Chapter 16
Mycorrhizal Studies in Temperate Rainforests of Southern Chile

Roberto Godoy and César Marín

16.1 Introduction

Old-growth temperate rainforests located in the mountain areas of the Southern Cone of America are often presented as global model ecosystems, as they have not been subject to chronic air pollution and have remained foristically stable throughout the Holocene (Armesto et al. 2009, 2010). In Chile, Andes and Coastal mountain ranges differ in terms of precipitation (Godoy and Oyarzún 1998; Godoy et al. 1999, 2001, 2003, 2009; Oyarzún et al. 1998, 2002, 2004, 2007, 2009, 2011; Staelens et al. 2003, 2005, 2009), and in the input of long-distance transported aerosols (Boy et al. 2014). These forests can be considered as unique, isolated biogeographic islands, as they have flora with representatives derived from Gondwanian elements, and extreme environmental, edaphic, and orographic conditions that are enhanced by seismic and volcanic activity. The Chilean Coastal mountain range served as a refugium for plants during the Last Glacial Maximum (Armesto et al. 2009), causing this area to have a high plant family endemism and a high number of isolated monotypic genera. The Coastal mountain range bedrock is highly weathered, and atmospheric nutrients coming from ocean processes have a significant influence on the biogeochemical dynamics of these forests (Boy et al. 2014). In contrast, nutrient inputs to the steep slopes of the Andes mountain range are mostly generated by young volcanic ash deposits and weathered basaltic volcanic scoria (Godoy et al. 2009).

The forests of the Chilean Coastal mountain range have developed in unique evolutionary and biogeochemical scenarios, where soil nutritional limitations and

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M. C. Pagano, M. A. Lugo (eds.), *Mycorrhizal Fungi in South America*,
Fungal Biology, https://doi.org/10.1007/978-3-030-15228-4_16

the dilution of marine salt aerosols have been prevalent (Armesto et al. 2009). The close connection between marine and terrestrial nutrient cycling in these forests has greatly shaped their ecosystem functioning (Hedin and Hetherington 1996; Weathers et al. 2000; Kennedy et al. 2002). Nutrient limitations make these coastal ecosystems extraordinarily sensitive to changes on biogeochemical cycles as a result of anthropogenic disturbances. In both mountain ranges there is a bedrock age gradient and therefore, a gradient of total weathering (Hedin and Hetherington 1996). The atmospheric and edaphic inputs of the Andes and Coastal mountain ranges are contrasting, thus allowing comparisons to be made at the micro-catchment scale (Oyarzún et al. 1998, 2004).

A regimen of natural disturbances maintains the population dynamics of southern Chile temperate rainforests (Godoy et al. 2009; Lara et al. 2014), which are altered by anthropogenic disturbances, resulting in genetically fragmented forest communities (Bekessy et al. 2002, 2004). Since 2005 Chile has experienced an extreme drought with a drastic drop in precipitation, causing an increased intensity and frequency of fires (Bowman et al. 2018). During the summer of 2017, fires affected 5000 km^2 of the Chilean central-southern region, affecting densely inhabited and important productive regions, as well as causing restricted access to several national parks in remote areas (Bowman et al. 2018).

16.2 Overview of Mycorrhizal Studies on Chilean Temperate Rainforests

The mycorrhizal symbiosis is one of the most common forms of mutualistic relationships, with crucial ecological and evolutionary roles on the terrestrial colonization of vascular plants (Brundrett and Tedersoo 2018). Moreover, about 92% of terrestrial plant species associate with mycorrhizal fungi (Brundrett and Tedersoo 2018). Mycorrhizal fungi improve plant survival and nutrient acquisition -mainly phosphorus and nitrogen- by creating large mycelial networks that access to both mobile and immobile forms of soil nutrients (Simard et al. 2012). Furthermore, mycorrhizal fungi influence several ecosystem processes such as plant productivity and biodiversity, soil aggregation, and carbon cycling (van der Heijden et al. 2008).

The first mycorrhizal studies on Chilean temperate rainforests classified the *Nothofagus* spp. forests as ectotrophic, and the native conifer forests as anectotrophic (Singer and Morello 1960; Singer et al. 1965; Singer 1969, 1970). Several morphoanatomical classification studies followed (Godoy and Mayr 1989; Carrillo et al. 1992; Godoy et al. 1994; Valenzuela et al. 1999, 2001), registering as many as 651 ectomycorrhizal (EM) fungi taxa exclusive to *Nothofagus* spp. (Garrido 1988), and concluding that the most abundant EM fungal orders on *Nothofagus* forests are: Boletales, Cortinariales, Gautieriales, and Russulales (Palfner and Godoy 1996a, b; Flores et al. 1997; Godoy and Palfner 1997; Palfner 2001; Nouhra et al. 2013).

Southern Chilean temperate rainforests are unique in that arbuscular mycorrhizal (AM) fungi associate with native conifers, as most of the flora, with the important exception of *Nothofagaceae* species, which are exclusively associated with EM fungi (Godoy et al. 1994; Fontenla et al. 1998; Palfner 2001, 2002; Castillo et al. 2006; Marín et al. 2016, 2017a, b, 2018a). Marín et al. (2016) registered 18 AM fungal species in three *N. pumilio* plots, which brought the number of AM fungal species described in Chile from 57 to 59 (Marín et al. 2017a). The vascular and fungal flora of southern Chile' temperate rainforests share the same climatic, geological, and evolutionary history, and the fungal flora is also characterized by a high endemism and a high number of monotypic families and genera (Palfner 2001; Marín et al. 2018b). According to descriptions and collections of EM fungi, the *Nothofagus* spp. forests of this region have a high diversity of Agaricales when compared with European *Fagus* forests (Garrido 1988; Valenzuela et al. 1999; Palfner 2001, 2002; Marín et al. 2017b). Molecular studies of soil fungi, particularly mycorrhizal fungi, are very recent in Argentinian and Chilean temperate rainforests (Nouhra et al. 2012, 2013; Tedersoo et al. 2014; Davison et al. 2015; Trierveiler-Pereira et al. 2015; Marín et al. 2017b; Truong et al. 2017, 2019).

These studies have found fungi new to science as well as pointed out vast understudied regions. Recent metagenomic studies in Chile examined soil fungi across the Andean and Coastal ranges, comparing *Nothofagus* spp. and native conifer forests, finding an inverse relationship between EM and saprotrophic fungal abundance (Marín 2018a). Another metagenomic study found more EM and saprotrophic fungi on less disturbed forests while more plant parasitic fungi were found in more disturbed forests (Marín 2017b). The survival of these forests is highly dependent on its mycorrhizal symbionts (Godoy et al. 1994; Marín et al. 2018a).

On the temperate rainforests of southern Chile, AM fungi make a significant contribution to the carbon and nitrogen cycling of soil organic matter (Etcheverría et al. 2009). This reinforces the imperative need to study the biodiversity, community composition, ecosystem roles, and eco-evolutionary parameters of the mycorrhizal symbiosis on temperate rainforests of the Southern Cone of America.

