representativo del *matorral chileno.*

Objetivo específico 4

Describir como el compartimento examinado de los hongos micorrícicos arbusculares (suelo vs raíz), la especie de planta hospedera, los factores físico-químicos del suelo y el ciclo anual determinan la diversidad y composición comunitaria de los hongos micorrícicos arbusculares en un bosque esclerófilo representativo del *matorral chileno*.

Capítulo II

Biogeography of plant mycorrhizal traits along a South American latitudinal gradient in Chile

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Article in preparation for Mycorrhiza

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Abstract

Mycorrhizal symbiosis is a key relationship between most plants and certain groups of soil fungi, central to plant nutrition, plant biotic interactions and ultimately to plant occurrence and survival. This interaction depends on the plant and taxa involved, described in four main plant mycorrhizal types; arbuscular mycorrhiza (AM), orchid mycorrhiza (OrM), ericoid mycorrhiza (ErM) and ectomycorrhiza (EcM). Besides, the frequency of occurrence of mycorrhizal symbiosis for each plant species (plant mycorrhizal status) will determine whether plants are always (obligately mycorrhizal; OM), sometimes (facultatively mycorrhizal; FM), or never (non-mycorrhizal plants; NM) colonized by mycorrhizal fungi. The mycorrhizal type (MT) and the mycorrhizal status (MS) are two plant mycorrhizal traits related to nutrient cycling, plant interactions and ecosystem processes. Therefore, studying their large-scale patterns can provide independent insights on ecological processes within and across biomes. Despite its relevance, there is no previous study about plant mycorrhizal traits along large gradients in the southern hemisphere. The aim of this study is to analyze the patterns of distribution of plant MT and MS in the Southern Hemisphere, specifically along a large continental gradient in Chile.

Specifically, we have considered all plants species in the region, across most prominent biome types (Desert, Mediterranean and Temperate) and in relation to plant species origin (native or endemic) and plant life forms (tree, shrub, sub-shrub, and annual, biennial or perennial herb) . Our results showed that AM type was by far the more frequent, in all latitudes, biomes (but with highest proportion in the desert), and among plant origins and life forms. The proportion of AM plant species decreases towards higher latitudes, while the other plant mycorrhizal types increases. In contrast, we found very few NM plant species, with highest proportions within biennial herbs. Regarding the plant origin, endemic plants showed higher proportion mycorrhizal symbiosis association than native ones, with a majority of obligate relationships. Finally, the relative proportion of EcM plants was highest within trees, where most trees showed OM status. Major gaps of empirical information on MT and MS can be found at lower and intermediate latitudes, in Desert and Mediterranean biomes and in species within families with dual species, such as Salicaceae. More empirical plant mycorrhizal data are needed in order to confirm and describe general patterns of mycorrhizal traits, and to understand their ecological role within the biomes and ecosystems of the Southern hemisphere.

Keywords

Plant mycorrhizal traits, mycorrhizal type, mycorrhizal status, arbuscular mycorrhiza, orchid mycorrhiza, ericoid mycorrhiza, ectomycorrhiza, obligately mycorrhizal, facultatively mycorrhizal, non-mycorrhizal plants.

1. Introduction

Mycorrhizal fungi are key belowground microorganisms capable of forming mycorrhiza, which is a symbiotic relation between fungal hyphae and plant roots. This symbiosis allows nutrients and water to be exchanged between plants and fungi (Smith and Read, 2008): while the fungal symbiont increases plant soil nutrient and water uptake, the plant symbiont provides the associated fungi with carbohydrates (Smith and Read, 2008) and fatty acids (Jiang et al., 2017; Keymer et al., 2017; Luginbuehl et al., 2017). Consequently, mycorrhizal associations can help plants to withstand abiotic and biotic stresses (Pozo et al., 2015). From a broader perspective, mycorrhizas improve many ecosystems processes and properties, such as plant productivity, plant diversity, soil aggregation, seedling survival, or carbon and nutrient cycling (van der Heijden et al., 2015). In addition, mycorrhizal fungi also connect coexisting plants forming common mycorrhizal networks (CMNs) allowing extensive exchange of nutrients and information (Simard et al., 2012). As such, they are currently recognized as fundamental components of plant communities (van der Heijden et al., 2008) and key modulators of ecosystem functioning (Bardgett and van der Putten, 2014).

Mycorrhizal symbiosis is expected to occur at a global scale in approximately 92% of the current known plant species (Brundrett and Tedersoo, 2018). To account for the variability and differences among different mycorrhizal associations, a trait-based approach can be adopted (Moora, 2014). The type of mycorrhizal symbiosis depends on the plant and fungal taxa involved, developing differential root symbiotic associations, each one considered as a plant mycorrhizal type (MT) (Moora, 2014). There are four main plant mycorrhizal types: arbuscular mycorrhiza (AM, approximately 78% of plants), orchid mycorrhiza (OrM, approximately 10%), ericoid mycorrhiza (ErM, approximately 2%) and ectomycorrhiza (EcM, approximately 2%) (Brundrett and Tedersoo, 2018). In contrast, plant mycorrhizal status (MS), focuses on the frequency of occurrence of mycorrhizal symbioses in plant species roots. Plant mycorrhizal status can be assigned depending on this frequency in, plants always, obligately mycorrhizal (OM); or sometimes, facultatively mycorrhizal (FM); or never colonized by mycorrhizal fungi, non-mycorrhizal (NM) plants (Moora, 2014; Smith and Read, 2008). Finally, MT and MS distribution patterns can indicate the relationship strength between plant and fungal mycorrhizal

communities, the role of the symbiosis in their dynamics and its potential applications in conservation and sustainable management of degraded ecosystems (Neuenkamp et al., 2018).

Mycorrhizal symbiosis can have an impact on plant species distribution by facilitating resources or alleviating biotic or abiotic stresses in communities, ecosystem and biomes (Gerz et al., 2018; Read, 1991; Read et al., 2004) and thus affecting their distribution along macroecological gradients (Bueno et al., 2017b; Menzel et al., 2016; Soudzilovskaia et al., 2017). While the theoretical framework of the mechanisms driving mycorrhizal patterns has been developed mostly in Europe (Read and Perez-Moreno, 2003), no study has so far analyzed these large-scale mycorrhizal traits in other continents (but see Swaty et al. 2016) in particular in the Southern hemisphere. In Europe, plant species change through latitudinal and altitudinal gradients; with lower latitudes dominated by AM and OM plants and an increased proportion of EcM, ErM, NM and FM plants towards high latitudes (Bueno et al., 2017b; Read et al., 2004). The relationship of mycorrhizal traits with habitat characteristics and other plant traits has also been explored (Hempel et al., 2013). For example, OM are positively related with higher temperatures and drier habitats and negatively associated with moist soils (Hempel et al., 2013). Also, in relation to plant species geographic niche, it has been shown that plants with wider geographical range are usually FM (Gerz et al., 2018), whilst plant species with different life spans (annuals, biennial and perennials) do not have a specific MS (Hempel et al., 2013). Also, different plant life forms might have different MS, for example annual plants living in seasonal environments are expected to be NM (Brundrett, 2009). Finally, in terms of plant MT, it has been theoretically proposed that desert plants are mostly AM, Mediterranean vegetation should have a mixture of EcM, ErM and AM, while temperate forests should be dominated by EcM with AM understory plants (Read et al., 2004). In terms of other plant traits, it is acknowledged that trees can be either EcM or AM (Smith and Read, 2008).

Southern hemisphere distribution of plant mycorrhizal traits is expected to be different from northern hemisphere patterns based on biogeographic and climatic drivers (Read, 1991; Read et al., 2004), although there is no information about large macroecological gradients in the southern hemisphere, including South America (Bueno et al., 2017a). Thus, the patterns from the northern hemisphere have been used for global projections (Read et al., 2004). Continental Chile is a territory of 4270 km long, between 17°35′ S and 56° S latitude (Bannister et al., 2012). The plant richness is approximately 4655 native plant species divided into 186 families and

1121 genera, with a species level endemism of 46% (Rodríguez et al., 2018). Contrary to the Northern hemisphere, Chile has a unimodal diversity gradient with higher richness at intermediate latitudes, in central Chile (Bannister et al., 2012). It also has a latitudinal climatic gradient, with increasing precipitation and decreasing temperature from north to south (di Castri and Hajek, 1976), what determines three major biomes: Desert in the North, Temperate in the South and Mediterranean ecosystems in between (Veblen et al., 2007). Despite the clear and interesting relationship between the Chilean latitudinal diversity and the potential role of mycorrhizal symbiosis along this gradient, very few studies exist regarding plant mycorrhizal traits in Chile (*e.g.* Carrillo et al. 1992; Godoy et al. 1994; Dhillion et al. 1995; Torres-Mellado et al. 2012), and none dealing plant mycorrhizal traits along latitudinal patterns in the Southern hemisphere.

Our general objective is to analyze the patterns of distribution of plant MT and MS in the Southern Hemisphere, specifically (1) considering all plants in continental Chile, (2) along its latitudinal gradient, (3) across Chilean biome types (Desert, Mediterranean and Temperate) and (4) in relation with plant origin (endemic or native) and life forms (tree, shrub, sub-shrub or perennial, biennial or annual herb) of Chilean plants. Thus, we have compiled all available plant mycorrhizal information about Continental Chilean plants up to now.

Due the patterns reported previously (Brundrett, 2009; Bueno et al., 2017b; Gerz et al., 2018; Hempel et al., 2013; Read et al., 2004), we expect:

Firstly, AM plants should be present in higher proportions, relative to the other plant mycorrhizal types. Accordingly, NM plants should be present in lower proportions than OM and FM.

Secondly, lower latitudes with warmer climates, showing abundant grasslands and shrublands, should be dominated by AM and OM plants. With latitudinal increment, proportions of AM and OM should decrease, while EcM, ErM, FM and OM should increase together with the replacement of grasslands and shrubland by temperate forest.

Regarding the distribution patterns across biomes, we expect for the MT trait the same as for the latitudinal gradient, considering the Desert biome as the lower latitude, Mediterranean as intermediate and Temperate as the higher latitude. While for the MS, the OM status should be more frequent in the Desert, with intermediate proportion in the Mediterranean biome and less frequent in the Temperate biome.

Finally, concerning the origin and life forms plant traits, we expect herbs to be mostly AM, OM and FM and trees mostly EcM and OM. Whilst native plants, with broader geographical ranges than endemic ones, should show higher proportions of FM than the latter, and consequently show a higher proportion of AM type.

2. Materials and methods

2.1. Plant distribution data for continental Chile

The plant distribution data were selected from the floristic database published in Bannister et al. (2012). This database covers 3787 plant species, which correspond to 81% of the richness defined for Chile (Rodríguez et al., 2018) and consists in a presence-absence matrix of the continental Chilean flora per latitudinal degree, from 18º to 56º south latitude (Bannister et al., 2012). Plant species traits regarding the origin (native and endemic) and life form (tree, shrub, sub-shrub, annual herb, biennial herb and perennial herb) were also extracted from Bannister et al. (2012). Plant taxonomy was updated according to *The Plant List* by changing the name of each plant species, genus or family to the current accepted name. Also, some plant life forms were absent for some species, thus this information was completed using the *Catálogo de Plantas Vasculares del Cono Sur* (Zuloaga et al., 2008) and Rodríguez et al. (2018). For determining the biome types, the classification of Read et al. (2004) and Veblen et al. (2007) was used: Desert (18º to 29º south latitude), Mediterranean (30º to 36º south latitude) and Temperate (37º to 56º south latitude). It was assumed that the plant species occurring within the latitudinal range for each biome, belongs to that particular biome. The new database consisted in 3168 plant species, divided in 758 genera and 163 plant families (Table 1). The updated database shows a higher proportion of native species versus endemic plant species. In terms of life forms, perennial herbs were most abundant, whilst the less abundant were the biennial (Table 1). The biome with highest abundance of plant species was the Mediterranean (Table 1).

Table 1: Number of plant families, genera and species; number and percentage of plants species by origin; number and percentage of plant species by life form and number of plant species by biome.

Number	Origin	Life form	Biome
Families: 163	Native: 1894 (50%)	Trees: 106 (3%)	Desert: 1398
Genera: 758	Endemic: 1274 (60%)	Shrubs: 507 (16%)	Mediterranean: 1591
Species: 3168		Sub-shrubs: 308 (10%)	Temperate: 1577
		Annual herbs: 483 (15%)	
		Biennial herbs: 31 (1%)	
		Perennial herbs: 1729 (55%)	
		Undefined: 4 (0.1%)	

2.2. Plant mycorrhizal dataset for continental Chile

Plants mycorrhizal types were obtained through a bibliographic review. For this, a search in the Web of Science (Database Web of Science Core Collection) was conducted for a timespan from 1988 to 2018 with the following search criteria: "mycorrhiza* AND Chile". The search retrieved 95 articles where only 10 articles had information on MT of Chilean plant species. Then, since some plants are shared with other South American countries a second search was performed in the Web of Science (Database Web of Science Core Collection) with the criteria: "mycorrhiza* AND Argentina" which yielded 5 articles about plant MT. Then four studies with large datasets on plant mycorrhizal types were explored in order to complete the MT of the continental Chilean plant species (Akhmetzhanova et al., 2012; Bueno et al., 2017b; Harley and Harley, 1987; Wang and Qiu, 2006). Even with this approach it was possible to know the MT of 13% of continental Chile plant species. As the total number of species covered by the literature were far less than half of the species described in Bannister et al. (2012), parallel hierarchical extrapolation approach was used (Brundrett, 2009), hereafter taxonomic approach according to Bueno et al. 2018, to complete the information gap of mycorrhizal traits. The taxonomic approach was developed by extrapolating the mycorrhizal data obtained at the species level to a higher taxonomic level, *i.e.* plant genus and family. This approach has been previously used in order to describe global patterns of mycorrhizal types (Brundrett, 2017, 2009) and it can be useful to describe mycorrhizal types patterns at regional or local scales when information on plant MT is far less than half of the plant list at the species level (Bueno et al. 2018). In addition, we explored

the mycorrhizal types described in four studies with large datasets on plant mycorrhizal types (Akhmetzhanova et al., 2012; Bueno et al., 2017b; Harley and Harley, 1987; Wang and Qiu, 2006) in order to complete those plant genera and families, where extrapolation from plant species or genus was not possible due the lack of information. When a plant genus had two or more species with more than one MT, then all those mycorrhizal types were considered for that genus. With this approach the mycorrhizal type for 74% of plant species at genus level was established. Following the same logic, information from plant genus to plant family was extrapolated, obtaining the mycorrhizal information for 99 % of the plant species. In some families most plant species were reported either as NM, AM or NM-AM, however exceptions with some species being EcM or EcM-like were found. This occurred for one single species in the families *Apiaceae, Caprifoliaceae, Caryophyllaceae, Cunoniaceae, Dryopteridaceae, Grossulariaceae, Ranunculaceae and Rubiaceae;* 3 species in *Goodeniaceae, Malvaceae and Sapindaceae;* and 7 species in the *Compositae* (Wang and Qiu, 2006). Since some of them might be classification errors or not really EcM (Brundrett, 2009), those families were considered as NM, AM or FM but not EcM. With this approach the mycorrhizal type for 99% of plant species was reached.

Once the dataset for MT was completed at species, genus and family level, this information was used to determine MS also at species, genus and family level. For this the criteria described by Moora (2014) was used, who considers EcM, ErM and OrM obligate biotrophs or OM, whilst AM might be either only AM or FM, thus OM and FM respectively.

2.3. Dataset analysis

In order to describe the MT and MS patterns of distribution in Chile, along the Continental Chilean latitudinal gradient, across Chilean biome types (Desert, Mediterranean and Temperate) and in relation with other traits (origin and life forms) of Chilean plants, the MT and MS plant mycorrhizal trait database at species, genus and family level of resolution was used.

Proportions were calculated using R 3.5.1 (R Core Team 2018), as well as graph construction.

3. Results

3.1. Distribution of MT and MS in continental Chile

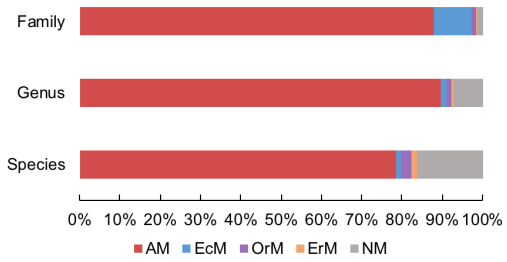
From the 3,168 species of plants compiled in the database, only 412 plant species (13%) were explored by direct observation of roots to assess their MT and consequently their MS (Table S1). In those 412 plants species, 393 (79%) were AM, 5 (1%) were EcM, 11 (3%) were OrM, 5 (1%) were ErM, 67 (16%) were NM (Figure 1a) and 1 was mycoheterothropic. It is worth noting that 2756 (87%) species had an undefined MT due the lack of direct observation of their roots. Thus, through the taxonomic approach it was possible to predict the MT of 2365 (74%) plant species at the genus level and 3158 (99%) plant species at family level, still leaving a proportion of plant species with an undefined status (Figure S1) at both, genus and family level. At the genus level, 2122 (90%) plant species were supposedly AM, 31 (2%) were EcM, 31 (1%) were OrM, 9 (0.4%) were ErM, 172 (7%) were NM (Figure 1a). Finally, at the family level, it was found that 3016 (88%) were AM, 48 (10%) were EcM, 43 (1%) were OrM, 10 (0.3%) were ErM and 41 (12%) were NM (Figure 1a).

In terms of patterns, it was found that at each taxonomic level explored, the AM type was the most frequent MT. The types EcM, OrM and ErM had similar proportions at the three resolution levels, whereas the NM proportion of plants diminished from species to family level, being the second most frequent MT only at the species and genus level of resolution (Figure 1a).

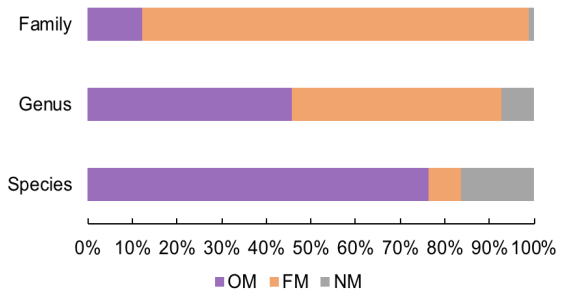
For the MS trait it was found at the species level, that 314 (77%) plant species were OM, 30 (7%) were FM and 67 (16 %) were NM (Figure 1b). The proportion of plant species within each plant MS changed depending on the taxonomic level considered (Figure 1b). At the genus level 1073 (46%) species were OM, 1100 (47%) were FM and 172 (7%) were NM. At the family level, 383 (12%) were OM, 2703 (87%) were FM and 41 (1%) were NM. The extrapolation of data from genus to family level resulted in the diminishing of the OM and the increasing of the FM status from species towards family level (Figure 1b). NM plants were always in lower proportion relative to OM and FM proportions considered together (Figure 1b) At species level NM was even higher than FM, while at higher taxonomic extrapolations this relative percentage were progressively reduced.

Figure 1: Mycorrhizal type (a) and mycorrhizal status (b) distribution in plant species of continental Chile. The proportions of both mycorrhizal traits are estimated by plant species (Species), through extrapolation of known trait for a plant species to genus level (Genus) and similarly from genus to family level (Family). AM: Arbuscular Mycorrhiza, EcM: Ectomycorrhiza, OrM: Orchid Mycorrhiza, ErM: Ericoid Mycorrhiza, NM: Non-Mycorrhizal, OM: Obligate Mycorrhiza and FM: Facultative Mycorrhiza.

(a)



(b)



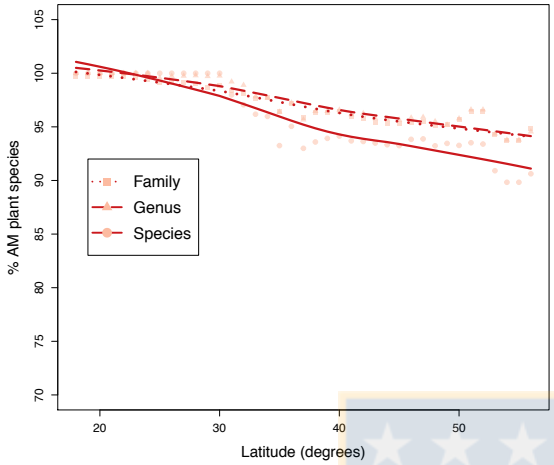
3.2. Distribution of MT and MS along the continental Chilean latitudinal gradient

The analysis of plant species MT changes along the Chilean latitudinal gradient showed clear patterns (Figure 2). Almost all mycorrhizal types showed a similar pattern independently of the level of resolution of the analysis (species, genus or family level) (Figure 2), only the family level curve in EcM type was remarkably different than the genus and species curves (Figure 2b). The proportion of AM type was always the highest MT in all latitudes at the three taxonomic levels (Figure 2). Also, the AM type showed higher proportion at lower latitudes than higher latitudes (Figure 2a). The opposite occurred for EcM type (at genus and species level of resolution) (Figure 2b) and ErM type (Figure 2d). Whilst OrM proportion also increased to higher latitudes, it reached a plateau between 40- and 50-degrees south latitude.

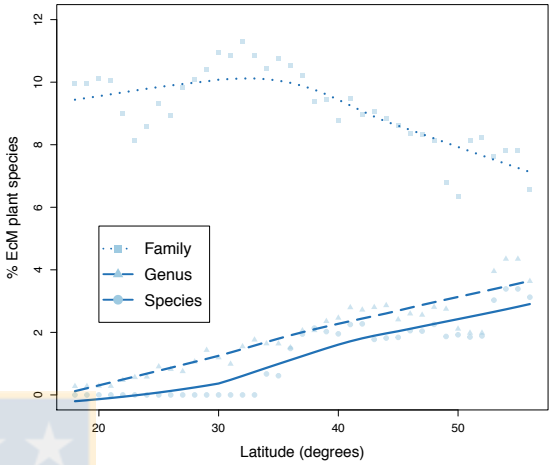
The plant species with an undefined MT also showed a similar pattern at the species and genus level of resolution, where lower latitudes had higher proportion of undefined MT in contrast to higher latitudes (Figure 3). On the contrary, at the family level the proportion of mycorrhizal undefined plant species was very low at all latitudes (Figure 3).

Figure 2: Mycorrhizal types distribution along the latitudinal gradient. (a) Arbuscular Mycorrhiza, (b) Ectomycorrhiza, (c) Orchid Mycorrhiza and (d) Ericoid Mycorrhiza. The proportions of each MT were represented by solid, dashed and dotted smooth regression lines for species, genus and family levels, respectively.

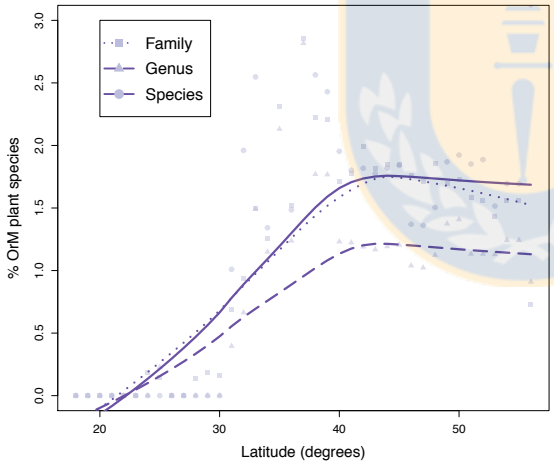
(a)



(b)



(c)



(d)

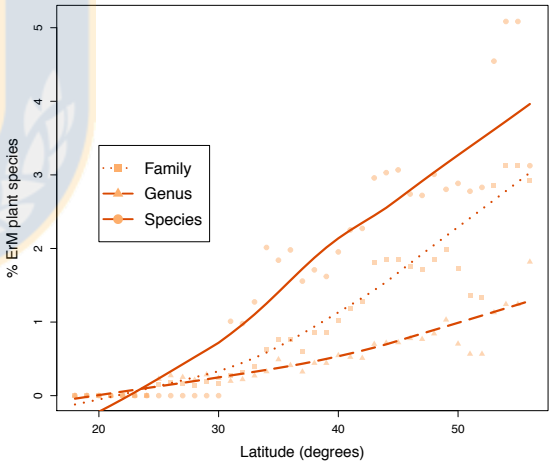
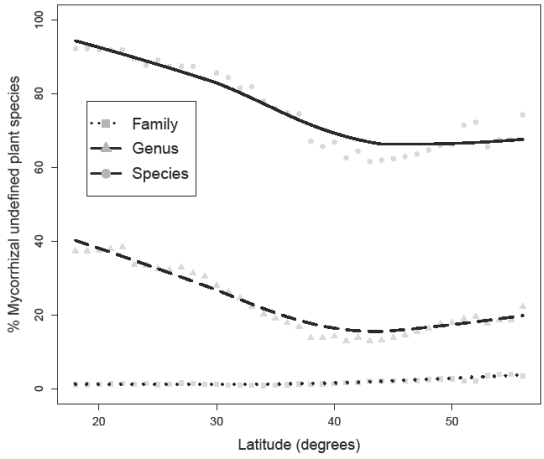


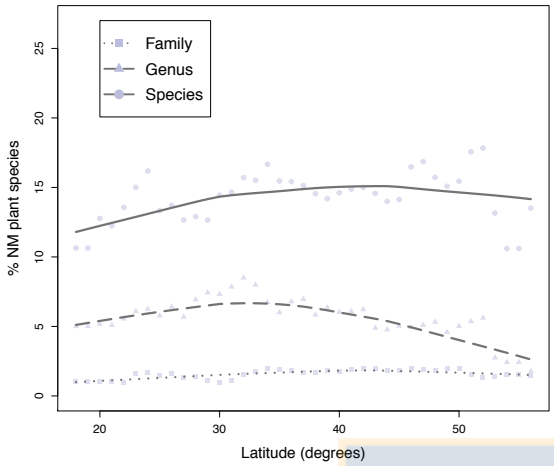
Figure 3: Proportion of mycorrhizal undefined plant species along the latitudinal gradient at different taxonomic levels of resolution. Trend lines were represented by solid, dashed and dotted smooth regression lines for species, genus and family levels, respectively.



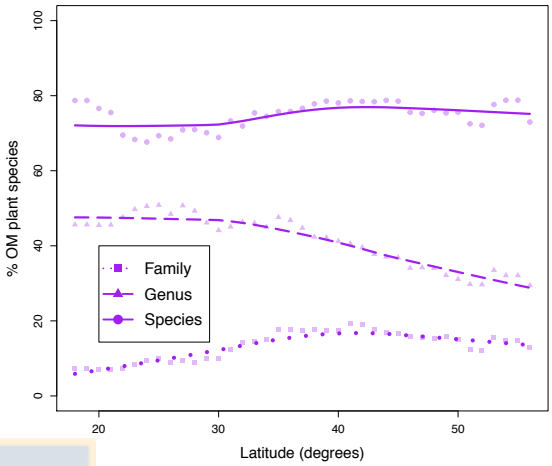
The analysis of plant species MS changes across the Chilean latitudinal gradient showed contrasting patterns depending on the taxonomic level considered. NM showed a similar trend at species and genus level with highest proportions at intermediate latitudes, while at family level the proportion were sharply reduced with no clear trend (Figure 4a). FM and OM showed complete opposite patterns, being the species and genus trends opposite between them as well (figure 4b, 4c). While the proportion of OM plants at species level increased, as at family level, the genus level decreased (Figure 4b). The opposite was found for FM plant (Figure 4c).

Figure 4: Mycorrhizal statuses along the latitudinal gradient. (a) Obligate Mycorrhiza, (b) Facultative Mycorrhiza and (c) Non-Mycorrhizal. The proportions of each MS were represented by solid, dashed and dotted smooth regression lines for species, genus and family levels, respectively.

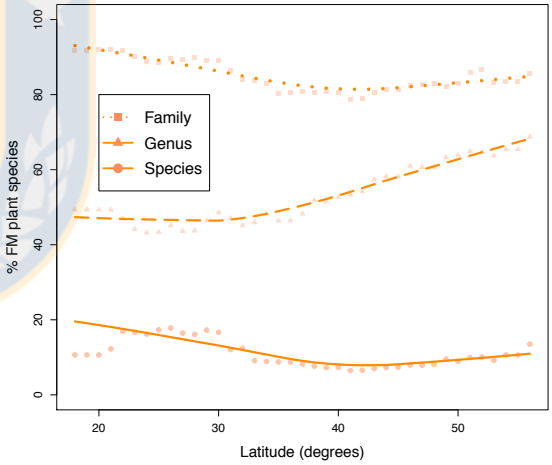
(a)



(b)



(c)



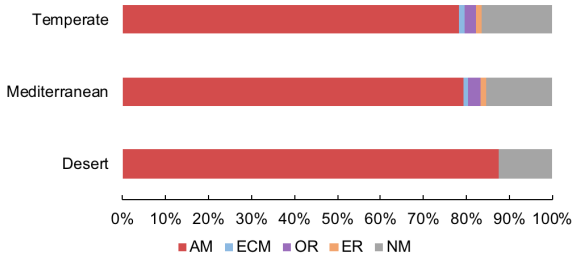
3.3. Distribution of MT and MS across Chilean biome types

The analysis of the distribution of the mycorrhizal types across biomes showed that the most frequent MT in all three biomes and at the tree taxonomic levels was the AM type (Figure 5a, 5c and 5e). In contrast, the proportion of the other three mycorrhizal types in the three biomes changed depending on the taxonomic level considered (Figure 5a, 5c and 5e). The most distinctive pattern found at the three taxonomic levels was a higher proportion of plants forming mycorrhiza in the Desert relative to the Mediterranean and Temperate biomes, while the Mediterranean and Temperate biomes showed similar proportions of plant mycorrhizal types (Figure 5a, 5c and 5e). Within the desert, only AM plants were found at the species level (and AM with EcM plants only at genus and family levels (Figure 5a).

