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# Diversity of mycorrhizal plant species and arbuscular mycorrhizal fungi in evergreen forest, deciduous forest and grassland ecosystems of Southern Chile

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## Summary

In the Valdivian rainforest region of the Southern Chilean Andes three main ecosystems are found: Primary evergreen forests, secondary deciduous forests, and grassland areas. The secondary forest and the grasslands are habitually the result of the clearance of the primary forest some 60 years ago. The secondary forest consists mainly of the deciduous tree species *Nothofagus alpina*; forest management practices such as crown thinning and clearance of the understorey are applied to the secondary forest to improve its economic value. The grasslands are used by extensive cattle grazing. Soils in this region are acid Andosols with high organic matter content, high exchangeable aluminum and low levels of available phosphorus. The main objective of this study was to investigate the diversity of arbuscular mycorrhizal (AM) plant species and of arbuscular mycorrhizal fungi (AMF) in these three ecosystems. The highest diversity with 53 plant species was found in the evergreen forest with 77.4% of them AM, while in the grassland 91% of the 22 plant species were AM. The deciduous forest had 11 plant species only and the lowest proportion of AM plant species (55%). Thirty-nine AM fungal species were found in total, of which most are being reported for the first time from Southern Chile. Thirteen fungal species were of the *Acaulospora* genus, 10 of *Glomus*, 4 species each of *Scutellospora* and *Archaeospora*, 3 species each of *Pacispora* and *Entrophospora*, and one species each of *Paraglomus* and *Diversispora*. AMF species were more abundant in the grassland (29 spp.) than in the evergreen forest (20 spp.) which is likely related to a higher relative proportion of AM plant species in the grassland. Four AMF species were present in all the ecosystems, and 15 species were apparently quite specific as they were only found in one of the ecosystems. Noteworthy was the lack of *Paraglomus* and *Scutellospora* spp. in any of the forest ecosystems, and the relatively higher presence of *Entrophospora* spores in those ecosystems. It was concluded that the diversity of the AMF species in the ecosystems is strongly influenced by the proportion of AM plant species in each ecosystem and that their diversity is not related to soil chemical properties.

## Introduction

In the area between 35ºS and 55ºS latitude in Southern Chile about 4,1 million ha are covered with a primary rain forest which is evergreen, and 1,5 million ha with a secondary forest which consist mainly of the deciduous tree *Nothofagus alpina*. This secondary forest and large grassland areas are the result of clearance and burning of the primary forest, some 60 years ago (CONAF et al., 1997). The secondary forest has multiple uses being the most important firewood production; it represents an important source of income for forest owners. The grassland areas are more or less intensively used for cattle production. Due to the economic, social, cultural and environmental interest in the evergreen forest, appropriate and sustainable forest management systems are now being developed which try to avoid the destruction or damage of its ecological components and which aim at conserving a biological diversity as high as possible (LARA et al., 2003).

The soil on which the evergreen forest grows has low pH with the associated, often high and toxic soluble aluminum concentrations and the very low phosphate availability (ÁLVAREZ et al., 2005). In this environment, mycorrhizal fungi are of primary importance for many if not the majority of plant species, and for the functioning of ecosystems (BAREA et al., 1997; SMITH and READ, 1997; van der HEIJDEN, 2002). There are two main types of mycorrhizas: Ectomycorrhiza (EC) which is formed by many forest tree species with a number of fungal species of the Basidiomycetes and Ascomycetes classes of fungi, and endomycorrhizas. EC is frequently found in forest plant species of the temperate zones, and Pinaceae and Fagaceae form this kind of symbiosis also in Southern Chile (GARRIDO, 1988; PALFNER, 2001). Of the endomycorrhizas, the arbuscular mycorrhiza (AM) type is by far the most important worldwide because it occurs with more than 60% of the species of the plant kingdom (TRAPPE, 1987). AM is commonly found in many, but not all forest species and in most herbaceous species, shrubs, ferns and grasses. The fungal species involved in the formation of AM belong to the Glomeromycota (SCHÜSSLER et al., 2001). Some plant families, like Ericaceae or Orchideaceae form very specific mycorrhizas (SMITH and READ, 1997) without which the plants cannot survive under natural conditions.

The main purpose of this study was to investigate the occurrence of AM plant species and arbuscular mycorrhizal fungal (AMF) species in the evergreen forest ecosystem of Southern Chile and the two ecosystems which developed from it when it was destroyed. This should give information in which way the diversity of mycorrhizal plants and AMF species is affected in a long term after slashing and burning the primary forest. Such information may have value for the future in conserving the diversity of a native soil microbiological resource like AMF species.

