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RESEARCH ARTICLE

Factors affecting arbuscular mycorrhizal fungi of Chilean temperate rainforests

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Abstract

While arbuscular mycorrhizal (AM) fungi in Chile have been widely documented in agro-ecosystems, there is a knowledge gap regarding AM fungal diversity in Chilean temperate rainforests. AM fungal communities of these forests are affected by several factors: the mountain systems of Chile (Coastal Range or Andes Mountains), the mycorrhizal dominance of the forest (either ectomycorrhizal -EM- or AM), soil chemistry, and altitude. We tested the effects of mountain system, mycorrhizal dominance, soil chemistry, and altitude on AM fungal diversity. From 7,120 AM fungal spores recovered, we identified 14 species, that were found in 41 soil samples collected from 14 plots located in EM and AM forests of the Coastal Range and Andes Mountains of Southern Chile. Mountain system and mycorrhizal dominance affected AM fungal community composition, although neither fungal richness nor abundance were affected. Soil Olsen available P, Ca, Mg, and Na were the edaphic variables structuring AM fungal community composition. There was no relationship between altitude and AM fungal richness, however at high altitudes there was higher abundance. Finally, with this and other studies, a total of 59 AM fungal species, many of which were previously registered exclusively in agroecosystems, are registered on the Chilean AM fungal species list.

Keywords: Atitude, arbuscular mycorrhizal fungi taxonomy, Chilean mountain systems, forest mycorrhizal dominance, soil chemistry, temperate rainforests

1. Introduction

Arbuscular mycorrhizal (AM) fungi, contained within the subphylum Glomeromycotina, are obligate symbiotic partners of up to 82% of land plants (Davison *et al*., 2015). This symbiosis is crucial for the plant communities' health and the functioning of nutrient cycles at the ecosystem level (Castillo *et al*., 2006; Etcheverría *et al*., 2009). A recent review (Castillo *et al*., 2016) shows that for Chile, 57 AM fungal species have been reported, yet these have mainly been documented in agroecosystems (as reported elsewhere, e.g. Aguilera *et al*., 2014, 2015). There is an important lack of knowledge about AM fungal species presence and community dynamics in Chilean native forests, particularly in southern temperate rainforests.

There are various factors that could affect the AM fungal diversity patterns of these forests. For example, the location of these forests in either the Coastal Range or the Andes Mountains could affect fungal diversity given that these mountain systems differ in terms of their geological, biogeochemical, and edaphic features (Armesto *et al.*, 2010). Second, the type of mycorrhiza associated with dominant vegetation could also affect fungal diversity patterns. Specifically, there are four main types of forest in Southern Chile: native coniferous-dominated forest, *Nothofagus* spp. (angiosperm) dominated forest, hygrophile forest, and Valdivian forest (Godoy *et al*., 1994). *Nothofagus* forests are mainly ectomycorrhizal (EM)-dominated (Palfner, 2001) while the other three forest types are predominately AM-dominated (Godoy *et al.*, 1994; Fontenla *et al.*, 1998; Castillo *et al.*, 2006) with subordinate plant species having EM associations. Conifers and species of *Nothofagus* have different altitudinal ranges spanning lowlands to the timberline (Godoy *et al*., 1994). Additionally, soil chemistry and altitude can greatly affect AM fungal diversity (Shi *et al.*, 2014; Davison *et al*., 2015; Yang *et al.*, 2016). Currently, base-line knowledge of diversity patterns of AM fungi of Chilean temperate rainforests, as well as how mountain system, mycorrhizal type of the dominant vegetation, soil chemistry, and altitude affect those patterns, remain unknown.

The Coastal Mountains and Andes Mountains in Southern Chile have contrasting geological histories and therefore edaphic conditions. The Coastal Mountains, which are considered a refugium for biodiversity (Armesto *et al*., 2010), have higher plant taxonomic and phylogenetic diversity than the Andes Mountains (Villagrán and Armesto, 2005). The bedrock of the Coastal Mountains is highly weathered (100 - 120 kyrs stand age); thus, nutrient inputs from the atmosphere are important (Kennedy *et al*., 2002), particularly at south of 37°S latitude were the Pacific Ocean plays a relevant subsidiary function in the nutrient dynamics of these forests (Kennedy *et al*., 2002). Meanwhile, most of the Andes Mountains were covered by glaciers during the Last Glacial Maximum (LGM); thus, young volcanic soils (<5 kyrs stand age; Villagrán and Armesto, 2005) that are richer in nutrients are found in this mountain system. In terms of belowground diversity, exchange of symbiotic microorganism was likely accelerated during the LGM in the biodiversity refugia that constituted the Chilean Coastal Range (Villagrán and Armesto, 2005). The Southern Chile Andean flora -less diverse than Coastal flora- had access to richer belowground biodiversity during concentration in refugia (Villagrán and Armesto, 2005). Therefore mycorrhizal associations should not represent functional restrictions to soil nutrient limitations at Andes (Marín *et al*., 2016).

There are several mycorrhizal association types including AM, EM, ericoid and orchidoid mycorrhiza.

Plant communities are usually dominated by a single mycorrhizal type (Soudzilovskaia *et al.*, 2015). In an ecosystem, the composition of plants with differing mycorrhizal associations can affect AM fungal diversity patterns. Recently, patterns of the mycorrhizal status of vegetation have been mapped at regional and global scales (Soudzilovskaia *et al.*, 2015; Bueno *et al*., 2017a). This being said, it is still unknown how mycorrhizal dominance, here defined as the mycorrhizal type associated with the dominant plant species in a forest, affects the diversity of AM fungi.

Forests of Southern Chile mainly have EM associations. Agaricales make up a large proportion of the mycorrhizal associations in *Nothofagus* spp. forests (Palfner, 2001), while hygrophile, Valdivian, and native coniferous forests house mainly AM fungi (Godoy and Mayr, 1989; Carrillo *et al.*, 1992; Godoy *et al.*, 1994; Castillo *et al*., 2006, 2016; Oehl *et al.*, 2011a, 2012; Marín *et al.*, 2016). In EM or AM dominated forests, other mycorrhizal types (either EM or AM) are not excluded; only they are found in lower proportions and are often associated with understory plants. It must be noted, however, that the influence of all of these factors- mountain system, mycorrhizal dominance, soil chemistry, and altitude- on AM fungi diversity and community structure, is dependent upon successful AM colonization and thus reproduction. Then, the effect of all those factors (mountain system, mycorrhizal dominance, soil chemistry, and altitude) must be weighted by the effect of AM fungi hyphae root colonization on AM fungi diversity.

This study targeted Chilean Coastal and Andean temperate EM and AM dominated forests and aimed at: (1) testing the effects of mountain system, mycorrhizal dominance, soil chemistry and altitude on AM fungal diversity patterns, weighting the effect of the colonization of roots by AM fungi, and (2) updating the morphological species list and available information for AM fungi of Chilean ecosystems.

2. Materials and Methods

2.1. Study sites.

A total of fourteen 30 x 30 m plots were sampled (Table 1) in six sites. Two sites in the Andes Mountains (San Pablo de Tregua Nature Reserve and Tolhuaca National Park) and four sites in the Coastal Range (Alerce Costero National Park, Nahuelbuta National Park, Los Ruiles Nature Reserve, and La Campana National Park), Chile, were selected. The selected sites included the four main types of old slow-growth temperate rainforest of south-central Chile: EM dominated forest (*Nothofagus* spp. including *N. alpina* (P. et E.) Oerst., *N. dombeyi* (Mirb.) Oerst., *N. nitida* (Phil.) Krasser, *N. alessandrii* Espinosa and *N. macrocarpa* (A. DC.) F.M. Vázquez & R. Rodr.), AM dominated forest (including the endemic conifers *Araucaria araucana* (Molina) K. Koch, *Fitzroya cupressoides* (Molina) Johnst. and *Saxegothaea conspicua* Lindl.), angiosperm dominated Valdivian forest (mixed broadleaf with some *N. nitida* (Phil.) Krasser trees and some *Weimannia trichosperma* CAV trees), forest where no single tree species was dominant, and hygrophile forest (mixed forest with some *Luma apiculata* (DC.) Burret and *Peumus boldus* Molina), where also no single tree species dominates.