16.3 Mycorrhizal Types on Southern Chile Temperate Rainforests

We analyzed the mycorrhizal type of plant species across 17 temperate rainforest plots (30 m × 30 m) on southern Chile, conducting the plant identification at the Herbarium of Universidad de Concepción, Chile, and after the species list provided by Rodriguez et al. (2018). The mycorrhizal type was determined by analysis of the mycorrhizal colonization of roots (i.e. fixation, root staining, and microscope quantification) (Koske and Gemma 1989). Five composite soil samples from the Ah horizon were collected and thoroughly mixed (litter and organic material removed; 0–20 cm depth, aprox. 1 kg each sample). Following Sadzawka et al. (2006), analyses included soil properties known to affect plant and fungal mycorrhizal communities: pH (KCl), conductivity, total C, total N, C/N ratio, available P (Olsen P at pH 8.5), exchangeable K, Ca, and Mg (extraction with CH_3COONH_4 1 mol/L at pH 7.0), and exchangeable Al (extracted with KCl 1/mol L).

From a total of 245 vascular plant species distributed on 17 temperate rainforest plots on southern Chile, we found that 208 species (85%) have mycorrhizal associations (Table 16.1; Fig. 16.1). A total of 187 plant species associated with AM fungi, 10 plant species with ericoid (ER) mycorrhizal fungi, seven plant species with EM fungi, and four plant species associated with orchid (OR) mycorrhizal fungi. A total of 37 plant species did not form any mycorrhizal association and are considered in the literature as typical non-mycorrhizal (NM) plants, such as epiphytic ferns and broadleaf herbs, parasitic plants (Loranthaceae and Misodendraceae), and species from the families Proteaceae, Caryophyllaceae, Cyperaceae, Juncaceae, and Brassicaceae (Fig. 16.1).

From the 31 species of Pteridophytic flora found, 23 (77%) present AM symbiosis, a similar result to other latitudes (Godoy et al. 1994). The eight NM fern species were mainly epiphytic plants belonging to the family Hymenophyllaceae, showing a similar result to a study on north Patagonian forests by Fernández et al. (2005). All the native conifer species of the region associate to AM fungi, and belong to the families Araucariaceae (one species), Cupressaceae (three species), and Podocarpaceae (three species), representing an exceptional association in comparison to the Northern hemisphere conifers (i.e. Pinaceae, Taxodiaceae), that predominately associate with EM fungi (Godoy and Mayr 1989). An aspect of particular interest is the formation of root nodules in Podocarpaceae and Araucariaceae with full AM colonization (Godoy and Mayr 1989). The populations of these endemic conifers are narrow-distributed and endangered. Particularly, conifers with long life-spans as *Fitzroya cupressoides* (3600 years; Lara and Villalba 1993) and *Araucaria araucana* (1000 years; Aguilera-Betti et al. 2017), may be highly susceptible to climate change.

The autochthonous EM plants of the genus *Nothofagus* on the 17 temperate rainforest plots investigated, included seven evergreen and deciduous species (Table 16.1). Some EM fungi taxa have a wide distribution while other more specialized types occur only on isolated localities (Palfner 2001; Marín et al. 2018b). The EM fungi taxa included epigeous, secotioid, and hypogeous forms.

ER mycorrhizal associations were found on a total of 10 understory plant species of Chilean temperate rainforests: nine species on the family Ericaceae and one species (*Empetrum rubrum*) on the family Empetraceae. ER plants are common on infertile and acidic soils, characterized by a high content of recalcitrant polyphenolic compounds, leading to a very slow decomposition of soil organic matter. Instrumental to the survival of ER plants in these ecosystems, are their mycorrhizal associations, that release soil nutrients through the degradation of a wide range of complex and recalcitrant organic substrates (Smith and Read 2008). Clemmensen et al. (2015) proposed that the ER fungal biomass may contribute to the large storage of soil organic matter in older and high-altitude temperate forests, especially in the treeline under extreme environmental conditions.

Table 16.1 Mycorrhizal type for the plant species of 17 plots of temperate rainforests in southern Chile

	Forest plot																
Plant species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Acaena ovalifolia	–	–	–	–	–	–	AM	AM	–	–	–	–	–	–	–	–	AM
Acaena pinnatifida	AM	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Acrisione cymosa	–	–	–	–	–	–	–	AM	–	–	–	–	–	AM	–	–	–
Adenocaulon chilense	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	AM	AM
Adesmia longipes	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Adesmia retusa	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	AM	–
Adiantum chilense	–	–	–	–	–	AM	–	–	–	–	–	–	–	–	–	–	–
Adiantum sulphureum	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Aextoxicon punctatum	–	–	–	–	AM	–	–	–	–	–	–	–	–	AM	–	–	–
Agrostis perennans	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Alstroemeria aurea	–	AM	–	–	AM	AM	–	–	–	–	–	–	–	–	–	–	–
Amomyrtus luma	–	–	–	–	–	–	AM	AM	AM	–	–	–	–	AM	–	–	–
Amomyrtus meli	–	–	–	–	–	–	AM	–	AM	–	–	–	–	AM	–	–	–
Antidaphne punctulata	–	–	–	NM	–	–	–	–	NM	–	–	–	–	NM	–	–	–
Arachnitis uniflora	–	–	–	–	–	AM	–	–	–	–	–	–	–	AM	–	–	–
Araucaria araucana	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Aristotelia chilensis	–	–	–	–	AM	AM	AM	–	–	–	–	–	–	AM	AM	–	–
Asplenium dareoides	NM	–	–	NM	–	–	NM	NM	NM	–	–	–	–	NM	NM	–	–
Aster vahlii	–	–	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–
Asteranthera ovata	–	–	–	–	–	–	–	–	–	AM	–	–	–	–	–	AM	–
Austrocedrus chilensis	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Azara integrifolia	–	–	–	–	AM	–	–	–	–	–	–	–	–	–	–	–	–
Azara lanceolata	–	–	–	–	–	–	AM	AM	AM	AM	–	–	–	AM	AM	AM	–
Azara microphylla	–	AM	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–

(continued)

Table 16.1 (continued)