## Materials and methods

### Study area

The study was carried out at the Experimental Station San Pablo de Tregua of the University Austral de Chile, Valdivia. It is located in the Panguipulli region, province of Valdivia in the Andean mountains (39º30'-39º38'S, 72º02'-72º09'W) at an altitude between 550 and 1600 m above sea level (a.s.l.). The locality has an approximate surface of 2180 ha. Almost 90 % of the site is covered by undisturbed evergreen primary forest with an excellent state of conservation. The primary forest is representative for Southern Chile; it belongs to the evergreen *Nothofagus dombeyi – Laureliopsis philippiana* subtype and is in an advanced state of maturity. Shadow tolerant tree species such as *Saxegothaea conspicua* and *Dasyphyllum diacanthoides* have begun to displace those plant species which cannot regenerate under shade, such as *N. alpina*. The soil at the site is an Andosol. It

derives from recent volcanic ashes and belongs to the Liquiñe soil series. The soil is a loamy sand, acidic to moderately acidic, deeply to moderately deeply developed (0.7-1.5 m) with good water infiltration capacity, good drainage, high water holding capacity and with high contents of organic matter (15-25%) in the upper part (LARA et al., 2002).

The land is mountainous, with a complex topography and few plane land surfaces. Fifty % of the forestland is made up of hills with a relatively low inclination (0-10º); more than 10% of the forestland is situated on hills with an inclination of more than 25º. The climate at the location is temperate with a mean annual temperature of 11º C, with a minimum of 5º C in August and a maximum of 20º C in February. The number of days with frost during the year ranges from 30 to 50. The annual rainfalls range between 4000 to 5000 mm, with 50% of the rain between May and August while the summers are short and dry with up to 200 mm per month. Most of the precipitation falls as snow, above 1000 m a.s.l.

### Ecosystems

Three ecosystems dominate the area and were selected for the study:

Evergreen forest ecosystem: This is the natural evergreen forest with the dominant trees having an estimated age of more than 200 years. The basal trunk area of the mature tree vegetation was 119 $m^2$ per ha in 2002, distributed over about 40-100 trunks per ha. The forest is dominated by *N. dombeyi, L. philippiana* and *S. conspicua* trees. The canopy is not completely closed. The degree of disturbance is low. The understorey has a rich community of epiphytes and climber plants.

Deciduous forest ecosystem: A small sector of the area, about 10% of the experimental location, has a secondary forest dominated by the deciduous tree species *N. alpina* which represents more than 90% of the tree trunks in number per ha. There are a few companion plant species such as *N. obliqua*, *N. dombeyi*, *Weinmannia trichosperma*, *L. philippiana*, *S. conspicua* and *Eucryphia cordifolia.* The estimated age of the trees is 50 years, and the basal trunk area was 30 $m^2$ per ha distributed over about 500 to 1000 trunks per ha. This basal area was obtained after a silvicultural management practice in 2002, which consisted of crown thinning and the reduction of the original basal trunk area per ha by 40%.

Grassland ecosystem: The same location also contains small patches with natural grasslands between the above-mentioned forest ecosystems. The grassland ecosystem has a high diversity of grasses and herbs. It is extensively used by cattle grazing.

### Root and soil sampling

Root and soil samples were collected at the end of autumn, in May 2003. Plant species were identified at the same time. The nomenclature of the plant species follows that of MARTICORENA and QUEZADA (1985). To establish the presence of the mycorrhiza type of the represented species in the vascular flora, root material from 3 individual specimen of each species was collected at each study site. Root samples were obtained at a soil depth down to 15 to 30 cm for the forest and bushy species. Fine roots were excavated starting from the trunk and working out towards the fine roots. Roots were collected from young and mature trees. Roots of the climbers, ferns, grasses and herbaceous species were collected at a soil depth from 0 to 20 cm.

Soil sampling for soil analyses and the first set of AMF spore separation and species identification was done in 5 plots of each ecosystem, in May 2003. Plots had been randomly established within each ecosystem area. The plots had been selected for nutrient recycling and for hydrological studies of the Institute of Botany of the Universidad Austral, Valdivia. Within each plot of 100 $m^2$ surface area, 15 samples were taken using a 20-cm-long tube sampler, and crossing the plot in a diagonal way. At the forest sites, the litter layer was cleared prior to soil sampling. Samples from each plot were bulked to give five replicates per ecosystem. Soil samples were brought to the lab and stored in a freezer until analyses. A second set of soil samples for the separation of spores and the identification of AMF species was taken in the ecosystems, in November 2004.

### Soil analysis methods

The key parameters of the chemical soil fertility in each ecosystem were determined: Soil pH was measured by glass electrode in a 1:2.5 soil:water suspension. Exchangeable aluminum (Al) and iron (Fe) were analysed by AAS after previous extraction with DTPA at pH 7.3 (SADZAWKA et al., 2000). Available phosphorus (P) was determined after extraction with a solution of 0.5 M $NaHCO_3$ at pH 8.5 (OLSEN and SOMMERS, 1982). Total P was determined according to DICK and TABATABAI (1977) and soil organic matter (OM) was analyzed using the dichromate oxidation method (WALKLEY and BLACK, 1934).