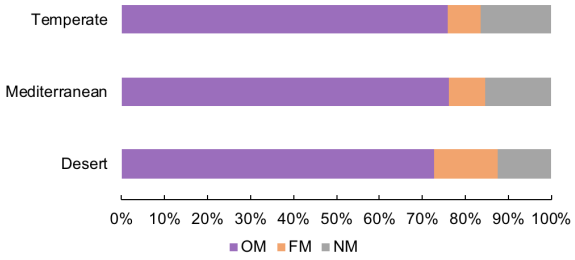
Mycorrhizal statuses across biomes analysis showed different patterns depending of the taxonomic level considered (Figure 5b, 5d and 5f). At the species level and in the three biomes, the most frequent MS was the OM (Figure 5b), whilst at the genus level, OM and FM were almost in the same proportion (Figure 5d) and at the family level, FM status was in higher frequency than the OM (Figure 5f). In accordance with the MT results, the proportion of NM plants was lower in the Desert biome at the three levels of resolution, whilst the proportions of NM plants were similar in the Mediterranean and Temperate biomes (Figure 5b, 5d and 5f).

Figure 5: Mycorrhizal type and mycorrhizal status distribution across Chilean biome types. The proportions of both mycorrhizal traits are estimated by plant species (a and b), through extrapolation of known trait for a plant species to genus level (c and d) and similarly from genus to family level (e and d). AM: Arbuscular Mycorrhiza, EcM: Ectomycorrhiza, OrM: Orchid Mycorrhiza, ErM: Ericoid Mycorrhiza, NM: Non-Mycorrhizal, OM: Obligate Mycorrhiza and FM: Facultative Mycorrhiza.

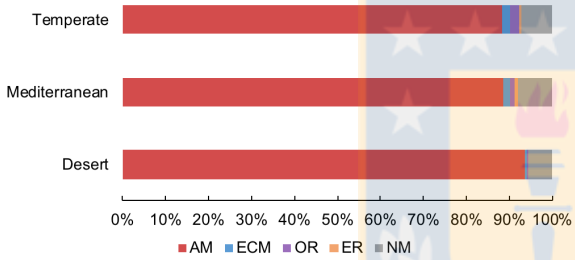
(a) MT at species level



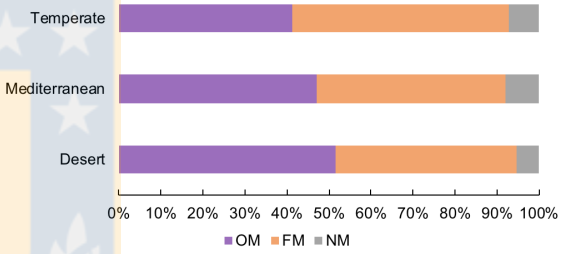
(b) MS at species level



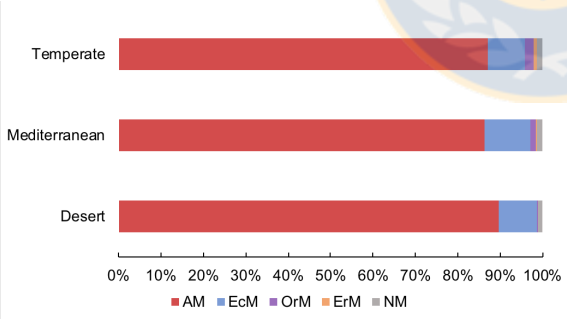
€ (genus)



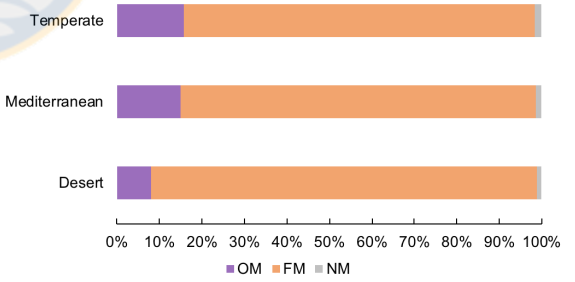
(d) (genus)



€ (family)



(f) (family)



3.4. Distribution of MT and MS in relation with other plant traits

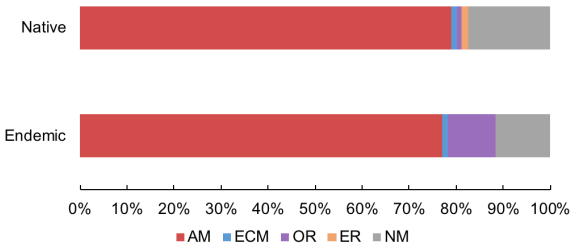
Plants with AM type were more frequent at the three taxonomic levels considered and independent of the origin (Figure 6a, 6c and 6e), followed by NM plants at the species (Figure 6a) and genus (Figure 6c) taxonomic level, whilst EcM plants were more frequent than NM at the family level (Figure 6e).

Considering the three taxonomic levels, endemic plants established mycorrhiza in a higher proportion than native plants, since native plants at the three taxonomic levels showed a higher proportion of NM plants (Figure 6a, 6c and 6e).

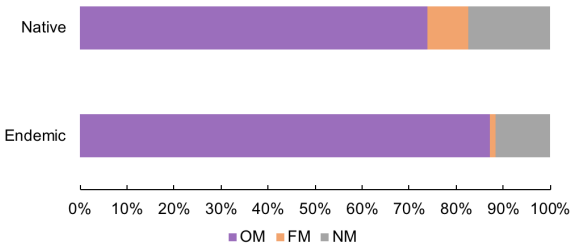
The analysis of the distribution of mycorrhizal statuses in relation with plant origin showed that independent of the taxonomic level considered, OM status was more frequent in endemic plants than in native plants, whilst the opposite occurred with FM and NM status (Figure 6a, 6c and 6e). There was also a high effect of the taxonomic level considered of the analysis in the proportions of FM and OM. For instance, at the species level, OM was considerably higher than FM (Figure 6b), in contrast to the family level (Figure 6e), where the opposite occurs.

Figure 6: Mycorrhizal type and mycorrhizal status distribution in native and endemic plants. The proportions of both mycorrhizal traits are estimated by plant species (a and b), through extrapolation of known trait for a plant species to genus level (c and d) and similarly to family level (e and d). AM: Arbuscular Mycorrhiza, EcM: Ectomycorrhiza, OrM: Orchid Mycorrhiza, ErM: Ericoid Mycorrhiza, NM: Non-Mycorrhizal, OM: Obligate Mycorrhiza and FM: Facultative Mycorrhiza.

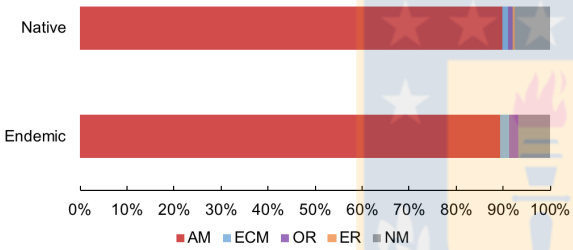
(a) MT at species level



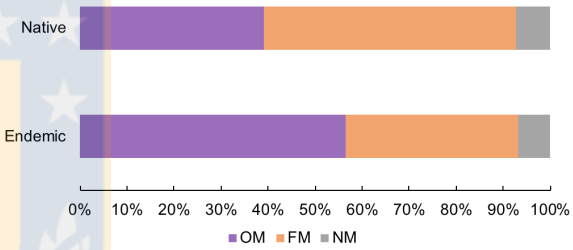
(b) MS at species level



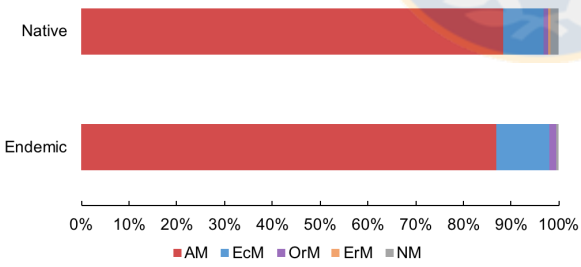
(c) (genus)



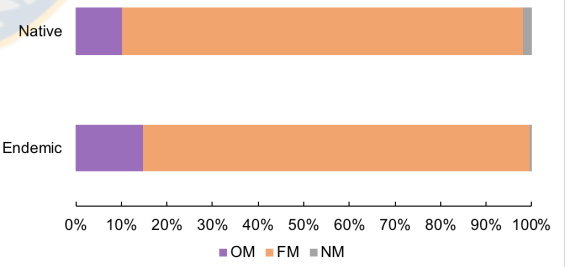
(d) (genus)



(e) (family)



(f) (family)

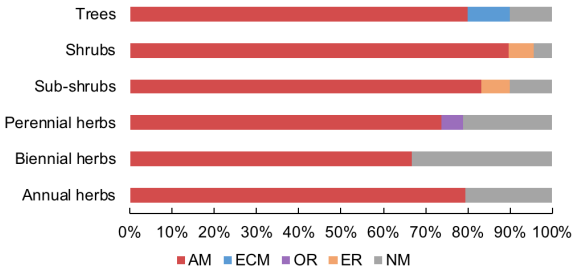


Concerning the distribution of MT across plant life forms, the most evident pattern was that AM plants were the most frequent across all plant life forms and at all taxonomic levels of the analysis (Figure 7a, 7c and 7e). Also, the EcM type was always in higher proportion in trees, however in the other plant life forms the proportions on EcM changed depending on the taxonomic level considered. Only perennial herbs formed OrM and only shrubs and sub-srubs formed ErM at the three taxonomic levels. The proportion of NM plants was highly variable across plant life forms and also depended on the taxonomic level considered.

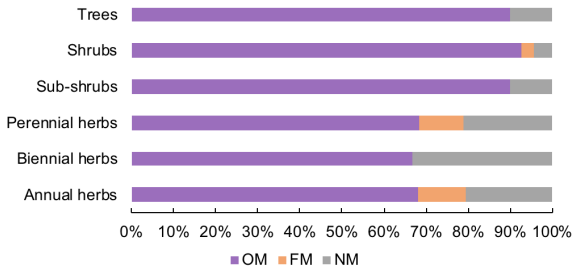
The analysis of MS across plant life forms showed that trees have a higher proportion of OM plants than FM and NM at the three taxonomic levels (Figure 7b, 7d and 7f). However, in the rest of plant life forms there were no clear patterns, since the proportion changes depending on the taxonomic level.

Figure 7: Mycorrhizal type and mycorrhizal status distribution in plant life forms. The proportions of both mycorrhizal traits are estimated by plant species (a and b), through extrapolation of known traits for a plant species to genus level (c and d) and similarly from genus to family level (e and d). AM: Arbuscular Mycorrhiza, EcM: Ectomycorrhiza, OrM: Orchid Mycorrhiza, ErM: Ericoid Mycorrhiza, NM: Non-Mycorrhizal, OM: Obligate Mycorrhiza and FM: Facultative Mycorrhiza.

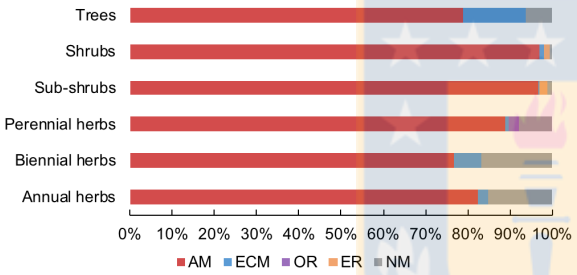
(a) MT at species level



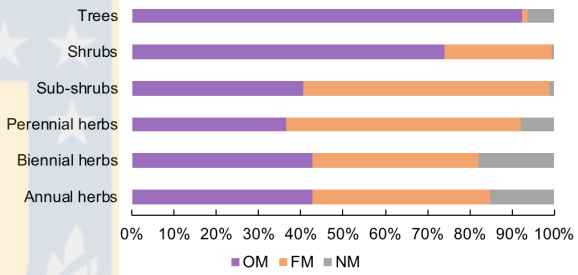
(b) MS at species level



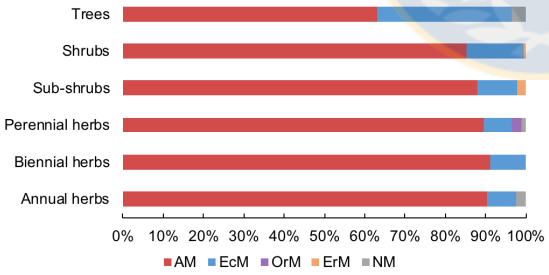
(c) (genus)



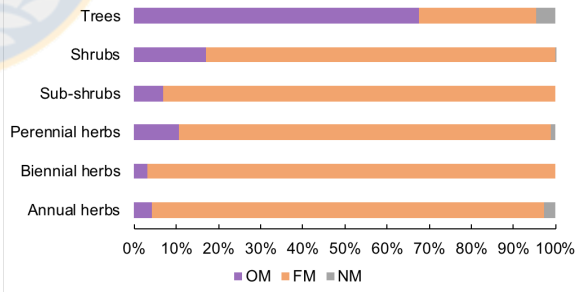
(d) (genus)



(e) (family)



(f) (family)



4. Discussion

With our analysis we were able to describe some patterns of distribution of two plant mycorrhizal traits for 68% of the Chilean known plant species, *i.e.* mycorrhizal type and status along the latitudinal gradient in continental Chile, across biome types and in relation with plant traits. To our knowledge, this can be considered the first latitudinal gradient analyses in the Southern hemisphere and in South America.

4.1. Distribution of MT and MS in continental Chile

According to our expectations the AM type resulted to be the most frequent MT relative to the other types present in the country independent of the level of taxonomic resolution of the analysis. The same occurred with the MS, where the NM status showed lower proportion than FM and OM considered together at all taxonomic levels, as well. With these results we think it is safe to state that the AM is the dominant MT and consequently most plants are either OM or FM but not NM. However, a final empirical proportion of either MT or MS cannot be established right now. At the species level, also named empirical (Bueno et al., 2018), our study could deal only with 13% of the known Chilean plant species of the database and the taxonomic approach has shown to be relative imprecise for higher categories within plant MT and MS (Bueno et al., 2018). Thus, the proportions of each MT and MS specially at higher taxonomic levels (*i.e.* genus, family), should be looked at with caution. More empirical data will allow to reveal a more complete and detailed information of plant mycorrhizal types and statuses along the studied gradient (Bueno et al., 2018).

It is worth noting that the types, OrM and ErM, had similar proportions at the three taxonomic levels, and EcM and NM plants changed more substantially its proportions towards a higher taxonomic level. The type OrM occurs only in the *Orchidaceae* and ErM in some subfamilies of the *Ericaceae* (Smith and Read, 2008) thus, the resolution level of the study should not have an effect of the proportions of these two types and consequently, we are not expecting changes in the trend of these two plant mycorrhizal types with future empirical information. In contrast EcM type, increases towards the family level, and is remarkably different from the genus and species level. This is due the fact that in Chile the only documented

EcM plants are those of the genus *Nothofagus* (Garrido, 1988). However, in Chile there are several families with species that might have EcM, since in other parts of the world have been documented. This stands for species in the families *Euphorbiaceae, Leguminosae, Myrtaceae, Nyctaginaceae, Polygonaceae, Rhamnaceae, Rosaceae* and *Salicaceae* (Brundrett, 2009). In fact, Garrido (1988), reported the presence of Cenococcum spp. hyphae like in samples of *Luma apiculata* (*Myrtaceae*), however the drawings and information provided does not allowed to discriminate the structures from dark septate endophyte hyphae that are common in plants (Newsham, 2011), thus, further studies are needed. The presence of *Amanita merxmuelleri* fruiting bodies has been observed bellow *L. apiculata* trees (Silva-Flores, personal observation), and that fungal genus is mostly forming EcM. All these provides evidence of the probable presence of EcM in plants apart from *Nothofagus* species.

4.2. Distribution of MT and MS along the Continental Chilean latitudinal gradient

Expectations regarding the MT and MS distribution along the latitudinal gradient were partially fulfilled. The AM type was always the dominating MT at all latitudes but a decrease in the proportion was found towards higher latitudes, while the opposite pattern occurred with EcM and ErM. Other studies have found the same pattern at higher latitudes (from 45º to 70º north latitude) in the northern hemisphere (Bueno et al., 2017b). Here we found a similar pattern, but from 18º to 56º south latitude. This study includes a desertic area, that has not been considered before in any study. Such a large latitudinal gradient comprised different ecosystems and biomes, hosting several environmental gradients that have proven to have an effect in changing the proportion of mycorrhizal types (Bueno et al., 2017). Here, the change in the proportion of mycorrhizal types has been shown to be potentially driven by environmental factors, which consequently leads to changes in vegetation structure. This might be related with plants niche expansion attributable to the mycorrhizal types (Gerz et al., 2018) and the different roles of prominent plant mycorrhizal types in plant communities and ecosystems (Phillips et al., 2013; Read and Perez-Moreno, 2003). Which environmental factors are affecting the MT patterns through the Southern hemisphere latitudinal gradient should be further investigated. Here, we also investigated the changes in the OrM type that resulted in a particular pattern where the proportion is low at lower latitude, increases to intermediate latitudes and reaches a plateau

toward higher latitudes. This trend reflects the increment of orchid species, reaching the higher diversity at 40º south latitude. Again, environmental conditions might produce a constrain in the OrM fungal species and thus no more orchid species can develop at higher latitudes due that constrain, however all this are hypothetical ideas that should be further investigated. Finally, it was not possible to find a consistent pattern with the MS trait in the latitudinal gradient since there is a clear effect of the level of resolution in the outcomes. More empirical data are needed in order to find clearer patterns in particular for plant mycorrhizal statuses (Bueno et al., 2018).

The analysis of the plant species with an undefined MT helped in detecting major gaps of empirical data. The higher proportion of plant species with unknown mycorrhizal information is at lower latitudes or the desert biome, followed by intermediate latitudes or mediterranean biome. Higher latitudes within the temperate biome, have the lowest proportion of undefined MT, even though the percentage of plant species with unknown mycorrhizal traits is higher than 60%. A major effort of basic research is needed in order to fill this knowledge gap and it will help in understanding the drivers for the plant distribution (Moora, 2014) and the mycorrhizal symbiosis role in ecosystem processes and diversity patterns (Philips et al., 2013; Tedersoo 2017). Ultimately, more knowledge could increase the success of conservation initiatives through a better understanding of ecological patterns (Tripp et al., 2017).

4.3. Distribution of MT and MS across Chilean biome types

According to our expectation the AM type showed a higher proportion in the Desert biome than in the other two biomes at all taxonomic levels. This suggests that the AM symbiosis might be relatively more important for plants in adapting to dry environments (Augé, 2001). Contrary to our expectations on this matter, OM status was not the most frequent status in the Desert biome, relative to the other biomes at the species and family levels, but was opposite at the genus level. Here, the contradictory patterns observed at different taxonomic levels are limiting the interpretation of the results, and highlight the need to keep extracting more empirical information to solve plant mycorrhizal status trends among biomes.

4.4. Distribution of MT and MS in relation with other plant traits

We explore the MT and MS in relation to plant origin and plant life forms. Contrary to our expectations, AM type was not more frequent in endemic plants. However, endemic plants form mycorrhiza (considering all types) more frequently than native plants, suggesting higher mycorrhizal dependence and plant-fungus specificity of these plants. These results aligned with finding higher proportions of FM plants with native than with endemic origin, whilst the opposite occurs for OM status. Native plants have a wider range of distribution than endemic plants. The ability of a plant to engage or not with mycorrhizal fungi, when necessary, allows native plants to cope with a wider range of ecological conditions (Gerz et al., 2018; Hempel et al., 2013). In contrast endemic plants are relatively obligately mycorrhizal plants, which are expected to live in less diverse habitat types, limited by the required coincidence of optimal conditions for mycorrhizal plants and fungi, constraining any potential effect of mycorrhizal fungi in plant niche range expansion or differentiation (Gerz et al., 2018). Further studies can test these conjectures.

Regarding life forms, not only herbs were mostly AM, but also trees and shrubs. EcM type was not more frequent than AM in trees, but it occurred at the highest proportion in this category of life form. In accordance to the known key role of EcM this might be related to a higher need of N than P (Philips et al 2013; Read & Moreno 2003).

This study is the first in showing distribution patterns of mycorrhizal traits in South America, and for this we focused on the flora of Chile, which is a country with an extraordinary latitudinal gradient. A limitation on this study was that empirical data are scarce thus, the taxonomic approach was used to complement the analyses, as recently suggested (Bueno et al 2018). We found several mismatches of information, when extrapolating from the species taxonomic level to genus or family taxonomic levels. These mismatches can indicate conflicting trait levels, calling for more empirical information to be collected to understand the patterns. However, when the same pattern occurred at the three taxonomic levels of resolution, the pattern appeared to be sound and can considered to be stable. Accordingly, we were able to find some patterns that are in accordance with the Northern Hemisphere and some others that might be exclusively from the Southern Hemisphere.

As a summary, we found that AM plants were the most frequent mycorrhizal type relative to the others along a large latitudinal gradient, in every biome, considering both plant origins and all plant life forms. We suspect that exploring the mycorrhizal traits in plant roots of certain plant species from plant families with other dual (AM+EcM) plant species (e.g *Salix* species from the Salicaceae family and *Luma apiculata* from the Myrtaceae family), would potentially provide more EcM plants to the Chilean flora. AM plants showed higher proportions than ErM, OrM and EcM plants at lower latitudes. AM plants decrease towards higher latitudes, while the other types increase. Major gaps of empirical information on MT can be found at lower and intermediate latitudes, which is equivalent to Desert and Mediterranean biomes. AM plants showed higher relative frequency in the Desert biome, than in the Mediterranean and Temperate biomes. In general, endemic plants form mycorrhizal symbioses at higher proportion than native plants and the majority of those relationships were obligate. Finally, only in trees it was possible to find a higher proportion of EcM plants relative to the other plant life forms. Importantly, most trees were OM in contrast to the other plant life forms, and this is given by obligately AM and EcM plants. These first results of a large latitudinal gradient in the southern hemisphere, highlights the potential relationship of plant mycorrhizal traits with the plant composition of biomes, origins of distribution of plant species and life forms in South America. More empirical plant mycorrhizal data are needed in order to confirm and describe general patterns of mycorrhizal traits, and to understand their ecological role within the biomes and ecosystems of the Southern hemisphere.

5. Acknowledgements

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7. Supplementary material

Table S1: Mycorrhizal type (MT) and mycorrhizal status (MS) of Chilean plant species from the latitudinal floristic database assessed by direct observation of plant roots (empirical approach according to Bueno et al. 2018). Plant classification, plant species origin (O), life form (LF) and biome where plant occur is specified. The country (C) where the study was performed is specified with the reference. O: N= Native, E=Endemic. LF: T=Trees, F=Shrubs, S=Sub-shrubs, A=Annual herbs, B=Biennial herbs and H=Perennial herbs. Biome: D=Desert, M=Mediterranean, T= Temperate. MT: AM=Arbuscular Mycorrhiza, OrM=Orchid Mycorrhiza, ErM=Ericoid Mycorrhiza, EcM=Ectomycorrhiza and MH=Mycoheterotrophic. MS: OM=Obligate Mycorrhiza, FM=Facultative Mycorrhiza and NM=Non-Mycorrhiza. C: A=Argentina, C=Chile, U=Uruguay, V=Venezuela and O=Other, outside South America.

Classification	**Plant species**	**O**	**LF**	**Biome**			**MT**	**MS**	**C**	**Reference**
Pteridophyta										
Aspleniaceae	*Asplenium dareoides*	N	H			T	AM	FM	C	7, 9, 21
Blechnaceae	*Blechnum asperum*	E	H			T	AM	OM	C	7
	Blechnum blechnoides	E	H			T	AM	OM	C	9
	Blechnum cordatum	N	S		M	T	AM	OM	C	7, 9
	Blechnum hastatum	N	H		M	T	AM	OM	C	7, 9
	Blechnum microphyllum	N	H		M	T	AM	OM	C	7
	Blechnum mochaenum	E	H		M	T	AM	OM	C	7
	Blechnum penna-marina	N	H			T	AM	OM	C-A	7, 21, 30
Cystopteridaceae	*Cystopteris fragilis*	N	H	D	M	T	AM	FM	O	41
Dennstaedtiaceae	*Histiopteris incisa*	N	H			T	AM	OM	O	41
	Hypolepis poeppigii	N	H		M	T	AM	OM	C	9
Dicksoniaceae	*Lophosoria quadripinnata*	N	S		M	T	AM	OM	C	9
Dryopteridaceae	*Polystichum chilense*	N	H		M	T	NM	NM	O	41
	Rumohra adiantiformis	N	H		M	T	AM	OM	C	21
Equisetaceae	*Equisetum bogotense*	N	H	D	M	T	AM	OM	A	13
Gleicheniaceae	*Sticherus quadripartitus*	N	H			T	AM	OM	C	21
Hymenophyllaceae	*Hymenophyllum pectinatum*	N	H		M	T	NM	NM	C	9

Classification	Plant species	O	LF	Biome			MT	MS	C	Reference
Pteridophyta										
Hymenophyllaceae	*Hymenophyllum peltatum*	N	H		M	T	AM	OM	O	41
	Hymenophyllum plicatum	N	H		M	T	NM	NM	C	21
Lycopodiaceae	*Lycopodium magellanicum*	N	H			T	AM	OM	C	21
Ophioglossaceae	*Ophioglossum lusitanicum*	N	H		M	T	AM	OM	O	41
	Ophioglossum vulgatum	N	H			T	AM	OM	O	41
Polypodiaceae	*Grammitis poeppigiana*	N	H			T	NM	NM	O	41
	Synammia feuillei	N	H		M	T	NM	NM	C	7, 21
Pteridaceae	*Adiantum chilense*	N	H	D	M	T	AM	OM	C	7
	Cheilanthes bonariensis	N	H	D			AM	OM	O	4
	Cheilanthes myriophylla	N	H	D			AM	OM	O	4
	Pellaea ternifolia	N	H	D	M	T	AM	OM	O	41
Salviniaceae	*Azolla filiculoides*	N	A	D	M	T	NM	NM	O	41
Schizaeaceae	*Schizaea fistulosa*	N	H			T	AM	OM	C	21
Gymnospermae										
Araucariaceae	*Araucaria araucana*	N	T			T	AM	OM	C	19, 21
Cupressaceae	*Austrocedrus chilensis*	N	T		M	T	AM	OM	C	19
	Fitzroya cupressoides	N	T			T	AM	OM	C	19, 21
	Pilgerodendron uviferum	N	T			T	AM	OM	C	19, 21
Ephedraceae	*Ephedra frustillata*	N	F		M	T	AM	OM	A	13
Podocarpaceae	*Lepidothamnus fonkii*	N	S			T	AM	OM	C	19
	Podocarpus nubigenus	N	T			T	AM	OM	C	19, 21
	Podocarpus salignus	E	T		M	T	AM	OM	C	19
	Prumnopitys andina	E	T		M	T	AM	OM	C	19

Classification	Plant species	O	LF	Biome			MT	MS	C	Reference
Gymnospermae										
Podocarpaceae	*Saxegothaea conspicua*	N	T			T	AM	OM	C	9, 19
Angiospermae										
Magnoliopsida										
Acanthaceae	*Stenandrium dulce*	N	H	D	M	T	AM	OM	A	15
Aextoxicaceae	*Aextoxicon punctatum*	N	T		M	T	AM	OM	C	7, 9
Aizoaceae	*Carpobrotus chilensis*	N	A	D	M	T	AM	OM	C	29
Amaranthaceae	*Atriplex atacamensis*	N	F	D			AM	OM	C	1
	Atriplex cristata	E	H	D			AM	OM	C	1
	Atriplex deserticola	N	H	D			AM	OM	C	1, 10
	Atriplex madaragae	N	H	D			AM	OM	C	1, 10
	Atriplex repanda	E	F	D	M		AM	OM	C	1, 10
	Chenopodium quinoa	N	A	D	M	T	AM	FM	O	25
	Dysphania ambrosioides	N	H	D	M	T	AM	FM	O	28
Anacardiaceae	*Schinus patagonicus*	E	F		M	T	AM	OM	A	12, 13
	Schinus polygama	N	F	D	M	T	AM	OM	C	7
Apiaceae	*Azorella amenighinoi*	N	H			T	AM	OM	A	13
	Azorella madreporica	N	S	D	M		AM	OM	C	8
	Azorella trifurcata	N	S			T	AM	OM	A	13
	Centella asiatica	N	H			T	AM	OM	O	32, 33
	Eryngium paniculatum	N	H		M	T	AM	OM	C-A	12, 21
	Laretia acaulis	N	S	D	M		AM	OM	C	8
	Lilaeopsis macloviana	N	H	D	M	T	AM	OM	A	13
	Mulinum spinosum	N	S	D	M	T	AM	OM	A	13

Classification	Plant species	O	LF	Biome			MT	MS	C	Reference
Angiospermae										
Magnoliopsida										
Apiaceae	*Osmorhiza chilensis*	N	H		M	T	AM	OM	C-A	7, 9, 12, 13, 21
	Pozoa coriacea	N	H		M	T	AM	OM	C	8
	Sanicula crassicaulis	N	H		M	T	AM	OM	C	7
Apocynaceae	*Cynanchum pachyphyllum*	E	H		M	T	AM	OM	C	7
	Diplolepis nummulariifolia	N	S		M	T	AM	OM	A	12
	Elytropus chilensis	N	F		M	T	AM	OM	C	7
Araliaceae	*Hydrocotyle bonariensis*	N	H	D	M	T	AM	OM	O	41
	Hydrocotyle poeppigii	E	H		M	T	AM	OM	C	7
	Raukaua laetevirens	N	T			T	AM	OM	A	12
Atherospermataceae	*Laurelia sempervirens*	E	T		M	T	AM	OM	C	7
	Laureliopsis philippiana	N	T		M	T	AM	OM	C	9
Berberidaceae	*Berberis congestiflora*	E	F			T	AM	OM	C	7
	Berberis darwinii	N	F		M	T	AM	FM	C-A	7, 12
	Berberis microphylla	N	F		M	T	AM	OM	C-A	7, 12, 17
	Berberis montana	N	F		M	T	AM	OM	C	21
	Berberis serratodentata	N	F			T	AM	OM	C	21
	Berberis trigona	N	F		M	T	AM	OM	C	21
Bignoniaceae	*Campsidium valdivianum*	N	F			T	AM	OM	C	7, 9
	Eccremocarpus scaber	N	F		M	T	AM	OM	C	7
Boraginaceae	*Cryptantha clandestina*	E	A	D	M	T	AM	OM	C	10
	Heliotropium curassavicum	N	H	D	M		AM	OM	O	41
	Phacelia secunda	N	H		M	T	AM	OM	C	8
	Tiquilia litoralis	N	S	D			AM	OM	C	10