2.2. Soil sampling

To identify AM fungal species assemblages and determine soil chemistry, bulk soil samples were taken in the 14 plots. In five randomly selected sub-sites within each plot, one 1,000 g soil sample was taken with a cleaned shovel after removing the O horizon (A horizon; approx. 20 cm x 20 cm x 20 cm). The samples were thoroughly mixed to obtain a composite sample for each plot -a total of 14 composite soil samples were obtained. In each composite sample,

three randomly selected root samples were separated for analysis of root colonization by AM fungi. Roots were carefully removed afterwards, and for each composite sample, three 25 g soil aliquots were taken for AM fungi spore isolation and determination. Another three 25 g soil aliquots were dried at ambient temperature and sieved to <2 mm for soil chemical analysis. A total of 42 root samples, 42 soil samples for spore isolation, and 42 soil samples for soil chemical analysis were obtained from the 14 plots examined in this study.

Table 1. Locations and characteristics of plots located in temperate rainforests in Southern Chile.

Site	Plot code	Coordinates	Altitude (m.a.s.l.)	Mycorrhizal dominance	Dominant tree species
Andes					
San Pablo	A1.EM	39°36.072´S 72°07.099´W	660	EM	*Nothofagus alpina* (P. et E.) Oerst.
de Tregua	A2.AM	39°36.080´S 72°05.794´W	770	AM	*Saxegothaea conspicua* Lindl.
Tolhuaca	A3.EM	38°12.298´S 71°49.044´W	1218	EM	*Nothofagus dombeyi* (Mirb.)
	A4.AM	38°11.989´S 71°48.644´W	1365	AM	*Araucaria araucana* (Molina) K. Koch
Coast					
Alerce	C1.EM	40°11.915´S 73°25.887´W	938	EM	*Nothofagus nitida* (Phil.) Krasser
Costero	C2.AM	40°11.768´S 73°26.108´W	933	AM	*Fitzroya cupressoides* (Molina) Johnst.
	C3.AM	40°11.777´S 73°26.129´W	928	AM	*Fitzroya cupressoides* (Molina) Johnst.
	C4.AM	40°11.775´S 73°26.162´W	924	AM	*Fitzroya cupressoides* (Molina) Johnst.
	C5.AM	40°10.182´S 73°32.941´W	673	AM	Valdivian forest
Nahuelbuta	C6.EM	37°48.952´S 73°00.538´W	1314	EM	*Nothofagus dombeyi* (Mirb.)
	C7.AM	37°47.275´S 72°59.870´W	1347	AM	*Araucaria araucana* (Molina) K. Koch
Los Ruiles	C8.EM	35°50.033´S 72°30.300´W	277	EM	*Nothofagus alessandrii* Espinosa
La Campana	C9.EM	32°58.008´S 71°07.215´W	1101	EM	*Nothofagus macrocarpa (A. DC.) F.M. Vázquez & R. Rodr.*
	C10.AM	32°58.500´S 71°07.847´W	580	AM	Hygrophile forest

Valdivian forest: high abundance of *Weimannia trichosperma* CAV and *Nothofagus nitida* (Phil.) Krasser. Hygrophile forest: high abundance of *Luma apiculata* (DC.) Burret and *Peumus boldus* Molina.

2.3. AM fungi isolation and identification

Spores were extracted from soils using wet sieving and sucrose density gradient centrifugation (Błaszkowski, 2012). Briefly, 25 g of soil were passed through sieves of 500, 125 and 32 µm and thoroughly washed with distilled water. The last soil portion was collected using 32 µm mesh, and the soil fraction between 500 and 125 µm was distributed onto plastic tubes. 25 mL of the spore suspensions were transferred to 50 mL centrifugation tubes. 25 mL of a 70% sugar solution were added to the bottom of the tubes, and then the tubes were centrifuged at 2,000 rpm for 2 min. After centrifugation, the samples were decanted, washed, and transferred to Petri dishes. Spores were carefully counted under the dissection microscope at up to 400-fold magnification. The number of AM fungal spores was expressed as spores in 100 g of dry soil. Finally, all spores found in each sample were mounted on microscope slides in polyvinyl alcohol-lactic acid glycerol (PVLG) medium mixed 1:1 (v/v) with Melzer´s reagent for their taxonomic identification. The spores were classified based on the Glomeromycota system of Oehl *et al.* (2011b, c). Identification reports (Błaszkowski, 2012; Oehl *et al.*, 2011b, c) and the homepage of the Swiss collection for AM fungi (SAF; http://www.agroscope.ch/saf) were also used.

2.4. Soil chemical analysis

Three subsamples of each of the 14 composite soil samples were used to measure pH (in 0.01 M $CaCl_2$); electrical conductivity (EC) and redox potential (Eh) were determined in a water solution (1/2.5) at 20 °C. Percentages of C (C) and N (N) were determined using a CN Elemental Analyzer (Elementar, Langenselbold, Germany). Plant available P (P_a) was determined by extraction in 0.5 M $NaHCO_3$ (pH 8.5) and dilution (1/2.5) in 10% HNO_3 using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, VARIAN, Palo Alto, U.S.A.). The cations (Al, Ca, K, Mg and Na) were determined by NH4OAc 1M extraction (multistandards in a matrix of NH_4OAc 1M, HNO_3 10% and ultra-pure water) by ICP-OES.

2.5. Root colonization by AM fungi

To test the effect of AM fungi hyphae root colonization on AM fungi diversity, three root samples from each composite sample of the 14 plots were randomly selected; -care was taken to ensure that the root samples were colonized by AM fungi; the dyeing methodology of Gemma *et al.* (1989) was followed to verify and evaluate colonization; these methodologies are described below. Young secondary roots were cut into 1 cm pieces and thoroughly washed with water. To remove the cytoplasm and nuclei from the host roots, the roots were transferred to tubes where KOH (2.5% w/v) was added. Then, the roots were incubated in this solution for 72 h. Afterwards, the KOH was removed and the roots were washed with water. The water was discarded and the roots were covered with HCl (1% w/w); the roots were incubated in this solution for 24 h to eliminate excess KOH. Excess HCl was also eliminated by thoroughly washing the roots with water. Then, trypan blue (0.05% w/v) was added to the roots for 24 h; afterwards, the trypan blue was carefully washed with abundant water. Colonization quantification was done by the grid line intersect method (Giovannetti and Mosse, 1980); specifically, roots were randomly located on Petri dishes with grid lines, where root colonization by AM fungi was quantified.

2.6. Statistical analysis

To test the effectiveness of AM spore sampling, species accumulation curves across soil samples and plots were calculated using the function *accumresult* of the *BiodiversityR* package (Kindt and Coe, 2005) in R 3.3.2 (R Development Core Team, 2016). To describe AM fungi diversity patterns in the 14 plots, several diversity indices accounting for alpha diversity, dominance, and evenness (Richness, S; Shannon, H´; Simpson, 1-D1; Inverse Simpson, D2; Evenness, J´; Berger, BP) were calculated using the function *diversityresult* of the *BiodiversityR* R package (Kindt and Coe, 2005). To test if mountain system, mycorrhizal dominance, or their interaction had any effect on the calculated diversity indices, mixed linear models (~ mountain system*mycorrhizal dominance, and site as a random factor to overcome spatial effects) were calculated using the *lme* function of the R package *nmle* (Pinheiro *et al*., 2016).