### Analyses of mycorrhizas

Roots were cleared and fungal structures were stained following the methods described by BRUNDRETT et al. (1996). Mycorrhizal structures were observed in a microscope at 50x magnification. Spores of AMF were extracted and separated from the soil using the wet-sieving and decanting procedure described by SIEVERDING (1991). Spores were transferred to Petri-dishes and those of the first sampling date (May 2003) were counted. Spores were isolated under a stereomicroscope and were fixed in polyvinyl alcohol-lactic acid-glycerol (PVLG) (KOSKE and TESSIER, 1983), and a mixture of PVLG and Melzer's reagent (BRUNDRETT et al., 1996) to obtain permanent specimen. For the taxonomic classification main morphological spore characteristics such as color, diameter, type and number of spore walls, and the morphology of the subtending hypha at the point of spore attachment were observed under a high-power light microscope at 100x and 400x magnification. For the species identification the instructions given by SCHENCK and PEREZ (1990)

**Tab. 1:** Characteristics of soils in the evergreen forest (EF), deciduous forest (DF) and grassland (GR) ecosystems[a]

| Ecosystem | pH | OM (%) | Al (mg $kg^{-1}$) | Fe (mg $kg^{-1}$) | P (mg $kg^{-1}$) | |
|---|---|---|---|---|---|---|
| | | | | | Available | Total |
| EF | 4.56 b | 21.85 ab | 321 a | 66.4 a | 6.11 a | 1763 b |
| DF | 5.40 a | 24.92 a | 376 a | 35.8 b | 3.64 b | 1487 c |
| GR | 5.37 a | 15.31 b | 341 a | 24.1 b | 2.93 b | 2095 a |

[a] Values are averages of five replicates per ecosystem. Treatment means followed by the same letter are not significantly different ($P \leq 0.05$).

and INVAM (International Culture Collection of Arbuscular and Vesicular-Arbuscular Endomycorrhizal Fungi, see internet homepage: www.invam.caf.wvu.edu) or in species descriptions were followed. *Diversispora* (WALKER and SCHÜSSLER, 2004) and *Pacispora* (OEHL and SIEVERDING, 2004) were identified using descriptions of spores of the species. All isolated specimen were deposited at the Laboratory of Plant Nutrition of the Universidad La Frontera, Temuco, Chile.

## Results

### Soil fertility

The pH was not different in soils under grassland and deciduous forest, whereas the soil under evergreen forest was significantly more acidic compared to the grassland (Tab. 1). With regard to the organic matter content, the soil under grassland had the lowest content. The grassland also showed the lowest available P content whereas the evergreen forest soil had the highest. In contrast, the total P content was highest in soil under grassland as compared to both forest systems. Exchangeable aluminum was not different in all the sites studied but Fe was much higher in the evergreen forest than in the other two ecosystems.

### Distribution of plant species and mycorrhizas

The evergreen forest had the most diverse botanical composition with 53 plant species (Tab. 2) of which 77.4% formed AM, 17% were non-mycorrhizal and only 5.6% formed EC including one species which was associated with ericoid mycorrhiza. Of the 18 tree species 12 were associated with AM, the 5 species of the Proteaceae were non-mycorrhizal. *Nothofagus dombeyi* of which some individuals had developed a large canopy forms EC. The understorey was not dense but the stability of the ecosystem permitted the presence of a rich community of 14 shrub species of which 13 were AM. The shrub of the Ericaceae family formed an ericoid mycorrhiza. The 6 climbers were all AM and only the epiphytic *Fascicularia bicolor* was non-mycorrhizal. Of the 8 ferns the *Asplenium* sp. and the Hymenophyllaceae were non-mycorrhizal, the others formed AM. Of the 6 herbaceous species the *Loasa* sp. was non-mycorrhizal.
The deciduous forest was dominated by *N. alpina* which is EC as was the other member of the Fagaceae. With the exception of the Proteaceae tree species, the other trees were AM. An understorey was almost absent. Two fern species were present, one of them AM, and the bromeliaceous species *Greigea landbecki* formed AM.
In the grassland 22 herbaceous and grass species were found, 20 of them were AM. The species of the Juncaceae and Polygonaceae were non-mycorrhizal.

**Tab. 2:** List of vascular plants and their mycorrhizal status in Chilean evergreen forest (EF), deciduous forest (DF) and grassland (GR) ecosystems.