Classification	Plant species	O	LF	Biome			MT	MS	C	Reference
Angiospermae										
Magnoliopsida										
Brassicaceae	*Lepidium didymum*	N	B	D	M	T	AM	OM	O	22
	Nasturtium officinale	N	H	D	M	T	AM	OM	O	22
	Rorippa palustris	N	H			T	AM	OM	O	13, 22, 41
Calceolariaceae	*Calceolaria biflora*	N	A		M	T	AM	OM	C-A	13, 21
	Calceolaria crenatiflora	N	H			T	AM	OM	A	12
	Calceolaria polyrhiza	N	H			T	AM	OM	A	12, 13
Calyceraceae	*Boopis gracilis*	N	A		M		NM	NM	A	13
	Nastanthus ventosus	N	H			T	AM	OM	C	8
Caprifoliaceae	*Valeriana carnosa*	N	H		M	T	AM	OM	A	13
	Valeriana laxiflora	N	H			T	AM	OM	A	12
Caryophyllaceae	*Silene andicola*	N	H			T	NM	NM	A	12
Celastraceae	*Maytenus boaria*	N	T	D	M	T	AM	OM	C-A	7, 12
	Maytenus chubutensis	N	F		M	T	AM	OM	A	12, 13
	Maytenus disticha	N	F		M	T	AM	OM	C	21
	Maytenus magellanica	N	T			T	AM	OM	C-A	9, 12
Ceratophyllaceae	*Ceratophyllum demersum*	N	H		M	T	NM	NM	O	23
Compositae	*Achyrocline tomentosa*	N	S	D			AM	OM	A	14
	Adenocaulon chilense	N	H		M	T	AM	OM	C	21
	Agoseris coronopifolia	N	A			T	AM	OM	A	13
	Baccharis nivalis	N	S			T	AM	OM	C	21
	Baccharis obovata	E	F		M	T	AM	OM	A	13
	Baccharis racemosa	N	F		M	T	AM	OM	C	7
	Baccharis sagittalis	N	S	D	M	T	AM	OM	C	7

Classification	Plant species	O	LF	Biome			MT	MS	C	Reference
Angiospermae										
Magnoliopsida										
Compositae	*Bidens andicola*	N	H	D			AM	OM	A	5
	Bidens pilosa	N	A	D	M	T	AM	OM	O	41
	Chaetanthera linearis	E	A	D	M		AM	OM	C	10
	Chaetanthera lycopodioides	N	H		M		NM	NM	C	8
	Chuquiraga oppositifolia	N	F		M		AM	OM	A	13
	Dasyphyllum diacanthoides	N	T		M	T	AM	OM	C	9
	Eclipta prostrata	N	A		M		AM	OM	O	32
	Encelia canescens	N	F	D			AM	OM	C	10
	Erigeron andicola	N	H		M	T	AM	OM	C	8
	Galinsoga parviflora	N	A	D	M	T	AM	OM	O	41
	Gamochaeta spiciformis	N	H		M	T	NM	NM	C	21
	Haplopappus glutinosus	N	S		M	T	AM	OM	A	13
	Helenium aromaticum	N	H	D	M		AM	FM	C	10
	Hypochaeris incana	N	H			T	AM	OM	A	13
	Hypochaeris tenuifolia	N	H		M	T	AM	OM	C	21
	Leptocarpha rivularis	E	F		M	T	AM	OM	C	7
	Leucheria achillaeifolia	N	H		M	T	NM	NM	A	12
	Mutisia decurrens	N	F		M	T	AM	OM	A	13
	Mutisia spinosa	N	S		M	T	AM	OM	A	13
	Nassauvia aculeata	N	H		M	T	AM	OM	A	13
	Nassauvia glomerulosa	N	F			T	NM	NM	A	13
	Nassauvia lagascae	N	H		M	T	NM	NM	C	8
	Noticastrum sericeum	N	H		M	T	AM	OM	O	41

Classification	Plant species	O	LF	Biome			MT	MS	C	Reference
Angiospermae										
Magnoliopsida										
Compositae	*Perezia carthamoides*	N	H		M		NM	NM	C	8
	Perezia linearis	N	H		M	T	AM	OM	A	13
	Perezia pedicularifolia	N	H			T	AM	OM	C	21
	Perezia recurvata	N	H		M	T	AM	OM	A	13
	Perityle emoryi	N	H	D	M		AM	OM	C	10
	Senecio acanthifolius	N	H			T	AM	OM	C	21
	Senecio bracteolatus	N	F		M	T	AM	OM	A	13
	Senecio bustillosianus	E	S		M		AM	OM	C	8
	Senecio chionophilus	N	S			T	AM	OM	C	21
	Senecio francisci	N	F		M		AM	OM	C	8
	Senecio sericeo-nitens	N	S			T	AM	OM	A	13
	Senecio subumbellatus	N	S		M	T	AM	OM	A	13
	Senecio trifurcatus	N	H			T	AM	OM	C	21
	Sigesbeckia serrata	N	A			T	AM	OM	A	5
	Solidago chilensis	N	H	D	M	T	AM	OM	A	12
	Symphyotrichum vahlii	N	H			T	AM	OM	C	7
	Tagetes minuta	N	A	D	M	T	AM	OM	O	22
	Triptilion achilleae	N	A			T	NM	NM	A	13
	Verbesina encelioides	N	A	D			AM	OM	O	41
Convolvulaceae	*Calystegia soldanella*	N	H		M	T	NM	FM	O	41
	Dichondra sericea	N	H	D	M	T	AM	OM	C	9
Coriariaceae	*Coriaria ruscifolia*	N	F		M	T	AM	OM	C	21
Cornaceae	*Griselinia ruscifolia*	N	F			T	AM	OM	C	9

Classification	Plant species	O	LF	Biome			MT	MS	C	Reference
Angiospermae										
Magnoliopsida										
Crassulaceae	*Crassula moschata*	N	A			T	NM	NM	O	41
Cunoniaceae	*Eucryphia cordifolia*	N	T		M	T	AM	OM	C	9
	Weinmannia trichosperma	N	T		M	T	AM	OM	C	9
Droseraceae	*Drosera uniflora*	N	H			T	AM	OM	C	21
Elaeocarpaceae	*Aristotelia chilensis*	N	T		M	T	AM	OM	C-A	7, 9, 12
Ericaceae	*Gaultheria caespitosa*	N	F-S			T	ER	OM	C	21
	Gaultheria mucronata	N	F		M	T	ER	OM	A	13
	Gaultheria phillyreifolia	N	F		M	T	ER	OM	C	9
	Gaultheria poeppigii	N	S		M	T	ER	OM	A	39
	Gaultheria pumila	N	F		M	T	ER	OM	C	9
Escalloniaceae	*Escallonia leucantha*	N	F			T	AM	OM	C	7
	Escallonia revoluta	E	T		M	T	AM	OM	C	7
	Escallonia rubra	N	F		M	T	AM	OM	A	12
Euphorbiaceae	*Dysopsis glechomoides*	N	H			T	AM	OM	C	9
	Euphorbia serpens	N	A	D	M	T	AM	OM	O	23
Geraniaceae	*Geranium magellanicum*	N	H			T	AM	OM	A	13
	Geranium sessiliflorum	N	H	D	M	T	AM	OM	A	12
Gesneriaceae	*Asteranthera ovata*	N	S			T	AM	OM	C	9
	Mitraria coccinea	N	F		M	T	AM	OM	C	7, 9
Grossulariaceae	*Ribes cucullatum*	N	F		M	T	AM	OM	A	13
	Ribes magellanicum	N	F			T	AM	OM	C-A	9, 12
	Ribes punctatum	N	F		M	T	AM	OM	C	7
	Ribes valdivianum	N	F			T	AM	OM	C	21

Classification	Plant species	O	LF	Biome			MT	MS	C	Reference
Angiospermae										
Magnoliopsida										
Gunneraceae	*Gunnera magellanica*	N	H		M	T	AM	OM	A	13
Hydrangeaceae	*Hydrangea serratifolia*	N	F		M	T	AM	OM	C	9
Lardizabalaceae	*Boquila trifoliolata*	N	F		M	T	AM	OM	C	7
	Lardizabala biternata	E	F		M	T	AM	OM	C	7
Lauraceae	*Persea lingue*	N	T		M	T	AM	OM	C	7
Leguminosae	*Acacia caven*	N	T	D	M	T	AM	OM	C	10
	Adesmia boronioides	N	F			T	AM	OM	A	13
	Adesmia corymbosa	N	H			T	AM	OM	A	13
	Adesmia filifolia	E	A	D	M		AM	OM	C	10
	Adesmia longipes	N	H			T	AM	OM	C	21
	Adesmia retusa	N	H			T	AM	OM	C	21
	Adesmia tenella	E	A	D	M		AM	OM	C	10
	Adesmia volckmannii	N	F		M	T	AM	OM	A	13
	Geoffroea decorticans	N	T	D	M		AM	OM	U	16
	Lathyrus magellanicus	N	H		M	T	AM	OM	A	13
	Lathyrus subandinus	E	H		M	T	AM	OM	C	21
	Lupinus microcarpus	N	A	D	M	T	AM	FM	O	35
	Prosopis alba	N	T	D			AM	OM	A	15
	Sophora microphylla	N	T			T	AM	OM	C	7
	Vicia nigricans	N	H		M	T	AM	OM	A	12, 13
	Vicia setifolia	N	A		M		AM	OM	C	21
Lentibulariaceae	*Pinguicula antarctica*	N	H			T	NM	NM	C	21
Linderniaceae	*Lindernia dubia*	N	A		M	T	AM	OM	O	23

Classification	Plant species	O	LF	Biome			MT	MS	C	Reference
Angiospermae										
Magnoliopsida										
Loasaceae	*Loasa bergii*	N	H			T	NM	NM	A	12
	Loasa sclareifolia	E	H		M	T	NM	NM	C	9
Loranthaceae	*Notanthera heterophyllus*	E	F		M	T	NM	NM	C	7
	Tristerix corymbosus	N	F	D	M	T	NM	NM	C	7
	Tristerix verticillatus	N	H	D	M	T	NM	NM	C	7
Malvaceae	*Corynabutilon ochsenii*	E	F			T	AM	OM	C	7
	Corynabutilon vitifolium	E	F			T	AM	OM	C	7
	Cristaria glaucophylla	E	H	D	M		AM	OM	C	10
	Tarasa humilis	N	H			T	AM	OM	A	13
Monimiaceae	*Peumus boldus*	E	T		M	T	AM	OM	C	7
Montiaceae	*Montia fontana*	N	A			T	NM	NM	O	41
	Montiopsis gayana	N	H		M	T	NM	NM	A	13
	Montiopsis polycarpoides	N	A	D			NM	NM	A	13
	Montiopsis sericea	E	H		M		NM	NM	C	8
Myrtaceae	*Amomyrtus luma*	N	T		M	T	AM	OM	C	9
	Amomyrtus meli	E	T			T	AM	OM	C	9
	Blepharocalyx cruckshanksii	E	T		M	T	AM	OM	C	7
	Luma apiculata	N	T		M	T	AM	OM	C	7, 9, 12
	Luma chequen	E	T		M	T	AM	OM	C	7
	Myrceugenia exsucca	N	T		M	T	AM	OM	C	7
	Myrceugenia parvifolia	E	T			T	AM	OM	C	7, 9
	Myrceugenia planipes	N	T		M	T	AM	OM	C	9
	Tepualia stipularis	N	F			T	AM	OM	C	7

Classification	Plant species	O	LF	Biome			MT	MS	C	Reference
Angiospermae										
Magnoliopsida										
Myrtaceae	*Ugni candollei*	E	F		M	T	AM	OM	C	21
Nothofagaceae	*Nothofagus alessandrii*	E	T		M		ECM	OM	C	14
	Nothofagus alpina	N	T		M	T	ECM	OM	C	9, 35
	Nothofagus antarctica	N	T			T	ECM	OM	C	7, 21
	Nothofagus betuloides	N	T			T	ECM	OM	C	35
	Nothofagus dombeyi	N	T		M	T	ECM	OM	C-A	7, 9, 12, 35
	Nothofagus nitida	E	T			T	ECM	OM	C	35
	Nothofagus obliqua	E	T		M	T	ECM	OM	C	7, 9, 35
	Nothofagus pumilio	N	T			T	ECM	OM	C	21, 35
Nyctaginaceae	*Boerhavia diffusa*	N	H	D	M	T	NM	NM	O	33
Onagraceae	*Camissonia dentata*	E	A	D	M	T	AM	OM	C	10
	Clarkia tenella	E	A		M	T	AM	OM	A	13
	Epilobium barbeyanum	N	H	D	M	T	AM	OM	A	13
	Epilobium ciliatum	N	H	D	M	T	AM	OM	A	13
	Fuchsia magellanica	N	F		M	T	AM	OM	C	9
	Oenothera odorata	N	A		M	T	AM	OM	A	13
Oxalidaceae	*Oxalis compacta*	N	A	D	M		AM	OM	C	8
	Oxalis dumetorum	E	H			T	AM	OM	C	7
	Oxalis valdiviensis	N	H		M	T	AM	OM	A	12
Phytolaccaceae	*Ercilla spicata*	E	F		M	T	AM	OM	C	7
Plantaginaceae	*Bacopa monnieri*	N	H	D	M		AM	FM	O	38
	Callitriche palustris	N	A		M	T	NM	NM	O	2
	Hippuris vulgaris	N	H			T	AM	OM	O	37

Classification	Plant species	O	LF	Biome			MT	MS	C	Reference
Angiospermae										
Magnoliopsida										
Plantaginaceae	*Melosperma andicola*	N	S		M	T	NM	NM	C	8
	Plantago australis	N	H		M	T	AM	OM	A	13
	Plantago barbata	N	H	D	M	T	AM	OM	A	13
	Plantago hispidula	E	A	D	M	T	AM	OM	C	10
Plumbaginaceae	*Armeria maritima*	N	H		M	T	AM	OM	A	13
Polygalaceae	*Polygala salasiana*	N	H		M	T	AM	OM	A	13
Polygonaceae	*Muehlenbeckia hastulata*	N	F	D	M	T	AM	OM	C	7
Portulacaceae	*Calandrinia caespitosa*	N	H		M	T	NM	NM	C	8
	Calandrinia ciliata	N	A	D	M	T	AM	OM	C	7
Primulaceae	*Pelletiera verna*	N	A			T	AM	OM	O	41
	Samolus latifolius	N	H			T	AM	OM	C	7
Proteaceae	*Embothrium coccineum*	N	T		M	T	NM	NM	C	7, 9
	Gevuina avellana	N	T		M	T	NM	NM	C	7, 9
	Lomatia dentata	N	T		M	T	NM	NM	C	7, 9
	Lomatia ferruginea	N	T			T	NM	NM	C	7, 9
	Lomatia hirsuta	N	T		M	T	NM	NM	C	7, 9, 21
Quillajaceae	*Quillaja saponaria*	E	T		M	T	AM	OM	C	20
Ranunculaceae	*Anemone multifida*	N	H			T	AM	OM	A	13
	Barneoudia major	N	H		M		AM	OM	C	8
	Halerpestes cymbalaria	N	H	D	M	T	AM	OM	O	2
	Ranunculus aquatilis	N	H	D	M	T	AM	FM	O	23
	Ranunculus biternatus	N	A			T	AM	OM	O	41
	Ranunculus minutiflorus	N	H		M	T	AM	OM	C	9

Classification	Plant species	O	LF	Biome			MT	MS	C	Reference
Angiospermae										
Magnoliopsida										
Rhamnaceae	*Colletia hystrix*	N	F	D	M	T	AM	OM	A	12
	Discaria articulata	N	F		M	T	AM	OM	A	12, 13
	Discaria chacaye	N	F		M	T	AM	OM	A	12
	Rhamnus diffusa	E	F		M	T	AM	OM	C	7
Rosaceae	*Acaena antarctica*	N	H			T	AM	OM	A	13
	Acaena magellanica	N	S	D	M	T	AM	OM	A-O	13, 41
	Acaena ovalifolia	N	H		M	T	AM	OM	C-A	9, 12
	Acaena pinnatifida	N	H		M	T	AM	OM	C-A	8, 12
	Acaena platyacantha	N	H			T	AM	OM	A	13
	Acaena splendens	N	H		M	T	AM	OM	A	13
	Fragaria chiloensis	N	H			T	AM	OM	A	12
	Geum magellanicum	N	H			T	AM	OM	A	13
Rubiaceae	*Galium antarcticum*	N	H			T	AM	OM	O	41
	Galium hypocarpium	N	H	D	M	T	AM	OM	C	7
	Nertera granadensis	N	H		M	T	AM	OM	C	7, 9
	Oreopolus glacialis	N	H		M	T	AM	OM	A	13
Salicaceae	*Azara integrifolia*	E	T		M	T	AM	OM	C	7
	Azara lanceolata	N	F			T	AM	OM	C	9
	Azara microphylla	N	T		M	T	AM	OM	C	7
Santalaceae	*Antidaphne punctulata*	E	S			T	NM	NM	C	7
	Lepidoceras chilense	E	S		M	T	NM	NM	C	7
	Myoschilos oblongum	N	F		M	T	AM	FM	C-A	7, 9, 1
Schoepfiaceae	*Arjona pusilla*	N	B			T	NM	NM	A	13

Classification	Plant species	O	LF	Biome			MT	MS	C	Reference
Angiospermae										
Magnoliopsida										
Schoepfiaceae	*Quinchamalium chilense*	N	H	D	M	T	AM	OM	A	13
	Quinchamalium excrecens	E	A	D			NM	NM	C	10
	Buddleja globosa	N	T		M	T	AM	OM	C	7
Scrophulariaceae	*Fabiana imbricata*	N	F	D	M	T	AM	OM	A	13
Solanaceae	*Nolana paradoxa*	E	H	D	M	T	AM	OM	C	10
	Schizanthus candidus	E	A	D			AM	OM	C	10
	Schizanthus pinnatus	E	A		M	T	AM	OM	O	2
	Solanum tuberosum	N	H	D	M	T	AM	OM	O	41
	Solanum valdiviense	N	F			T	AM	OM	C	7
	Ovidia andina	N	F			T	AM	OM	C	12
Thymelaeaceae	*Ovidia pillopillo*	E	F			T	AM	OM	C	7, 9, 21
	Lippia turbinata	N	S	D			AM	OM	A	15
Verbenaceae	*Rhaphithamnus spinosus*	N	T		M	T	AM	OM	C	7
	Viola arvensis	N	A		M	T	AM	FM	O	23
Violaceae	*Viola atropurpurea*	N	H		M		AM	OM	C-O	8, 41
	Viola cotyledon	N	H		M	T	AM	OM	A	13
	Viola maculata	N	H			T	AM	OM	C-A	7, 12, 13
	Viola philippii	N	H		M		NM	NM	C	8
	Cissus striata	N	F		M	T	AM	OM	C	7
Vitaceae	*Balbisia gracilis*	N	S		M	T	AM	OM	A	13
Vivianiaceae	*Drimys andina*	N	T			T	AM	OM	C	9
Winteraceae	*Drimys winteri*	N	T		M	T	AM	OM	C	7, 9

Classification	Plant species	O	LF	Biome			MT	MS	C	Reference
Angiospermae										
Liliopsida										
Alstroemeriaceae	*Alstroemeria aurea*	N	H		M	T	AM	OM	C-A	7, 21, 12
	Luzuriaga polyphylla	E	S			T	AM	OM	C	7
	Luzuriaga radicans	N	S		M	T	AM	OM	C	7, 9
Amaryllidaceae	*Gilliesia graminea*	E	H		M		AM	OM	C	40
	Gilliesia montana	E	H		M	T	AM	OM	C	40
	Miersia chilensis	E	H		M	T	AM	OM	C	40
	Solaria atropurpurea	E	S		M		AM	OM	C	40
	Solaria miersioides	E	H		M	T	AM	OM	C	40
	Speea humilis	E	H		M		AM	OM	C	40
Bromeliaceae	*Fascicularia bicolor*	E	H			T	NM	NM	C	7, 9
	Greigia sphacelata	E	H			T	AM	OM	C	7
Corsiaceae	*Arachnitis uniflora*	N	H		M	T	MH		C	7
Cyperaceae	*Carex aphylla*	N	H		M	T	NM	NM	A	12
	Carex canescens	N	H			T	NM	NM	O	41
	Carex capitata	N	H			T	NM	NM	A-O	2, 13, 30
	Carex fuscula	E	H			T	NM	NM	C-A	7, 13, 30
	Carex gayana	N	H	D	M	T	NM	NM	A	13
	Carex macloviana	N	H		M	T	NM	NM	A	13
	Carex maritima	N	H	D	M	T	NM	NM	A	13
	Carex subantarctica	N	H			T	NM	NM	A	13
	Carex trifida	N	H			T	NM	NM	A	30, 41
	Eleocharis pachycarpa	N	H			T	NM	NM	A	13
	Eleocharis quinqueflora	N	H	D	M	T	NM	NM	O	23

Classification	Plant species	O	LF	Biome			MT	MS	C	Reference
Angiospermae										
Liliopsida										
Cyperaceae	*Ficinia nodosa*	N	H		M	T	AM	OM	O	41
	Isolepis inundata	N	H		M	T	AM	OM	C	7
	Isolepis ranko	N	A			T	NM	NM	O	41
	Schoenoplectus americanus	N	H	D	M	T	AM	OM	O	27
	Schoenoplectus pungens	N	H	D	M	T	AM	OM	O	26
	Schoenus rhynchosporoides	N	H			T	NM	NM	C	7, 21
	Uncinia phleoides	E	H		M	T	NM	NM	C	7
	Uncinia tenuis	N	H			T	AM	FM	C	7, 21
Dioscoreaceae	*Dioscorea brachybotrya*	E	H			T	AM	OM	C	7
	Dioscorea bryoniifolia	E	H		M		AM	OM	C-O	6, 21
Iridaceae	*Libertia chilensis*	N	H		M	T	AM	OM	C	7
	Olsynium junceum	N	H	D	M	T	AM	OM	A	13
	Sisyrinchium arenarium	E	H		M	T	AM	OM	C	21
	Sisyrinchium graminifolium	E	H	D	M	T	AM	OM	C	10
Juncaceae	*Juncus balticus*	N	H	D	M	T	AM	FM	O	11
	Juncus bufonius	N	A	D	M	T	AM	FM	O	23
	Juncus bulbosus	N	H			T	NM	NM	O	40
	Juncus effusus	N	H		M	T	AM	FM	O	23, 26, 36
	Juncus microcephalus	N	H		M	T	NM	NM	C	7
	Juncus pallescens	N	H		M	T	NM	NM	C	7
	Juncus planifolius	E	H			T	NM	NM	C	21
	Juncus procerus	N	H		M	T	NM	NM	C	7, 9
	Juncus scheuchzerioides	N	H	D	M	T	AM	FM	C	7, 9

Classification	Plant species	O	LF	Biome			MT	MS	C	Reference
Angiospermae										
Liliopsida										
Juncaceae	*Juncus stipulatus*	N	H	D	M	T	NM	NM	A	13
	Juncus tenuis	N	H		M	T	AM	FM	O	23
	Luzula campestris	N	H			T	AM	FM	O	23
	Luzula racemosa	N	H	D	M	T	AM	OM	C	21
Juncaginaceae	*Triglochin maritima*	N	H	D	M	T	NM	NM	A-O	13, 41
	Triglochin palustris	N	H	D	M	T	NM	NM	A-O	13, 41
Orchidaceae	*Bipinnula fimbriata*	E	H		M		OR	OM	C	24, 31
	Bipinnula plumosa	E	H		M		OR	OM	C	31
	Chloraea bletioides	E	H		M	T	OR	OM	C	24
	Chloraea chrysantha	E	H		M	T	OR	OM	C	24
	Chloraea crispa	E	H		M	T	OR	OM	C	24
	Chloraea fonkii	N	H			T	OR	OM	C	21
	Chloraea gavilu	E	H		M	T	OR	OM	C	24
	Chloraea grandiflora	E	H		M	T	OR	OM	C	24
	Chloraea longipetala	E	H		M	T	OR	OM	C	24
	Chloraea piquichen	N	H			T	OR	OM	A	12
	Codonorchis lessonii	N	H			T	OR	OM	C	7
Philesiaceae	*Lapageria rosea*	E	F		M	T	AM	OM	C	7
	Philesia magellanica	N	F			T	AM	OM	C	21
Poaceae	*Agrostis glabra*	N	H		M	T	AM	OM	A	13
	Agrostis idahoensis	N	H		M		AM	FM	O	2
	Agrostis magellanica	N	H			T	AM	FM	O	41
	Agrostis perennans	N	H			T	AM	OM	C	41

Classification	Plant species	O	LF	Biome			MT	MS	C	Reference
Angiospermae										
Liliopsida										
Poaceae	*Aristida adscensionis*	N	A	D	M		AM	OM	O	41
	Bromus catharticus	N	B	D	M	T	AM	OM	A	13
	Bromus setifolius	N	H	D	M	T	AM	OM	A	13
	Chusquea culeou	N	F		M	T	AM	OM	C-A	7, 9, 12
	Chusquea montana	N	H			T	AM	OM	C	21
	Chusquea quila	N	F		M	T	AM	OM	C	7
	Chusquea uliginosa	E	H			T	AM	OM	C	7, 21
	Cortaderia araucana	N	H		M	T	AM	OM	A	13
	Cortaderia selloana	N	H			T	AM	OM	C	15
	Deschampsia antarctica	N	H			T	AM	FM	O	41
	Deschampsia danthonioides	E	A	D	M		AM	OM	O	18
	Distichlis spicata	N	H	D	M	T	AM	FM	A-O	13, 41
	Festuca contracta	N	H			T	NM	NM	O	41
	Festuca monticola	N	H			T	AM	OM	C	21
	Festuca pallescens	N	H			T	AM	OM	A	13
	Hordeum comosum	N	H	D	M	T	AM	FM	A-C	8, 12, 13
	Hordeum pubiflorum	N	H	D	M	T	AM	OM	A	13
	Hordeum vulgare	N	A			T	AM	FM	C-O	8, 21
	Nassella philippii	N	H		M	T	AM	OM	V	3
	Paspalum dilatatum	N	H		M		AM	OM	O	41
	Phleum alpinum	N	H		M	T	AM	OM	O	41
	Phragmites australis	N	H	D	M	T	AM	FM	O	23
	Poa holciformis	N	H		M		NM	NM	A	13

Classification	Plant species	O	LF	Biome			MT	MS	C	Reference
Angiospermae										
Liliopsida										
Poaceae	*Poa lanuginosa*	N	H		M	T	AM	OM	A	13
	Poa ligularis	N	A			T	AM	OM	A	13
	Poa obvallata	N	H		M	T	AM	OM	C	21
	Trisetum spicatum	N	H	D	M	T	AM	FM	O	23
	Vulpia australis	N	A	D	M	T	AM	OM	A	13
Potamogetonaceae	*Potamogeton lucens*	N	H			T	NM	NM	O	23
	Stuckenia pectinata	N	H	D	M	T	NM	NM	O	23
	Zannichellia palustris	N	H	D	M	T	AM	OM	O	23
Tecophilaeaceae	*Zephyra elegans*	E	H	D			AM	OM	C	10
Typhaceae	*Typha angustifolia*	N	H		M	T	AM	FM	O	41
	Typha domingensis	N	H	D	M	T	AM	FM	O	27, 38

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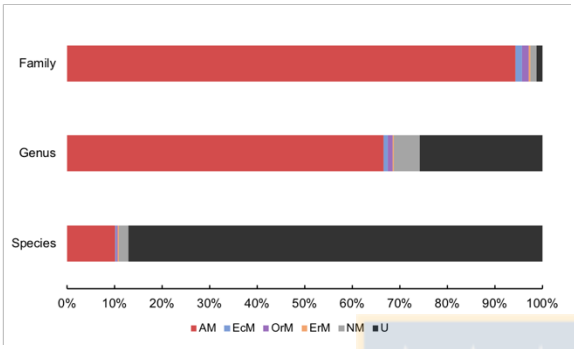
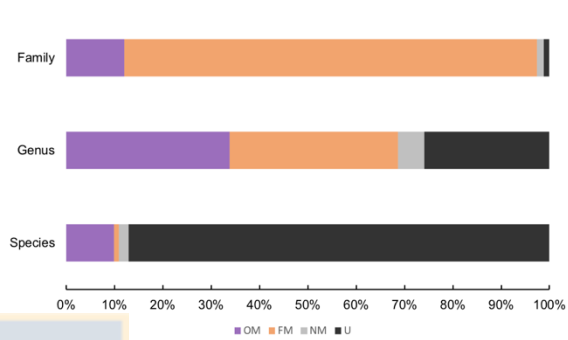
Figure S1: Mycorrhizal type (a) and mycorrhizal status (b) distribution in plant species of continental Chile. The proportions of both mycorrhizal traits are estimated by plant species (Species), through extrapolation of known trait for a plant species to genus level (Genus) and trough extrapolation of known trait for a plant genus to family level (Family). AM: Arbuscular Mycorrhiza, EcM: Ectomycorrhiza, OrM: Orchid Mycorrhiza, ErM: Ericoid Mycorrhiza, NM: Non-Mycorrhizal, OM: Obligate Mycorrhiza, FM: Facultative Mycorrhiza and U: Undefined type (a) or status (b).

(a)



(b)



Capítulo III

Mycorrhizal type of dominant trees in the sclerophyllous forest of the Mediterranean Chilean matorral

Article in review in Journal of Soil Science and Plant Nutrition

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Abstract

The Chilean matorral is one of the five worldwide Mediterranean-type ecosystems (MTEs), most of which are threatened by anthropogenic impact. Within this framework, mycorrhizal fungi (MF) are considered fundamental to restore degraded MTEs. However, gaps in basic knowledge, such as the mycorrhizal types of the dominant plant species, still limit the efficient application of MF in restoration programs on local and regional scales. In this study, we aimed to identify the mycorrhizal type of characteristic trees of the sclerophyllous forest, the most common plant formation of the Chilean matorral, by direct observation of the roots. The trees explored were *Peumus boldus*, *Lithrea caustica*, *Quillaja saponaria*, *Cryptocarya alba, Kageneckia oblonga* and *Escallonia pulverulenta*. The presence of diagnostic mycorrhizal structures, as well as the frequency (F%) and intensity (M%) of MF colonization and the presence of dark septate endophytes (DSE) were recorded. We also evaluated whether F% and M% are affected by soil factors. We establish that *C. alba, E. pulverulenta, P. boldus*, *Q. saponaria* and *K. oblonga* are AM plants. *L. caustica* showed some AM structures, however

further analyses are recommended to accurately assess their mycorrhizal type. DSE structures were present in *C. alba, P. bol*dus and *Q. saponaria*. F% was between 77% and 99%, whilst M% was between 1% and 36%. None of the soil factors investigated had a significant effect on the F% and M%. The next step would be to study the diversity of arbuscular mycorrhizal fungi associated with plants of the Chilean matorral.