Rényi alpha diversity and evenness profiles of each plot were calculated using the function *renyiresult* of the *BiodiversityR* package (Kindt and Coe, 2005). Rényi diversity profile values (H-alpha) are calculated based on the relative abundance of each species and using a scale parameter (alpha), ranging from zero to infinity (Kindt and Coe, 2005). Rényi profiles are directly related to richness (S) and to the Shannon (H´), Simpson (D1) and Berger (BP) indices. Thus, in a Rényi profile, the H-alpha values reflect diversity (i.e., community A is more diverse than community B if A is always plotted above B; Kindt and Coe, 2005). In

the profile, community A is more diverse or has more evenness than community B, if the former is above and never intersects with the latter.

To calculate the alpha, beta, and gamma diversity (measured as contribution to the Simpson index, 1-D1) of the plots, the function *contribdiv* in the R package *vegan* (Oksanen *et al*., 2015) was used. The function *vegdist* of the R package *vegan* (Oksanen *et al*., 2015) was used to calculate Bray-Curtis dissimilarity, an ecological distance used to generate heatmaps of the samples and species.

To identify which of the 11 edaphic variables best predict the AM fungi community composition, Canonical Correspondence Analysis (CCA) for AM fungi was conducted using the function *cca* of the R package *vegan* (Oksanen *et al*., 2015). The order of the final variables was selected using backward model selection.

2.7. Update on AM fungi of Chilean ecosystems

Using the information presented in Castillo *et al*. (2016) as a baseline, the list of AM fungal species present in different Chilean ecosystems was updated to include temperate rainforest ecosystems. In addition to Castillo *et al*. (2016), three more sources of information were added: Paulino (2006), Marín *et al.* (2016) and this study. With this information, a comprehensive list of AM fungal species presence in various ecosystems was compiled.

3. Results

3.1. Soil analysis and AM fungal communities

Contents of N and P_a were significantly affected by mountain system and mycorrhizal dominance, respectively (Supplemental table 1, Link: https://drive.google.com/open?id=0B6U25wfht2s1bUFTbEZTY0taM1k). Coastal N content (average of 6.200%) was significantly lower (t-value: -2.713, p-value: 0.053) than Andean N content (average of 17.383%). Content of P_a was significantly higher (t-value:1.815, p-value: 0.012) in EM forests (average of 19.703 mg/Kg) than in AM forests (average of 13.586 mg/Kg). Neither mountain system, mycorrhizal dominance, nor their interaction had any effect on the remaining nine soil chemistry variables.

With 7,120 AM fungi spores sampled and identified in the 42 soil samples from 14 plots (three technical replicates by plot obtained from one composite sample by plot), the sampling effort was sufficient both at the sample level (Figure 1a) and at the plot level (Figure 1b). A total of 14 AM fungi species were identified in the 14 studied plots (Figure 2), and these species belong to six genera: six species belong to *Acaulospora* (Acaulosporaceae), three to *Glomus* (Glomeraceae), two to *Claroideoglomus* (Entrophosporaceae), one to *Cetraspora* (Gigasporaceae), one to *Scutellospora* (Scutellosporaceae), and one to *Simiglomus* (Glomeraceae). One *Acaulospora* and one *Glomus* species could potentially be undescribed species (Figure 2). The most abundant AM species, irrespective of mountain system and mycorrhizal dominance were: *Glomus* sp CL1, *Acaulospora laevis*, and *Claroideoglomus claroideum* (Figure 2). Interestingly, the ecological similarity represented on Figure 2 did not reflected the phylogenetic relatedness of the species -thus, species within the same genus were not grouped together by ecological distance (Figure 2). Also, plots C5.AM, C8.EM, C9.EM and C10.AM were ecologically close (Figure 2), mainly by the high abundance of *Glomus* sp CL1 on these plots.

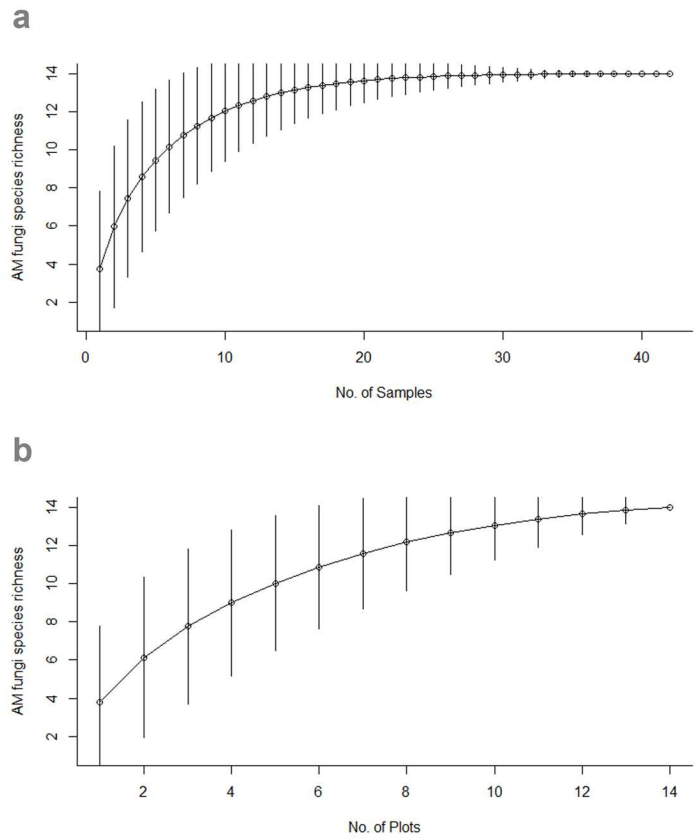

Figure 1. a. Arbuscular mycorrhizal fungi species richness accumulation curve based on number of soil samples. Bars represent permutations (1000). **b**. Arbuscular mycorrhizal fungi species richness accumulation curve based on number of plots. Bars represent permutations (1000).

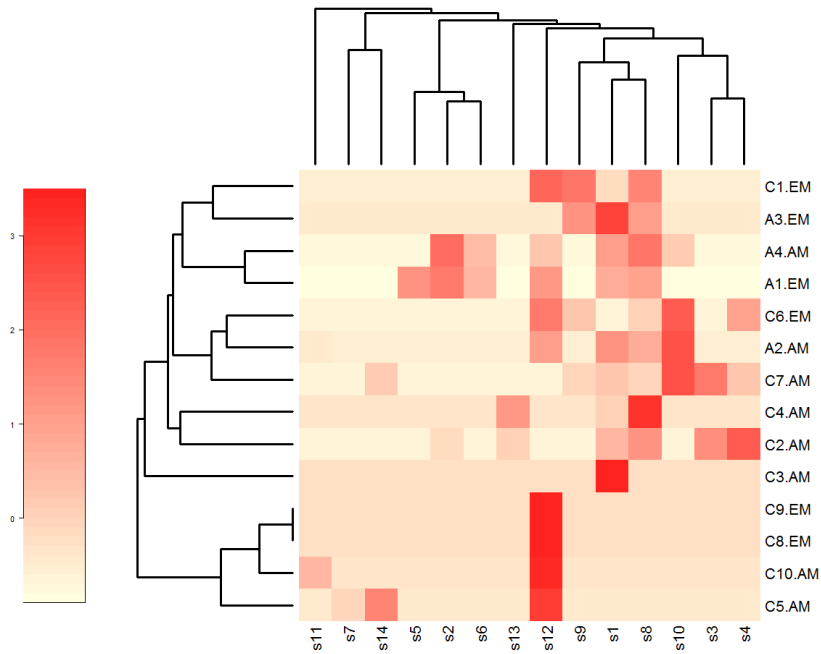

Figure 2. Heatmap and clustering (Bray-Curtis dissimilarity) of the 14 arbuscular mycorrhizal fungi species described in the 14 study plots located in temperate rainforests of Southern Chile. Species legend: s1: *Acaulospora laevis*, s2: *Acaulospora paulinae*, s3: *Acaulospora punctate*, s4: *Acaulospora scrobiculata*, s5: *Acaulospora sieverdingii*, s6: *Acaulospora* sp CL1, s7: *Cetraspora gilmorei*, s8: *Claroideoglomus claroideum*, s9: *Claroideoglomus etunicatum*, s10: *Glomus badium*, s11: *Glomus diaphanum*, s12: *Glomus* sp CL1, s13: *Scutellospora calospora*, s14: *Simiglomus hoi*.