| Plant type | Scientific name | Family | Ecosystem[a] | | |
|---|---|---|---|---|---|
| | | | EF | DF | GR |
| Trees | *Aextoxicom punctatum* R. et P. | Aextoxicaceae | AM | - | - |
| | *Amomyrtus luma (*Mol.*)* Legr. et Kause | Myrtaceae | AM | - | - |
| | *Amomyrtus meli* (Phil.) Legr. et Kausel | Myrtaceae | AM | - | - |
| | *Dasyphyllum diacanthoides* (Less.) Cabr. | Asteraceae | AM | - | - |
| | *Drimys winteri* J.R. et G. Forster | Winteraceae | AM | - | - |
| | *Embothrium coccineum* J.R. et G. Forster | Proteaceae | N | - | - |
| | *Eucryphia cordifolia* Cav. | Eucryphiaceae | AM | AM | - |
| | *Gevuina avellana* Mol. | Proteaceae | N | - | - |
| | *Laureliopsis philippiana* (Looser) Schodde | Monimiaceae | AM | AM | - |
| | *Lomatia dentata* (R. et P.) R. Br. | Proteaceae | N | - | - |
| | *Lomatia ferruginea* (Cav.) R. Br. | Proteaceae | N | N | - |
| | *Lomatia hirsuta* (Lam.) Diels ex Macbr. | Proteaceae | N | - | - |
| | *Luma apiculata* (DC.) Burret | Myrtaceae | AM | - | - |
| | *Maytenus magellanica* (Lam.) Hook. f. | Celastraceae | AM | - | - |
| | *Myrceugenia planipes* (H. et A.) Berg | Myrtaceae | AM | - | - |
| | *Nothofagus alpina* (P. et E.) Oerst | Fagaceae | EC | EC | - |
| | *Nothofagus dombeyi* (Mirb.) Oerst | Fagaceae | EC | EC | - |
| | *Nothofagus obliqua* (Mirb.) Oerst | Fagaceae | - | EC | - |
| | *Saxegothaea conspicua* Lindl. | Podocarpaceae | AM | AM | - |
| | *Weinmannia trichosperma* Cav. | Cunoniaceae | AM | AM | - |
| Shrubs | *Aristotelia chilensis* (Mol.) Stuntz | Elaeocarpaceae | AM | - | - |
| | *Azara lanceolata* Hook. f. | Flacourtiaceae | AM | - | - |
| | *Berberis buxifolia* Lam. | Berberidaceae | AM | - | - |
| | *Berberis linearifolia* Phil. | Berberidaceae | AM | - | - |
| | *Chusquea culeou* Desv. | Gramineae | AM | - | - |
| | *Desfontainia spinosa* R. et P. | Desfontainiaceae | AM | - | - |
| | *Drimys andina* (Reiche) R.A. Rodr. et Quez. | Winteraceae | AM | - | - |
| | *Fuchsia magellanica* Lam. | Onagraceae | AM | - | - |
| | *Gaultheria phyllireifolia* (Pers.) Sleumer | Ericaceae | ER | - | - |
| | *Griselinia ruscifolia* (Clos.) Taub | Cornaceae | AM | - | - |
| | *Myoschilos oblonga* R. et P. | Santalaceae | AM | - | - |
| | *Myrceugenia parvifolia* (DC.) Kausel | Myrtaceae | AM | - | - |
| | *Ovidia pillo-pillo* (Gay) Meisn. | Thymeleaceae | AM | - | - |
| | *Ribes magellanicum* Poir. | Saxifragaceae | AM | - | - |

Tab. 2 (Continued)