Keywords

Arbuscular mycorrhiza, mycorrhizal type, Mediterranean-type ecosystem, Chilean matorral, sclerophyllous forest, restoration.

1. Introduction

The Chilean matorral is located in Central Chile from 30 to 36º southern latitude across the plains and lower altitudes of coastal and Andean mountains (Armesto et al., 2007). It is one of the five Mediterranean-type ecosystems worldwide (MTEs) which as a whole are considered to be a biodiversity hotspot (Myers et al., 2000), characterized by high plant species richness and a high proportion of endemic taxa (Cowling et al., 1996; Rundel and Cowling, 2013). Most MTEs, due to their favorable climate and fertile soils, are threatened by extensive land-use change (Underwood et al., 2009), and therefore, their conservation is of increasing concern. Since mycorrhizal fungi (MF) are efficient promoters of plant nutrition and mitigate biotic and abiotic stress in the root-soil system, they are considered key microorganisms in the restoration concepts of degraded habitats, especially in the MTEs (Harris, 2009). MF form a symbiosis with approximately 90 percent of known plant species (Brundrett and Tedersoo, 2018). Several types of mycorrhizae can be distinguished based primarily on climate zone and vegetation type: arbuscular mycorrhiza (AM), ectomycorrhiza (EcM), orchid mycorrhiza (OrM), and ericoid mycorrhiza (ErM) (Brundrett and Tedersoo, 2018). The MF structures constitute a highly dynamic and efficient extension of the plant feeder roots and thus not only enhance nutrient and water uptake in exchange for photosynthates (Smith and Read, 2008) and lipids (Jiang et al., 2017; Keymer et al., 2017; Luginbuehl et al., 2017) but also increase plant resistance to pathogens and herbivores or drought (Pozo et al., 2015). Consequently, mycorrhizal symbiosis has a positive effect on plant fitness, plant diversity and soil aggregation (van der Heijden et al., 2015), key factors for the recovery of disturbed MTEs.

Research on the functional diversity of mycorrhizal symbiosis in the Chilean matorral, despite its high plant diversity with at least 1,500 species (Bannister et al., 2012), is still scarce. From the few studies conducted directly in this ecosystem, the mycorrhizal types of approximately 71 plant species (Singer, 1969; Moser and Horak, 1975; Garrido, 1985, 1988; Casanova-Katny et al., 2011;; Torres-Mellado et al., 2012), the identity of some MF associated with native orchids (Herrera et al., 2016) and the variation in the degree of fungal specificity among some orchid species (Steinfort et al., 2010; Herrera et al., 2016; Mujica et al., 2016) have been investigated.

After the proclamation published by Bueno et al. (2017) to encourage filling the gaps in basic knowledge about the diversity, distribution and functioning of mycorrhizae in South America, the Chilean matorral appears to be a perfect candidate for this goal. Among the different plant formations, the most common one is the evergreen sclerophyllous forest (Luebert and Pliscoff, 2017), characterized by the dominant trees *Peumus boldus* Molina (Monimiaceae), *Lithrea caustica* Hook. & Arn. (Anacardiaceae), *Quillaja saponaria* Molina (Quillajaceae), *Cryptocarya alba* (Molina) Looser (Lauraceae) and *Kageneckia oblonga* Ruiz & Pav. (Rosaceae). Knowing the mycorrhizal type of the dominant plant species is important to understand plant distribution and community structure (Moora, 2014), and consequently the baseline for the restoration concepts of degraded ecosystems, which involve the root-soil compartment (Neuenkamp et al., 2018). Determining the mycorrhizal type known in other plant species of the same family offers a valuable suggestion (Brundrett, 2009) before direct observation of the diagnostic fungal structures in roots (Brundrett, 2009). Following this approach and based on studies where the diagnostic structures used to classify the mycorrhizal type were explicitly stated (Brundrett, 2009), the mycotrophy in most of the trees in the sclerophyllous forest is expected to be AM (Fontenla et al., 1998, 2001; Gehring and Connell, 2006). Whether the intensity of AMF colonization is affected by soil factors as has been shown in other habitats (Soudzilovskaia et al., 2015), but it is still unknown for the Chilean matorral. Thus, the aim of this study was to verify the presence, frequency and intensity of AM by direct observation of the roots in the dominant tree species of the sclerophyllous forest plant formation of the Chilean matorral and assess the possible relation with the soil factors.

2. Materials and methods

2.1. Sampling area

The sampling area is located in a mountain chain with east-west orientation of the Central Depression (between the Andes and the Coastal Cordillera) in the O´Higgins Region of Central Chile (34º26`S, 70º59`W). The Central Depression is characterized by fertile soils, high agricultural activity, dense human population and Mediterranean seasonal climate (warm/dry summers and cold/wet winters) (Armesto et al., 2007). Dominant trees present in this habitat were identified as *P. boldus, L. caustica, Q. saponaria, C. alba, K. oblonga* and *Escallonia pulverulenta* (Ruiz & Pav.) Pers. (Escalloniaceae). These species were selected to examine their mycorrhizal type.

2.2. Root sampling, processing and staining procedure

In December 2017, the fine roots of three randomly selected individuals of each of the six trees species were collected, yielding a total of 18 samples. The trees were all similar in height (approximately 2 meters), and the distance between them was at least 10 meters to ensure the independence of the samples (Maherali and Klironomos, 2012). Litter and soil were gently removed by hand, and at least 50 1 cm pieces of fine roots found between 1 and 10 cm depth were collected tracing them from the primary stem. In the field, the material of each plant species collected was placed individually in absorbent paper and then in a resealable plastic bag. Each bag was properly labeled to keep track of the plant species in the laboratory. During the sampling campaign and transport to the laboratory, the bags with the collected material were kept in a cooler on ice (Orchard et al., 2017). Before staining, the roots were carefully washed with tap water in the laboratory to remove the excess of soil and debris and placed in 50-mL plastic tubes. To clear the strongly impregnated and opaque fine roots, we used a previously customized method for our samples (Silva-Flores, unpublished), which consists of a combination of staining protocols adapted from Pitet et al. (2009) and Vierheilig et al. (1998). The roots were cleared in 10% KOH solution overnight. The KOH was discarded, and the roots were washed with tap water. After a bleaching step of 1 hour with 10% H2O2, the samples were acidified for 1 hour

with 5% acetic acid and subsequently stained for 72 hours in a mixture of 5% black-blue Pelikan ink and 5% acetic acid. E. pulverulenta was stained for 72 hours in 5% black Lamy ink in 5% acetic acid. A final de-staining step was performed for all the samples in acetic glycerol at 4 ºC.

2.3. Root analysis

We recorded the presence and absence of the following AM structures in the roots: appressoria at the entry point, intercellular hyphae, intracelullar hyphae, hyphal coils, vesicles and arbuscules. In addition, the presence and absence of dark septate endophytes was recorded. The frequency of mycorrhiza (F%) and the intensity of mycorrhizal colonization (M%) in the root system was calculated as described by Trouvelot et al. (1986). Stained roots of each individual plant species (3 x 6 = 18) were placed in a Petri dish with acetic glycerol. Under a dissecting microscope (GX Microscopes, model XTL101T), thirty 1-cm-long stained fine root segments from each individual of each plant species were mounted in parallel on two microscopic glass slides (15 segments each) with acetic glycerol and observed at 40x magnification under a compound microscope (Euromex Oxion, model OX.3015). The F% was calculated as follows:

$$F\% = \left(\frac{n}{N}\right) x\ 100$$

N is the total number of observed root fragments, and n is the number of root fragments with mycorrhizal structures. M% was calculated by assigning each root fragment to a relative mycorrhizal colonization class ranging from 0 to 5, where 0 is 0% mycorrhiza, 1 is <1%, 2 is < 10%, 3 is <50%, 4 is >50% and 5 is >90%. Then, the following equation was used:

$$M\% = \frac{(95n5+70n4+30n3+5n2+n1)}{N}$$

Finally, mycorrhizal structures were observed in detail under the compound microscope at increasing magnification (100x, 400x and 1000x) and documented by digital micrographs using a Euromex camera (model CMEX3).

2.4. Soil factors analysis

Rhizospheric soil was collected next to each plant sampled for the root analysis. The leaf litter was removed, and approximately 1 kg of soil from 1 to 10 cm depth was extracted and placed in a plastic bag using a shovel. The samples were stored at 4º C overnight before being transferred to the Las Garzas laboratory (Escuela Agrícola Las Garzas, O´Higgins Region, Chile) where the soil analyses were performed using the protocol suitable for Chilean soils (Sadzawka et al., 2006). The parameters analyzed were pH, electrical conductivity (EC), proportion of soil organic matter (SOM), soil available phosphorous (P), total nitrogen (N), and soil available potassium (K).

2.5. Statistical analysis

To test the effect of the soil factors on mycorrhizal frequency (F%) and on the intensity of mycorrhizal colonization (M%), and since both parameters correspond to percentages, we analyzed the data fitting a beta regression model using the betareg package (Cribari-Neto and Zeileis, 2010). We also used this model to analyze the differences in the F% and M% between plant species. Post hoc analyses were conducted by running the same beta regression model and changing the baseline reference level for each of the levels of the factor analyzed (Zuur et al., 2010). We also analyzed whether each soil factor was different between the plant species using a one-way analysis of variance (ANOVA). All these analyses were performed using R 3.5.1 (R Core Team, 2018).

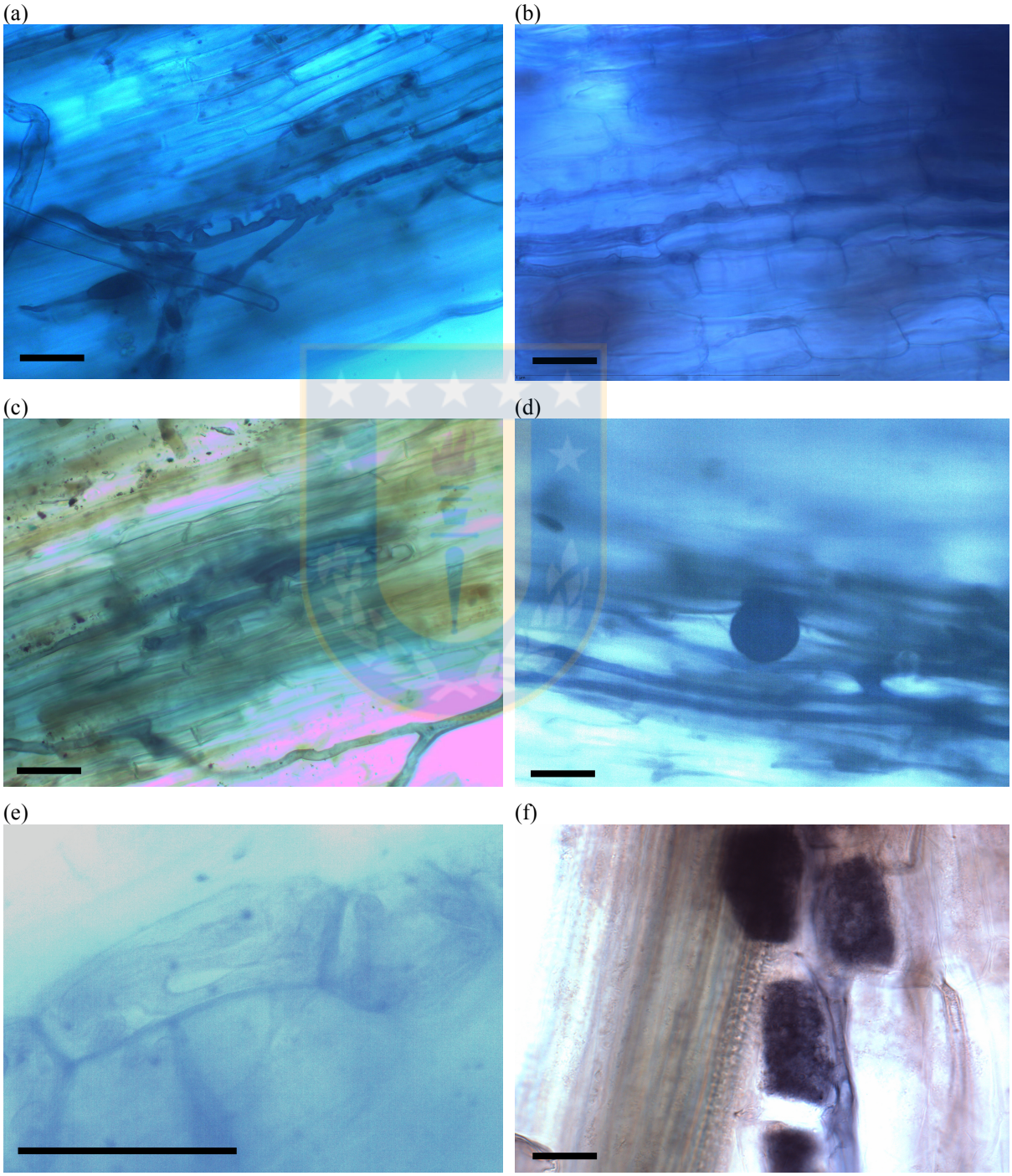
3. Results

The fine roots of all the plant species studied showed the presence of one or more AM structures (Table 1, Figure 1). In L. caustica, the least differentiation of AM structures could be observed with only intercellular aseptate hyphae present (Table 1, Figure 1C). In contrast, in *C. alba*, all the important diagnostic mycorrhizal structures (appressoria at the entry point, intercellular hyphae, intracelullar hyphae, hyphal coils, vesicles and arbuscules) were visible (Table 1, Figure 1D). While intercellular aseptate hyphae (Table 1) could be found in the rootlets of all species examined, vesicles were only found in *C. alba* and arbuscules only in *E. pulverulenta* and *C. alba* (Table 1).

Table 1. Presence (+) and absence (-) of mycorrhizal structures, mycorrhizal frequency (F%) and mycorrhizal colonization (M%) of arbuscular mycorrhizae in plant species of the sclerophyllous forest. Arbuscular mycorrhizal structures: AP = appressorium, IEH = intercellular hyphae, IAH = intracelullar hyphae, HC = hyphal coils, V = vesicles, A = arbuscules. DSE = F% and M% are means ± SE. Letters in F% and M% values show significantly differences between plant species.

Family	Plant Species	AP	IEH	IAH	HC	V	A	F%	M%	DSE
Anacardiaceae	*Lithrea caustica*	-	+	-	-	-	-	77 ± 5.1b	1 ± 0.1a	-
Escalloniaceae	*Escallonia pulverulenta*	+	+	+	+	-	+	80 ± 3.3b	33 ± 2.6b	-
Lauraceae	*Cryptocarya alba*	+	+	+	+	+	+	94 ± 2.2a	36 ± 4.6b	+
Monimiaceae	*Peumus boldus*	+	+	+	+	-	-	94 ± 1.1a	32 ± 2.2b	+
Quillajaceae	*Quillaja saponaria*	+	+	+	-	-	-	94 ± 2.2a	36 ± 6.2b	+
Rosaceae	*Kageneckia oblonga*	+	+	+	-	-	-	84 ± 2.9b	34 ± 5.1b	-

Figure 1. Arbuscular mycorrhizal structures in the fine roots of trees of the sclerophyllous forest. (a) appressoria at an entry point on the root surface of *Quillaja saponaria*; (b) intercellular hyphae in *Lithrea caustica*; (c) intracellular hyphae in *Kageneckia oblonga*; (d) vesicle in *Cryptocarya alba*; (e) hyphal coil in *Peumus boldus*; (f) arbuscules in *Escallonia purverulenta*; Scale bar for all Figures except E: 20 µm (400× magnification). Scale bar for E: 20 µm (1000× magnification).



The F% in the root systems ranged from 77% to 94% with a mean value of 87% (Table 1). The lowest F% was found in *L. caustica* and the highest in *C. alba, P. boldus* and *Q. saponaria*. In addition, the M% ranged from 1% to 36% with a mean value of 29% (Table 1). The highest M% was found in *C. alba* and *Q. saponaria* and the lowest in *L. caustica*. The statistical analysis showed a significant difference in the F% ($z = 9.338$, $P < 0.0001$) and M% ($z = -4.788$, $P < 0.0001$) between plant species (Table 1). Post hoc analysis showed that in terms of F%, *P. boldus, C. alba* and *Q. saponaria* were not different between each other. However, they differed from *L. caustica, K. oblonga* and *E. purverulenta*, which at the same time did not differ between each other (Table 1). Only *L. caustica* had a different M% from the other plant species (Table 1). The soil factors for each plant species are summarized in Table 2. In this study, the pH, SOM and P were significantly different between the plant species. However, none of the soil factors investigated had a significant effect on the F% or M% (Table 3).

Table 2. Mean values and standard error of rhizospheric soil factors for each tree species of the sclerophyllous forest investigated. '*', '**' and '***' indicate statistical significance at alpha equals 0.1, 0.05 and 0.001 respectively.

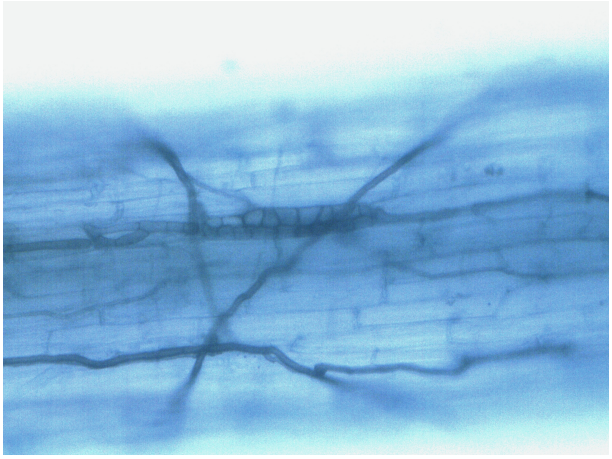
Plant species	**pH** ***	**EC** (mmhos/cm)	**SOM** (%)*	**N** (mg/kg)	**P** (mg/kg)**	**K** (mg/kg)
Lithrea caustica	6.47±0.03	0.09±0.02	6.40±1.04	18.67±2.19	25.33±6.33	356.00±60.80
Escallonia pulverulenta	5.87±0.07	0.10±0.01	9.07±0.43	22.67±0.88	10.67±4.18	236.67±49.78
Cryptocarya alba	6.47±0.12	0.77±0.35	7.23±1.10	16.00±1.00	25.00±3.00	236.00±36.91
Peumus boldus	6.93±0.89	0.09±0.003	4.43±0.39	17.33±1.76	34.33±4.84	393.00±36.56
Quillaja saponaria	7.07±0.19	0.84±0.43	5.70±1.16	19.33±0.88	16.67±1.76	317.67±68.74
Kageneckia oblonga	5.87±0.12	0.10±0.003	7.67±0.41	21.00±1.53	5.33±0.88	194.67±11.98

Table 3. Effect of soil factors, *i.e.*, pH, electrical conductivity (EC), soil organic matter percentage (SOM), total nitrogen (N), available phosphorus (P) and available potassium on a) mycorrhizal frequency (%F) and b) mycorrhizal intensity (%M). Beta regression model with z and p values shown derived from the analysis.

	a) %F		**b) %M**	
	z	p value	z	p value
(Intercept)	-0.090	0.928	-1.236	0.216
pH	1.187	0.235	1.394	0.163
EC (mmhos/cm)	0.738	0.461	-0.182	0.856
SOM (%)	-1.025	0.305	1.056	0.291
N (mg/kg)	-0.919	0.358	-0.465	0.642
P (mg/kg)	-0.624	0.532	-0.856	0.392
K (mg/kg)	-0.361	0.718	-1.393	0.164

Finally, dark septate endophytes (DSE) were found in three out of six plant species, diagnosed primarily by the presence of intracellular structures and by the DSE hyphae on the root surface (Figure 2).

Figure 2. Dark septate endophyte hyphae and dictyospore-like intracellular structure in a root of *Peumus boldus*. Scale bar 20 µm (400× magnification).



4. Discussion

The first aim of this study was to verify the AM mycorrhizal type in the roots of the dominant trees of a sclerophyllous forest plant formation of the Chilean matorral. In all six plants species explored, we found one or more diagnostic AM structures. Aside from the DSE structures in *C. alba, P. boldus* and *Q. saponaria*, no evidence of other types of mycorrhizal symbiosis could be observed.

The presence of arbuscules is the primary diagnostic structure used to unequivocally classify a plant as AM (Brundrett, 2009). Thus, based on our results, E. pulverulenta and C. alba can be classified as AM with certainty. Two of the tree species examined in our study have been previously surveyed for their mycorrhizal type by Garrido (1988). He classified E. pulverulenta (Escalloniaceae) as forming ericoid mycorrhizae. However, the drawing provided does not show the dense hyphal coils, which are typical for this type of mycorrhiza. Similarly, C. alba was described as AM by the same author, but the staining protocol was not provided, and the corresponding line drawing shows septate structures, unlike typical AM elements. In addition, the material examined by Garrido (1988) was collected more than two degrees further south (approximately 37° south latitude, surroundings of Concepción) where climate, soil conditions and vegetation are different from those prevailing in our study area. We consider our findings more reliable and representative for the Mediterranean sclerophyllous forest of Central Chile.

We did not find arbuscules in *P. boldus, Q. saponaria, K. oblonga* and *L. caustica*, which might be due to seasonal effects or to the phenology (age) of the root that has an effect on mycorrhizal development (Brundrett, 2009). *P. boldus, Q. saponaria* and *K. oblonga* demonstrated hyphae in several forms: appressoria, intercellular and intracellular hyphae. Hyphal coils were also found in P. boldus. In addition, a regular colonization was found, since the F% for these three species was between 84% and 94%. Thus, our findings provide solid evidence that *P. boldus, Q. saponaria* and *K. oblonga* form AM. It is worth noting that the AM type for *P. boldus* was previously reported by Carrillo et al., (1992). However, the roots examined by Carrillo et al., (1992) correspond to plant individuals sampled in a temperate forest (approximately 40º south latitude). Thus, our results are representative for the Chilean matorral. In addition, Godoy et al., (1991) proved that Q. saponaria is able to form AM symbiosis in a greenhouse. However, since the inoculation experiment does not necessarily reflect the natural

mycorrhizal type of a plant species, our results provided the first evidence for *Q. saponaria* in their natural environment.

L. caustica was the plant with less diagnostic AM structures, since only intercellular aseptate hyphae were found in 77% of the root fragments examined, and the M% was extremely low (1%). This plant species belongs to the Anacardiaceae. *Schinus patagonicus* (Phil.) I.M. Johnst. from the same family has been proven to form AM (Fontenla et al., 1998, 2001). However, since we found only one AM related structure, low M% and the lowest F% compared to the other species, it could be possible that this species is Non-Mycorrhizal (NM) and is only able to form an endophytic Glomeromycotan Fungus Colonization (GFC) (Brundrett, 2009). A more extensive survey of the fine roots of this species in space and time would be needed to answer this question. Another approach, also suggested by Brundrett (2009), could be to grow the plants from seeds and use the soil from natural habitats where the older roots were found to explore the complete developmental stages, including young fine roots. In addition, molecular analyses could be employed to detect arbuscular mycorrhizal fungi (AMF) in field-collected roots (Öpik et al., 2014).

We detected the presence of DSE in some samples. DSE in the roots in combination with AM have been previously reported (*e.g.*, Casanova-Katny et al., 2011) and it is not unusual that they appear in roots with variable and low intensity of AM (Brundrett, 2009), as we observed in our samples. The sclerophyllous forest is a semiarid environment with stressful seasonal droughts, and in this context, DSE have proven to be important for plants to manage water deficiency (Santos et al., 2017).

We also aimed to quantify the F% and M% and describe whether these two parameters were affected by the soil factors. Although we found significant differences in the F% and M% between plant species, these differences could not be explained by soil factors as has been reported in another study for a similar ecosystem (Neffar et al., 2015). Several other factors might be affecting the F% and M%, such as plant age (Kessler et al., 2010) and climatic variations (Mekahlia et al., 2013).

Finally, another important further step would be to identify the AMF species associated with the plant species investigated. AMF species composition will help to reveal the structure of specific communities and the presence of common mycorrhizal networks (van der Heijden and Horton, 2009). Since the Chilean matorral is increasingly threatened by human-related

factors (Underwood et al., 2009), the habitat loss that is affecting the vegetation might be recovered by reforestation close to existing stands of the same plant species, which may serve as an inoculum sources of compatible mycorrhizal symbionts. Independently of why AMF are associated with the plants (mutualistic symbionts or endophytic GFC) they require each other to sustain the ecosystem, since a plant with an AMF as GFC might serve as a bridge between two plants that could relate to those AMF as mutualistic symbionts. All of these merit future study.

5. Conclusion

Through the direct observation of roots, we could establish that the mycorrhizal type of C. alba, E. pulverulenta, P. boldus, Q. saponaria and K. oblonga is AM. Even though L. caustica showed some AM structures, and the literature also suggests that they should be AM, further analysis is recommended to accurately assess their mycorrhizal type. We also suggest future diversity analysis of AMF associated with this ecosystem to apply the knowledge in restoration initiatives in the sclerophyllous forest of the Chilean matorral, which is one of the five threatened MTEs.

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Capítulo IV

Factors affecting arbuscular mycorrhizal fungi spore density in the Chilean Mediterranean-type ecosystem

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Abstract

Arbuscular mycorrhizal fungi (AMF) are highly important for plant communities in dry or seasonally dry ecosystems, such as the South American Mediterranean-type ecosystem (MTE), considered a biodiversity hotspot. While AMF hold potential for sustainable MTE management and conservation, they have been under investigated on this ecosystem and little is known about AMF spore bank dynamics. In this study we analyzed the effect of physico-chemical soil factors, phytobiont species, and seasonality on the AMF spore soil density in two sites of a representative sclerophyllous forest (Malloa and San Vicente). We sampled soil once per season during one year and beneath four representative tree species for each site. The results show a strong season effect at both sites, while physical-chemical parameters differed between sites. At Malloa, clay content and electrical conductivity were positively correlated with spore density, while available phosphorous showed a negative correlation. At San Vicente, clay content and total nitrogen were positively correlated with spore density, while soil organic matter showed a negative

effect. Overall, spore number reached a minimum value in winter and higher values during the growing season at both sites. These results indicate a strong regulation of AMF spore density by seasonal climate, while physico-chemical soil properties exert a host-independent but site-specific effect in both forests.

Keywords

spores, arbuscular mycorrhizal fungi, Mediterranean-type ecosystem, Chilean matorral, seasonality, spore bank

1. Introduction

The only South American Mediterranean-type ecosystem (MTE) (Arista *et al.,* 2017) is located in Central Chile, approximately between 30º and 36º South latitude (Armesto *et al.,* 2007). This MTE is also known as the Chilean matorral and is considered a biodiversity hotspot because it holds a high level of plant species endemism and at the same time is threatened by several anthropogenic factors (Cowling *et al.,* 1996; Myers *et al.,* 2000). The Chilean matorral, as most Mediterranean ecosystems, is in urgent need of novel concepts of conservation and sustainable management due to degradation by human land uses, especially agriculture (Underwood *et al.,* 2009). In this context, research on arbuscular mycorrhizal fungi (AMF) (Phylum Glomeromycota) (Tedersoo *et al.,* 2018) is relevant from several points of view. Firstly, mycorrhizal symbiosis is present in most terrestrial plants, driving essential or macro nutrient exchanges between both symbionts (Smith and Read 2008); the mycobiont facilitates and enhances soil nutrient uptake by the phytobiont, in return for plant carbohydrates (Smith and Read, 2008) and lipids (Keymer *et al.,* 2017). In addition, mycorrhizal associations have been shown to mitigate abiotic and biotic stress suffered by plants, such as drought, pathogens, and herbivores (Pozo *et al.,* 2015). Overall, mycorrhizal symbiosis has a significant influence on plant fitness, as well as on several ecosystem processes: carbon, nitrogen, and phosphorous cycles; regulation of plant diversity; soil aggregation; and seedling survival (van der Heijden *et al.,* 2015). Consequently, it has been demonstrated, that AMF have a role in restoration of degraded natural ecosystems (Neuenkamp *et al.,* 2018), such as the Chilean matorral.

AMF spores can be considered as a dormant structure that is formed as a response to an unfavorable change in the environment of the fungi (Lennon and Jones, 2011). This dormant structure constitutes a spore bank which is dynamic partially because of the ability of the spores of entering and breaking dormancy (sporulation and germination respectively) in response to changes in environmental conditions (Lennon and Jones, 2011). In fact, AMF spore bank can be highly variable between and within different ecosystems and being affected by different soil physical, chemical and biological factors (Smith and Read, 2008). Many studies across different ecosystems have investigated the effect of these factors on spore density at both, spatial (*e.g.* Carvalho *et al.,* 2003; He *et al.,* 2004; Cuenca and Lovera 2010) and temporal (*e.g.* Allen *et al.,* 1998; Lugo and Cabello 2002; Escudero and Mendoza 2005) scales. Particularly, for

Mediterranean ecosystems, such as salt marsh and the maquis in Portugal, it has been shown that soil organic matter is positively correlated with spore density in the maquis but not in the salt marsh (Carvalho *et al.,* 2003). Another study in a Mediterranean agroecosystem in Jordan showed that the spore density is not affected by pH, electrical conductivity, clay and silt percentage (Mohammad *et al.,* 2003). However, soil organic matter and phosphorous positively and negatively affect spore density respectively (Mohammad *et al.,* 2003). Moreover, increasing the nitrogen content has proven to negatively affect AMF spore density in a Mediterranean agroecosystem from Italy (Avio *et al.,* 2013). In terms of biological factors, Varela-Cervero *et al.,* (2016) showed that plant host species in Spain, can have a significant effect on AMF spore density. Finally, spore density temporal changes in two Mediterranean ecosystems (Spain and Australia), has shown that spore number is relatively high during the growing season (spring-summer-autumn) (Smith, 1980; López-Sánchez and Honrubia, 1992) and decreased to a minimum in winter (López-Sánchez and Honrubia, 1992). Surprisingly, neither AMF spore density nor their expected drivers, such as season, soil physical or chemical factors, or phytobiont species, have been extensively studied in Chilean Mediterranean areas (Marín *et al.,* 2017; Benedetti *et al.,* 2018). To determine which factors, affect the AMF spore bank in the Chilean matorral can have important implications for conservation strategies. The spore bank represents not only an AMF biodiversity source, but also a reserve of biodiversity in terms of mycorrhizal associations, plant species, communities and ecosystems, as well as ecosystem processes (Lennon and Jones, 2011).