3.2. Effects of mountain system, mycorrhizal dominance, soil chemistry, and altitude.

Richness and abundance of AM fungi was not higher solely on any of the mountain systems/mycorrhizal dominances examined: EM and AM dominated forests of both the Coastal and Andean Mountains had the highest AM fungi richness and abundance values (Supplemental table 2, Link: https://drive.google.com/open?id=0B6U25wfht2s1bkZwR3VtYmlQZWc). Meanwhile, two Coastal EM forests (C8.EM and C9.EM) located at the northern distribution of this study (Table 1) had extremely low AM fungal richness and abundance (Supplemental table 2a), when compared to Coastal AM and Coastal and Andean EM forests. These two Coastal *Nothofagus* forests presented extreme environmental conditions (low precipitation), and the plant cover by the ectomycorrhizal *Nothofagus* spp. (>95%) was higher than in the other *Nothofagus* spp. plots. The diversity indices calculated also reflect this pattern (Supplemental table 2a). Furthermore, the AM fungi alpha diversity from the Rényi diversity profile (Figure 3a) was also highest in EM and AM forests of the Coastal and Andean Mountains. But two plots had 0 alpha diversity, and one Coastal AM forest had the lowest AM fungi

alpha diversity. No significant differences were seen in the Rényi evenness profile (Figure 3b) except for plot C7.AM, which was slightly less even that the other plots, and the two plots with 0 alpha diversity, which logically had complete evenness. Mountain system, mycorrhizal dominance, and their interaction did not affect any AM diversity measurement (Supplemental table 2b). The community composition of AM fungi was indeed affected by mountain system and mycorrhizal dominance; Coastal EM, Andean EM and Andean AM forests tended to have a similar AM fungal community composition (Figure 4).

Despite their proximity and having similar altitudinal and edaphic conditions to those of Coastal EM forests -with high plant cover of ectomycorrhizal *Nothofagus* species, within the same site Coastal AM forests had very different AM fungal community compositions (Figure 4). Despite being highly distant and contrasting in altitude (824 m difference), plots C8.EM and C9.EM had the exact same community composition: just species *Glomus* sp CL1 was present. Both Andean AM and EM plots had a more between and within similar community structure, given by *Acaulospora* and *Claroideoglomus* species.

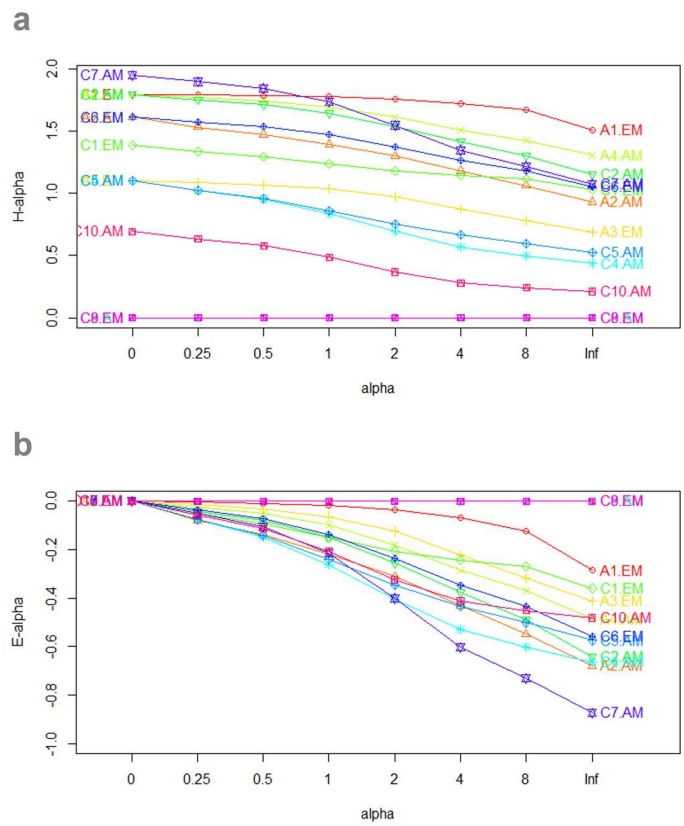

Figure 3. Rényi diversity and evenness profiles of arbuscular mycorrhizal fungi per plot located in temperate rainforests in Southern Chile. In a Rényi profile, community A is more diverse or has more evenness than community B, if the former is above and never intersecting with the latter. **a**. Rényi alpha diversity profile. **b**. Rényi evenness profile.

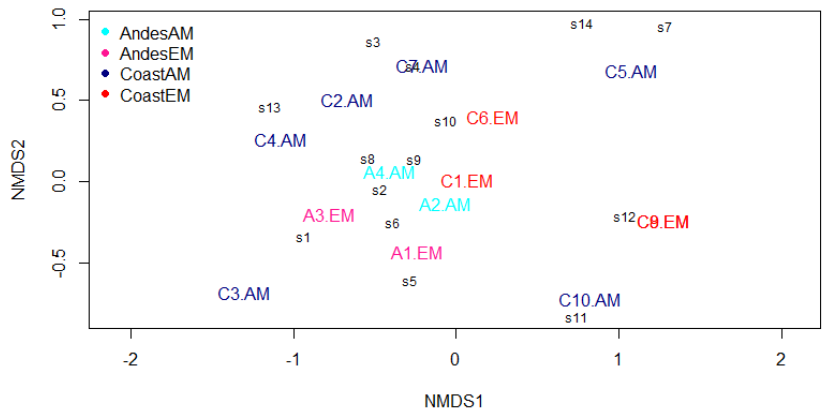

Figure 4. Non-metric multidimensional scaling (NMDS) of the arbuscular mycorrhizal fungal communities found in the 14 study plots. The plots were located in temperate rainforests in Southern Chile in either the Andes or the Coastal Mountains, and mycorrhizal dominance (AM or EM) of the forest was determined. Species legend: s1: *Acaulospora laevis*, s2: *Acaulospora paulinae*, s3: *Acaulospora punctate*, s4: *Acaulospora scrobiculata*, s5: *Acaulospora sieverdingii*, s6: *Acaulospora* sp CL1, s7: *Cetraspora gilmorei*, s8: *Claroideoglomus claroideum*, s9: *Claroideoglomus etunicatum*, s10: *Glomus badium*, s11: *Glomus diaphanum*, s12: *Glomus* sp CL1, s13: *Scutellospora calospora*, s14: *Simiglomus hoi.*

Soil P_a, Ca, Mg, and Na (in that order) were the main edaphic variables affecting AM fungal community compositions (Figure 5). Content of P_a and Mg had the largest effects on the AM fungal communities of Coastal EM forests while content of Ca and Na had the greatest effects on the AM fungal communities of Andean EM and AM forests (Figure 5). There was no relationship between altitude and AM fungal richness (Figure 6a), but with higher altitude there was higher AM fungi abundance (number of spores) (Figure 6b). At high altitude, abundance was highest in three of the four forest combinations examined: Coastal AM, Coastal EM, and Andean AM forests (Figure 6b).