| Plant type | Scientific name | Family | Ecosystem[a] | | |
|---|---|---|---|---|---|
| | | | EF | DF | GR |
| Climbers and epiphytics | *Asteranthera ovata* (Cav.) Hanst. | Gesneriaceae | AM | - | - |
| | *Campsidium valdivianum* (Phil.) Skottsb. | Bignoniaceae | AM | - | - |
| | *Fascicularia bicolor* (N. et Z.) | Bromeliaceae | N | - | - |
| | *Hydrangea integerrima* Engl. | Hydrangeaceae | AM | - | - |
| | *Hydrangea serratifolia* (H. et A.) F. Phil. | Hydrangeaceae | AM | - | - |
| | *Luzuriaga radicans (*R. et P.) | Philesiaceae | AM | - | - |
| | *Mitraria coccinea* Cav. | Gesneriaceae | AM | - | - |
| Ferns | *Asplenium dareoides* A.N. Desv. | Aspleniaceae | N | N | - |
| | *Blechnum hastatum* Kaulf. | Blechnaceae | AM | - | - |
| | *Blechnum blechnoides* (Keyserl.) | Blechnaceae | AM | AM | - |
| | *Blechnum chilense* (Kaulf.) Mett. | Blechnaceae | AM | - | - |
| | *Hymenophyllum pectinatum* Cav. | Hymenophyllaceae | N | - | - |
| | *Hymenophyllum* sp. | Hymenophyllaceae | N | - | - |
| | *Hypolepis poeppigii* (Kunze) R.A. Rodr. | Dennstaedtiaceae | AM | - | - |
| | *Lophosoria quadripinnata* (J.F. Gmel.) C. Chr. | Lophosoriaceae | AM | - | - |
| Herbs | *Acaena ovalifolia* R. et P. | Rosaceae | - | - | AM |
| | *Achillea millefolium* L. | Asteraceae | - | - | AM |
| | *Agrostis capillaris* L. | Poaceae | - | - | AM |
| | *Dichondra sericea* Sw. | Convolvulaceae | - | - | AM |
| | *Dysopsis glechomoides* (Rich.) Muell. Arg | Euphorbiaceae | AM | - | - |
| | *Fragaria chiloensis* (L.) Duch. | Rosaceae | - | - | AM |
| | *Greigea landbeckii* (Lechlerex Phil.) Phil. Ex F. Phil. | Bromeliaceae | AM | AM | - |
| | *Holcus lanatus* L. | Poaceae | - | - | AM |
| | *Hypericum perforatum* L. | Clusiaceae | - | - | AM |
| | *Hypochaeris radicata* L. | Asteraceae | - | - | AM |
| | *Juncus procerus* E. Meyer | Juncaceae | - | - | N |
| | *Loasa sclareifolia* Juss. | Loasaceae | N | - | - |
| | *Lotus uliginosus* Schkuhr. | Fabaceae | - | - | AM |
| | *Medicago* sp. | Fabaceae | - | - | AM |
| | *Mentha piperita* L. | Labiatae | - | - | AM |
| | *Mentha spicata* L. | Labiatae | - | - | AM |
| | *Nertera granadensis* (Mutis ex R.f) Druce | Rubiaceae | AM | - | - |
| | *Osmorrhisa chilensis* H. et A. | Apiaceae | AM | - | - |
| | *Plantago lanceolata* L. | Plantaginaceae | - | - | AM |
| | *Poa annua* L. | Poaceae | - | - | AM |
| | *Prunella vulgaris* L. | Lamiaceae | - | - | AM |
| | *Ranunculus minutiflorus* Bert. Ex Phil. | Ranunculaceae | - | - | AM |
| | *Rumex acetosella* L. | Polygonaceae | - | - | N |
| | *Senecio vulgaris* L. | Asteraceae | - | - | AM |
| | *Solanum* sp. | Solanaceae | AM | - | - |
| | *Taraxacum officinale* Weber | Asteraceae | - | - | AM |
| | *Trifolium pratense* L. | Fabaceae | - | - | AM |
| | *Trifolium repens* L. | Fabaceae | - | - | AM |
| Nr. total | | | 53 | 11 | 22 |
| Nr. species with Arbuscular Mycorrhiza (AM) | | | 41 | 6 | 20 |
| Nr. species without mycorrhizas (N) | | | 9 | 2 | 2 |
| Nr. species with EC and ER Mycorrhizas | | | 3 | 3 | 0 |

[a]AM: arbuscular mycorrhizal; N: Non-Mycorrhizal; EC: Ecto-Mycorrhizal; ER: Ericoid Mycorrhizal; (-) Species absent

### AMF species and distribution in ecosystems

In total, 39 AMF species could be distinguished on the basis of morphological criteria (Tab. 3) from the sampling in autumn; the additional sampling in late spring did not result in more AMF species but some of the species could only be identified after having obtained additional specimen from the second sampling. Thirty four species were identified unequivocally according to descriptions in the literature. Short descriptions of the 5 unknown AMF species are given in Tab. 4. The major number of species of AMF was observed in the grassland (29), followed by the evergreen forest (20), and the deciduous forest with only 14 species. In the three ecosystems studied, 13 *Acaulospora* spp., 4 *Archaeospora* spp., one *Diversispora* sp., 3 *Entrophospora* spp., 10 *Glomus* spp., 3 *Pacispora* spp., one *Paraglomus* sp., and 4 *Scutellospora* spp. were isolated. No *Gigaspora* sp. was observed in any sampling.

In the total study area a third of the AMF species belonged to the genus *Acaulospora*, and a quarter to the genus *Glomus*, the rest being of other genera. While this relative distribution in number of species in these two genera was more or less the same in the evergreen forest

and the deciduous forest, there was a strong deviation in the grassland ecosystem where more than 40% of the species belonged to *Acaulospora* and only 21% to *Glomus*. Species of *Paraglomus* and *Scutellospora* were not present in any of the forest ecosystems. Four AMF species were found in all the three ecosystems: *Acaulospora alpina*, *A. mellea*, *G. etunicatum* and *G. macrocarpum*. Many of the other species appeared to be highly specialized, hence occurring in only one of the three ecosystems.

The total number of AMF spores was highest in the evergreen forest with 3164 spores per 100 g dry soil, significantly lower in the grassland with 1001 spores and significantly lower still in the deciduous forest with 456 spores. Counting the spores of each individual AMF species was laborous because some species have very similar spore morphology, making difficult their differentiation under a stereo-microscope at low magnification. Therefore, for comparing the relative occurrence of spores of the AMF genera in the three ecosystems we pooled all spore counts of the species of each genus (Fig. 1). While in the evergreen forest the number of *Acaulospora* and *Glomus* spores were each about one-third of the total spore number, the relative number of *Acaulospora* spores decreased in the deciduous forest and increased in the grassland ecosystem. By contrast, the relative numbers of *Glomus* spores increased in the deciduous forest and decreased in the grassland. *Scutellospora* spores were only present in the grassland. Noteworthy was also the relatively higher spore number of *Entrophospora* in the two forest ecosystems than in the grassland.