	Forest plot																
Plant species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Baccharis magellanica	–	–	–	–	–	–	AM	–	–	–	–	AM	–	–	–	–	–
Baccharis nivalis	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Baccharis racemosa	–	–	–	–	–	AM	AM	–	–	–	–	–	–	–	–	–	–
Baccharis sagittalis	–	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Baccharis sphaerocephala	–	–	–	–	–	–	AM	AM	AM	–	–	–	–	–	–	–	–
Berberis congestiflora	–	–	–	–	–	AM	–	–	–	–	–	–	–	–	–	–	–
Berberis darwinii	–	–	AM	–	AM	–	AM	AM	AM	–	–	–	–	–	–	–	–
Berberis microphylla	–	–	AM	–	AM	–	–	–	–	–	–	–	–	–	–	–	–
Berberis montana	AM	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	AM
Berberis serratodentata	–	–	–	–	–	–	–	–	–	–	–	AM	–	–	–	–	AM
Berberis trigona	–	AM	–	–	–	–	–	–	–	–	–	–	AM	–	–	–	AM
Blechnum asperum	–	–	–	AM	–	AM	–	–	AM	–	–	–	–	–	AM	–	–
Blechnum blechnoides	–	–	–	–	–	–	–	–	–	–	–	–	–	AM	–	–	–
Blechnum chilense	–	–	AM	AM	–	–	AM	–	AM	AM	–	–	–	–	–	–	AM
Blechnum hastatum	–	–	–	AM	AM	AM	AM	AM	–	–	–	–	–	AM	–	–	–
Blechnum magellanicum	–	–	–	–	–	–	AM	–	AM	AM	–	AM	AM	–	–	–	–
Blechnum microphyllum	–	–	–	–	AM	–	–	–	–	–	–	–	–	–	–	–	–
Blechnum mochaenum	–	–	–	–	AM	–	–	–	–	–	–	–	–	AM	–	–	–
Blechnum penna-marina	–	AM	AM	–	–	–	–	AM	–	–	–	–	–	–	–	AM	AM
Blepharocalyx cruckshanksii	–	–	AM	AM	–	–	–	–	–	–	–	–	–	–	–	–	–
Boquila trifoliolata	–	–	–	AM	AM	AM	AM	AM	AM	AM	–	–	–	AM	–	–	–
Buddleja globosa	–	–	–	–	AM	–	–	–	–	–	–	–	–	–	–	–	–
Calandrinia ciliata	–	–	–	–	AM	–	–	–	–	–	–	–	–	–	–	–	–
Calceolaria biflora	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Caldcluvia paniculata	–	–	–	–	–	–	–	–	AM	–	–	–	–	AM	AM	–	–

	Forest plot																
Plant species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Campsidium valdivianum	–	–	–	AM	–	–	–	–	AM	AM	AM	–	AM	–	AM	–	–
Carex fuscula	–	–	NM	NM	–	–	–	–	–	–	NM	–	–	–	–	–	–
Carex sp.	–	–	NM	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Centella asiatica	–	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Chascolytrum subaristatum	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Chloraea gaudichaudii	OR	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Chloraea sp.	–	–	–	–	–	–	–	OR	–	–	OR	OR	OR	–	–	–	–
Chrysosplenium valdivicum	–	–	–	–	–	–	–	–	–	–	–	–	–	AM	–	–	–
Chusquea culeou	–	AM	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Chusquea montana	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	AM
Chusquea montana f. *nigricans*	–	–	–	–	–	–	–	–	–	–	–	–	AM	–	–	–	–
Chusquea quila	–	–	–	AM	AM	AM	AM	AM	AM	AM	AM	AM	–	AM	AM	–	–
Chusquea uliginosa	–	–	AM	–	–	–	–	–	–	–	AM	–	–	–	–	–	–
Cissus striata	–	–	–	AM	AM	AM	–	–	–	–	–	–	–	AM	–	–	–
Codonorchis lessonii	–	–	–	–	OR	–	OR	–	OR	–	–	–	–	–	–	–	OR
Coriaria ruscifolia	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Corynabutilon ochseni	–	–	–	–	AM	–	–	–	–	–	–	–	–	–	–	–	–
Corynabutilon vitifolium	–	–	–	–	AM	–	–	AM	–	–	–	–	–	–	–	–	–
Cynanchum pachyphyllum	–	–	–	–	AM	–	AM	AM	AM	–	–	–	–	–	–	–	–
Cyperus sp.	NM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Dasyphyllum diacanthoides	–	–	–	–	–	–	–	–	AM	AM	–	–	–	–	AM	–	–
Desfontainia fulgens	–	–	–	–	–	–	–	AM	AM	AM	–	AM	–	–	–	AM	–
Desmaria mutabilis	–	–	–	–	–	–	–	–	NM	–	–	–	–	–	–	–	–
Dioscorea brachybotrya	–	–	–	–	AM	–	–	–	–	–	–	–	–	–	–	–	–

(continued)

Table 16.1 (continued)

	Forest plot																
Plant species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Dioscorea bryoniifolia	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Discaria chacaye	–	–	AM	–	AM	–	–	–	–	–	–	–	–	–	–	–	–
Drimys andina	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	AM
Drimys winteri	–	–	AM	AM	AM	–	AM	–	AM	AM	–	AM	AM	–	–	–	–
Drosera uniflora	–	–	–	–	–	–	–	–	–	–	AM	–	–	–	–	–	–
Eccremocarpus scaber	–	–	–	–	–	AM	–	–	–	–	–	–	–	–	–	–	–
Eleocharis macrostachya	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Elytropus chilensis	–	–	–	–	AM	–	AM	AM	AM	–	–	–	–	AM	–	–	–
Embothrium coccineum	–	NM	NM	–	–	–	NM	NM	–	–	–	NM	NM	–	–	NM	NM
Empetrum rubrum	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	ER
Epilobium ciliatum	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	AM
Ercilla volubilis	–	–	AM	–	AM	–	–	–	–	–	–	–	–	–	–	–	–
Eryngium paniculatum	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Escallonia alpina	–	–	–	–	–	–	–	–	–	–	–	–	AM	–	–	–	AM
Escallonia leucantha	–	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Escallonia revoluta	–	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Escallonia rosea	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	AM
Eucryphia cordifolia	–	–	–	–	–	–	AM	AM	–	–	–	–	–	AM	AM	–	–
Fascicularia bicolor	–	–	–	NM	NM	–	–	NM	NM	NM	–	–	–	NM	–	–	–
Festuca monticola	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Fitzroya cupressoides	–	–	–	–	–	–	–	–	–	–	–	AM	–	–	–	–	–
Fragaria chiloensis	–	AM	AM	–	–	–	–	AM	–	–	AM	–	–	–	–	–	–
Francoa appendiculata	–	–	–	–	–	AM	–	–	–	–	–	–	–	–	–	–	–
Fuchsia magellanica	–	–	–	–	–	–	–	–	AM	–	–	–	–	–	–	AM	–
Galium hypocarpium	–	–	–	–	AM	–	–	–	–	–	–	–	–	–	–	–	–