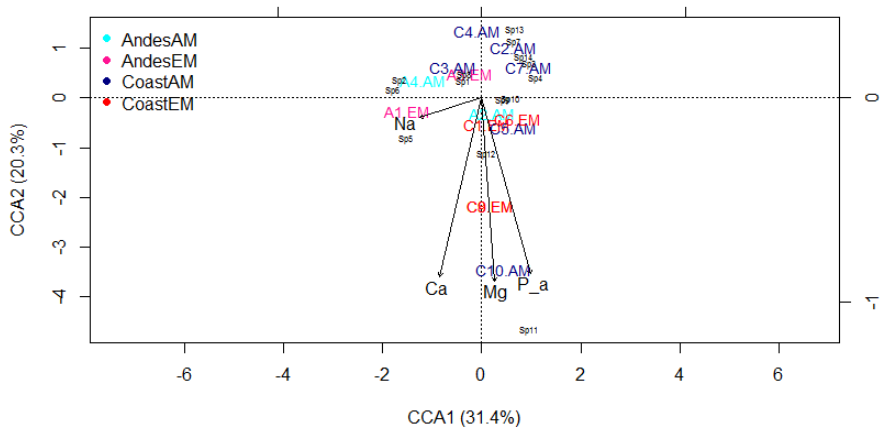

Figure 5. Canonical Correspondence Analysis (CCA) model for the arbuscular mycorrhizal fungal communities of the 14 study plots. The plots were located in temperate rainforests in Southern Chile in either the Andes or the Coastal Mountains, and mycorrhizal dominance (AM or EM) of the forest was determined. Model: ~ P_a + Ca + Mg + Na; F: 1.741, significance: 0.039 (ANOVA for CCA, 1000 permutations; the order of the soil chemical variables was selected via backward model selection, the variance is shown in brackets).

3.3. Effect of AM root colonization on AM fungal diversity.

All the previous results were not affected by AM fungi hyphae root colonization, as neither AM fungal richness nor abundances of spores were related with root colonization by AM fungi, although the percentage of roots colonized by AM fungi was lower in Coastal AM plots (Supplemental figure 1, Link:

https://drive.google.com/open?id=0B6U25wfht2s1SU1NbVBZdWxEMTA).

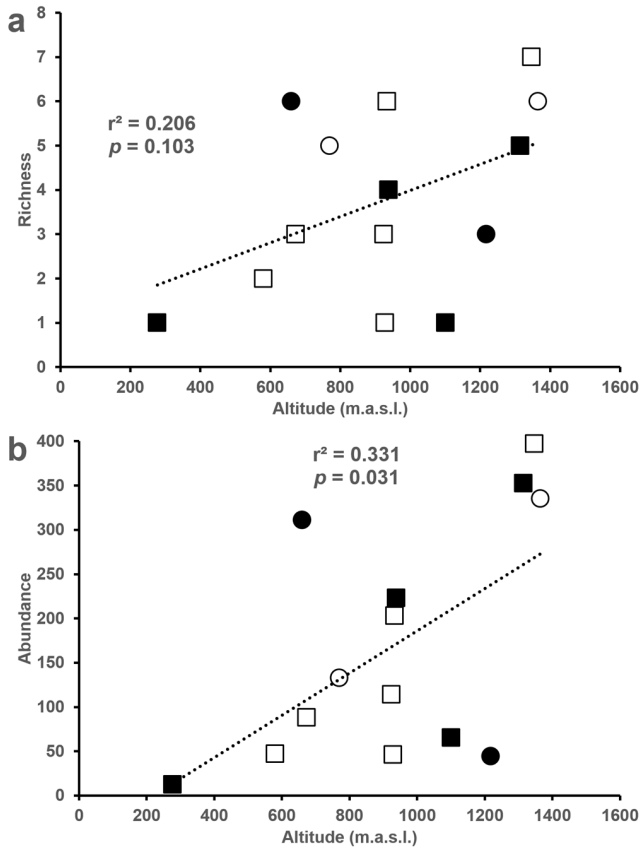

Figure 6. a. Relationship between altitude (m.a.s.l.) and arbuscular mycorrhizal fungi species richess by mountain system and by mycorrhizal dominance combination (Andes EM (●), Andes AM (○), Coast EM (■), Coast AM (□)). **b**. Relationship between altitude (m.a.s.l.) and arbuscular mycorrhizal fungi abundance (number of spores) by mountain system and by mycorrhizal dominance combination (Andes EM (●), Andes AM (○), Coast EM (■), Coast AM (□)).

3.4. Update on AM fungi of Chilean ecosystems

From 57 AM fungal species registered by Castillo *et al*. (2016), two more species were added for a total of 59 AM fungi species, belonging to 21 genera, registered for Chile (Table 2) -a 3.5% increase in the AM fungal species registered for Chile. Importantly, 20 registered AM fungal species found in anthropogenically intervened ecosystems (Castillo *et al*., 2016) were also present in pristine EM and AM forests (Table 2) -thus, 35.1% of registered species, which were thought to be exclusive from anthropogenic environments are also present on pristine forests. Two AM fungi species recently discovered were also found on this study: *Acaulospora punctata* (Oehl *et al*., 2011a) and *Ambispora reticulata* (Oehl *et al*., 2012). These results provide new information on AM fungal species presence by ecosystem type (two new ecosystem types) and AM fungal species (two new AM fungal species).

Table 2. Updated arbuscular mycorrhizal (AM) fungal species list for Chilean ecosystems. The majority of the information in this table can be found in Table 5 of Castillo *et al*. (2016); (DOI: http://dx.doi.org/10.4067/S0718-95162016005000036) however, two types of ecosystems were added: AM forest, which corresponds to native coniferous forests dominated by AM fungi, and high altitude Scrubland. Ectomycorrhizal (EM) forest corresponds to *Nothofagus* forests dominated by EM fungi. Numbers in parenthesis indicate where the information comes from: (1), Paulino (2006); (2), Marín *et al.* (2016); (3), this study.

AM species	**AM forest**	EM forest	**Scrubland**	Grassland	Horticultural	Wheat rotation	Wheat
**Acaulospora*							
*Ac. alpina*Oehl, Sýkorová & Sieverd. (2)		X (2)		X	X		
Ac. cavernata Blaszk.				X			
Ac. colossica P.A. Schultz, Bever & J.B. Morton		X		X		X	
Ac. dilatata J.B. Morton		X		X		X	
Ac. koskei Blaszk.		X		X		X	
Ac. laevis Gerd. & Trappe (2, 3)	**X (3)**	X (2, 3)	**X (2)**	X	X	X	X
Ac. longula Spain & N.C. Schenck		X		X	X		X
Ac. mellea Spain & N.C. Schenck (1)	**X (1)**	X		X		X	
Ac. nivalis Oehl, Palenz., I.C. Sánchez, Kuss, Sieverd. & G.A. Silva				X			
Ac. paulinae Blaszk. (2, 3)	**X (3)**	**X (2, 3)**		X	X		
Ac. punctata Oehl, Palenz., I.C. Sánchez, G.A. Silva, C. Castillo & Sieverd. (2, 3)	**X (3)**	X (2)	**X (2)**	X			
Ac. scrobiculata Trappe (1, 2, 3)	**X (1, 3)**	**X (2)**		X			
Ac. sieverdingii Oehl, Sýkorová & Blaszk. (3)		**X (3)**					X
Ac. spinosa C. Walker & Trappe (2)		X (2)			X	X	
Ac. thomii Blaszk.				X	X	X	
Acaulospora spp. (2, 3)	**X (3)**	**X (2)**		X	X	X	X
**Ambispora*							
Am. gerdemannii C. Walker, Vestberg & A. Schüssler							X
***Am. reticulata* Oehl & Sieverd.**		**X (2)**					
Ambispora spp.							X
*Archaeospora							
Ar. leptoticha J.B. Morton & D. Redecker		X				X	
Ar. myriocarpa Oehl, G.A. Silva, B.T. Goto & Sieverd. (2)		**X (2)**					X
Ar. trappei J.B. Morton & D. Redecker (1, 2)	**X (1)**	X (2)		X	X	X	X
Ar. undulata Sieverd., G.A. Silva, B.T. Goto & Oehl.					X		
Archaeospora spp. (1)	**X (1)**	X		X		X	X
*Cetraspora							
Ce. gilmorei Oehl, F.A. de Souza & Sieverd. (3)	**X (3)**			X			X
Cetraspora spp.					X		X
*Claroideoglomus							
Cl. claroideum C. Walker & A. Schüssler (2, 3)	**X (3)**	X (2, 3)	**X (2)**	X	X	X	X
Cl. etunicatum C. Walker & A. Schüssler (2, 3)	**X (3)**	X (3)	**X (2)**	X	X	X	X
Cl. lamellosum C. Walker & A. Schüssler						X	
**Diversispora*							
Di. spurca C. Walker & A. Schüssler		X		X	X	X	
Di. epigaea A. Schüssler, Krüger, C. Walker					X		
**Dominikia*							

continued...