**Tab. 3:** Genera and species of the Glomeromycota found in the evergreen forest (EF), deciduous forest (DF) and grassland (GR) ecosystems in Southern Chile.

| Genus | Species name | Ecosystem[a] | | |
|---|---|---|---|---|
| | | EF | DF | GR |
| *Acaulospora* | *Acaulospora alpina* Oehl, Sykorova & Sieverd. | + | + | + |
| | *Acaulospora cavernata* Blaszk. | - | - | + |
| | *Acaulospora colossica* P.A. Schultz, Bever & J.B. Morton | + | - | + |
| | *Acaulospora dilatata* J.B. Morton | + | - | + |
| | *Acaulospora koskei* Blaszk. | + | - | + |
| | *Acaulospora laevis* Gerd.&Trappe | + | - | + |
| | *Acaulospora longula* Spain & N.C.Schenck | - | + | + |
| | *Acaulospora mellea* Spain & N.C. Schenck | + | + | + |
| | *Acaulospora paulinae* Blaszk. | - | - | + |
| | *Acaulospora scrobiculata* Trappe | - | - | + |
| | *Acaulospora spinosa* C. Walker & Trappe | + | - | - |
| | *Acaulospora thomii* Blaszk. | - | - | + |
| | *Acaulospora* sp. A | - | + | + |
| *Archaeospora* | *Archaeospora leptoticha* (N.C. Schenck & G.S. Sm.) J.B. Morton & D. Redecker | - | + | - |
| | *Archaeospora trappei* (R.N. Ames&Linderman) J.B. Morton & D. Redecker emend Spain | + | - | + |
| | *Archaeospora* sp. A | - | + | + |
| | *Archaeospora* sp. B | + | - | + |
| *Diversispora* | *Diversispora spurca* (C.M. Pfeiff., C. Walker & Bloss) C. Walker & A. Schüssler | + | - | + |
| *Entrophospora* | *Entrophospora baltica* Blasz. | - | + | - |
| | *Entrophospora infrequens* (I.R. Hall) R.N. Ames & R.W. Schneid. | + | + | - |
| | *Entrophospora schenckii* Sieverd. & S. Toro | + | - | + |
| *Glomus* | *Glomus brohultii* R.A. Herrera, Ferrer & Sieverd. | - | - | + |
| | *Glomus claroideum* N.C. Schenck & G.S. Sm. emend C. Walker & Vestberg | + | + | - |
| | *Glomus diaphanum* J. B. Morton & C. Walker | - | - | + |
| | *Glomus etunicatum* W.N Becker & Gerd. | + | + | + |
| | *Glomus fasciculatum* (Thaxt.) Gerd. & Trappe emend. C. Walker & Koske | - | + | - |
| | *Glomus geosporum* (T.H. Nicolson & Gerd.) C. Walker | + | - | + |
| | *Glomus invermaium* I.R. Hall | + | - | - |
| | *Glomus laccatum* Blaszk. | - | - | + |
| | *Glomus macrocarpum* Tul.& C. Tul. | + | + | + |
| | *Glomus rubiforme* (Gerd. & Trappe) R.T. Almeida & N.C. Schenck | + | + | - |
| *Pacispora* | *Pacispora dominikii* (Blaszk.) Oehl & Sieverd. | - | - | + |
| | *Pacispora* sp. A | + | - | - |
| | *Pacispora* sp. B | + | + | - |
| *Paraglomus* | *Paraglomus occultum* (C. Walker) J.B. Morton & D. Redecker | - | - | + |
| *Scutellospora* | *Scutellospora auriglobosa* (I.R. Hall) C. Walker & F.E. Sanders emend. C. Walker & I.R. Hall | - | - | + |
| | *Scutellospora calospora* (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders | - | - | + |
| | *Scutellospora dipurpurascens* J.B. Morton & Koske | - | - | + |
| | *Scutellospora weresubiae* Koske & C. Walker | - | - | + |
| Nr. of species | | 20 | 14 | 29 |

[a] (-): absence or (+): presence of arbuscular mycorrhizal fungal species.