	Forest plot																
Plant species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Gamochaeta spiciformis	NM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Gaultheria caespitosa	ER	–	–	–	–	–	–	–	–	–	ER	–	–	–	–	–	–
Gaultheria insana	–	–	–	–	–	–	–	–	–	–	–	–	ER	–	–	–	–
Gaultheria mucronata	ER	–	ER	–	–	–	ER	ER	–	–	–	ER	–	–	–	–	–
Gaultheria myrtilloides	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	ER
Gaultheria phillyreifolia	–	ER	–	–	–	–	ER	–	–	ER	–	–	–	–	–	–	–
Gaultheria poeppigii	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	ER
Gaultheria poeppigii var. *linifolia*	–	–	ER	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Gaultheria pumila	–	ER	ER	–	–	–	–	–	–	–	–	ER	–	–	–	–	ER
Gaultheria sp.	–	ER	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Gavilea odoratissima	–	–	–	–	–	–	–	–	–	–	–	–	–	OR	–	–	–
Geranium robertianum	–	–	–	–	AM	–	–	–	–	–	–	–	–	–	–	–	–
Gevuina avellana	NM	–	NM	–	NM	–	NM	NM	–	–	–	–	–	NM	–	–	–
Gleichenia quadripartita	–	–	–	–	–	–	AM	–	–	–	AM	–	–	–	–	–	–
Gleichenia squamulosa	–	–	AM	–	–	–	–	–	–	–	–	AM	–	–	–	–	–
Grammitis magellanica	–	–	–	–	–	–	NM	–	–	–	–	–	–	–	–	–	NM
Greigia landbeckii	–	–	–	–	–	–	–	–	AM	–	–	–	–	–	–	AM	–
Greigia sphacelata	–	–	–	–	AM	–	AM	–	–	–	–	–	–	AM	–	–	–
Griselinia scandens	–	–	–	–	–	–	AM	–	AM	–	–	–	–	–	–	–	–
Gunnera magellanica	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	AM
Gunnera tinctoria	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	AM	–
Hydrangea serratifolia	–	–	–	–	–	AM	–	–	AM	–	–	–	–	AM	–	–	–
Hydrocotyle poeppigii	–	–	–	–	AM	–	–	AM	AM	–	–	–	–	AM	AM	–	–
Hymenophyllum caudiculatum	–	–	–	–	–	–	–	–	–	NM	–	NM	–	NM	–	–	–

(continued)

Table 16.1 (continued)

	Forest plot																
Plant species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Hymenophyllum pectinatum	–	–	–	–	–	–	–	–	–	NM	–	–	–	NM	–	–	–
Hymenophyllum peltatum	–	–	–	–	–	–	–	–	–	–	–	–	–	NM	–	–	–
Hymenophyllum plicatum	NM	–	–	–	–	–	–	–	–	–	–	–	–	NM	–	–	–
Hymenophyllum seselifolium	–	–	–	–	–	–	–	NM	–	–	–	–	–	NM	–	–	–
Hypochaeris tenuifolia	–	–	–	–	–	–	–	–	–	–	–	AM	–	–	–	–	–
Hypolepis poeppigii	–	–	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–
Juncus microcephalus	–	–	NM	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Juncus pallescens	–	–	NM	NM	–	–	–	–	–	–	–	–	–	–	–	–	–
Juncus planifolius	–	–	–	–	–	–	–	–	–	–	NM	–	–	–	–	–	–
Juncus procerus	–	–	NM	NM	–	–	–	–	–	–	–	–	–	–	–	–	–
Lagenophora hariotti	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	AM
Lapageria rosea	–	–	–	–	AM	–	AM	–	–	–	–	–	–	AM	–	–	–
Lardizabala biternata	–	–	–	–	AM	AM	–	–	–	–	–	–	–	–	–	–	–
Lathyrus subandinus	AM	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Latua pubiflora	–	–	–	–	–	–	–	–	AM	–	–	–	–	–	–	–	–
Laurelia sempervirens	–	–	–	–	AM	AM	AM	–	–	–	–	–	–	AM	–	–	–
Laureliopsis philippiana	–	–	–	–	–	–	–	–	AM	AM	–	–	–	AM	AM	–	–
Lepidoceras chilense	–	–	–	NM	NM	–	–	–	–	–	–	–	–	NM	–	–	–
Leptinella scariosa	–	–	–	–	–	–	–	AM	–	–	–	–	–	–	–	–	–
Leptocarpha rivularis	–	–	–	–	AM	AM	–	–	–	–	–	–	–	–	–	–	–
Libertia chilensis	–	–	AM	–	–	–	AM	AM	–	–	–	–	–	–	–	–	–
Lobelia tupa	–	–	–	–	–	–	AM	–	–	–	–	–	–	–	–	–	–
Lomatia dentata	–	–	–	–	NM	–	NM	–	–	–	–	–	–	NM	–	–	–
Lomatia ferruginea	–	–	–	–	–	–	NM	NM	NM	NM	–	NM	–	–	NM	–	–
Lomatia hirsuta	NM	NM	NM	–	–	NM	NM	–	–	–	–	–	–	–	–	–	–

	Forest plot																
Plant species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Lophosoria quadripinnata	–	–	–	–	–	–	–	–	AM	AM	–	–	–	AM	AM	–	–
Lotus pedunculatus	–	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Luma apiculata	–	–	–	AM	AM	AM	AM	–	AM	AM	–	–	–	AM	–	–	–
Luma chequen	–	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Luzula racemosa	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Luzuriaga polyphylla	–	–	–	AM	–	–	AM	–	–	–	–	–	–	AM	AM	–	–
Luzuriaga radicans	–	–	–	AM	AM	–	–	AM	AM	AM	–	–	–	AM	–	–	–
Lycopodium gayanum	–	–	–	–	–	–	–	–	–	–	–	AM	AM	–	–	–	–
Lycopodium magellanicum	–	–	–	–	–	–	–	–	–	–	–	AM	AM	–	–	–	AM
Lycopodium paniculatum	–	–	–	–	–	–	–	–	–	–	–	AM	AM	–	–	AM	AM
Maytenus boaria	–	–	–	AM	AM	AM	–	–	–	–	–	–	–	–	–	–	–
Maytenus disticha	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	AM
Maytenus magellanica	AM	AM	–	–	–	–	–	–	–	–	–	–	AM	–	–	AM	–
Megalastrum spectabile	–	–	–	–	–	–	–	–	–	–	–	–	–	–	AM	–	–
Misodendrum brachystachium	–	–	–	–	–	–	NM	–	–	–	–	–	–	–	–	–	–
Misodendrum linearifolium	–	–	–	–	NM	–	–	–	–	–	–	–	–	–	–	–	–
Mitraria coccinea	–	–	–	–	–	AM	AM	AM	AM	AM	–	–	–	AM	AM	–	–
Muehlenbeckia hastulata	–	–	–	AM	AM	–	–	–	–	–	–	–	–	–	–	–	–
Mutisia spinosa	AM	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Myoschilos oblonga	AM	–	AM	–	–	AM	–	–	–	–	–	AM	AM	–	–	–	–
Myrceugenia chrysocarpa	–	–	–	–	–	–	–	–	–	AM	–	–	–	–	–	–	–
Myrceugenia exsucca	–	–	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–
Myrceugenia parvifolia	–	–	AM	–	–	–	AM	–	–	–	–	–	–	AM	–	–	–
Myrceugenia planipes	–	–	–	–	–	–	–	AM	AM	–	–	–	–	AM	AM	AM	–

(continued)

Table 16.1 (continued)