Do. aurea Błaszk., Chwat, G.A. Silva & Oehl							X
**Entrophospora*							
En. infrequens R.N. Ames & R.W. Schneid. emend. Oehl & Sieverd. (1)	**X (1)**	X					
**Funneliformis*							
Fu. coronatus Giovann.					X	X	
Fu. geosporus C. Walker		X		X	X		
Fu. monosporus Gerd. & Trappe					X		
Fu. mosseae C. Walker & A. Schüssler					X	X	X
**Gigaspora*							
Gigaspora spp.					X	X	
**Glomus*							
Gl. ambisporum G.S. Sm. & N.C. Schenck						X	
***Gl. badium* Oehl, D. Redecker & Sieverd. (2, 3)**	**X (3)**	**X (2, 3)**	**X (2)**				
Gl. brohultii Sieverd.				X			
Gl. clavisporum Almeida & N.C. Schenck					X		
Gl. diaphanum J.B. Morton & C. Walker (3)	**X (3)**			X	X	X	X
Gl. macrocarpum Tul. & C. Tul.		X		X	X	X	X
Gl. pallidum I.R. Hall					X		
Gl. vesiculiferum C. Walker & A. Schüssler					X		
Glomus spp. (1, 3)	**X (1, 3)**	**X (3)**		X		X	X
**Intraspora*							
In. schenkii Sieverd. & S. Toro		X		X		X	
**Pacispora*							
Pa. dominikii Oehl & Sieverd.				X	X	X	X
Pacispora spp.		X				X	
**Paraglomus*							
P. occultum J.B. Morton & D. Redeker				X	X	X	X
P. laccatum Blaszk.				X			
**Racocetra*							
Ra. weresubiae Koske & C. Walker				X			
**Rhizoglomus*							
R. aggregatum N.C. Schenck & G.S. Sm.					X		
R. fasciculatum C. Walker & Koske		X			X	X	X
R. intraradices N.C. Schenck & G.S. Sm. (2)			**X (2)**		X	X	X
R. invermaium I.R. Hall (2)		X (2)		X		X	X
R. microaggregatum Koske, Gemma & Olexia							X
**Sacculospora*							
Sa. baltica Blaszk.		X					
**Sclerocystis*							
Sc. rubiformis Gerd. & Trappe		X				X	
Sc. sinuosa Gerd. & Bakshi							X
Sclerocystis spp.						X	
**Scutellospora*							
Sc. auriglobosa C. Walker & R.E. Sanders				X			
Sc. calospora C. Walker & R.E. Sanders (2, 3)	**X (3)**	**X (2)**		X		X	X
Sc. dipurpurescens J.B. Morton & Koske				X	X	X	
Scutellospora spp.						X	
**Septoglomus*							
Se. constrictum Sieverd., G.A. Silva & Oehl							X
**Simiglomus*							
Si. hoi Sieverd., G.A. Silva & Oehl (2, 3)	**X (3)**		**X (2)**			X	X

In bold: new additions (with respect to Castillo *et al.*, 2016) of AM fungal species presence by ecosystem type and species. Two new arbuscular mycorrhizal fungi species were registered with this update. Fungal genera are underlined and preceded by an asterisk.

4. Discussion

While Chilean coastal forests have greater plant diversity and are typically poorer in soil nutrients than Andean forests (Armesto *et al.*, 2010), here we did not find any effect of mountain system on fungal richness or abundance. Rather AM community composition was affected by mountain system- a result similar to the idea proposed by Armesto *et al.* (2010) for microbial communities. As coastal forests served as biodiversity refugia for species originating in Andean forests, the belowground community likely recovered its original state after the reconnection of formerly glacially separated areas. Despite this, ecological interactions of microbial communities would have changed given the contrasting environments (Armesto *et al*., 2010). Interestingly, and contrary to our previous studies on temperate rainforests (Marín *et al*., 2016), the ecological similarities between AM fungal species did not correspond to its phylogenetic relatedness. Rather, this result is like what we have found on agroecosystems (Aguilera *et al*., 2017). Thus, while Marín *et al*. (2016) study was a geographically restricted study, both Aguilera *et al*. (2017) and this study used a broad ecosystem and geographic range, respectively. Thus, it may be possible that phylogenetic relatedness of AM species reflects also its ecological interactions only when comparing geographically close and/or similar ecosystems, while ecological functions of AM species on comparisons from distant and different ecosystems can be performed by non-related species (Davison *et al*., 2015).

Edaphic factors also seem to be scale-dependent: in Marín *et al.* (2016), when comparing closely located plots, P_a, pH and Al saturation were the main edaphic factors structuring AM fungal communities, while in this study, just P_a remained but Ca, Mg and Na replaced pH and Al saturation. Could be deduced that a small scale, besides P_a, limiting edaphic factors structure AM fungal communities, as pH and Al saturation in Marín *et al.* (2016), cations (Ca, Mg and Na) structure these communities at a regional scale (this study), and basic nutrients (N and organic C) at a global scale (Davison *et al*., 2015). A global study on AM fungal diversity patterns (Davison *et al*., 2015) shows that despite AM fungi are locally adapted, they are broadly distributed and present low endemism. Furthermore, in our study, a single non-described species (*Glomus* sp. CL1) was highly abundant and grouped four highly separated coastal plots (C5.AM, C8.EM, C9.EM and C10.AM), thus, reinforcing this idea, given the contrasting edaphic parameters and the distance between these plots. In our previous studies (Marín *et al*., 2016; Aguilera *et al.*, 2017) we also found that described and non-described AM fungal species grouped together highly contrasting ecosystems/treatments. Also, further studies are necessary to clarify the taxonomic status of these non-described AM fungi species.

Regarding the community structure of the studied plots, interestingly, the Andean plots grouped both between them and within EM and AM forests, and this grouping was given by six AM fungal species of the genus *Acaulospora* and *Claroideoglomus*, none of which was specifically related to a single plot. Contrastingly, both Coastal EM and Coastal AM plots had highly different community structure, except for plots C8.EM and C9.EM which had a single common species in low abundance. The community structure of Coastal plots was often given by one or two AM fungal species related to a single plot; these species belonged to all the described genus on this study. This leads to conclude that the AM fungal community structure of Andean plots -which is highly similar between and within EM and AM forests- is given by related and common species, while the Coastal AM fungal community structure -highly different between and within EM and AM forests- is given by non-related and specific species.

This contrast between Andean and Coastal AM fungal community structures is given by edaphic parameters: while Andean communities were more related to Na, Coastal EM Andean plots were more related to Mg and P_a, and there was no clear pattern regarding Coastal AM plots. *Glomus* sp CL1 which grouped plots C5.AM, C8.EM, C9.EM and C10.AM, was highly related to Mg content. Meanwhile, some *Acaulospora* species were related to Na content.

Forest mycorrhizal dominance -the dominant mycorrhizal type of the forest- was related to AM fungal community composition/structure (conjunct measure of species richness and abundance) but not to AM fungal community richness or abundance. Furthermore, community structure, more than simply richness or abundance, reflects the ecological cooperative and competitive interactions within each community (Davison *et al.*, 2015). The effects of mycorrhizal dominance on AM fungal communities needs to be further studied to clarify how 'mycorrhizal traits' (Moora, 2014) influence the total soil fungal community as well as specific fungal guilds.