Consequently, on this study, we aimed to explore for the first time, the effect of plant host, season, and physico-chemical soil factors on spore density of AMF present in two sites of a representative sclerophyllous forests of the Chilean matorral. According to background studies previously presented for other MTEs, we expect that (1) soil organic matter, phosphorous and nitrogen will be the more important soil factors affecting AMF spore density; (2) that plant host identity will have a role in explaining the effect on soil spore density and (3) that seasonality will have an effect on spore density with a minimum value in winter, and higher values during the plant growing season (spring-summer-autumn).

2. Materials and Methods

2.1. Study site

The study area is located in Libertador Bernardo O´Higgins region of central Chile and is characterized by a typical Mediterranean climate with relatively warm, dry summers (December-February) and cold, wet winters (June-August). Field work was conducted from summer 2015 to spring 2016. On this time period mean precipitation was 101 mm in summer, 194 mm in autumn, 162 mm in winter and 40 mm in spring. While the mean air temperature was 21º C in summer, 13º C in autumn, 11º C in winter and 18º C in spring. Finally mean 1o cm soil temperature was 22º C in summer, 15º C in autumn, 11º C in winter and 19º C in spring. Two sites of a representative sclerophyllous forests where chosen for the study and corresponded to an evergreen vegetation with trees and shrubs. The first site was located at Malloa (hereafter M site) (34º26`S, 70º57`W) and was dominated by *Peumus boldus* Molina (Monimiaceae), *Lithrea caustica* Hook. & Arn. (Anacardiaceae), *Kageneckia oblonga* Ruiz & Pav. (Rosaceae), and *Escallonia pulverulenta* (Ruiz & Pav.) Pers. (Escalloniaceae). This site had a north-east facing slope, being relatively dry. The second forest was located at San Vicente (hereafter SV site) (34º26`S, 70º59`W) and was dominated by *P. boldus*, *L. caustica, Quillaja saponaria* Molina (Quillajaceae) and *Cryptocarya alba* (Molina) Looser (Lauraceae). This site had a south-west facing slope, which was more humid than Malloa, due to less sun exposure. In general, plant phenology was similar among all the plant species. On average, vegetative growth started in spring (September-November), followed by flowering-bud formation and flowering during summer (December-February), fruiting in autumn (March-May), whereas there was no growing activity during winter (June-August) (Orshan, 1989).

2.2. Sampling procedure

In each site (M and SV), samples were collected from three individuals, of each of four plant species, on four seasons, obtaining 96 samples in total (2 sites x 3 individuals x 4 plant species x 4 seasons = 96 samples). The sampling area in each site consisted in one plot of approximately 100 x 100 m. Plant individuals were randomly selected however were all similar in height

(approximately 2 meters), and the distance between them was at least 10 meters to ensure the independence of the samples (Maherali and Klironomos, 2012). The same plant individuals were sampled in each season. All samples consisted of 2.5 kg of rhizospheric soil and most were collected during a three-day period each season; autumn 2016 (April), winter 2016 (June), and spring 2016 (September); only the summer samples were collected in early summer (December 2015) in SV site and in mid-summer (January-2016) in M. Soil sampling was performed with a shovel from a depth of 1–10 cm after removal of leaf litter, and soil was collected in plastic bags. After sampling a plant species, the shovel was cleaned using water and paper towel in order to avoid cross contamination. Additionally, three cylindrical soil cores were collected per sample, to calculate soil bulk density. Immediately after field work, the samples were transported to the laboratory and stored at 4ºC for further analyses.

2.3. Soil chemical analysis

The day after sampling, 1 kg of each soil sample was used for chemical analyses at Las Garzas laboratory (*Escuela Agrícola Las Garzas*, Chile), based on a soil protocol recommended for Chilean soils (Sadzawka *et al.,* 2006). Briefly, each sample was homogenized, air dried, and sieved at <2 mm mesh size. Then pH, soil organic matter (SOM), soil available phosphorous (P), total nitrogen (N), and soil available potassium (K) were determined; pH was obtained using a pH-meter in a 1:2.5 soil water suspension; SOM was obtained through soil oxidation using dichromate in an acid medium and with colorimetric determination of the reduced chromate; P (P-Olsen) was obtained by extracting phosphorous with a solution of sodium bicarbonate (0.5 mol/L, pH 8.5) and with colorimetric determination using molybdenum blue; N was determined with the Kjeldahl method (Sadzawka *et al.,* 2006). Finally, K content was obtained through the method of saturation extract and measured by absorption and atomic emission spectrophotometry with lanthanum.

2.4. Soil physical analysis

Electrical conductivity (EC) was also measured for each sample at *Las Garzas* laboratory after extracting soil with water in a proportion of 1:2.5. Another 1 kg of each soil sample was used

to determinate particle size distribution using the hydrometer method (Bouyoucos, 1962), and particle density (ρs) by the method of the pycnometer (Blake and Hartge, 1986a). Finally, bulk density (ρb) was determined by the cylinder method (Blake and Hartge, 1986b). In order to determine ρs and particle size distribution, the soil samples were air dried and sieved at <2 mm mesh size; ρb was determined using undisturbed soil samples from soil cores which were dried in an oven at 105 °C for 24 h. Particle size distribution was used to estimate the soil content of sand, silt, and clay.

2.5. Spore isolation and spore density quantification

Spore isolation was performed with the remaining 0.5 kg of each soil sample. Soil was homogenized; larger stones and roots removed and only 100 mL of soil was selected for spore isolation. For this, a customized method of wet-sieving-decanting and sucrose centrifugation was performed (Gerdemann and Nicolson, 1963; Brundrett *et al.,* 1996). The final material was poured with water in a plastic 100 mm diameter Petri dish marked with a 5 mm square grid to separate stereomicroscope fields for spore counting. Direct spore counting of each sample was performed. Only intact and apparently healthy spores were counted under a stereomicroscope at 40x magnification using a four-digit tally counter. Sporocarps and spore clusters were considered as 1 spore.

2.6. Statistical analysis

To analyze the relative importance of seasonality and physical, chemical, and biological drivers on AMF spore density, we used generalized mixed models (GLMM) with a negative binomial error distribution. For investigating how soil physical (EC, proportion of sand, silt, and clay, ρs, and ρb) and chemical variables (pH, SOM, P, N, and K) influenced the soil AMF spore density, the models included season, plant species, and plant individual replicates as random factors. Due to collinearity problems among predictors (correlation higher than 0.6 and VIF values higher than 3) (Zuur *et al.,* 2010) we omitted K, and proportion of silt and sand from the analyses. To compare the differences among sites on the soil properties, we ran linear mixed models (LMM) with each soil property as response, and season and species as random factors.

To analyze the effect of the host plant identity and season on soil AMF spore density, a negative binomial GLMM with plant individual replicates as a random factor was used. *Post hoc* analyses were carried out by running the same GLMM models, but changing the baseline reference level for each of the levels of the factor analyzed (Zuur *et al.,* 2010).

Model assumptions, including over-dispersion, were visually inspected and met the validation criteria; residual dispersion around one, and normal distributed model residuals (Zuur *et al.,* 2010). All analyses were performed in R version 3.4.2 (R Core Team, 2018) using the package *lme4* (Bates *et al.,* 2015) and the function *nb.glme* for the negative binomial GLMM.

3. Results

3.1. Physico-chemical soil properties effect on soil spore density

The mean spore density in the Malloa site was 617 spores per 100 cm 3 of soil, with a maximum and minimum density of 3012 and 92 spores per 100 cm 3 of soil, respectively. In the San Vicente site, the mean spore density was 600 spores per 100 cm 3 of soil, with a maximum and minimum density of 2574 and 124 spores per 100 cm 3 of soil, respectively. Sporocarps or spores cluster were found only in two samples, thus, the results are neither under nor over estimating the spore density in soil.

When comparing the relative average values from both sites, we found a significant difference in pH, nutrients, and slightly on silt proportion (Table 1). M site showed significant lower average values for pH, and P, while in SV site, the relative lower values were for SOM, N, and silt (Table 1). Other factors, such as EC, content of sand and clay, ρs, ρb, or K, did not differ between sites, when plant host species and seasonal variability was accounted for (Table 1). Following the average values of sand, silt, and clay (Table 1), both M and SV site presented sandy-clay soil textures.

Soil spore density was significantly affected by physico-chemical factors at both study sites. Contrary to our expectation, not only SOM, P and N affected the spore bank, but also other factors at each site. At M site, we found a positive correlation of spore density with EC ($p < 0.05$), and clay ($p < 0.01$), and a negative correlation with P ($p < 0.05$) (Table 2; Figure 1a, 1b, and 1c), while in SV site, N ($p < 0.01$) and clay ($p < 0.05$) showed a positive and SOM ($p < 0.01$), a negative significant correlation with AMF spore densities in the soil (Table 2; Figure 1d, 1e, and 1f).

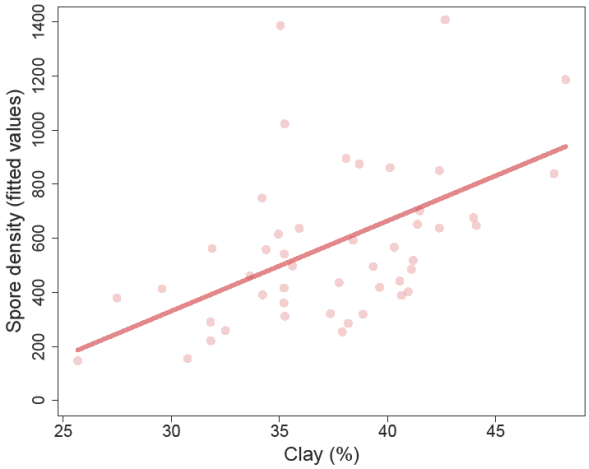
Table 1. Mean values, standard error, and range of physico-chemical parameters for both sites of the sclerophyllous forest investigated. '*', '**' and '***' indicate statistical significance at alpha equals 0.1, 0.05 and 0.001 respectively, after linear mixed models with species and season as crossed random factors.

	a) Malloa		b) San Vicente	
	Mean ± SE	Range	Mean ± SE	Range
pH * **	**5.854 ± 0.039**	**5.3-6.6**	**6.750 ± 0.055**	**6.2-7.7**
EC (mmhos/cm)	0.121 ± 0.006	0.06-0.22	0.240 ± 0.043	0.07-1.62
SOM (%) **	**7.496 ± 0.278**	**4.2-13.2**	**6.404 ± 0.415**	**3.6-16.9**
**N (mg/kg) ** **	**26.458 ± 0.992**	**14-47**	**22.438 ± 1.010**	**14-48**
P (mg/kg) * **	**13.542 ± 1.333**	**4-41**	**27.417 ± 1.553**	**12-59**
K (mg/kg)	330.583 ± 19.799	160-605	328.229 ± 15.465	159-607
SAND (%)	46.853 ± 1.063	32.3-62.8	49.823 ± 0.878	40.4-66.9
SILT (%) *	**15.577 ± 0.530**	**9.7-23.7**	**15.240 ± 0.385**	**10.3-20.9**
CLAY (%)	37.570 ± 0.700	25.7-48.2	35.004 ± 0.592	20.7-41.2
ρs	2.433 ± 0.022	2.1-3.0	2.512 ± 0.017	2.2-2.7
ρb	1.014 ± 0.020	0.6-1.3	1.042 ± 0.018	0.7-1.3

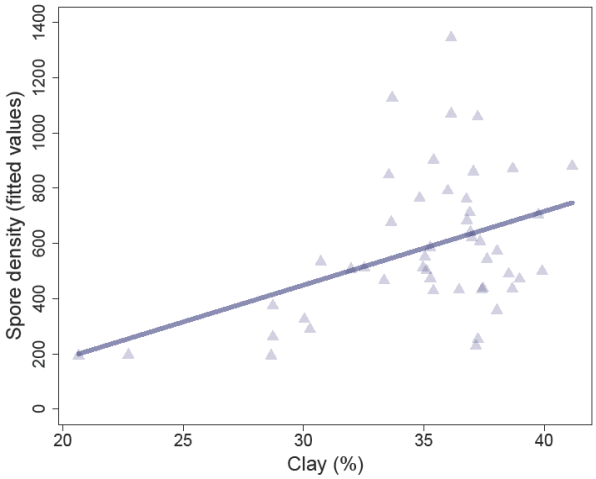
Table 2. Soil spore densities in relationship to physico-chemical soil properties. *i.e.* pH, electrical conductivity (EC), soil organic matter percentage (SOM), total nitrogen (N), available phosphorus (P), the proportion of clay (Clay), soil particle density (ρs) and soil bulk density (ρb) in Malloa (a) and in San Vicente (b). Generalized linear mixed model with negative-binomial error distribution were used, with the seasons, plant species and plant individual identities as random factors. Due to collinearity among predictor variables, K concentration, proportion of silt and sand were left out of the analyses. *Chisq* and *p* values shown derived from the analyses of deviance tables.

	a) Malloa			b) San Vicente		
	Chisq	Df	p value	Chisq	Df	p value
(Intercept)	2414.752	1	0.000	1808.768	1	0.000
pH	0.125	1	0.724	0.388	1	0.533
EC (mmhos/cm)	**5.744**	**1**	**0.017**	2.636	1	0.104
SOM (%)	1.588	1	0.208	**7.756**	**1**	**0.005**
N (mg/kg)	0.134	1	0.714	**7.909**	**1**	**0.005**
P (mg/kg)	**4.645**	**1**	**0.031**	0.330	1	0.566
Clay (%)	**9.360**	**1**	**0.002**	**4.690**	**1**	**0.030**
ρS	0.317	1	0.574	0.436	1	0.509
ρb	0.063	1	0.802	0.077	1	0.782

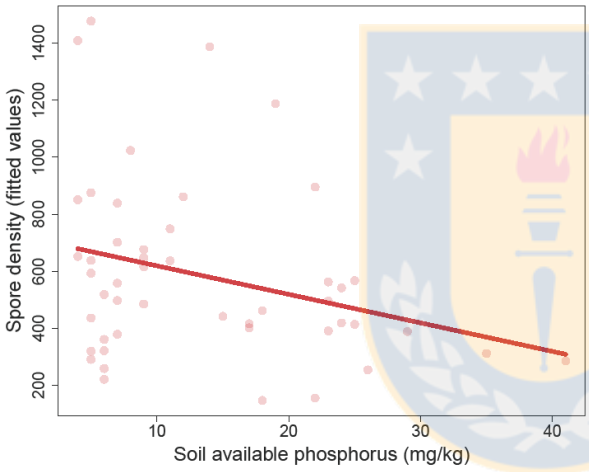
a)


Spore density (fitted values)
0
200
400
600
800
1000
1200
1400
25
30
35
40
45
Clay (%)

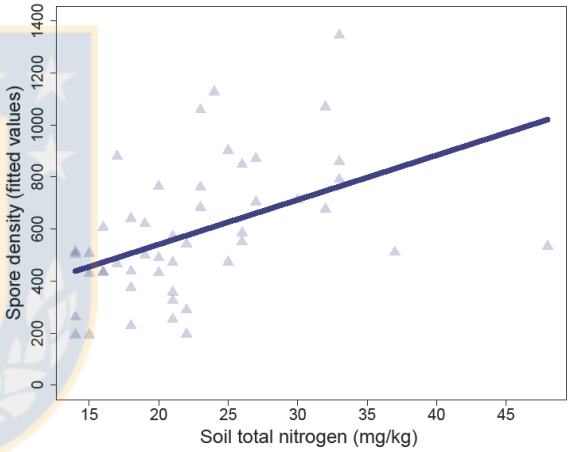
d)


Spore density (fitted values)
0
200
400
600
800
1000
1200
1400
20
25
30
35
40
Clay (%)

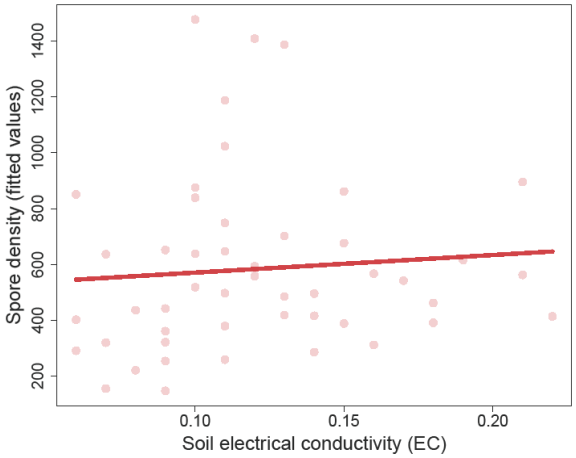
b)


Spore density (fitted values)
200
400
600
800
1000
1200
1400
10
20
30
40
Soil available phosphorus (mg/kg)

e)


Spore density (fitted values)
0
200
400
600
800
1000
1200
1400
15
20
25
30
35
40
45
Soil total nitrogen (mg/kg)

c)


Spore density (fitted values)
200
400
600
800
1000
1200
1400
0.10
0.15
0.20
Soil electrical conductivity (EC)

f)

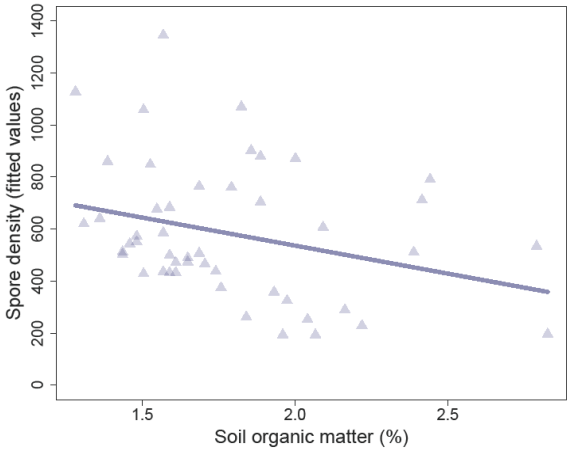

Spore density (fitted values)
0
200
400
600
800
1000
1200
1400
1.5
2.0
2.5
Soil organic matter (%)

Figure 1. Spore density in relation to the most relevant edaphic factors after generalized mixed negative binomial models (see table 1) for the Malloa site (red circles, left column): proportion of soil clay (a), soil phosphorus (b), and soil electrical conductivity (c), and for the San Vicente site (blue triangles, right column): proportion of soil clay (d) soil total nitrogen (e) and organic matter (f). Seasons, plant host species identities and the plant individual identities were used as random factors.

3.2. Host plant species and seasonality effect on soil spore density

Soil spore density was significantly affected by plant host species only at SV site($p < 0.001$), but not at M site (Table 3). However, at SV site, only average spore density of L. caustica was significantly higher than values of the other three plant species (Figure 2b). At M site, the highest spore density was found under K. oblonga, and the lowest was found under P. boldus (Figure 2a), however the values were not statistically significant.

Season was a significant factor modulating soil spore density at both study sites (Table 4). The lowest spore median density was found in winter, while higher values were found during the plant growing season (spring-summer-autumn) for both sites (Figure 2c and 2d).

Table 3. Soil spore densities in relationship to host plant species and season, in Malloa (a) and in San Vicente (b) Generalized linear mixed model with negative-binomial error distribution were used, with the plant individual identities as a random factor. *Chisq* and *p* values shown derived from the analyses of deviance tables.

	a) Malloa			b) San Vicente		
	Chisq	Df	p value	Chisq	Df	p value
(Intercept)	559.753	1		1091.438	1	0.000
Plant host	5.121	3	0.163	**25.743**	**3**	**0.000**
Season	**24.971**	**3**	**0.000**	**17.439**	**3**	**0.001**

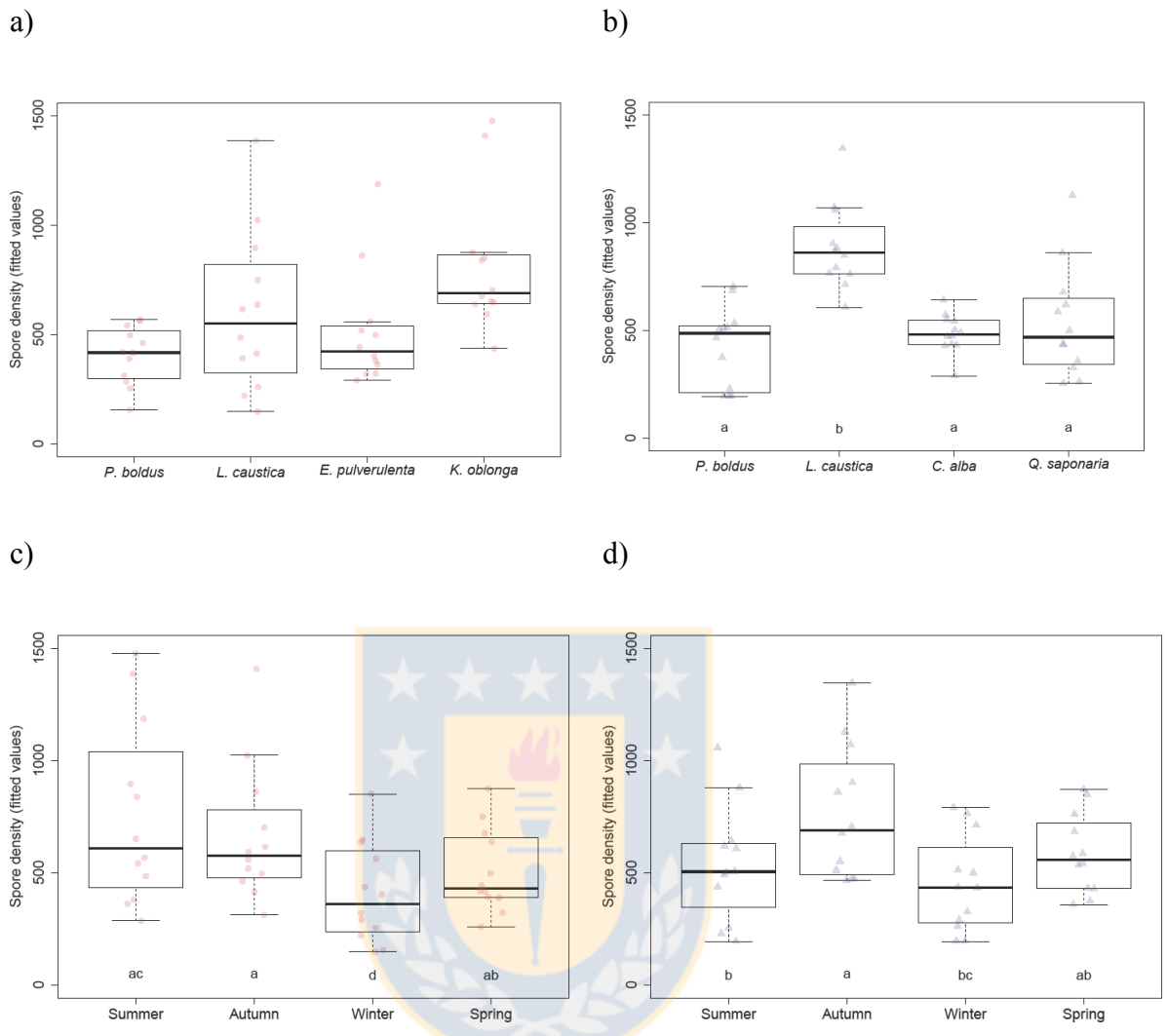


Figure 2. Spore density relationship to studied plant species and the four seasons after generalized mixed negative binomial models (see table 2). In the Malloa site (red circles, left column), the plant host species studied (a) and the four seasons (c); and in San Vicente (blue triangles, left column), the plant species studied (b) and the seasons (d). Letters indicate significant differences (alpha < 0.05) among the seasons and the plant host species. The plant individual identities were used as a random factor.

4. Discussion

This study represents the first analysis on the drivers that influences AMF spore abundance in the Chilean matorral, a highly diverse ecosystem threatened by the expansion of human activities. We investigated whether soil physico-chemical factors, season, and plant host identity have relevant effects on AMF spore abundance in the soil. We provided evidence for a strong site-specific regulation of physico-chemical soil factors on spore density dynamics. In M site, we found spore density to be positively correlated with conductivity and clay content, but negatively correlated with P content. In SV site soil N and clay contents showed positive correlations with AMF spore density while SOM was negatively correlated with AMF spore density. In contrast, spore bank density regulation was found to be host-independent at both sites, with the exception of *L. caustica* at SV site. Finally, spore numbers were lower in winter (cold, wet season) relative to the higher values during the plant growing season (spring-summer-autumn) at both sites, indicating a strong regulation by season.

4.1. Physico-chemical soil properties effect on soil spore density

Several soil physico-chemical factors showed a strong effect on changing AMF spore density in the soils of the sclerophyllous forests. We expected that only SOM, P and N had a significant effect on the spore density. However, P was only important in M site, while SOM and N was important in SV site. Moreover, and contrary to expectations clay content and EC resulted to have also an important role in spore density. This suggests that within the Chilean matorral soil, heterogeneity can be high. In fact, between our two study-sites, we found significant differences in pH, SOM, N, P, and silt content, indicating that soil environment can be partially responsible for the differences found in AMF soil spore banks.

Among the factors affecting the AMF spore bank, clay had a significantly strong, positive effect at both sites. This means that at higher proportion of clay in the soil both, AMF sporulation might be stimulated and/or spore germination might be inhibited, consequently increasing the spore number. There is no direct evidence that proves both statements however, clay proportion is known to determine the water holding capacity of soils (Blume *et al.,* 2016). According to several studies, soil moisture is positively correlated with AMF root colonization

(de Oliveira and de Oliveira, 2005; 2010) which in turn is related with high spore production (Sivakumar, 2013), Thus, indirectly clay content might have a positive or negative effect on spore density depending on soil moisture.

At M site, P and EC show a significant effect on spore density, with P content being negatively and EC positively correlated. Conductivity corresponds to the total salt concentration of soil (Blume *et al.,* 2016). Several studies have found positive correlations between soil salt concentration and spore number (Hildebrandt *et al.,* 2001; Landwehr *et al.,* 2002; Evelin *et al.,* 2009, Bencherif *et al.,* 2015), and this is in accordance with the idea of AMF forming dormant structures in order to persist in unfavorable environmental conditions (Bencherif *et al.,* 2015). In fact, it has been demonstrated that AMF sporulation is stimulated in saline conditions (Hirrel 1981; Aliasgharzadeh *et al.,* 2001), although germination can also be inhibited (McMillen *et al.,* 1998), resulting in an increase of the local AMF spore bank. Also, a high proportion of the AMF species present in the sclerophyllous forest of the South American Mediterranean ecosystem, might be of the *Glomeraceae* family (Redecker *et al.,* 2013), since *Glomus spp*., are common in other Mediterranean ecosystems (Sánchez-Castro *et al.,* 2012) and are tolerant to high levels of salinity (Aliasgharzadeh *et al.,* 2001; Krishnamoorthy *et al.,* 2014).

Soil P content at M site was negatively correlated with spore density. It has been suggested that increasing soil P, decreases or even inhibits AM symbiosis (Muthukumar and Udaiyan, 2002). High soil P concentration could interrupt the life cycle of the AMF, inhibiting spore production (Mohammad *et al.,* 2004) and consequently decreasing spore density in the soil. This result is in accordance with several other studies analyzing natural AMF spore communities in other ecosystems (Panwar *et al.,* 2011; Sivakumar 2013) and MTEs (Mohammad *et al.,* 2003), as well as in experimental approaches (Mohammad *et al.,* 2004).

At SV site, both N and SOM had a significant effect on spore density. N was positively correlated with spore density. It is known that the nitrification process changes ammonium to nitrate releasing hydrogen-ions and consequently producing more acidic soils (Blume *et al.,* 2016). The SV site mean pH was almost neutral, thus a slight decrease in soil pH might be mediated by an increase of nitrogen associated processes (*e.g.* nitrification). Thus, an acid pH could favor the sporulation process as a survival strategy, which has been suggested to occur in unfavorable conditions (Bencherif *et al.,* 2015). However, this is a speculation that should be further investigated. Finally, the positive correlation between N and spore density was in

accordance with what it has been experimentally demonstrated in cereal cultivars (Cornejo *et al.,* 2008; 2009).

SOM was negatively correlated with spore density. An inverse relation between organic matter and AMF P uptake has been observed in other studies (Ravnskov *et al.,* 1999). Thus, the increase of SOM would decrease P uptake by AMF. This in turn might impair AM symbiosis, since the plant would be unable to exchange sugar and lipids due the lack of P. Consequently, AMF would not complete their life cycle and would not sporulate. Without spore input, the spore bank would eventually decrease, since germination, decomposition, and predation of existing spores would continue to occur. Again, this should be further investigated.

Finally, it is worth mentioning that even though both sites correspond to sclerophyllous forests, there are different soil physico-chemical factors affecting the sporulation and/or germination of AMF, changing the spore bank density. Whether the AMF community at each site is composed by the same species, with different adaptations (ecotypes) or by different species responding to different soil conditions, deserves further research.

4.2. Host plant species effect on soil spore density

The effect of phytobiont identity was not in concordance with our expectations. Seven out of eight plant species did not have significantly different spore densities. The only exception came from the SV site, in which *L. caustica* had a significantly higher spore density in its rhizosphere than the other plant species, while at M site, spore banks beneath *L. caustica* did not differ from the other plant species. Varela-Cervero *et al.,* (2016) reported plant host species having a significant effect on AMF spore density. However, only one out of five plant species had a significantly lower spore number, and this was explained by the different phenology of that single species in relation to the others. In our study, the six species evaluated had all the same phenology (Orshan 1989), thus it is expected that all plant species behave similarly regarding sporulation dynamic. The exception in SV site might be due to a local soil feature. In fact, the average soil N value for *L. caustica* in San Vicente was relatively higher than the other three plant species. This might explain the higher spore density, since here we found that N was positively correlated with AMF spore density.