**Tab. 4:** Morphological characteristics of spores of non-identified arbuscular mycorrhizal fungal species in Southern Chilean forest ecosystems

| Species | Characteristics of spores |
|---|---|
| *Acaulospora* sp. A | Yellow to light brown, globose spores, 100-125 µm in diam., with 2-2.5 µm thick yellow outer wall which is ornamented with pits, 1-2 µm deep and 2-5 µm wide. |
| *Archaeospora* sp. A | Subhyaline to dull cream yellow, globose to subglobose spores, 100-120 x 150-160 µm in diam., with a 1-3 µm thick outer wall which is ornamented with a pentagonal to hexagonal reticulum, openings 5-10 x 10-20 to 27-37 x 25-50 µm wide, and ridges about 2-3 µm wide and 2-3 µm high; germinal wall 3-layered and 1-2.5 µm thick. |
| *Archaeospora* sp. B | Dull white globose spores, 130-180 µm in diam., with a up to 5 µm thick outer wall which is ornamented with tiny thin spines. The next (germinal) wall is ornamented with a crenulate (pitted wave-like) structure and is 3-6 µm thick. |
| *Pacispora* sp. A | Light yellow to orange yellow, globose to subglobose spores, 100-125 µm in diam. with a 3-layered outer thin wall and a 3-layered inner wall. Outer layer ornamented with edged warts 5-10 µm broad at the bases, and up to 7.5 µm high and formed in a distance of 5-7 µm to each other. |
| *Pacispora* sp. B | Yellow to yellow orange subglobose tear like spores, 87-105 x 125-150 µm in diam. with a 3-layered outer wall and a 3-layered inner wall. The outer wall is hyaline to white and 4-5 µm thick in total. The inner wall is light yellow and has a reticulate ornamentation on the outer layer; the openings of the reticulum are 5-7.5 µm wide and 2.5 µm deep. |

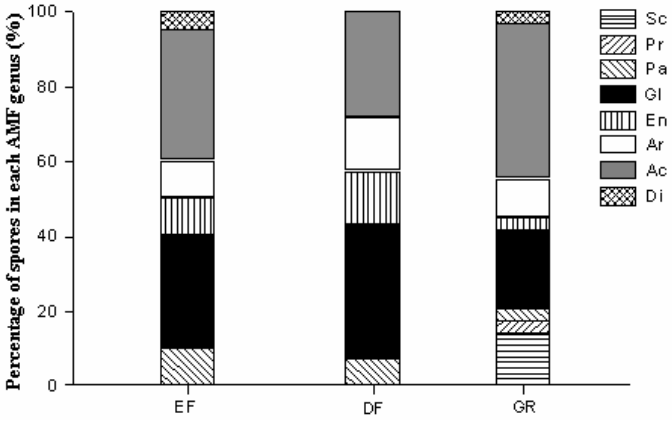


**Fig. 1:** Relative number of spores belonging to the different arbuscular mycorrhizal fungal (AMF) genera, in the evergreen forest ecosystem (EF), deciduous forest ecosystem (DF) and grassland ecosystem (GR). Total spore number in each ecosystem = 100%. Fungal genera are: Sc: *Scutellospora*; Pr: *Paraglomus*; Pa: *Pacispora*; Gl: *Glomus*; En: *Entrophospora*; Ar: *Archaeospora*; Ac: *Acaulospora*; Di: *Diversispora*.

## Discussion

It was not surprising that the evergreen forest had a higher plant species diversity than the grassland vegetation and the secondary deciduous forest (Tab. 2). The main timber producing trees of both the forest ecosystems formed ectomycorrhiza. Trees of the Fagaceae are known EC species also in the temperate zones (SMITH and READ, 1997). Myrtaceae can form dual associations of EC and AM (http://www.ffp.csiro.au/research/mycorrhiza/ozplants.html, 2005); the two Myrtaceae *Amomythus* and *Myrceugenia* were AM in our study. In both the forest ecosystems the relative number of non-mycorrhizal tree species was about the same. The non-mycorrhizal Proteaceae were only found in the evergreen forest and not in the deciduous forest, however. The understorey vegetation in the evergreen forest was almost completely AM with the exception of an epiphyte and 3 ferns which were non-mycorrhizal. The understorey vegetation was poor in the deciduous forest.

The natural grassland had a diverse plant species community which was somewhat lower in number of plant species than that of temperate natural grasslands in regions of Europe and North America where similar studies on the mycorrhizal status were performed earlier (e.g. READ and HASELWANDTER, 1981; MILLER, 1987). Of the two non-mycorrhizal plant species found was *Rumex acetocella* (Polygonaceae) reported earlier to be non-mycorrhizal (HARLEY and HARLEY, 1987) although other *Rumex* spp. can be AM. *Juncus procerus* was non-mycorrhizal; other Juncaceae can form AM (SILVA et al., 2001).

Even though ectomycorrhizal tree species represented the main trunk area per ha in both the forest ecosystems, a surprisingly high number of AM plant species could survive even in view that there must have been competition between EC and AM in the soil. Strong dominance of ectomycorrhizal hyphal networks in the soil can be detrimental for the establishment of AM understorey species (BOOTH, 2004) which may be applicable also for the Chilean deciduous forest with a higher number of trunks of EC trees per ha. In the Chilean evergreen forest, however, many of the AM understorey species grow directly in the crown area of the dominant ectomycorrhizal *N. dombeyi* trees. This may indicate that in the evergreen forest with a relative lower number of stems of EC trees per ha, the competition between the two mycorrhizal types was low.