The AM fungal communities were structured by several edaphic factors; a pattern that has been found in global scale studies (Davison *et al.*, 2015). Although higher altitudes have been shown to have lower total fungal diversity (Shi *et al*., 2014; Yang *et al.*, 2016), and this holds even at short elevational gradients (Marín *et al*., 2016), in our study AM fungal richness was not affected by altitude. Interestingly, though, AM fungal abundance (number of spores) increased with altitude irrespective of the mycorrhizal dominance of the forests or mountain system. The altitudinal range of the plots covered more than 1,000 m, from 277 m.a.s.l. to 1,365 m.a.s.l., i.e., which is the natural altitudinal range of these forests in Southern Chile. It is worth to mention that none of these results -effects of mountain system, mycorrhizal dominance, edaphic parameters and altitude and AM fungal diversity- seems to be affected by root colonization, as there was no relationship between the percentage of roots colonized by AM fungi hyphae and AM fungal richness and abundance. This result is in accordance with previous studies (Mafaziya and Madawala, 2015; Aguilera *et al.*, 2017). Finally, and based on the work of Castillo *et al.* (2016) we updated the species list of AM fungi species in Chile. The list now includes a total of 59 species belonging to 21 genera. Interestingly, we also found that most of these species are present both in agroecosystems and in southern Chilean temperate rainforests.

5. Conclusions

Mountain system and mycorrhizal dominance did affect ecological interactions (composition) of AM fungi, although richness and abundance were not affected. Coastal EM and AM forests presented a highly different AM fungal community structure, where one or two single non-related species were related to a single plot and soil Mg and P_a, while Andean EM and AM forests presented a similar community structure given by related species and soil Na. This underpins the idea that Coastal ecosystems served as a belowground biodiversity refugia (Villagrán and Armesto, 2005), as a more complex and specific AM fungal community structure is found in the Coast Mountains when compared to the Andes. Thus, this is precisely the effect of ´mountain system´ on the AM fungal community structure. While less pronounced, the effect of the forest mycorrhizal dominance (EM and AM) was especially stronger in systems with almost complete EM (or AM) plant cover. There was no relationship between altitude and AM fungal richness, but with higher altitude there was higher AM fungi abundance. Finally, we updated to 59 AM fungi species registered in Chile. Future studies of AM fungi in Chile should employ metagenomic and bioinformatic methods to obtain a

comprehensive view of AM fungal diversity in Chilean ecosystems (Bueno *et al*., 2017b). Furthermore, it is crucial to determine the relationships between soil chemical variables, mycorrhizal colonization, and plant diversity, to establish a broader view of ecosystem functioning.

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Supplemental table 1. a. Soil chemical characteristics of the study plots located in temperate rainforests in Southern Chile. **b.** Mixed linear models of differences in the corresponding soil chemical variables among plots showing the effects of mountain system (MS; Andes and Coast), mycorrhizal dominance (MD; ectomycorrhizal, EM; arbuscular mycorrhizal, AM), and their interaction (MS:MD). Site was a random factor in all models.

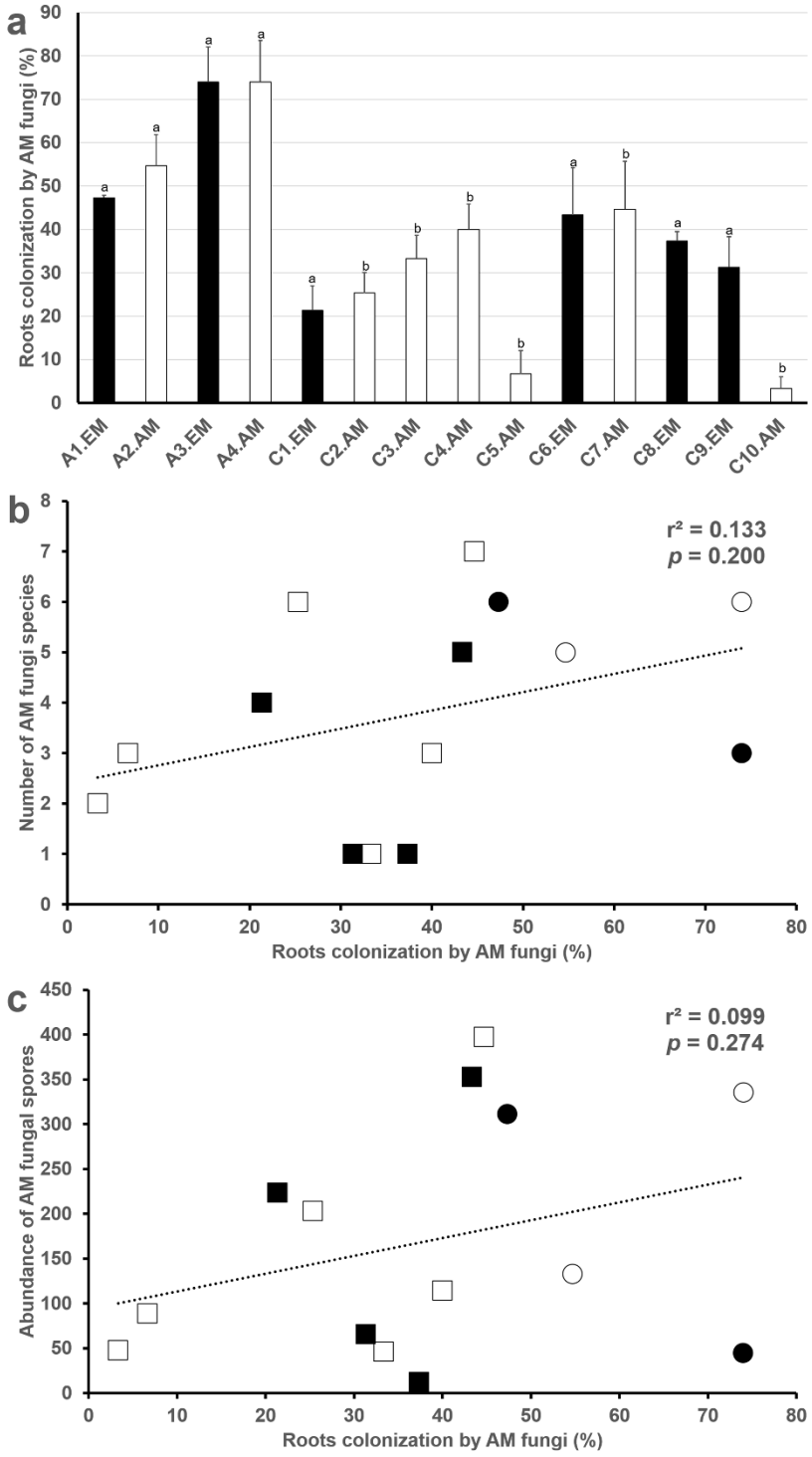
a. Plot	pH	EC (µS/cm)	Eh (mV)	N (%)	C (%)	P_a (mg/Kg)	Al (mg/Kg)	Ca (mg/Kg)	K (mg/Kg)	Mg (mg/Kg)	Na (mg/Kg)
A1.EM	4.33±0.006c	23.9±0.200a	83±0.707b	1.125±0.012c	19.643±0.052d	19.567±0.399d	96.552±2.604d	990.398±22.510d	101.537±0.694b	136.586±4.330d	60.107±0.444d
A2.AM	4.37±0.175c	35.5±0.265b	86±0.000c	1.331±0.000d	20.847±0.156d	19.258±0.088c	37.666±2.587a	1531.023±120.158d	198.635±13.592d	268.287±4.370d	55.167±2.664c
A3.EM	4.31±0.006c	30.8±0.200a	75±3.536b	0.774±0.002c	17.389±0.163d	17.567±1.039c	89.929±4.711d	749.62±23.378c	129.926±5.495c	119.399±4.310b	60.162±0.166d
A4.AM	4.87±0.082d	39.8±0.751c	34±0.707a	0.486±0.001b	11.653±0.091d	8.633±0.302a	86.58±6.974c	857.383±16.029c	128.621±1.193c	62.974±4.870a	43.235±0.444a
C1.EM	3.04±0.060a	62.2±0.058d	153±0.707d	0.281±0.001b	9.210±0.076c	16.283±1.208b	31.385±0.250a	246.55±7.074a	86.599±1.315a	83.472±3.040a	69.902±15.179d
C2.AM	2.99±0.040a	99±3.853d	154±0.707d	0.266±0.021a	9.002±0.816b	8.708±0.362a	73.954±0.083c	441.669±35.308b	112.258±5.934b	126.318±2.990c	50.672±0.389c
C3.AM	3±0.010a	51.2±0.252c	169±0.707d	0.281±0.005b	10.363±0.543c	9.567±0.201b	48.868±3.302b	305.824±11.937a	108.503±1.472b	110.168±3.000b	49.377±0.722c
C4.AM	3.09±0.006a	31.3±0.557b	152±0.000d	0.231±0.015a	7.815±0.049b	6.267±0.208a	147.649±6.744d	356.976±13.686a	92.741±1.665a	91.242±3.090a	47.258±1.360b
C5.AM	3.15±0.025b	49±1.000c	149±3.536c	0.188±0.009a	6.131±0.159a	8.217±0.791a	179.21±0.444d	249.251±20.537a	93.074±0.972a	97.384±3.150b	56.333±1.655d
C6.EM	4.08±0.104b	30.4±2.479a	93±1.414c	0.538±0.013c	11.155±0.362c	23.113±0.788d	47.86±1.891b	802.604±25.860c	179.857±2.636d	119.732±4.080c	37.999±4.211a
C7.AM	4.13±0.070b	19.3±1.473a	78±2.828b	0.267±0.001a	7.219±0.163b	16.975±0.613c	38.878±3.302b	369.945±15.862b	91.464±1.221a	68.395±4.130a	47.564±6.270b
C8.EM	4.53±0.023d	32.8±1.812b	45±4.497a	5.579±0.040d	0.243±0.002a	9.325±0.032b	49.839±0.949c	586.931±22.093b	120.185±0.527c	132.395±0.083c	41.681±1.332a
C9.EM	5.81±0.084d	92.2±2.844d	6±1.700a	5.312±0.053d	0.328±0.009a	32.363±0.089d	20.313±2.165a	3047.755±12.071d	374.847±0.944d	547.369±3.802d	45.159±3.847b
C10.AM	5.63±0.095d	141.3±0.946d	12±2.449a	7.287±0.094d	0.539±0.008a	31.063±0.132d	7.021±0.416a	3623.355±195.293d	837.107±0.056d	758.213±2.470d	44.437±4.649a
b.											
MS	0.021^{ns}	0.785^{ns}	0.009^{ns}	0.754^{ns}	**8.872***	0.031^{ns}	0.284^{ns}	0.026^{ns}	0.286^{ns}	0.225^{ns}	1.370^{ns}
MD	1.220^{ns}	0.526^{ns}	0.932^{ns}	0.641^{ns}	1.411^{ns}	**11.810***	0.557^{ns}	1.474^{ns}	1.426^{ns}	1.409^{ns}	1.579^{ns}
MS:MD	4.239^{ns}	<0.001^{ns}	1.606^{ns}	0.477^{ns}	0.214^{ns}	0.083^{ns}	1.630^{ns}	0.715^{ns}	0.146^{ns}	0.037^{ns}	0.850^{ns}
	AIC=22.589	AIC=114.509	AIC=109.371	AIC=48.346	AIC=69.132	AIC=76.887	AIC=122.797	AIC=169.776	AIC=148.234	AIC=141.995	AIC=87.608