	Forest plot																
Plant species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Nertera granadensis	–	–	AM	–	–	–	–	AM	AM	AM	–	–	–	AM	–	–	–
Notanthera heterophylla	–	–	–	–	–	NM	–	–	–	–	–	–	–	–	–	–	–
Nothofagos obliqua	–	–	–	–	EM	EM	–	EM	–	–	–	–	–	EM	–	–	–
Nothofagus alpina	–	–	–	–	–	–	–	EM	–	–	–	–	–	–	–	–	–
Nothofagus antarctica	–	–	EM	–	–	–	–	–	–	–	EM	–	–	–	–	–	–
Nothofagus betuloides	–	–	–	–	–	–	–	–	–	–	–	–	EM	–	–	EM	–
Nothofagus dombeyi	EM	–	EM	–	–	–	EM	–	EM	–	–	–	–	–	–	EM	–
Nothofagus nitida	–	–	–	–	–	–	–	–	–	EM	–	–	–	–	–	–	–
Nothofagus pumilio	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	EM	EM
Oreobolus obtusangulus	–	–	–	–	–	–	–	–	–	NM	–	–	–	–	–	–	–
Osmorhiza chilensis	–	AM	–	–	AM	AM	AM	AM	–	–	–	–	–	AM	–	–	–
Ourisia sp.	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ovidia andina	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	AM	AM
Ovidia pillopillo	–	–	AM	–	–	–	AM	AM	AM	AM	–	–	–	AM	–	–	–
Oxalis arenaria	–	–	–	–	AM	AM	–	–	–	–	–	–	–	–	–	–	–
Oxalis dumetorum	–	–	–	–	AM	–	–	AM	–	–	–	–	–	–	–	–	–
Perezia pedicularifolia	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Persea lingue	–	–	–	–	AM	AM	AM	–	–	–	–	–	–	AM	–	–	–
Peumus boldus	–	–	–	–	–	AM	–	–	–	–	–	–	–	–	–	–	–
Philesia magellanica	–	–	–	–	–	–	–	AM	–	AM	–	AM	–	–	–	–	–
Pilea elliptica	–	–	–	–	–	–	–	–	–	–	–	–	–	–	AM	–	–
Pilgerodendron uviferum	–	–	–	–	–	–	–	–	–	–	AM	–	–	–	–	–	–
Pinguicula antarctica	–	–	–	–	–	–	–	–	–	–	–	NM	–	–	–	–	–
Poa obvallata	AM	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Podocarpus nubigenus	–	–	–	–	–	–	–	AM	AM	AM	–	AM	–	–	–	–	–

	Forest plot																
Plant species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Podocarpus salignus	–	–	–	–	–	–	AM	AM	–	–	–	–	–	AM	–	–	–
Polypodium feuillei	NM	–	–	–	NM	NM	NM	–	NM	–	–	–	–	NM	–	–	–
Polystichum plicatum	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Polystichum sp.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	AM	–	–
Potentilla sp.	–	–	–	–	–	–	–	–	–	–	–	–	–	AM	–	–	–
Pseudopanax laetevirens	–	–	–	–	–	–	–	AM	AM	AM	–	–	–	AM	–	–	–
Pseudopanax valdiviensis	–	–	–	–	–	–	–	–	AM	–	–	–	–	AM	–	AM	–
Pteris semiadnata	–	–	–	–	–	–	–	–	–	–	–	–	–	–	AM	–	–
Ranunculus peduncularis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	AM
Rhamnus diffusus	–	–	–	AM	AM	AM	–	–	–	–	–	–	–	AM	–	–	–
Rhaphithamnus spinosus	–	–	–	–	AM	–	AM	AM	AM	–	–	–	–	AM	–	–	–
Ribes magellanicum	–	–	–	–	–	–	–	AM	AM	AM	–	–	–	–	–	AM	AM
Ribes punctatum	–	–	AM	–	AM	–	–	–	–	–	–	–	–	–	–	–	–
Ribes valdivianum	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Rubus geoides	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	AM
Rubus radicans	–	–	–	–	–	–	–	–	AM	–	–	–	–	–	–	–	–
Rumohra adiantiformis	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Samolus latifolius	–	–	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–
Sanicula crassicaulis	–	–	–	–	AM	AM	–	–	–	–	–	–	–	–	–	–	–
Sanicula graveolens	–	–	–	–	–	–	–	–	–	–	–	–	–	AM	–	–	–
Sarmienta repens	–	–	–	NM	NM	NM	–	–	–	–	–	–	–	NM	–	–	–
Saxegothaea conspicua	–	–	–	–	–	–	–	AM	AM	AM	–	–	–	–	–	AM	–
Schinus polygamus	–	–	AM	–	–	AM	–	–	–	–	–	–	–	–	–	–	–
Schizaea fistulosa	–	–	–	–	–	–	–	–	–	–	AM	–	–	–	–	–	–

(continued)

Table 16.1 (continued)

	Forest plot																
Plant species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Schoenus rhynchosporoides	–	–	NM	–	–	–	–	–	–	–	NM	–	–	–	–	–	–
Scirpus inundatus	–	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Senecio acanthifolius	–	AM	–	–	–	–	–	–	–	–	–	AM	AM	–	–	–	–
Senecio chionophilus	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Senecio trifurcatus	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	AM
Sisyrinchium arenarium	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Solanum krauseanum	–	–	–	–	–	–	–	–	AM	–	–	–	–	–	–	–	–
Solanum valdiviense	–	–	–	–	AM	–	–	–	–	–	–	–	–	–	–	–	–
Sophora cassioides	–	–	–	–	–	AM	–	–	–	–	–	–	–	–	–	–	–
Stellaria arvalis	–	–	–	–	AM	–	–	–	–	–	–	–	–	–	–	–	–
Tepualia stipularis	–	–	AM	AM	–	–	AM	–	–	–	–	–	–	–	–	–	–
Tristerix corymbosus	–	–	–	NM	NM	NM	–	–	–	–	–	–	–	NM	–	–	–
Tristerix verticillatus	–	–	–	–	–	NM	–	–	–	–	–	–	–	–	–	–	–
Ugni candollei	–	–	–	–	–	–	–	–	AM	AM	–	AM	–	–	–	–	–
Ugni molinae	–	–	–	–	–	–	AM	AM	–	–	–	–	–	–	–	–	–
Uncinia phleoides	–	–	–	–	NM	NM	–	–	NM	NM	–	–	–	NM	NM	–	–
Uncinia tenuis	–	–	NM	–	–	–	–	–	NM	–	–	NM	–	–	NM	NM	–
Valeriana lapathifolia	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	AM	AM
Veronica officinalis	–	–	–	–	–	–	–	NM	NM	–	–	–	–	–	–	–	–
Vicia setifolia	–	AM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Viola buchtienii	–	–	–	–	–	–	–	AM	AM	–	–	–	–	AM	–	–	–
Viola maculata	–	–	–	–	AM	–	–	–	–	–	–	–	–	–	–	–	–
Viola reichei	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	AM	AM
Viola rubella	–	–	–	–	–	–	AM	–	–	–	–	–	–	–	–	–	–
Weinmannia trichosperma	–	–	–	–	–	–	–	–	AM	AM	–	–	AM	–	AM	–	–