4.3. Season effect on soil spore density

According to expectations, we found that season had a significant effect on AMF spore density. In general, spore density reached a minimum value in winter and higher values during the growing season of the plant (spring-summer-autumn) in both forests. This further supports the idea of strong spore density seasonality for the sclerophyllous forest of the South American MTE.

At M site, and in concordance with our expectations, winter density was significantly lower than in the other seasons. This result may indicate that in winter, the spore bank importantly decreases, probably as a consequence of less AM symbiotic activity in the roots and consequently lower sporulation than during the growing season (spring to autumn) (Smith, 1980; López-Sánchez and Honrubia, 1992).

In contrast, at SV site, winter density was significantly lower than autumn and spring, but unexpectedly not lower, when compared to summer. This site has a south-western facing slope and M site a north-eastern facing slope. This means that SV has less sun exposure, that might reduce photosynthetic yield and, as a consequence, less carbon flow to benefit AMF and therefore, less AMF sporulation (Smith and Read, 2008). It is important to highlight the similar pattern between M and SV, with a relatively high value in autumn, a decrease in winter, and a slight increase in spring, but not higher than autumn. The relatively different status of spore density in summer between both sites might be explained by the time of sampling. We could not rule out the possible effect of summer sampling, for San Vicente the sampling time was early summer (December 2015), while in Malloa it was January 2016. Whether a higher spore density, similar to Malloa, might exist in San Vicente in January should be further investigated.

In our study, seasonal changes are evident, which is in accordance with previous studies in Mediterranean and other regions (Smith, 1980; López-Sánchez and Honrubia, 1992; Sivakumar, 2013; Zangaro *et al.,* 2013). Only in Varela-Cervero *et al.,* (2016), where the winter season was not considered, the authors found that season did not affect AMF soil spore density. Thus, it is important to consider all seasons, particularly winter, since this season is usually the rainy season in an MTE; rainy seasons have been found to have a significantly low spore production (Lugo and Cabello, 2002; Cuenca and Lovera, 2010), likely due to an excess of water

that favors spore germination and/or spore destruction by microorganisms (Guadarrama and Álvarez-Sánchez, 1999).

5. Conclusion

Our study is the first that analyzes spatial and temporal drivers affecting soil AMF spore bank in the South American MTE, a baseline for filling in knowledge gaps regarding South American mycorrhizal research (Bueno *et al.,* 2017). Our results show that for this MTE, the spore density dynamic is strongly regulated by season, but not by host plant species. Additionally, a site-specific regulation of soil physico-chemical factors occurs in the spore bank. Since the Chilean matorral MTE is a biodiversity hotspot, our results are relevant for conservation. Describing the factors that affect the AMF spore bank density and knowing in which cases spore density decreases serves as a reference ecosystem to preserve not only AMF species, but also mycorrhizal associations, plant species, communities and ecosystems, as well as ecosystem processes (Lennon and Jones 2011). Further studies on AMF in the Chilean matorral should continue on describing diversity and community composition of AMF and the factors that shape those AMF communities. Also, evaluation of other soil parameters that are also important in affecting AMF ecology (*i.e.* base cations) should be explored, as well as AMF root colonization dynamic and factors that might affect it. With this a better understanding of AMF importance on the Chilean matorral will be achieved.

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Capítulo V

Factors that determine the molecular arbuscular mycorrhizal fungal community structure in the sclerophyllous forests of the Mediterranean Chilean matorral

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Abstract

Arbuscular mycorrhizal fungi (AMF) form a mutualistic relationship with 71% of the known plant species. This relationship called arbuscular mycorrhiza (AM), positively and directly influences plants and AMF fitness and have an indirect effect on diversity and community composition of both integrant of the association. The Chilean matorral is a biodiversity hotspot and AMF have the potential to be used for restoration purposes, however they have been overlooked and consequently ecological patterns are unknown, hampering the application in conservation initiatives. On this study it was analyzed the effect of AMF compartment (root or soil sample type), host plant species, season and physico-chemical soil factors on AMF diversity and community composition in two sites of a representative sclerophyllous forest. The results show a strong regulation of AMF community, by AMF compartment (root or soil) and host plant species but not by season. Also, pH and SOM resulted to shape AMF communities overall, however other soil factors affected differentially each site. Moreover, 173 VT were found,

where 20 are new and exclusive from this ecosystem where endemic plants to Chile were explored. VT from the genera *Glomus*, *Claroideoglomus* and *Paraglomus* were the most abundant. Those genera, together with *Acaulospora, Archeospora, Ambispora* and *Diversispora* were also frequent and abundant in at least one AMF compartment or host plant species. This study represents the first for this ecosystem in the Chilean matorral and the findings supports the idea of an AMF soil pool, relatively stable during the year, with plant species filtering the AMF from soil to form an AM with several species that are shared between all plant species, but with a few that are specific for each plant. It is discussed how these results might be used for restoration purposes.

Keywords

arbuscular mycorrhizal fungi, arbuscular mycorrhiza, Mediterranean-type ecosystem, Chilean matorral, seasonality, sclerophyllous forest, Illumina MiSeq, virtual taxa

1. Introduction

Arbuscular mycorrhiza (AM) is a mutualistic symbiosis between arbuscular mycorrhizal fungi (AMF) (Phylum Glomeromycota) (Tedersoo et al., 2018) and plant roots. It has been estimated that approximately 71% of known plant species form this association. (Brundrett and Tedersoo, 2018). In this symbiosis the AMF supply the host plant with nutrients in exchange for carbohydrates (Smith and Read, 2008) and fatty acids (Jiang et al., 2017; Keymer et al., 2017; Luginbuehl et al., 2017), enhances plant immunity against pathogens and increases tolerance to abiotic stress (Pozo et al., 2015). In consequence, AM symbiosis has proven to have an important effect on plant communities in terms of seedling survival, plant productivity and diversity (van der Heijden et al., 2015). Due to all the previously mentioned benefits of AMF on plants, is that AM symbiosis is increasingly being recognized as key factor for restoration of the function and biodiversity of degraded ecosystems (Harris, 2009). In fact, a recent study has found that AMF inoculation promotes plant growth and plant richness in field conditions (Neuenkamp et al., 2018).

The South American Mediterranean-type ecosystem (MTE) is located in Central Chile and is one of the five MTEs in the world which altogether are considered a biodiversity hotspot (Cowling et al., 1996; Myers et al., 2000). They hold a high plant species richness with high percentage of endemism (Cowling et al., 1996; Rundel and Cowling, 2013) and are severely threatened ecosystems because of anthropogenic influence (Underwood et al., 2009). Due to this, the MTEs are a main conservation concern. For the Mediterranean Basin MTE, AMF has proven to be important in improving vegetation restoration programs (Barea et al., 2011). For example, a long-term improvement of plant performance and soil properties has been demonstrated when plants are inoculated with native AMF in contrast to an exotic inoculum (Requena et al., 2001). Currently, the application of AMF in the Chilean matorral with restoration purposes might not be feasible since local information is scarce (Silva-Flores et al., accepted). In fact, very little research has been performed about presence and diversity of AMF in plant species of the Chilean matorral (Casanova-Katny et al., 2011; Torres-Mellado et al., 2012; Benedetti et al., 2018) and on the factors that shapes the communities (Marín et al., 2016). None of these studies has used molecular tools in order to describe AMF communities, which have proven to be more efficient than morphological identification of spores in terms of

describing AMF community patterns (Öpik et al., 2014). In fact, next generation sequencing (NGS) approaches are an excellent tool for characterizing AMF communities in detail including detection of rare taxa that are hard to find by other means (*e.g.* Sanger sequencing) (Hart et al., 2015).

In other Mediterranean ecosystems some studies have shown that certain variables might regulate AMF molecular diversity and community composition. Host plant species, seasons, AMF compartment (soil or root) and physico-chemical soil factors have proven significant in shaping AMF communities. Both richness and community composition have proven to be regulated by AMF compartment, while host plant species only regulates the richness and the community composition of AMF living in plant roots but not in soil (Varela-Cervero et al., 2015, 2016a). The same occurs with seasons, which only play a role in shaping AMF community composition in the root compartment (Varela-Cervero et al., 2016b). Also, the most common genus in plant roots has proven to be *Glomus*, while in soil is *Paraglomus, Pacispora, Scutellospora, Diversispora* y *Claroideoglomus* (Varela-Cervero et al., 2015). In contrast the most infrequent genus reported in both soil and roots has been *Archeospora* (Varela-Cervero et al., 2015). Soil conditions as pH and SOM has been proposed as factors related with community composition changes (Varela-Cervero et al., 2016b).

Thus, on this study, we aimed to explore for the first time, the effect of AMF compartment, plant host species, season, and physico-chemical soil factors on the diversity and community composition of AMF present at two sites of a representative sclerophyllous forest of the Chilean matorral using NGS to describe the communities. We expected that (1) AMF compartment would have a strong effect on AMF diversity and community composition; (2) plant host identity and seasons would have an intermediate role since they would determine only the root AMF richness and community composition and (3) that some, but not all physico-chemical soil factors would have an effect on AMF diversity and community composition. All these expectations should opply for both sites of the sclerophyllous forest explored.

2. Materials and Methods

2.1. Study site

The study was performed in Central Chile which is characterized by Mediterranean climate with contrasting seasons of cold rainy winters and warm dry summer. Field work was conducted from summer 2015 until spring 2016, specifically in the VI Region of *Libertador Bernardo O´Higgins* and the climatic conditions during this period are detailed in Table 1. Two sites of a representative sclerophyllous forest were sampled. The first site is located at *Malloa* (hereafter, M site) (34°26`S, 70°57`W) and the most common trees are *Peumus boldus* Molina (Monimiaceae), *Lithrea caustica* Hook. & Arn. (Anacardiaceae), *Kageneckia oblonga* Ruiz & Pav. (Rosaceae), and *Escallonia pulverulenta* (Ruiz & Pav.) Pers. (Escalloniaceae). This site has a north-east sun exposure being consequently dry in contrast to the second site of study. The second site is located at *San Vicente* (hereafter, SV site) (34°26`S, 70°59`W) and the most common trees are *P. boldus, L. caustica, Quillaja saponaria* Molina (Quillajaceae) and *Cryptocarya alba* (Molina) Looser (Lauraceae). This site has a south-west sun exposure, being moister than M site. On average, all the trees investigated had similar phenology. Vegetative growth starts in spring (September-November), followed by flower-bud formation and flowering during summer (December-February), fruiting in autumn (March-May) and no growth during winter (June-August) (Orshan, 1989). Also, the six plants species are known to associate with AMF (Silva-Flores et al., in review).

Table 1: Climatic parameters from "El Tambo" meteorological station (34°47`S, 70°98`W), which is the nearest station to the study site.

	SUMMER	AUTUMN	WINTER	SPRING
Total Precipitation (mm)	10,1	194,4	161,6	40,3
Relative humidity (%)	60,6	79,2	74,9	59,8
Mean air temperature (ºC)	20,5	12,6	10,5	17,6
Absolute Max air temperature (ºC)	34,7	30,8	30,4	35,4
Absolute Min air temperature (ºC)	7,3	-1,6	-0,5	2,7
Mean 10 cm soil temperature (ºC)	22,2	15,2	11,1	19,2
Absolute Max 10 cm soil temperature (ºC)	26,1	21,7	18	29,5
Absolute Min 10 cm soil temperature (ºC)	17,2	8,6	7,7	13,2
Mean surface temperature (ºC)	20,7	13,3	10,3	20,8

2.2. Sampling

At both sites (M and SV), two AMF compartments (rhizosphere soil and roots, hereafter sample type) were collected from three individuals of each of four host plant species (hereafter plant species) during four seasons (2 sites x 2 sample types x 3 individuals x 4 plant species x 4 sampling times = 192), obtaining 96 root samples and 96 soil samples (Table S1). The samples were collected during a two-day campaign in each season: autumn 2016 (April), winter 2016 (June), and spring 2016 (September), with the exception of the summer samples, which were collected in early summer (December 2015) at SV site and in mid-summer (January-2016) at M site. First, samples for molecular analysis was collected. For this, leaf litter was removed, then soil was collected with a single use plastic spoon from topsoil (1-10 cm depth), placed in a paper envelope and then placed in a plastic grip seal bag. Roots were collected following the main root of the tree to the finer roots; they were cut with a knife, placed in a paper towel and then in a plastic grip seal bag. All samples were properly labeled in order to trace them back in the laboratory. For physico-chemical soil analysis 2.5 kg of rhizosphere soil and three cylindrical soil cores were also collected. Immediately after field work, the samples were taken to the laboratory. There, roots were cleaned from soil by hand under tap water, dried with a paper towel and stored in a new dry paper towel in a plastic grip seal bag adding silica-gel in a box at room temperature for further processing. Soil samples were stored at room temperature also adding silica-gel into the bag. Silica gel used to dry soil and root samples was changed twice

after the samples were packed to ensure that samples were completely dry. Thus, finally, samples stored consisted in 2 g of rhizosphere soil and 20 cm of roots for molecular analyses; and 2.5 kg of rhizosphere soil and three cylindrical soil cores in order to perform physico-chemical analysis.

2.3. Soil chemical analysis

Soil chemical analyses were conducted as described in Silva-Flores et al. (accepted), as follows. The day after sampling, 1 kg of each soil sample was used for chemical analyses at *Las Garzas* laboratory (*Escuela Agrícola Las Garzas, VI Region, Chile*), based on a soil protocol recommended for Chilean soils (Sadzawka et al., 2006). Briefly, each sample was homogenized, air dried, and sieved at <2 mm mesh size. Then pH, soil organic matter (SOM), soil available phosphorous (P), total nitrogen (N), and soil available potassium (K) were determined. pH was measured in a 1:2.5 soil:water suspension; SOM was extracted by soil oxidation using dichromate in an acid medium and quantified colorimetrically; P (P-Olsen) was extracted with a solution of sodium bicarbonate (0.5 mol/L, pH 8.5) and quantified colorimetrically using molybdenum blue; N was measured with the Kjeldahl method (Sadzawka et al., 2006)**.** Finally, K was extracted and quantified by absorption and atomic emission spectrophotometry with lanthanum.

2.4. Soil physical analysis

Soil physical analyses were conducted as described in Silva-Flores et al. (in review), as follows. Electrical conductivity (EC) was measured for each sample at Las Garzas laboratory after extracting soil with water in a ratio of 1:2.5. Another 1 kg of each soil sample was used to determine particle size distribution using the hydrometer method (Bouyoucos, 1962), and particle density (ρs) by the method of the pycnometer (Blake and Hartge, 1986a). Finally, bulk density (ρb) was determined by the cylinder method (Blake and Hartge, 1986b). In order to determine ρs and particle size distribution, the soil samples were air dried and sieved at <2 mm mesh size; ρb was determined using undisturbed soil samples from soil cores which were dried

in an oven at 105 °C for 24 h. Particle size distribution was used to estimate the soil content of sand, silt, and clay.

2.5. Molecular analysis

DNA was extracted from 250 mg of dried soil and 70 mg of dried roots using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA) following the manufacturer protocol with some modification as described in Saks et al. (2014). Amplification of AMF sequences was performed targeting the small subunit (SSU) of the ribosomal RNA (rRNA) gene, since is the most common marker used for Glomeromycotina biodiversity surveys (Hart et al., 2015). The primers WANDA (5' CAGCCGCGGTAATTCCAGCT 3') (Dumbrell et al., 2011) and AML2 (5' GAA CCC AAA CAC TTT GGT TTC C 3') (Lee et al., 2008) were used. Amplicon sequencing was performed with Illumina MiSeq 2x300 bp paired end protocols at the Estonian Genome Center, University of Tartu, Estonia.

2.6. Bioinformatical analysis

The Illumina MiSeq sequence data were processed with the following workflow: Raw sequences were cleaned using an in-house pipeline from the Plant Ecology Laboratory of the Botany Department of the University of Tartu (Vasar et al., 2017). First, it was checked whether forward reads had WANDA primer and reverse reads had AML2 primer sequences. For this, it was allowed one nucleotide error for both forward and reverse reads. An average quality ≥ 30 for both sequences was used. For Illumina, the quality is measured between 0 and 40 were 30 means on average 1 error per 1000 nucleotides. Then, FLASh (v1.2.10, Magoč and Salzberg, 2011) was used to pair reads together, for this we used a minimum of 10 base pair overlap with 75% sequence identity. It was tested whether sequences were at least of 450bp long in order to be used in the following steps. Finally, chimera sequences were detected and discarded using USEARCH (v7.0.1090, Edgar et al., 2011).

After cleaning the sequences, an open-reference operational taxonomic unit (OTU) picking approach was used to match reads against AMF taxa and generate the OTU delimitation (Bik et al., 2012). For this purpose, first BLAST+ (v2.5.0) was used to perform a BLAST search

of the cleaned sequences against the public database Maarja*AM* (Öpik et al., 2010). This database is based mainly on SSU rRNA gene sequences of published AMF sequences detected from environment. It uses a unique concept of Virtual Taxa (VT) as nomenclature of OTU (Öpik et al., 2014). A VT is a phylogenetically related group of SSU rRNA gene sequences with a sequence similarity threshold ≥97%. This concept gathers in the database all the published phylogroups designations of AMF as a particular VT in order to make comparable all the information available (Öpik et al., 2014). Illumina reads were assigned to a particular VT using ≥ 97% identity, 95% alignment threshold and a BLAST e-value ≤ 1e-50. Sequences that did not match against sequences of the Maarj*AM* database with the above criteria were used for a BLAST search in the INSDC (REF) database with a ≥ 90% identity, 90% alignment threshold and a BLAST e-value ≤ 1e-50 to find potential new VT. Sequences shorter than 500 nucleotides and with homopolymers were removed. Then a phylogenetical analysis was performed in order to determine whether the potential Glomeromycotina sequences were novel VT. For this the sequences were clustered at 97% similarity level using USEARCH (v7.0.1090, Edgar et al., 2011). Then from each cluster, four random sequences were selected and aligned with all sequences available in *MaarjAM* database using MAFFT multiple sequence alignment from JALVIEW version 2.10.4b1 (Waterhouse et al., 2009).

2.7. Statistical data analysis

No AMF sequences were obtained from 12 root samples and 5 soil samples, leaving 175 samples for further analyses. Also, 19 singleton taxa were removed since it might represent sequencing artifacts (Tedersoo et al., 2010) and thus are not useful for conducting diversity analyses were dominant community patterns wants to be described (Vasar et al., 2017). In order to investigate sampling efficiency, individual-based rarefaction curves were constructed using the function rarefy() of the package vegan (Oksanen et al., 2018). For visualization of shared and unique number of VT between sample type, plant species and season in both sites, Venn diagrams were drawn using the function venn.diagram() of the package VennDiagram in R (Chen and Boutros, 2011). In order to analyze the VT richness variation in relation to each sample type, plant species, season and soil physico-chemical variables, a generalized linear model with a negative binomial error was performed for each site using the function glm.nb from the package MASS

in R (Venables and Ripley, 2002). Post hoc analyses were conducted to detect the differences between the levels of the statistically significant variables. This was performed by running the same glm.nb models done previously, but changing the baseline reference level for each of the levels of the variable analyzed (Zuur et al., 2010). Since sample type was significant in SV site, a PERMANOVA testing the effect of plant species and season on the AMF community composition for SV site in each sample type was performed. To analyze AMF community composition, a matrix of VT relative abundances per sample was used. We ran a PERMANOVA to test the effect of sample type, plant species, season and physico-chemical soil variables on the AMF community composition for each site, using the function adonis() from the package vegan (Oksanen et al., 2018). The significances of the variables were assessed through 9999 permutations and as a measure of distance between communities, the Bray-Curtis dissimilarity was used. Since sample type was significant in each site, a PERMANOVA testing the effect of plant species and season on the AMF community composition for each site and sample type was performed. Nonmetric multidimensional scaling (NMDS) was used to illustrate the differences in community composition regarding different sample type, plant species and seasons for each site. For this, the monoMDS() function of the package vegan was used (Oksanen et al., 2018). Ellipses indicating one standard deviation around group centroid were shown on NMDS figures for each level of the corresponding variable visualized using the function ordiellipse() of the package vegan in R (Oksanen et al., 2018).

To identify which VT was more abundant and frequent in each sample type, plant species and season, an indicator species analysis was performed using the indval() function from labdsv package (Roberts, 2016). Those VT with an indicator value index equal or higher than 25% were considered indicator species of the corresponding level explored (Dufrene and Legendre, 1997).

All statistical analyses were carried out using R (v.3.5.1; R Core Team, 2018) in the RStudio environment (v.1.1.383).

3. Results

3.1. Illumina sequencing data

A total of 19,474,734 raw reads from 175 samples were obtained. After quality filtering we obtained 8,562,915 (44%) reads. BLAST against Maarj*AM* database retrieved 818,405 (4%) Glomeromycotina reads. After removing singleton VT, a total of 153 VT in 818,386 reads were obtained: 110 Glomeraceae, 8 Paraglomeraceae, 9 Diversisporaceae, 9 Claroideoglomeraceae, 6 Acaulosporaceae, 6 Archaeosporaceae, 3 Gigasporaceae, 1 Ambisporaceae and 1 Pacisporaceae (Table 1). Also 20 new Glomeromycotina VT were found, distributed as follows: 11 Glomeraceae, 1 Archaeosporaceae, 4 Claroideoglomeraceae, 1 Paraglomeraceae, 1 Gigasporaceae, 1 Ambisporaceae and 1 Diversisporaceae. Thus, in this study a total of 173 VT were found.

Rarefaction analysis indicates for the M site that the number of AMF reads per sample were not sufficient to produce asymptotic estimates of VT richness per sample (Figure 1a and 1b), while in SV site most of the samples reached an asymptotic curve (Figure 2a and 2b).

Figure 1. Rarefaction analyses for AMF showing VT accumulation curves for (a) soil samples and (b) root samples for the four plants species and the four seasons in M site. Each line represents an individual sample. The black line represents the median number of reads in every case. Plants names: *Peumus boldus, Lithrea caustica, Escallonia purverulenta* and *Kageneckia oblonga.*

(a) (b)

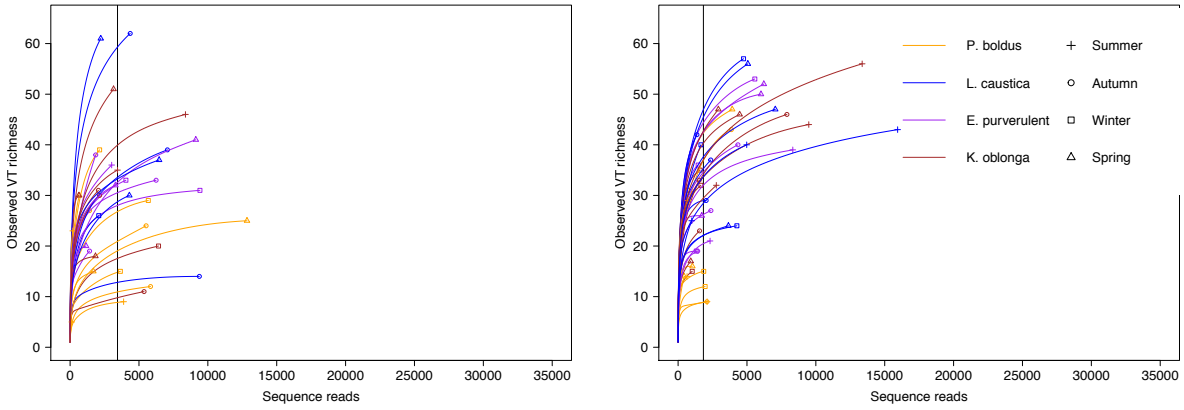
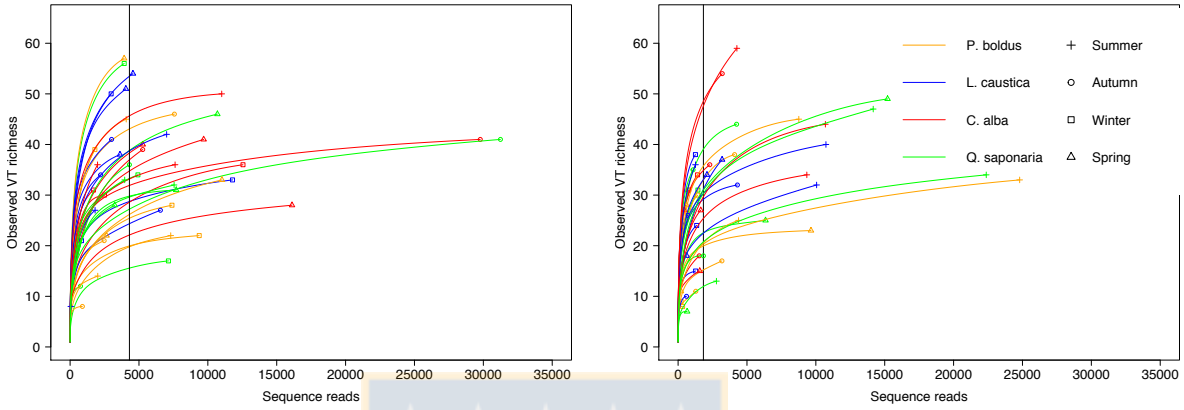


Figure 2. Rarefaction analyses for AMF showing VT accumulation curves for and (a) soil samples and (b) root samples for the four plants species and the four seasons in SV site. Each line represents an individual sample. The black line represents the median number of reads in every case. Plants names: *Peumus boldus, Lithrea caustica, Cryptocarya alba* and *Quillaja saponaria.*

(a) (b)

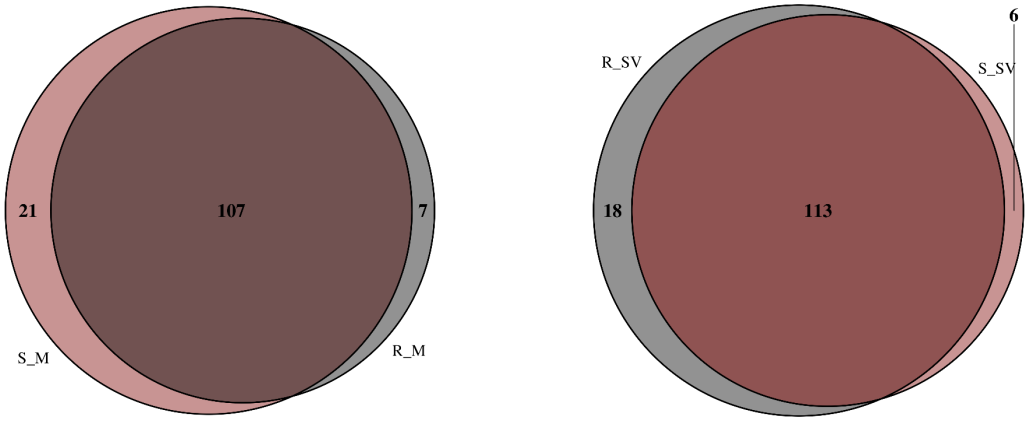


3.2. AMF community structure

In M site, the total number of AMF VT found was 135, whilst in SV site was 137 (Figure 3). Most of the VT were shared between soil and root. However, the number of VT in soil was higher for M site than SV site whilst the opposite occurred for root VT.

Figure 3. Venn diagrams representing the number of VT unique to and shared between different sample type in M (a) and SV (b) site. S: soil and R: root.

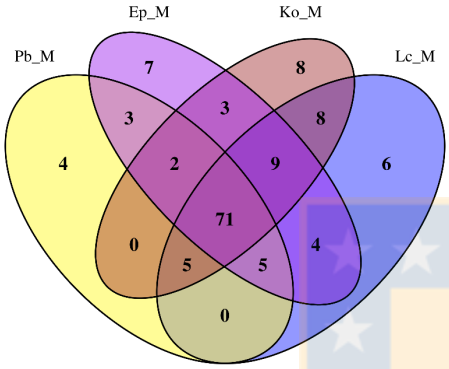
(a) (b)



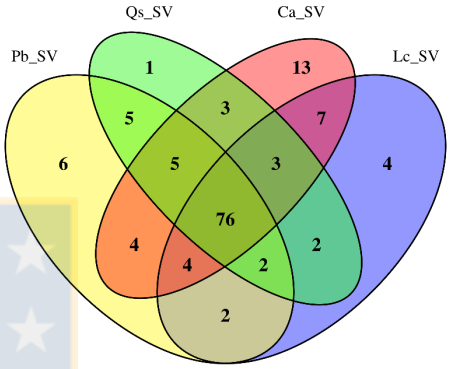
The distribution of VT in the plant species showed that 56% of the VT in each site were shared between the four plants species (Figure 4a and 4b). Finally, regarding the season variable, in M site 56% of the VT were shared, whilst in SV site was 61% (Figure 4c and 4d).