a: Means ± SE are shown (n=3). Letters represent quartiles. b: F^p, *p*-value: *<0.05, ns=non-significat. Significant factors in bold.

Supplemental table 2. a. Diversity indices and contribution to the diversity (alpha, beta, and gamma diversity, Simpson's index) of the arbuscular mycorrhizal fungal communities of the 14 study plots located in temperate rainforests in Southern Chile. **b.** Mixed linear models showing the effects of mountain systems (MS; Andes and Coast), mycorrhizal dominance (MD; ectomycorrhizal, EM; arbuscular mycorrhizal, AM), and their interaction (MS:MD) on the respective diversity indices. Site was a random factor in all models.

a. Plot	Richness (S)	Abundance (N)	Shannon (H´)	Simpson (1-D1)	Inverse Simpson (D2)	Evenness (E´)	Berger (BP)	Contribution to Simpson (1-D1)		
All plots	14	2373.333	2.283	0.879	8.243	0.7	0.194	Alpha	Beta	Gamma
A1.EM	6d	311.000d	1.774d	0.827d	5.791d	0.982d	0.222a	0.827d	0.055a	0.882d
A2.AM	5c	132.667c	1.392c	0.727c	3.664c	0.804a	0.394b	0.727c	0.113a	0.840b
A3.EM	3b	44.333a	1.035b	0.622b	2.646b	0.938c	0.504c	0.622b	0.243c	0.865c
A4.AM	6d	335.333d	1.693d	0.800d	5.009d	0.906c	0.271a	0.800d	0.081a	0.882c
C1.EM	4c	223.333c	1.235c	0.693c	3.255c	0.860b	0.358b	0.693c	0.125b	0.818b
C2.AM	6d	203.000c	1.641d	0.785d	4.648d	0.860b	0.317a	0.785d	0.137b	0.922d
C3.AM	1a	46.000a	0.000a	0.000a	1.000a	1.000d	1.000d	0.000a	0.828d	0.828b
C4.AM	3b	114.333b	0.836b	0.500b	1.999b	0.769a	0.647c	0.500b	0.384c	0.884d
C5.AM	3b	88.333b	0.860b	0.529b	2.123b	0.787a	0.592c	0.529b	0.269c	0.799a
C6.EM	5c	352.667d	1.472c	0.746c	3.943c	0.871c	0.350b	0.746c	0.108a	0.854c
C7.AM	7d	397.333d	1.732d	0.787d	4.686d	0.807a	0.342a	0.787d	0.140b	0.927d
C8.EM	1a	12.333a	0.000a	0.000a	1.000a	1.000d	1.000d	0.000a	0.678d	0.678a
C9.EM	1a	65.333b	0.000a	0.000a	1.000a	1.000d	1.000d	0.000a	0.678d	0.678a
C10.AM	2a	47.333a	0.486a	0.308a	1.445a	0.813b	0.810d	0.308a	0.428d	0.736a
b.										
MS	1.357^{ns}	0.203^{ns}	2.114^{ns}	2.302^{ns}	2.945^{ns}	0.456^{ns}	2.424^{ns}	2.302^{ns}	2.786^{ns}	1.420^{ns}
MD	0.730^{ns}	0.004^{ns}	0.267^{ns}	0.202^{ns}	0.087^{ns}	5.316^{ns}	0.020^{ns}	0.202^{ns}	0.075^{ns}	2.133^{ns}
MS:MD	0.006^{ns}	0.311^{ns}	0.004^{ns}	0.019^{ns}	0.011^{ns}	0.017^{ns}	0.002^{ns}	0.019^{ns}	0.004^{ns}	2.087^{ns}
	AIC=59.529	AIC=143.773	AIC=36.413	AIC=21.634	AIC=54.374	AIC=-6.242	AIC=19.594	AIC=21.634	AIC=17.822	AIC=-10.938

a: Letters represent quartiles. b: F^{p}, *p*-value: ns=non-significat.



Supplemental figure 1. a. Percentage of roots colonized by AM fungi. Roots were randomly selected from composite samples. Andean forest: left side of the figure; Coastal forests: right side of the figure. Black bars: EM forests; white bars: AM forests. Letters indicate significative (<0.05) differences between the combination of the two treatments by a TukeyHSD test. Bars indicate SE. **b.** Relationship between root colonization by AM fungi and number of AM fungi species. **c.** Relationship between root colonization by AM fungi and abundance of AM fungal spores.