Species list after Rodriguez et al. (2018). Mycorrhizal types: arbuscular mycorrhizal (AM), ectomycorrhizal (EM), ericoid (ER), orchid (OR), and non-mycorrhizal (NM). The plots were located on the Andean mountain range (AR), the Chilean Central Valley (CV), and the Coastal mountain range (CR) and were dominated by: (1) *Austrocedrus chilensis* (AR); (2) *Araucaria araucana* (AR); (3) *Nothofagus antarctica* (CV); (4) *Blepharocalyx cruckshanksii* (CV); (5) *Nothofagus obliqua* (CV); (6) *Peumus boldus* (CV); (7) *Nothofagus dombeyi* and *Eucryphia cordifolia* (CR); (8) *Nothofagus alpina* (CR); (9) *Weinmannia trichosperma* (CR); (10) *Nothofagus nitida* (CR); (11) *Pilgerodendron uviferum* (CR); (12) *Fitzroya cupressoides* (CR); (13) *Nothofagus betuloides* (CR); (14) *Aextoxicon punctatum* (AR); (15) *Luma apiculata* (AR); (16) *Nothofagus dombeyi* (AR); and (17) *Nothofagus pumilio* (AR)

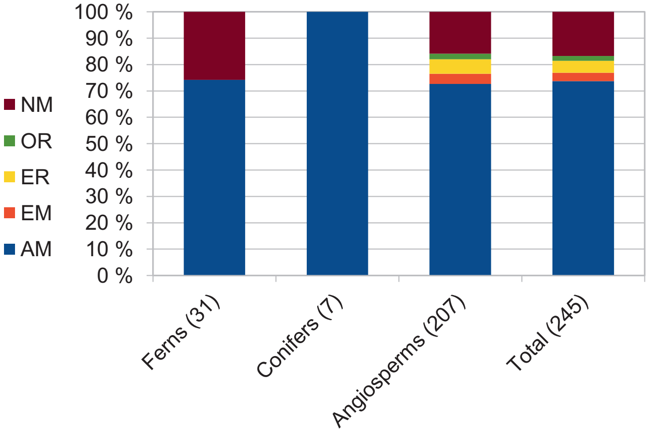

Fig. 16.1 Proportion of mycorrhizal types by different plant groups. Mycorrhizal types: arbuscular mycorrhizal (AM), ectomycorrhizal (EM), ericoid (ER), orchid (OR), and non-mycorrhizal (NM)

OM mycorrhizal associations were found only in four plant species of the family Orchidaceae. Pereira et al. (2018) studied the terrestrial orchid *Codonorchis lessonii*, endemic to southern Chile and Argentina, showing on the plant the presence of fungal binucleate cells and DNA material belonging to the families Ceratobasidiaceae and Tulasnellaceae. Fungal isolates belonging to Ceratobasidiaceae grew at a higher rate than those from Tulasnellaceae (Pereira et al. 2018). Phylogenetic analyses showed that different fungal partners associate with this orchid, suggesting relatively low specificity (Pereira et al. 2018).

A total of 10 vascular plant species presented fungal-bacterial tripartite associations: six species from Fabaceae (AM fungi + *Rhizobium*), two species from Rhamnaceae (AM fungi + *Frankia*), and two plant species from Gunneraceae (AM fungi + Cyanobacteria) (Carú 1993). These plants with tripartite associations, as well as several of the 37 NM plant species, are prevalent as pioneer plants on degraded soil or are known to colonize new substrates (for example after a volcanic event), having the role of ecosystem engineers on degraded ecosystems (Zúñiga-Feest et al. 2010).

The soils of the 17 plots sampled were generally acidic (Table 16.2), with low nitrogen and phosphorous content, and in some plots, extremely high concentrations of aluminum. Under these extremely harsh conditions for plant growth, the mycorrhizal associations play a key role on enhancing plant nutrition (Etcheverría et al. 2009; Marín et al. 2018a), and on giving the plant resistance to phytotoxic aluminum concentrations (Aguilera et al. 2017).

Table 16.2 Soil physicochemical parameters of 17 plots of temperate rainforests in southern Chile. The plot numbers correspond to the same plots as in Table 16.1

Plot No.	pH (KCl)	Cond. (uS/cm^{-1})	TC (%)	TN (%)	C/N	Av. P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Al (ppm)
1	5.60	48	0.87	0.06	14.50	1.9	25	212	25	74
2	4.70	69	9.00	0.43	20.93	4.7	79.00	658	92.00	261
3	4.30	213	13.74	1.31	10.49	2.6	218	698	167	1469
4	4.70	395	9.33	0.94	9.93	6.7	130	3546	901	130
5	5.13	209	11.51	0.98	11.74	3.5	233	4637	716	358
6	6.01	304	10.09	0.76	13.28	52.5	1309	7613	705	14
7	3.24	115	10.98	0.43	25.53	7.4	143	249	71	1790
8	3.98	124	9.1	0.58	15.69	2.7	131	661	113	1002
9	3.61	69	10.99	0.68	16.16	1.0	202	950	139	768
10	3.29	223	14.77	0.92	16.05	8.3	210	285	146	1745
11	3.47	223	7.91	0.50	15.82	20.3	81	254	51	639
12	3.35	126	6.3	0.19	33.16	1.0	90	137	73	752
13	3.15	224	8.71	0.39	22.33	16.7	171	200	93	1355
14	4.55	257	13.47	0.92	15.01	6.0	107	2171	488	721
15	4.27	122	9.53	0.81	12.01	9.0	69	361	84	1043
16	4.61	156	11.97	0.43	28.01	7.0	164	43	48	2230
17	4.06	113	10.85	0.33	33.01	9.0	120	204	61	1375

16.4 Mycorrhizal Bioweathering

In terrestrial ecosystems, crucial nutrients for plant nutrition as phosphorous and base cations, largely come from the *in situ* weathering of the bedrock or from weathering elsewhere and subsequent atmospheric deposition (Boy and Wilcke 2008; Boy et al. 2008). Bioweathering is the physicochemical process by which rocks are degraded by the direct and/or indirect actions of biota (Burford et al. 2003). The role of soil biota in weathering-related processes was largely ignored (Berner 1992). Mycorrhiza represents the most direct connection between photosynthesis and weatherable mineral surfaces, resulting in the lowest energy costs (Hoffland et al. 2004; Gadd 2007). Mycorrhiza, unlike plant roots, can explore small and nutrient-specifc mineral areas, and unlike bacteria, have a direct source of energy. Mycorrhiza degrades minerals by physical mechanisms such as tigmotropism, and by chemical processes such as acidolysis, complexolysis, redox reactions, and metal precipitation (Banfield et al. 1999; Burford et al. 2003; Hoffland et al. 2004; Rosling et al. 2004; Gadd 2007; van Schöll et al. 2008; Finlay et al. 2009; Taylor et al. 2009; Smits and Wallander 2017).