Figure 4. Venn diagrams representing the number of VT unique to and shared between different plant species in M (a) and SV (b) site and season in M (d) and SV (e). Pb: *Peumus boldus,* Lc: *Lithrea caustica,* Ep: *Escallonia purverulenta,* Ko: *Kageneckia oblonga,* Ca: *Cryptocarya alba* and Qs: *Quillaja saponaria.*

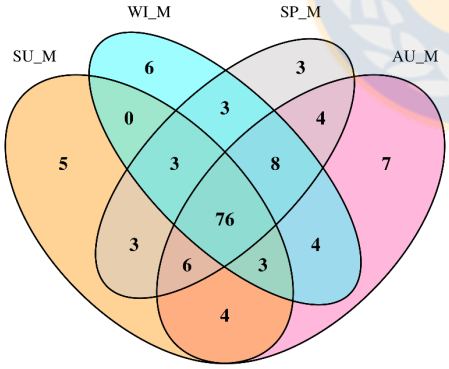
(a)



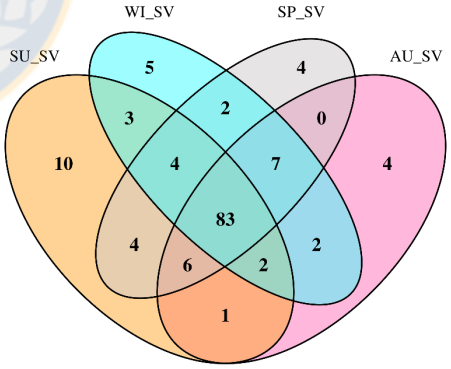
(b)



(c)



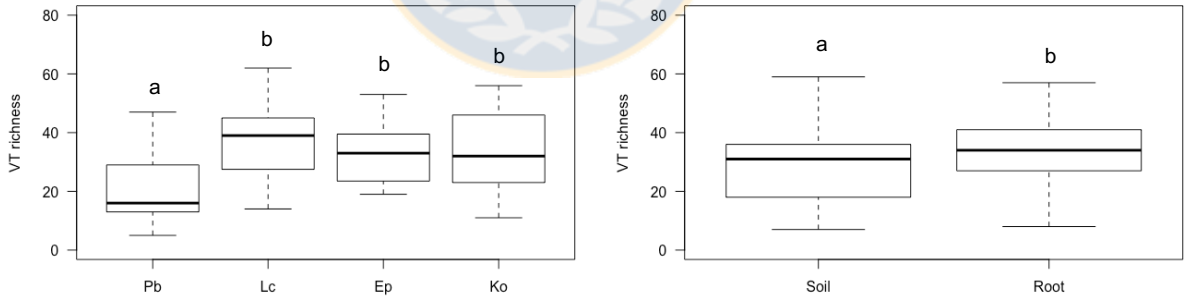
(d)



At M site the mean richness of VT per sample was significantly affected by plant species (Df=3, X^2=29.733, P<0.0001; Figure 5a), but not by sample type (Df=1, X^2=1.028, P=0.3106), season (Df=3, X^2=3.163, P=0.3672; Figure 3b), or the interaction between variables. Post hoc analysis of plant species effect on VT richness showed that only *P. boldus* showed a lower mean VT richness per sample compared to the other plant species (Figure 3c). In SV site the mean richness of VT per sample was significantly affected by sample type (Df=1, X^2=4.6162, P<0.05; Figure 5b) and the interaction between season and sample type (Df=3, X^2=9.2184, P<0.05), but not by season (Df=3, X^2=2.7537, P=0.4312), plant species (Df=3, X^2=7.4083, P=0.0599), or the other interactions between variables. Since sample type was significant only at SV, it was tested whether plant species and seasons had an effect on AMF richness only in roots and only in soil samples. Neither plant species nor seasons had a significant effect on VT richness neither for root samples nor soil samples in SV.

Figure 5. Boxplots of the relationship between variables with a significant effect upon VT richness after generalized negative binomial models. (a) Plant species in the M site, where the letters indicate significant differences (P < 0.0001) among the different levels and (b) sample type in the SV site where the letters indicate significant differences (P < 0.05). Pb: *P. boldus,* Lc: *L. caustica,* Ep: *E. pulverulenta* and Ko: *K. oblonga.*

(a) (b)



In terms of physico-chemical soil variables, for M site the pH, K and ρb had a significant effect on VT richness (Table 2a, Figure 6). Whilst, for SV site pH, SOM, P, K and clay content resulted to affect VT richness (Table 2b, Figure 7). In M, pH was positively correlated with VT richness (Figure 4a) in contrast to SV (Figure 5a). On the other hand, K was negatively correlated to VT richness in both sites (Figure 4b and 5d). Finally, only ρb was positively correlated with VT richness in M site, in contrast to SOM, P and clay which were negatively correlated with VT richness in SV site. Since sample type was significant only in SV, it was tested which physico-chemical soil factors had an effect on AMF richness only in roots and only in soil. The results showed that in roots only pH produces a statistically significant effect on VT richness (Df= 1, X^2=4.7616, P<0.05), while for soil only SOM had an effect (Df= 1, X^2=4.7463, P<0.05).

Table 2. VT richness in relation to physico-chemical soil variables *i.e.* pH, electrical conductivity (EC), soil organic matter percentage (SOM), total nitrogen (N), available phosphorus (P), available potassium (K), proportion of sand, silt and clay, soil particle density (ρs) and soil bulk density (ρb) in Malloa (a) and in San Vicente (b). Generalized linear model with negative-binomial error distribution were used. *Chisq* and *p* values shown derived from the analyses of deviance tables.

	a) M			**b) SV**		
	X^2	**Df**	**p value**	**X^2**	**Df**	**p value**
pH	**20.577**	**1**	**0.000**	**11.839**	**1**	**0.000**
EC (mmhos/cm)	0.014	1	0.905	3.804	1	0.051
SOM (%)	1.460	1	0.227	**6.067**	**1**	**0.014**
N (mg/kg)	1.419	1	0.234	0.113	1	0.737
P (mg/kg)	0.032	1	0.859	**8.596**	**1**	**0.003**
K (mg/kg)	**6.886**	**1**	**0.009**	10.371	1	**0.001**
Sand (%)	1.030	1	0.310	3.199	1	0.074
Silt (%)	0.172	1	0.678	0.796	1	0.372
Clay (%)	0.475	1	0.491	**9.574**	**1**	**0.002**
ρS	0.091	1	0.763	1.687	1	0.194
ρb	**5.329**	**1**	**0.021**	0.954	1	0.329

Figure 6. VT richness per sample in relation to the significant physico-chemical soil variables after a generalized linear model with a negative binomial error (see table 2) for the M site. The red line indicates whether the relation between variables is positive or negative.

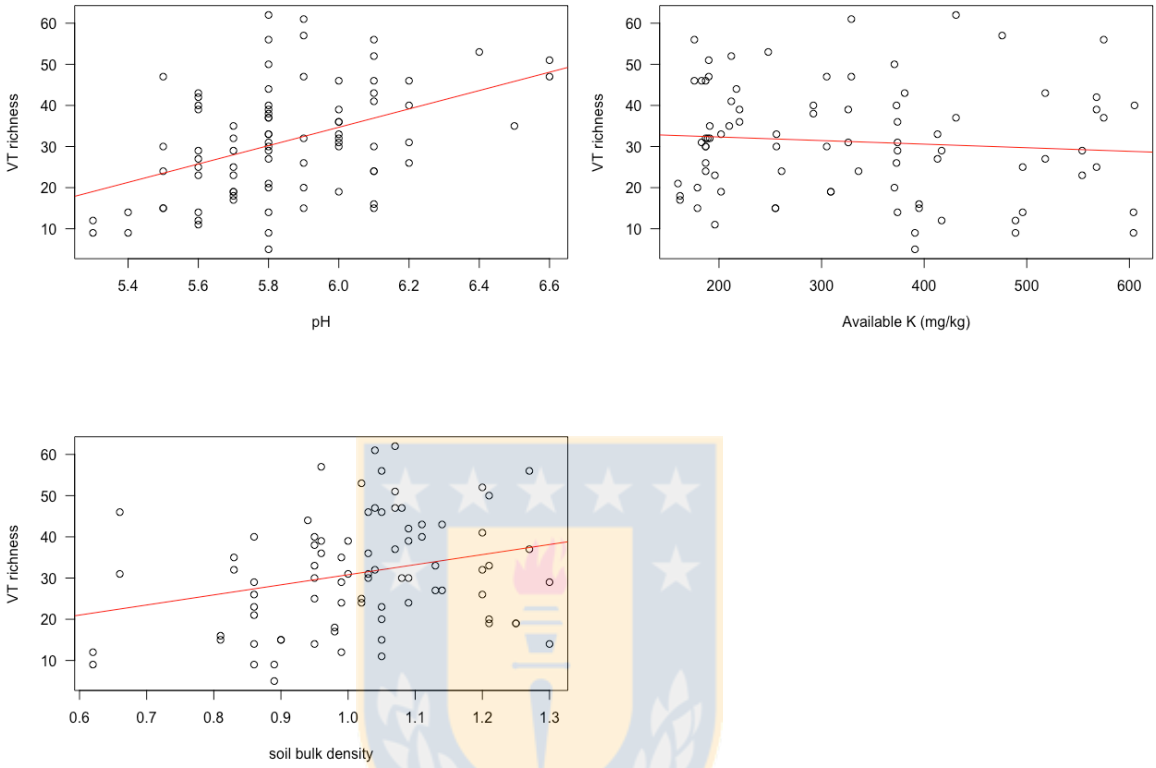
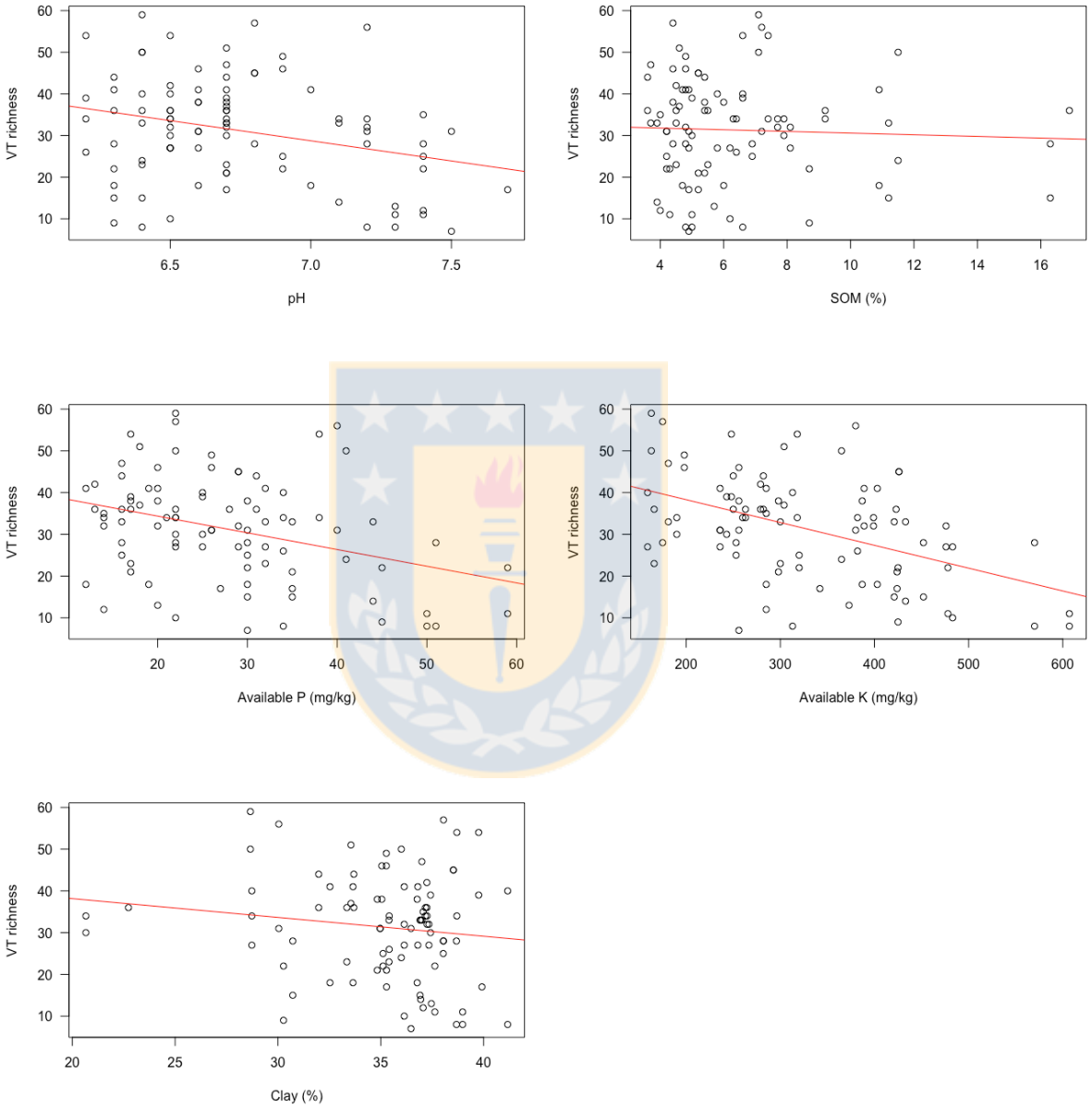
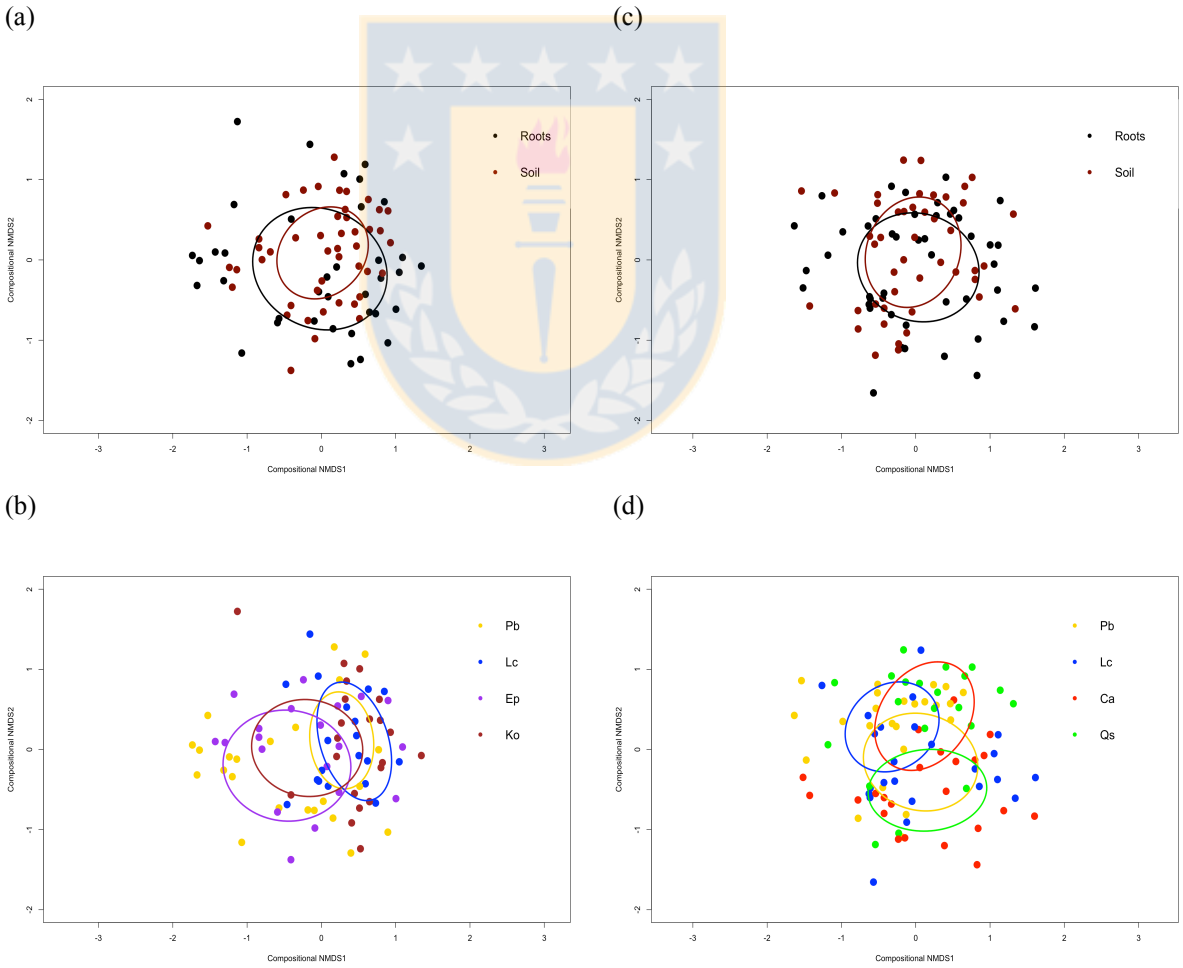


Figure 7. VT richness per sample in relation to the significant physico-chemical soil variables after a generalized linear model with a negative binomial error (see table 2) for the SV site. The red line indicates whether the relation between variables is positive or negative.



The PERMANOVA analysis for the M site, showed that only sample type (Df=1, pseudoF=0.7198, P<0.01, Figure 8a) and plant species (Df=1, pseudoF=2.5331, P<0.01, Figure 8b) had an effect on the AMF community composition. Sample type explained 3% of the AMF community composition variation, while plant species explained and additional 10%. Interactions between the variables were not significant. For the SV site also only sample type (Df=1, pseudoF=0.5785, P<0.01, Figure 8c) and plant species (Df=1, pseudoF=2.2732, P<0.0001, Figure 8d) had an effect on the AMF community composition, explaining also 3% and 10% of the variation respectively.

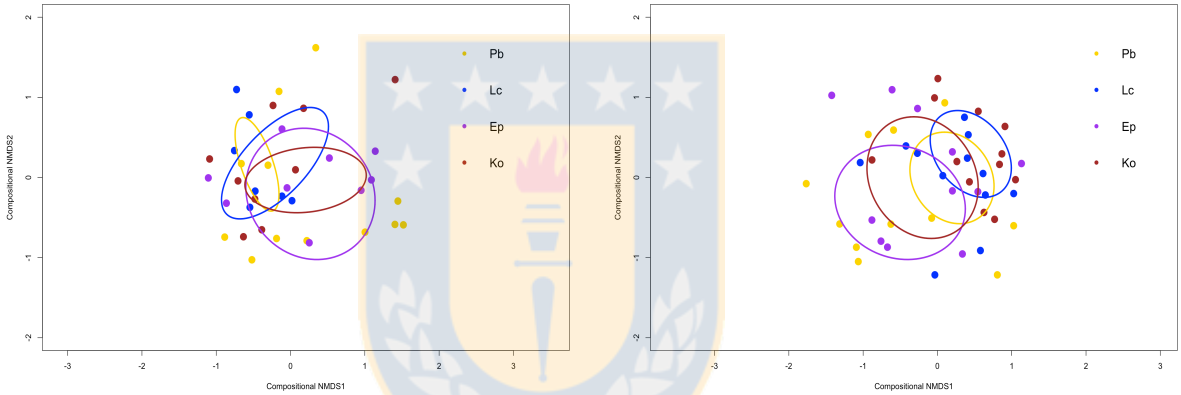
Figure 8. Nonmetric multidimensional scaling analysis to indicate the effects of (a) sample type and (b) plant species in M and (c) sample type and (d) plant species in SV on AMF community composition. Pb= *P. boldus,* Lc=*L. caustica,* Ep=*E. pulverulenta,* Ko=*K. oblonga,* Ca=*C. alba* and Qs=*Q. saponaria.*



Since sample type significantly affected the community composition variation in both sites, a PERMANOVA for each sample type was performed. In M site, root samples community composition was regulated only by plant species (Df=3, pseudoF=1.4598, P<0.05, Figure 9a) explaining 12% of the variation and not by season. In soil samples, the same occurred, only plant species had a significant effect (Df=3, pseudoF=1.7187, P<0.0001, Figure 9b), explaining 15% of the variation.

Figure 9. Nonmetric multidimensional scaling analysis to indicate the effects of (a) plant species in root samples and (b) plant species in soil samples in M site on AMF community composition. Pb= *P. boldus,* Lc=*L. caustica,* Ep=*E. pulverulenta,* Ko=*K. oblonga.*

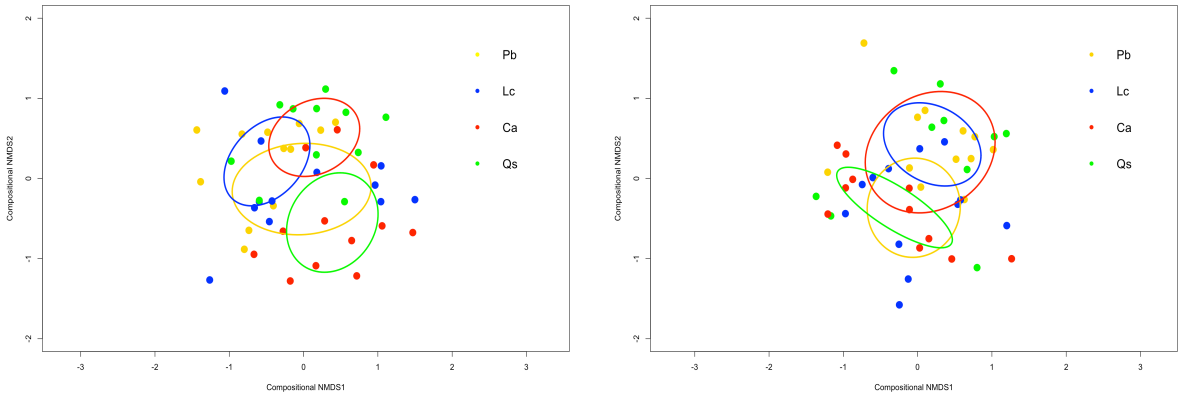
(a) (b)



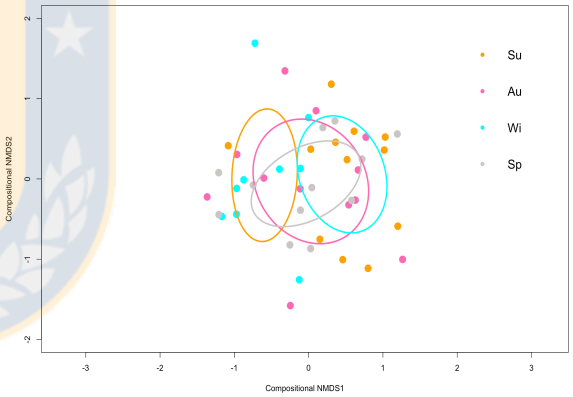
In SV site, root samples community composition was regulated only by plant species (Df=3, pseudoF=1.3961, P<0.01, Figure 10a) explaining 12% of the variation, while in soil samples was regulated by both, season (Df=3, pseudoF=0.9663, P<0.05, Figure 10b) and plant species (Df=3, pseudoF=1.4490, P<0.001, Figure 10c), explaining 10% and 15% of the variation respectively.

Figure 10. Nonmetric multidimensional scaling analysis to indicate the effects of (a) plant species in root samples, (b) plant species in soil samples and (c) seasons in soil samples in SV site on AMF community composition. Pb= *P. boldus,* Lc=*L. caustica,* Ca=*C. alba,* Qs=*Q. saponaria.* Su=Summer, Au=Autumn, Wi=Winter and Sp=Spring.

(a) (b)



(c)

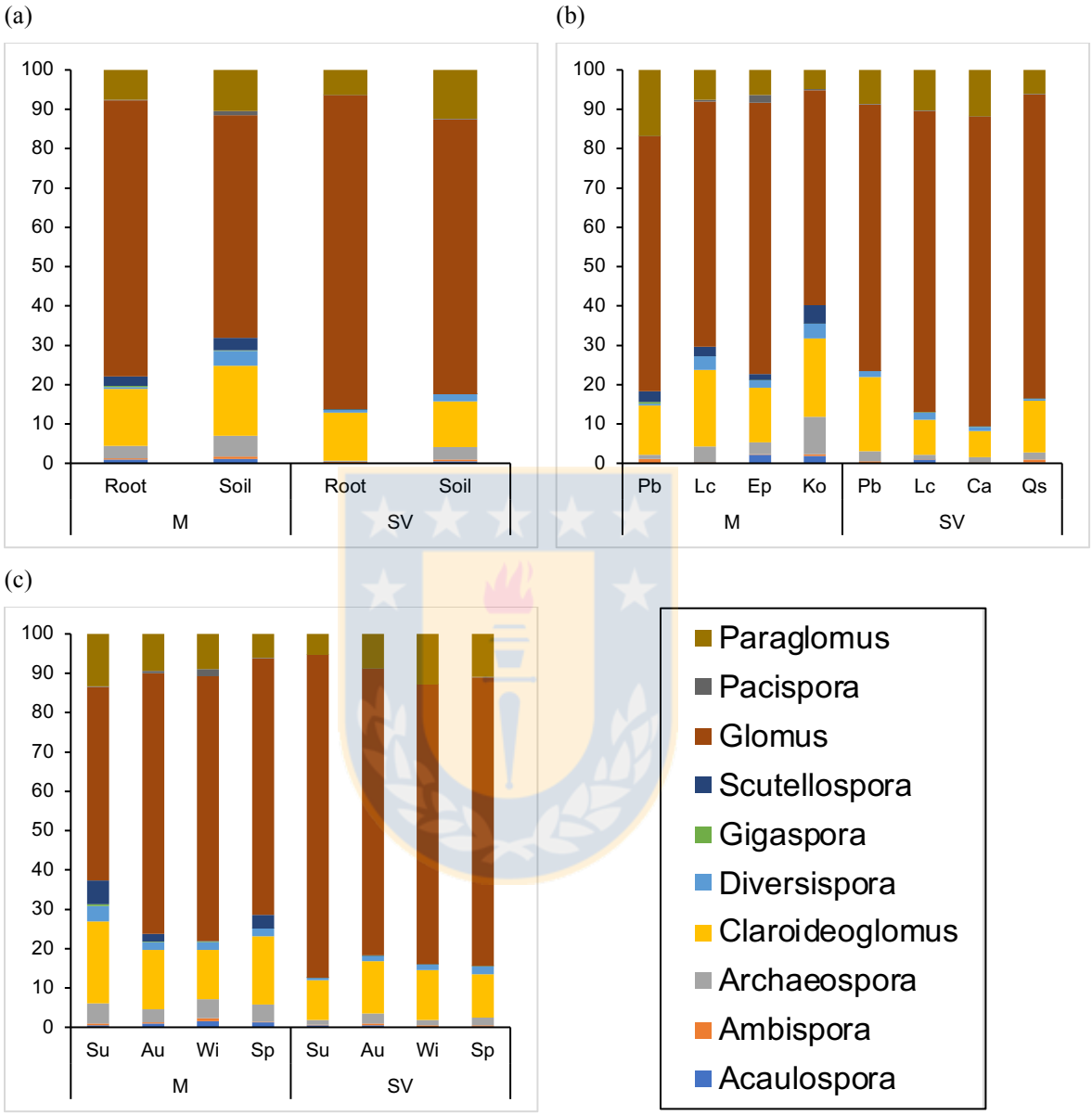


Physico-chemical soil variables also contributed in explaining the variation in the community composition. In M site, SOM (Df=1, pseudoF=0.5217, P<0.05), N (Df=1, pseudoF=0.5301, P<0.05), K (Df=1 pseudoF=0.5118, P<0.05) and sand content (Df=1, pseudoF=0.4964, P<0.05) explained 2.1%, 2.2%, 2.1% and 2% of the AMF community composition variation respectively. In SV site, soil pH (pseudoF=0.018, P<0.05), SOM (pseudoF=0.034, P<0.0001) and K (pseudoF=0.027, P<0.05) explained 2%, 3.4% and 3% of the variation respectively.

The distribution of AMF genera relative abundance between sample type, plant species and season in both sites showed similar patterns (Figure 11). *Glomus* VT were more commonly

present in both sample type (Figure 11a), all plant species (Figure 11b) and all seasons (Figure 11c) in both sites, followed by *Claroideoglomus* and *Paraglomus.* However, *Glomus* was more abundant in roots than soil samples while *Claroideoglomus* and *Paraglomus* were more abundant in soil than roots (Figure 11a).

Figure 11. Mean relative abundance of AMF genera by (a) sample type, (b) plant species and (c) season in both sites. (b) Pb= *P. boldus,* Lc=*L. caustica,* Ep=*E. pulverulenta,* Ko=*K. oblonga,* Ca=*C. alba* and Qs=*Q. saponaria.* (c) Su=summer, Au=autumn, Wi=winter and Sp=spring.



Six dominant VT occurred in the dataset; these VT were present with a relative abundance greater than 10% in at least one sample type (Figure 12). The same six VT were dominant in at least one plant species as well (Figure 13) and in at least one season (Figure 14). However, two more VT were also dominant in at least one plant species (Figure 13). *Glomus* VT199 (related to *G. hoi* / *G. macrocarpum*) was a dominant taxon in SV in sample type, plant species and season (Figure 12a, 13a and 14a). *Claroideoglomus* VT56 was dominant in M site in root and soil (Figure 12b), in three plant species (Figure13b) and in all seasons (Figure 14b). However, it was also dominant for *P. boldus* and winter season in SV site (Figure 13b and 14b). *Glomus* VT160 was dominant in M site in both sample type (Figure 12c), in two plant species (Figure 13c) and in two seasons (Figure 14c). *Glomus* VT177 was dominant in SV root samples (Figure 12d), two plant species (Figure 13d) and one season (Figure 14d). Moreover, this taxon was almost exclusively present only in SV site (Figure 12d, 13d and 14d), in contrast to *Glomus* VT108, that was almost exclusively present in M site (Figure 12e, 13e and 14e). More in detail, *Glomus* VT108 was a dominant taxon in M site, in root and soil (Figure 12e), in all plant species (Figure 13e), and in three seasons (Figure 14e). *Paraglomus* IH1 was a dominant taxon in soil samples (Figure 12f) and in winter and spring (Figure 14f) of SV site. Whilst it was also dominant in *P. boldus* (Figure 13e) and in summer (Figure 14e) for M site. Finally, *Glomus* VT155 was a dominant taxon in *Q. saponaria* (Figure 13g) and *Glomus* VT123 was a dominant taxon in *C. alba* (Figure 13h), both plant species present in SV site.

Figure 12. Bar plots showing the mean relative abundance percentage of dominant AMF VT for the sample type in both sites of the study. Whiskers correspond to standard error. M=Malloa and SV=San Vicente. S=soil and R=roots.