Ecosystem age, which reflects the ecological succession estate, was related to the degree of bioweathering in a mesocosm experiment on temperate rainforests in southern Chile (Marín 2018a), showing also higher bioweathering degree on EM-dominated forests. Furthermore, hyphae channels were seen on phyllosilicate minerals (Fig. 16.2; Marín 2018a). The degree of fungal bioweathering increased

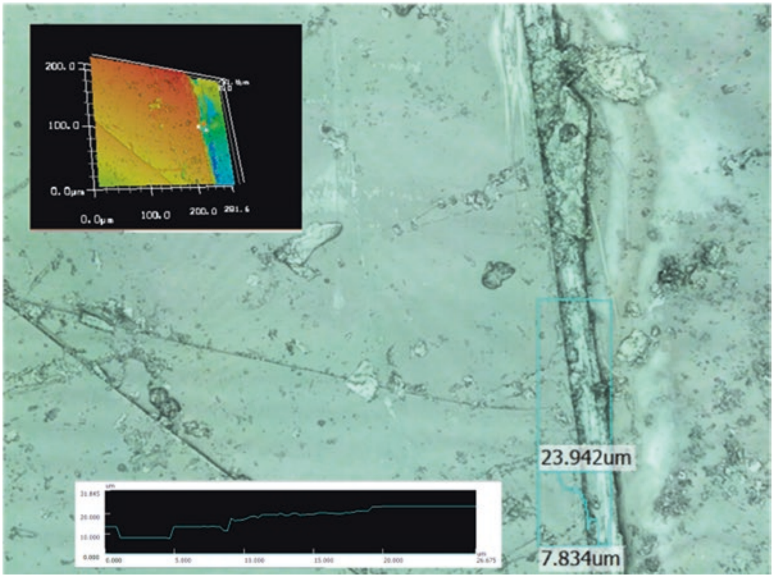

Fig. 16.2 Channels formed by mycorrhizal fungi on Muscovite after one year of exposition in a *Fitzroya cupressoides* forest, Alerce Costero National Park, southern Chile. Photo taken with a Confocal Laser Microscope at 5000×

over time, showing that this is a relevant biogeochemical process on terrestrial ecosystems (Marín 2018a).

16.5 Mycorrhizal Fungi as Ecological Restoration Tools

Up to now forest restoration using native trees has shown limited success (Thomas et al. 2014). Among the potential factors influencing this low success is the lack of integrating underground processes essential for tree survival. Mycorrhizal symbiosis is a key interaction directly affecting plant nutrition and resistance to abiotic and biotic stressors (Godoy et al. 2014), and thus should constitute a fundamental tool for ecological restoration (Lara et al. 2014). Specifically, the whole ´symbiome´ (Tripp et al. 2017) -plants and its symbionts- should be considered in restoration efforts. However, mycorrhizal fungi are rarely subjects of conservation programs, as mycorrhizal biogeography is an incipient area (Tedersoo 2017). In fact, the effects of anthropogenic disturbances on the distribution of plants and its mycorrhizal fungi largely remains to be studied, especially in regions with harsh environmental conditions as the temperate rainforests of southern Chile (Bueno et al. 2017).

AM fungi have been shown to be essential for the survival of the native conifer *Araucaria araucana* after fires, providing access to the remaining available soil-nutrients (Paulino et al. 2009; Lara et al. 2014; Cortés 2016) or even by maintaining glomalin production without mycelia growth (Rivas et al. 2012, 2016). Similarly, endemic EM fungi as the Basidiomycete *Descolea antarctica* seem to be crucial for the recovery of post-fire *Nothofagus alpina* seedlings (Palfner et al. 2008). Despite the conservation threats, restoration plans of Chilean native plant species are still incipient (Lara et al. 2014). However, suggestions as considering systems with native plants growing with their local rhizosphere and AM fungi under greenhouse conditions have emerged (Godoy et al. 2014; Lara et al. 2014; Marín et al. 2018a). It is important to emphasize that endangered plant species should preferably be grown in nurseries with local mycorrhizal fungal symbionts, as local mycorrhizal fungi guarantee greater growth and resistance to environmental stress (Godoy et al. 1994; Marín et al. 2018a). Greenhouse experiments involving mycorrhizal fungal inoculation of both *Nothofagus* spp. (Garrido 1988; Godoy et al. 1995; Marín et al. 2018a), and native conifer (Godoy et al. 1994) species, have shown significant effects on the plant growth rate, biomass, and seedling survival.

16.5.1 Nursery Experiment

In southern Chile, with the objective of producing native flora seedlings, an assay of simple and combined mycorrhizal inoculations with *Pisolithus tinctorius* and *Laccaria laccata* on *Nothofagus alpina* and *N. obliqua* was developed. Simultaneously, the potential of litter applications as a natural source of mycorrhizal incoculum for plant production programs was tested.

Seedlings were obtained under sterile germination conditions in culture chambers, and later transported to the nursery. At the end of the assay (16 weeks), several plant morphometric variables were measured, discerning statistical differences with the Tukey test (p value <0.05).

For *N. alpina*, the treatments involving *P. tinctorius* + *L. laccata* (with and without fertilization) and also litter, resulted on significantly higher values of the morphometric variables (except for the length of the radical systems) (Table 16.3). In contrast, all treatments on *N. obliqua* resulted on significantly higher values of the morphometric variables (Table 16.3). The quality index was significantly higher in respect to the control for the combined inoculations and litter treatments on *N. alpina*, while it was significantly higher for all treatments on *N. obliqua* (Table 16.3).

16.5.2 Reforestation Experiment

A reforestation experiment with the *Nothofagus alpina* and *N. obliqua* seedlings of the previous nursery assay was developed. Plantations were installed on two different sites (Folilco and Riñihue), of the premontane Andean region in southern Chile. After 23 weeks of the plantations, the plants were harvested and transported to the laboratory for the measurement of morphometric parameters and the estimation of

Table 16.3 Inoculation assay (simple and combined) with *Pisolithus tinctorius* and *Laccaria laccata* on two *Nothofagus* species. Treatments: (1) *P. tinctorius* without fertilizer; (2) *P. tinctorius* with fertilizer; (3) *P. tinctorius* and *L. laccata* without fertilizer; (4) *P. tinctorius* and *L. laccata* with fertilizer; (5) litter; (6) control

Treatment	Stem diameter (mm)	Stem length (cm)	Root length (cm)	Fresh stem weight (g)	Fresh root weight (g)	Dry stem weight (g)	Dry root weight (g)
Nothofagus alpina							
1	3.6	14.15	13.13	2.66	1.50	0.73	0.31
2	3.98[a]	19.78[a]	15.65	3.35	1.76[a]	0.91	0.33
3	4.43[a]	25.55[a]	16.2	4.83[a]	1.84[a]	1.25[a]	0.43[a]
4	5.83[a]	36.90[a]	16.68	9.37[a]	3.28[a]	2.49[a]	0.68[a]
5	5.05[a]	29.85[a]	17.50[a]	6.18[a]	2.06[a]	1.68[a]	0.48[a]
6	3.40	13.57	15.8	2.10	1.14	0.58	0.26
Nothofagus obliqua							
1	4.00[a]	33.52[a]	16.40[a]	5.45[a]	1.45[a]	1.40[a]	0.33[a]
2	4.53[a]	41.10[a]	14.55[a]	7.90[a]	2.42[a]	1.94[a]	0.41[a]
3	5.00[a]	51.45[a]	15.95[a]	10.77[a]	2.93[a]	2.73[a]	0.53[a]
4	5.58[a]	57.45[a]	18.45[a]	13.02[a]	3.07[a]	3.46[a]	0.65[a]
5	3.95[a]	34.43[a]	15.37[a]	6.04[a]	1.90[a]	1.41[a]	0.27[a]
6	2.35	17.08	11.63	1.30	0.53	0.32	0.10

Values correspond to the average of 20 plant individuals. [a]denotes statically significant differences between the control and the treatments (Tukey test, p value <0.05)

the quality index (Ritchie 1984). The combined inoculation of *Pisolithus tinctorius* and *L. laccata* in both *Nothofagus* species showed overall excellent results (Table 16.4). According to the quality index, the best treatment for *N. obliqua* was co-inoculation with fertilization, and for *N. alpina* it was co-inoculation without fertilization (Table 16.4). These results show clear advantages of mycorrhizal co-inoculations for the re-establishment of crucial native flora of the temperate rainforests of southern Chile.

16.6 Conclusions and Future Directions

Mycorrhizal fungal communities of the temperate rainforests of Southern Chile are affected by the mountain system in which they are located (Andes and Coastal mountain ranges), the mycorrhizal dominance of the forest (either ectomycorrhizal -EM- or arbuscular mycorrhizal-AM), soil chemistry, and altitude (Marín et al. 2017a; Marín 2018a). Mycorrhizal fungi are important ecosystem components as they play central roles in nutrient cycling, maintenance of biodiversity, and ecosystem productivity (van der Wal et al. 2013; Bardgett and van der Putten 2014; Peay et al. 2016). Mycorrhizal fungal communities can be highly diverse (Tedersoo et al. 2014), and their diversity is affected by edaphic and climatic conditions, as well as

Table 16.4 Reforestation from seedlings of an Inoculation assay (simple and combined) with *Pisolithus tinctorius* and *Laccaria laccata* on two *Nothofagus* species. Treatments: (1) *P. tinctorius* without fertilizer; (2) *P. tinctorius* with fertilizer; (3) *P. tinctorius* and *L. laccata* without fertilizer; (4) *P. tinctorius* and *L. laccata* with fertilizer; (5) litter; (6) control

Treatment	Stem diameter (mm)	Stem length (cm)	Root length (cm)	Fresh stem weight (g)	Fresh root weight (g)	Dry stem weight (g)	Dry root weight (g)	Quality Index
Nothofagus alpina – Folilco								
1	5.00	23.1[a]	13.20	7.40	7.60	7.40	5.50	0.27
2	7.7[a]	35.5[a]	15.5[a]	18.0[a]	13.6[a]	13.6[a]	10.1[a]	0.50
3	9.5[a]	50.3[a]	15[a]	23.1[a]	20.85[a]	16.7[a]	14.3[a]	0.57
4	7.6[a]	36.9[a]	15.1[a]	15.3[a]	15.50	11.0[a]	10.30	0.43
5	9.5[a]	55.4[a]	17.9[a]	23.0[a]	19.3[a]	16.9[a]	15.8[a]	0.55
6	4.10	17.00	12.30	8.00	7.90	6.30	6.20	0.29
Nothofagus alpina – Riñihue								
1	4.90	30.00	12.7[a]	8.6[a]	8.00	6.60	6.1[a]	0.20
2	18.1[a]	40.4[a]	17.8[a]	16.6[a]	14.3[a]	12.3[a]	10.3[a]	0.44
3	8.0[a]	35.3[a]	19.9[a]	14.9[a]	11.8[a]	10.5[a]	9.9[a]	0.45
4	9.9[a]	54.8[a]	19.6[a]	28.9[a]	24.4[a]	17.4[a]	15.2[a]	0.30
5	7.2[a]	38.1[a]	16.2[a]	13.3[a]	13.4[a]	9.8[a]	9.0[a]	0.35
6	4.10	24.60	9.40	5.80	7.70	7.60	4.96	0.20
Nothofagus obliqua – Folilco								
1	7.50	59.20	16.9[a]	19.70	9.60	15.40	8.0[a]	0.29
2	9.3[a]	62.20	21.50	27.70	15.70	21.3[a]	14.70	0.52
3	8.80	77.3[a]	22.00	24.50	19.00	19.00	14.00	0.37
4	11.1[a]	84.9[a]	22.20	37.3[a]	26.7[a]	27.5[a]	23.3[a]	0.65
5	8.9[a]	78.1[a]	22.2[a]	32.6[a]	18.60	27.80	16.60	0.49
6	7.80	55.40	19.70	24.10	17.50	16.70	13.00	0.41
Nothofagus obliqua – Riñihue								
1	7.9[a]	64.1[a]	21.3[a]	25.7[a]	14.30	17.7[a]	9.9[a]	0.33
2	8.5[a]	60.40	17.90	29.0[a]	17.1[a]	18.2[a]	11.1[a]	0.40
3	8.7[a]	80.4[a]	19.00	31.9[a]	19.8[a]	20.5[a]	12.8[a]	0.35
4	11.5[a]	94.3[a]	17.50	51.0[a]	25.7[a]	31.0[a]	16.0[a]	0.56
5	9.4[a]	77.4[a]	21.0[a]	39.4[a]	20.9[a]	24.9[a]	13.5[a]	0.45
6	6.30	53.80	16.80	16.80	10.50	10.90	7.10	0.20

Values correspond to the average of 20 plant individuals. [a]denotes statically significant differences between the control and the treatments (Tukey test, p value <0.05). Quality Index after Ritchie (1984)

biotic factors such as plant diversity (Tedersoo et al. 2014; Davison et al. 2015). However, how these abiotic and biotic factors interact and affect mycorrhizal fungal communities -and the mycorrhizal symbiosis overall- remains to be throughly studied (Truong et al. 2017), especially on the temperate rainforests of South America (Bueno et al. 2017).

The diversity and function of soil biota under scenarios of climate change provides fundamental information on the ecosystem processes that take place over long periods. Such questions cannot be addressed by traditional approaches which are commonly limited to 2–3 years due to funding and logistical restrictions (Amano and Sutherland 2013). Thus, scientific collaboration represents an opportunity to tackle the role of soil biota, particularly mycorrhizal fungi, in future studies of biogeochemical cycles in pristine temperate rainforests of South America (Truong et al. 2017, 2019; Oeser et al. 2018).

Acknowledgments R.G and C.M. were funded by the project Fondecyt 1190642. C.M. was funded by the Universidad de O'Higgins post-doctoral research fund. Special thanks to Dr. Jens Boy for support in the laboratory at the Institute of Soil Science, Leibniz Universität Hannover, Germany.

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