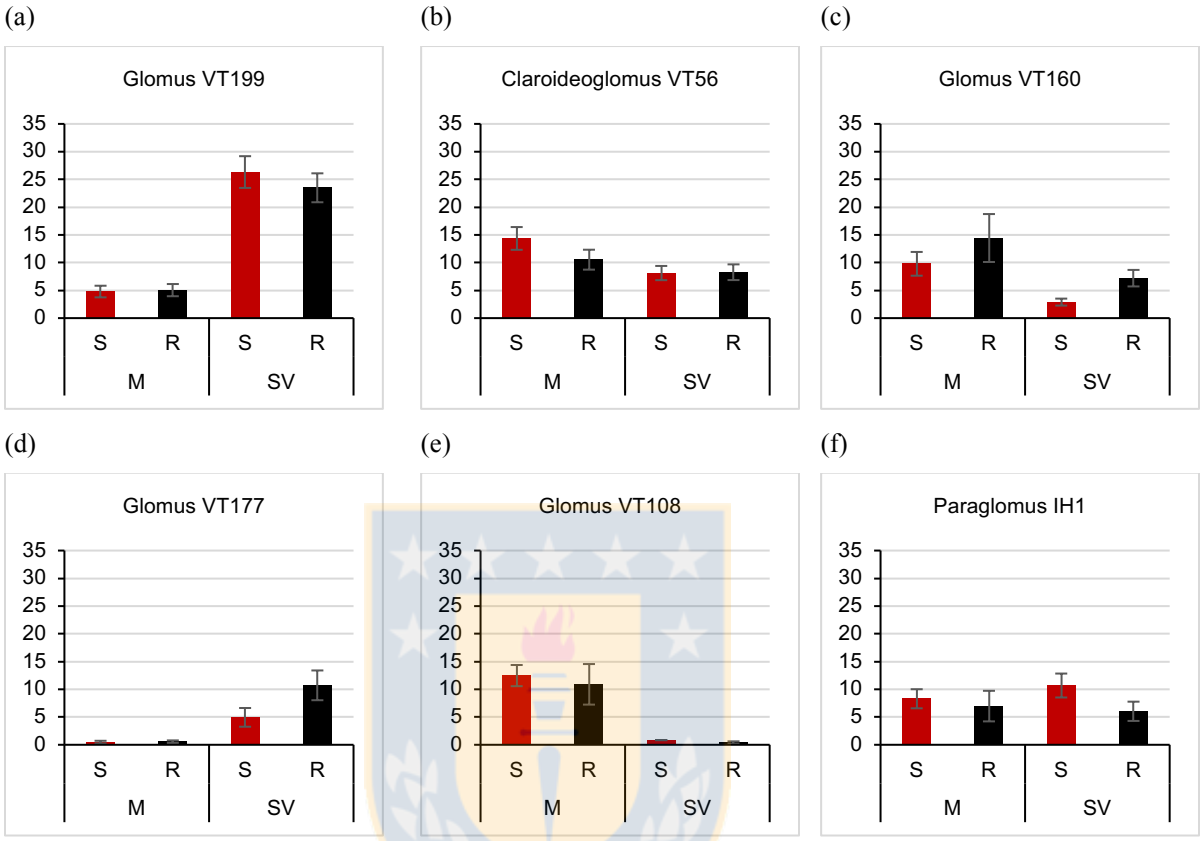


Figure 13. Bar plots showing the mean relative abundance percentage of dominant AMF VT for plant species in both sites of the study. Whiskers correspond to standard error M=Malloa and SV=San Vicente. Pb= *P. boldus,* Lc=*L. caustica,* Ep=*E. pulverulenta,* Ko=*K. oblonga,* Ca=*C. alba* and Qs=*Q. saponaria.*

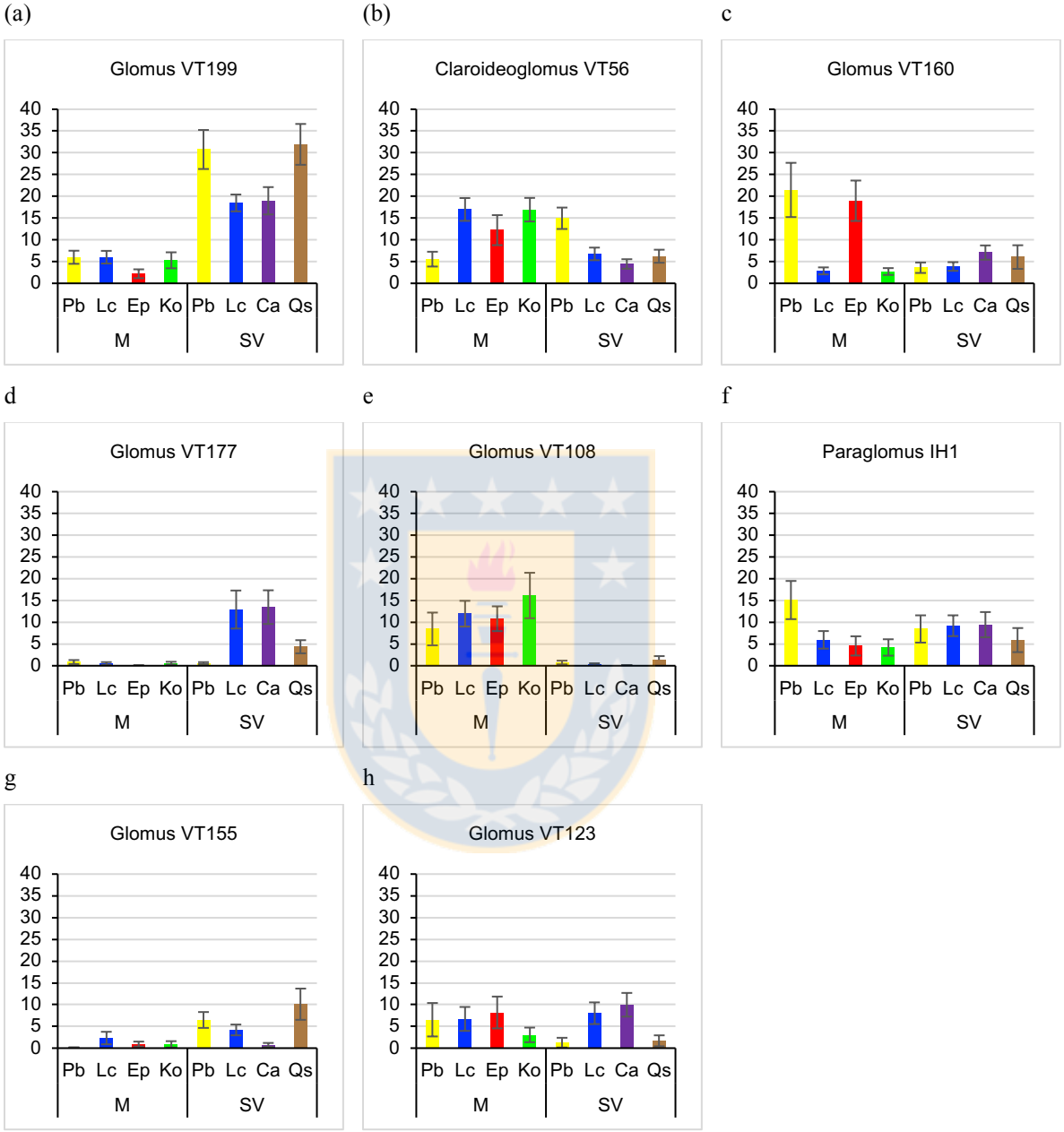
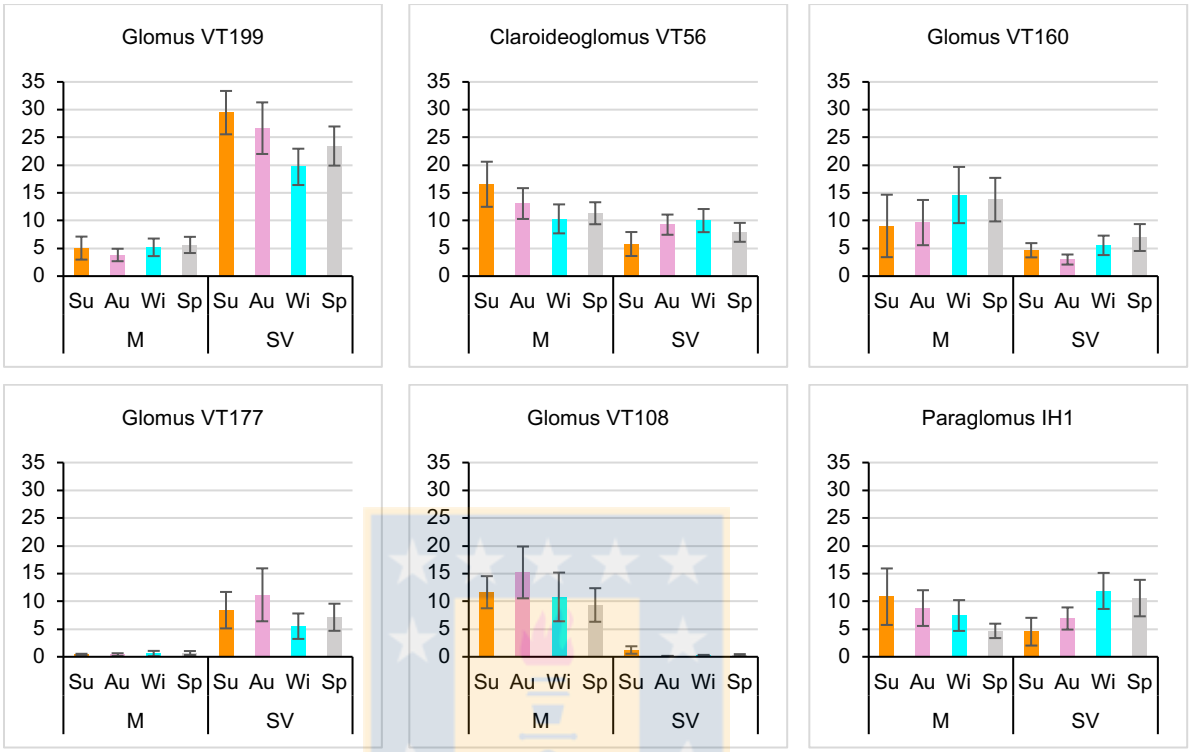


Figure 14. Bar plots showing the mean relative abundance percentage of dominant AMF VT for season in both sites of the study. Whiskers correspond to standard error. M=Malloa and SV=San Vicente. Su=summer, Au=autumn, Wi=winter and Sp=spring.



Indicator species analysis showed contrasting patterns for sample type between sites (Table 3). In M site, root samples had 5 indicator VT and soil samples had 14 (Table 3a), whereas in SV, root samples had 17 indicator species and soil samples had 2 (Table 3b). Indicator species in M root samples were all from *Glomus* VT (Table 3a), whilst M soil samples and SV root samples had more VT (Table 3a and 3b). Indicator species analysis for plant species showed one taxon for *P. boldus* in SV and also one but different taxon for *P. boldus* in M site (Table 4a and 4b). *L. caustica* in M site had five indicator species, only from the *Glomus* VT (Table 4a) in contrast to SV site that had three indicator species from *Glomus* and *Acaulospora* VT (Table 4b). *E. purverulenta* from M site also had also *Glomus* (2) and *Acaulospora* (1) VT as indicator species (Table 4a). *C. alba* only had *Glomus* VT (7 indicator species), in contrast to *Q saponaria* that had three different VT as indicator species (Table 4b). Remarkably, *K. oblonga* had only one indicator species from the *Archaeospora* VT (Table 4a). Finally, in M site indicator species were present only in summer and spring, with three *Diversispora* and one *Glomus* VT respectively (Table 5a). Indicator species in SV site were present only in summer, and the five belonged to *Glomus* VT (Table 5b).

Table 3. Significant indicator VT for sample type in both sites of the study. a) M=Malloa site, b) SV=San Vicente site. C=compartment, VT= virtual taxa, IV= indicator value, P=probability. R=roots, S=soil.

	a) M					b) SV			
C	**VT**	**Genus**	**IV**	**P**	**C**	**VT**	**Genus**	**IV**	**P**
R	GCL.2	Glomus	0.54	0.001	**R**	VT160	Glomus	0.74	0.004
	SS.G1	Glomus	0.44	0.001		GCL.2	Glomus	0.66	0.001
	MO.G66	Glomus	0.41	0.003		VT177	Glomus	0.64	0.037
	VT256	Glomus	0.36	0.035		VT23	Acaulospora	0.57	0.049
	VT115	Glomus	0.30	0.001		SS.G1	Glomus	0.57	0.001
						VT73	Glomus	0.55	0.048
						VT156	Glomus	0.52	0.001
						VT142	Glomus	0.52	0.030
						VT180	Glomus	0.50	0.001
						VT340	Claroideoglomus	0.50	0.047
						GCL.8	Glomus	0.44	0.001
						VT62	Diversispora	0.30	0.034
						VT395	Glomus	0.29	0.002
						VT69	Glomus	0.29	0.031
						VT153	Glomus	0.28	0.012
						VT163	Glomus	0.27	0.030
						VT115	Glomus	0.25	0.005
S	VT54	Diversispora	0.74	0.001	**S**	VT155	Glomus	0.63	0.014
	VT380	Diversispora	0.66	0.001		VT245	Archaeospora	0.50	0.001
	VT245	Archaeospora	0.64	0.001					
	VT281	Paraglomus	0.60	0.003					
	VT67	Glomus	0.60	0.011					
	VT193	Claroideoglomus	0.59	0.005					
	VT60	Diversispora	0.58	0.002					
	VT306	Diversispora	0.57	0.001					
	VT93	Glomus	0.50	0.042					
	VT62	Diversispora	0.50	0.001					
	VT155	Glomus	0.49	0.009					
	INTA.9	Glomus	0.35	0.003					
	VT287	Glomus	0.33	0.022					
	VT402	Claroideoglomus	0.25	0.016					

Table 4. Significant indicator VT for the different plant species in both sites of the study. a) M=Malloa site, b) SV=San Vicente site. PS=Plant species, VT= virtual taxa, IV= indicator value, P=probability. Pb= *P. boldus,* Lc=*L. caustica,* Ep=*E. pulverulenta,* Ko=*K. oblonga,* Ca=*C. alba* and Qs=*Q. saponaria.*

		a) M					b) SV		
PS	**VT**	**Genus**	**IV**	**P.**	**PS**	**VT**	**Genus**	**IV**	**P**
Pb	VT409	Glomus	0.34	0.028	**Pb**	VT56	Claroideoglomus	0.51	0.002
Lc	VT53	Glomus	0.44	0.014	**Lc**	VT113	Glomus	0.31	0.046
	VT93	Glomus	0.44	0.007		VT26	Acaulospora	0.24	0.026
	VT253	Glomus	0.41	0.020		VT114	Glomus	0.20	0.021
	VT96	Glomus	0.39	0.006					
	VT398	Glomus	0.25	0.035					
Ep	VT30	Acaulospora	0.48	0.005	**Ca**	VT123	Glomus	0.63	0.002
	VT143	Glomus	0.47	0.002		VT177	Glomus	0.56	0.010
	VT159	Glomus	0.41	0.002		VT142	Glomus	0.49	0.008
						VT372	Glomus	0.43	0.010
						IS.Gl2	Glomus	0.42	0.002
						VT167	Glomus	0.29	0.015
						GCL.8	Glomus	0.27	0.023
Ko	VT338	Archaeospora	0.46	0.006	**Qs**	VT193	Claroideoglomus	0.63	0.019
						VT67	Glomus	0.57	0.006
						VT155	Glomus	0.50	0.014
						VT402	Claroideoglomus	0.42	0.002
						VT283	Ambispora	0.41	0.008
						VT180	Glomus	0.28	0.016
						VT196	Glomus	0.26	0.049

Table 5. Significant indicator VT for seasons in both sites of the study. a) M=Malloa site, b) SV=San Vicente site. S=season, VT= virtual taxa, IV= indicator value, P=probability. Su=summer and Sp=spring.

		a) M					b) SV		
S	**VT**	**Genus**	**IV**	**P**	**S**	**VT**	**Genus**	**IV**	**P**
Su	VT54	Diversispora	0.52	0.018	**Su**	VT199	Glomus	0.47	0.007
	VT380	Diversispora	0.43	0.022		VT155	Glomus	0.45	0.031
	VT60	Diversispora	0.38	0.044		VT222	Glomus	0.39	0.022
						VT234	Glomus	0.38	0.021
						VT196	Glomus	0.28	0.022
Sp	VT69	Glomus	0.38	0.010					

4. Discussion

This is the first study on AMF communities and their determining factors in the sclerophyllous forest of the South American MTE, also known as Chilean matorral. Several patterns could be detected and some of them met the expectations.

The AMF compartments, meaning soil or root sample type, had a strong effect on shaping the AMF community composition in both sites, while AMF richness was affected by sample type only at SV site. This pattern has been found for other ecosystems (Hempel et al., 2007) also of the Mediterranean type (Sánchez-Castro et al., 2012; Torrecillas et al., 2012; Varela-Cervero et al., 2015). It has been suggested that this difference might come from roots of a particular plant species being capable of forming mycorrhiza only with some AMF present in the soil, and thus AMF communities in the soil compartment presenting higher number of AMF species. However, this explanation would stand only for M site were total soil richness resulted in 128 VT vs 114 in roots. On the contrary in SV site roots had 131 VT vs 119 in soil. Saks et al. (2014) reported higher VT richness in roots than soil, attributing this to a methodological problem coming from less DNA available from soil samples than root samples and suggesting to use larger soil samples in order to have same amount of DNA for both types of samples and thus making a better comparison. However, our rarefaction analysis showed that for SV soil samples almost all samples reached a plateau, thus the sampling effort was efficient, and having more DNA will not significantly increase the yield of VT. Thus, a possible explanation for roots having AMF that are not present in the soil is the presence of AMF that does not produce spores (Clapp et al. 1995; Oehl et al. 2004). Moreover, it is worth to remark that these AMF are probably present in all the plant species of SV and in all seasons. Another explanation might be the differential biomass production in root vs soil, which is the case of some species in the Glomeraceae, producing more biomass within the root than outside (Smith et al. 2000; Hart and Reader 2002).

Contrary to the expectations, plant host identity had a strong effect in shaping community composition, considering both sample types together and separated, while AMF richness was affected by plant host species only at the M site and only considering both sample types. This difference might come from the ability of some plant species to form mycorrhiza only with certain AMF present in the soil (Bever 2002; Bever et al. 2009). And the pattern is

enhanced when the effect of plant species is maintained even splitting the sample types. This pattern has been previously reported for other Mediterranean environments as well (Alguacil et al., 2009; Sánchez-Castro et al., 2012; Torrecillas et al., 2012; Varela-Cervero et al., 2015).

Contrary to our expectation, the season variable did not have an effect on shaping AMF richness and community composition in any of the two sites. However, when the analyses were performed on root and soil samples separately, the season variable showed an effect on community composition of soil samples of SV site. It has been shown for forest with understory herbaceous AM plants, that soil AMF community does not change during the growing season (Davison et al., 2012). Here, a difference was found between winter and summer samples, while spring and autumn shared AMF species. Also, VT richness was not affected by seasons. Both results suggest that the significant effect of season came from changing the relative abundance but not the disappearing of an AMF species. Thus, our result support the idea of this ecosystem having a relatively constant AMF community in both soil and root samples across all sampling times.

The effect of plant host and sample type but not season can be further explored with the indicator species analysis, where it is shown the species that are highly frequent and abundant within a group. Regarding plant species at M site, *Glomus* species were in *P. boldus* and *L. caustica,* while *E. pulverulenta* had *Glomus* and *Acaulospora* species and *K. oblonga* only *Archeospora.* Plant species in SV showed *Claroideoglomus* spp. in *P. boldus, Glomus* and *Acaulospora* in *L. caustica,* while *C. alba* had *Glomus* and *Q. saponaria* had *Claroideoglomus, Glomus and Ambispora.* At M site root samples had only *Glomus,* while soil samples had *Diversispora, Archeospora, Paraglomus* and *Claroideoglomus.* In contrast at SV, roots had *Glomus Acaulospora, Claroideoglomus* and *Diversispora,* while soil had *Glomus* and *Archaeospora.*

In contrast to other studies, we found an overall richness of 173 VT, which is the highest number reported so far relative to other Mediterranean ecosystems (Torrecillas et al., 2012; Varela-Cervero et al., 2015) and to other ecosystems (Davison et al., 2012). Moreover, 20 new VT were found which is supposed to owe to the fact that all species but *E. pulverulenta* are endemic to Chile. Also 6 VT resulted to be the abundant (>10% of reads) at least in one level of each factor explored. Those VT were from the *Glomus* clade, *Claroideoglomus* and *Paraglomus.* Moreover, two more *Glomus* VT were also abundant in at least one plant species.

pH resulted to have a significant effect on VT richness in both sites. Regarding community composition, it was significantly affected by SOM in both sites. However, there were also site-specific effects of other soil factors. pH can indirectly affect AMF communities by regulating nutrient availability (Xu et al., 2017). An inverse relation between SOM and AMF P uptake has been observed (Ravnskov et al., 1999). Thus, the increase of SOM would decrease P uptake by AMF. This in turn might impair AM symbiosis, since the plant would be unable to exchange sugar and lipids due the lack of P. In consequence, AMF community composition changes.

In summary, this study is the first on AMF communities and the factors that determine it in the sclerophyllous forest of the Chilean matorral. AMF communities are strongly shaped by AMF compartment (root or soil) and host plant species but not by season. This supports the idea of an AMF soil pool, relatively stable during the year, with plant species filtering the AMF from soil to form an AM with several species that are shared between all plant species, but with a few that are specific for each plant. Also, pH and SOM are shaping AMF communities, however other soil factors affected differentially each site. 173 VT were found, where 20 are new and exclusive from this ecosystem where endemic plants to Chile were explored. VT from the genera *Glomus*, *Claroideoglomus* and *Paraglomus* were the most abundant. Those genera, together with *Acaulospora, Archeospora, Ambispora* and *Diversispora* were also frequent and abundant in at least one AMF compartment or host plant species.

These results are a useful starting point for restoration purposes, and should be considered as as a reference natural system. Now a major part of the AMF community of the sclerophyllous forest is known and it is also known that soil AMF might serve as an AMF bank to plant species along the four seasons. Thus, in case of a sclerophyllous forest being affected by fire, a screening of the AMF community of the affected soil could be compared with the AMF community found here and concluded whether the inoculation with AMF is or is not necessary before reforestation. When necessary, soil from reference ecosystem can be used to inoculate plants to restore both, AMF and plant communities. Additionally, at least pH and SOM should be monitored and manipulated in order to regulate the AMF richness and community composition.

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Capítulo VI

1. Discusión general

En la presente tesis se tenía como objetivo general identificar los factores que determinan la abundancia, diversidad y composición comunitaria de los hongos micorrícicos arbusculares de un bosque esclerófilo representativo del *matorral chileno.*

Para conseguir este objetivo se realizaron variados análisis. Debido a los pocos estudios realizados en el *matorral chileno*, primero se hizo un búsqueda y análisis en base a bibliografía que diera luz de la distribución de los tipos y estatus micorrícicos de las plantas asociadas a este bioma. Con este análisis queda en evidencia que gran parte de las plantas presentes en Chile forman AM. Se llegó a esta conclusión a través de una aproximación empírica y una extrapolación jerárquica en base de varias categorías taxonómicas, sin embargo, la proporción exacta solamente puede ser conocida a través de la recolección de mas datos empíricos a nivel de especie (Bueno et al., 2018). El análisis a escala latitudinal evidencia el mismo patrón previamente descrito, en donde el tipo AM es siempre el mas abundante, sin embargo, va disminuyendo su proporción hacia latitudes mayores, mientras que los otros tipos comienzan a aumentar. Luego se realiza un análisis dividiendo el país en biomas: desierto, mediterráneo y bosque templado y nuevamente el tipo AM es siempre el mas frecuente, siendo su proporción mas alta en el desierto. Por último, las plantas endémicas son mas dependientes de la simbiosis micorrícica que las nativas y estas son mayormente plantas con micorriza obligada. Además, el tipo AM también es el más frecuente en las distintas formas de vida, esto es hierbas, arbustos y árboles. Todos estos resultados en conjunto, permiten fuertemente apoyar que el *matorral chileno* es principalmente del tipo AM, independiente de la forma de vida de las plantas y de su origen. En general la proporción de AM es similar comparada con el bosque templado y menor que el desierto, pero hay un patrón latitudinal, que probablemente esté asociado al gradiente latitudinal de precipitación y temperatura que genera un gradiente de condiciones edáficas y que a su vez condiciona los tipos de micorriza que pueden estar presentes, generando por último un recambio de especies de plantas en este gradiente. Esto confirma los que se ha propuesto previamente de forma teórica, en donde tanto hongos como plantas son capaces de coexistir en distintos ambientes gracias a la simbiosis micorrícica (Smith and Read, 2008). No obstante, el

análisis de estos datos con datos ambientales podría explicar detalladamente los cambios a escala latitudinal. Otro resultado importante de destacar es la urgencia en realizar mas estudios en las micorrizas del desierto y del mediterráneo, ya que están frecuentemente presentes, pero poco se ha estudiado en relación al bosque templado.

En segundo lugar, se eligió el bosque esclerófilo para iniciar la descripción de patrones comunitarios de HMA y los factores que los regulan, dado que es la formación vegetacional mas común o característica del bosque esclerófilo. Además, este bosque se ubica principalmente en sititos con distinta exposición a horas de sol, por lo que se eligieron ambas laderas de una montaña para describir de forma mas completa las comunidades de HMA asociadas a este bosque. En los bosques esclerófilos elegidos las plantas dominantes resultaron ser *Peumus boldus, Lithrea caustica, Quillaja saponaria, Cryptocarya alba, Kageneckia oblonga* y *Escallonia pulverulenta*. Al revisar la base de datos construida en el primer capítulo se observó que no existía evidencia empírica en este ecosistema que corroborara el tipo de micorriza de estas plantas, y la aproximación taxonómica indicaba que eran AM. Es por esto se procedió a explorar la presencia de MA en las raíces de estas plantas. Todas las plantas exploradas tenían presencia de estructuras de HMA sin embargo en *L. caustica* la frecuencia e intensidad fue muy baja y las estructuras asociadas a la raíz eran muy superficiales, por lo que más estudios serían necesarios para poder asegurar su micorriza de tipo MA.

Una vez confirmado que todas estas especies se asocian de alguna forma con HMA, se procedió a determinar la abundancia de estos hongos a partir de la abundancia de esporas en el suelo rizosférico de cada planta en los dos sitios de bosque esclerófilo elegido. Los resultados demostraron un fuerte efecto estacional en la abundancia de esporas, mientras que los factores físico-químicos del suelo que regulan la abundancia de esporas son sitios dependientes. Siendo importantes en Malloa, el contenido de arcilla, la conductividad eléctrica y el fósforo. Mientras que en San Vicente fueron importantes la el contenido de arcilla, el nitrógeno total y la materia orgánica del suelo fueron importantes. Por otra parte, la especie de planta hospedera no tiene mayor efecto en regular la abundancia de esporas. Esto indica que las estaciones regulan la esporulación y germinación de esporas en, mientras que la especie de planta hospedera se asocia con aquello disponible en el suelo de forma general y no especialista. No obstante, esto ocurre solamente en cuanto a abundancia, pudiendo cambiar al revisar la diversidad y composición comunitaria de los HMA, tanto presentes en raíces como en el suelo.

Dicho lo anterior se realizó el último estudio en donde se buscaba describir como el compartimento de los HMA (suelo vs raíz), la especie de planta hospedera, los factores físico-químicos del suelo y las estaciones del año regulaban la diversidad y composición comunitaria de los HMA en dos bosques esclerófilos representativos del *matorral chileno*. En contraste a la abundancia de esporas en el suelo, existe una fuerte determinación de la comunidad de HMA por el tipo de compartimento (raíz o suelo) y la especie de planta hospedera, pero no por la variable estación. El pH y el contenido de materia orgánica en el suelo fueron los factores mas importantes en regular la comunidad de HMA en general, habiendo factores del suelo que afectaron diferencialmente en cada sitio. Se observó una alta riqueza, mas alta que lo descrito para otros sistemas mediterráneos (173 vs 107 TV) (Varela-Cervero et al., 2015), en donde los géneros mas abundantes fueron *Glomus, Claroideoglomus y Paraglomus*. Estos géneros junto con *Acaulospora, Archeospora, Ambispora* y *Diversispora* fueron los mas abundantes y frecuentes en las raíces, suelo, o alguna especie de planta.

Estos resultados son los primeros en describir las comunidades de HMA y los factores que los regulan para el bosque esclerófilo, perteneciente al *matorral chileno* y vienen a confirmar la importancia en influenciar a la comunidad de plantas de estos bosques. Además, sirven como información ecosistémica de referencia para comparar el nivel de degradación que tuvo por ejemplo un fenómeno de perturbación (ejemplo: incendios o agricultura convencional).

Futuras estrategias de revegetación debiesen considerar plántulas nativas asociadas a los HMA, ya que estas permitirán apoyar el establecimiento de la plántula y servir como una "isla de recursos", sirviendo como fuente de inóculo para el área circundante. La inoculación puede ser vía consorcios a partir de suelo no perturbado, que ha demostrado tener efectos positivos en el crecimiento de plantas y en estimular la riqueza de una comunidad (Neuenkamp et al., 2018). Sería interesante en un futuro, caracterizar otros bosques esclerófilos, ya que el que se caracterizó en esta tesis están en la depresión intermedia. Los bosques esclerófilos tienen distribución desde la costa a la precordillera por lo que se podrían encontrar nuevos TV y generar mas bases de datos referenciales. Los resultados muestran la presencia de especies de HMA cosmopolitas, pero también especies propias, las cuales deben estar aportando al mantenimiento de ecosistemas y que también son importantes de preservar en conjunto con las distintas especies de plantas. Por otra parte, seguir caracterizando las comunidades de HMA en otras formaciones vegetacionales del *matorral chileno*, probablemente entregue aún mas nuevas especies de HMA.

Por último, considerar la eficiencia en el uso de agua de las plantas inoculadas con HMA es un punto importante a considerar en futuras investigaciones, considerando que el *matorral chileno* tiene un verano cálido y seco, en donde probablemente los HMA puedan estar cumpliendo un rol importante en mejorar el desempeño de las plantas. Para esto, estudios que consideren la ecofisiología de plantas y HMA en conjunto serían valiosos (Barea et al., 2011).

2. Conclusión general

La hipótesis de estudio es rechazada ya que la abundancia de esporas es determinada por las estaciones del año, mientras que la diversidad y composición comunitaria es determinada por el hábitat del HMA y la especie de planta hospedera. Además, tanto la abundancia de esporas, como la diversidad y composición comunitaria de HMA en el suelo y en las raíces de plantas del bosque esclerófilo se ve determinada por distintos factores edáficos.

Se concluye que mientras que la esporulación y germinación de esporas ocurre diferenciadamente a lo largo de las estaciones del año, las distintas especies de plantas se asocian a una pequeña parte de especies de hongos de forma preferencial, y en mayor grado a la mayoría del pool de especies disponibles en el suelo. Al haber una diversidad y composición comunitaria de HMA diferenciada por especies de plantas indica la importancia de estos organismos como simbiontes de las plantas y por lo tanto su necesidad de ser considerados en planes de restauración futuros para mejorar el desempeño de las plantas y preservar también la diversidad de HMA en los suelos del *matorral chileno*.

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