The deciduous forest had a significantly lower AMF spore concentration and lower diversity of AMF species (Tab. 3) than the evergreen forest or the grassland. It is not likely that the slight differences in soil chemical properties between the two forest systems, such as increased organic matter in the deciduous forest as compared to the evergreen forest, have much to do with the ecosystem differences in the AMF communities. This is because the soil characteristics (Tab. 1) of the deciduous forest were very similar to those of the grassland ecosystem which had a high AMF diversity. Hence, the reason for the differences in the AMF populations must be related to the AM plant species community which was proportionally highest in the grassland followed by the evergreen forest and finally the deciduous forest, respectively. We relate the low number of AM plant species and AMF species in the deciduous forest to the dominating presence (in terms of stem number per ha) and competition of EC tree species.

Thirty-nine species of the Glomeromycota were found at the research location, 13% of them had not been previously described to our knowledge. This is not a surprisingly high number of unknown species, given that this was the first survey of AMF species in Southern

Chile and given that so far only about 170 AMF species have been described worldwide in the phylum Glomeromycota (INVAM; see: www.invam.caf.wvu.edu). OEHL et al. (2003) detected a total of 45 AMF species in 8 field sites in agro-ecosystems in Central Europe, of which about 20% were not previously described. The relative number of tentatively new species in our study was thus about the same. We identified the AMF species on the basis of their spore morphology and did not apply molecular biological tools to detect them in the roots. The identification method on the basis of spore morphology is appropriate for a survey of AMF in a region, as there is no reason to believe that molecular biological tools are more efficient in detecting AMF species in field soils. Both the methods have disadvantages as explained by SANDERS (2004). Mycorrhizal species may seasonally sporulate (PRINGLE and BEVER, 2002). We believe that we discovered most of the AMF species present in the ecosystems at the study location because the times of sampling corresponded to the end of the growing season (autumn) and to a warm season at the end of the spring.

In both the forest ecosystems *Acaulospora* spp., *Glomus* spp. and the rest represented each about one third of the relative species number. *Acaulospora* spp. dominated in the grassland ecosystem in Southern Chile which is in contrast to European grasslands where *Glomus* spp. clearly represent the majority of the AMF community (BLASZKOWSKI, 1993; OEHL et al., 2005). The relative proportion of spores of *Entrophospora* spp. was higher in the forest ecosystems than in the grassland, and there is another study (JOHNSON and WEDIN, 1997) reporting that *Entrophospora* spp. were only formed in a native tropical forest ecosystem and not in grasslands. To our knowledge no reliable information is available about the occurrence of AMF species in deciduous forests, anyway.

Only 4 of the 39 AMF species were found in all the three ecosystems, and thus can be considered able and fit to survive and form spores under very diverse conditions. These 4 fungal species must be considered ecological generalists. *Glomus etunicatum* is known as such a generalist from European studies (BLASZKOWSKI, 1993; OEHL et al., 2004) and *G. macrocarpum* is frequent in European grasslands and elsewhere in the world. The other two generalists were *Acaulospora* spp. which is not surprising as species of this genus occur frequently in acidic tropical soils (SIEVERDING, 1989). *Acaulospora alpina* is known to occur at high mountainous regions of the Swiss Alps (OEHL et al., 2006). The number of species found in only one of the three ecosystems investigated was relatively high with 19 AMF species. Also other studies from European grasslands and agroecosystems (OEHL et al., 2004; 2005) and from tropical ecosystems (JOHNSON and WEDIN, 1997) report that some fungal species occur only under specific ecological conditions. As yet it is not possible to relate their occurrence to specific ecological factors. However, it is an interesting finding of our study that *Paraglomus* and all *Scutellospora* spp. were only isolated from the grasslands. These grassland plots were in the neighborhood of the forests so that a wider distribution could have been expected. *Paraglomus occultum* and *Scutellospora* spp. were often reported from grassland savanna areas with acidic soils from Colombia and Venezuela (SIEVERDING and TORO, 1986; HERRERA-PEDRAZA et al., 2001). We assume that *Paraglomus* and *Scutellospora* spp. prefer open, un-shaded areas like grasslands.

It is as yet unknown what function the different AMF species play in each of the investigated ecosystems. It is assumed that a high diversity of AMF species is likely to be more beneficial for a plant community than a low number (VAN DER HEIJDEN et al., 1998). Whether or not the low plant species diversity and low number of AM plant species in the deciduous forest ecosystem was related to the relatively low fungal species diversity remains unknown. It is known, however, that a diverse fungal population is very helpful in the establishment of seedlings of AM forest species (LANDIM, 2003).

We can conclude from this study that the only way to maintain a high plant species diversity with its associated AM plant species and AMF community is by conserving the native rainforest ecosystem. Grasslands with a broad plant species community appear to be good alternatives to the native evergreen rainforests in conserving a high biodiversity of AMF but it is clear that this is at the expense of the forest vegetation diversity. The deciduous secondary forest with an almost mono-specific *N. alpina* cover appears to be economically attractive in the first view but results in an ecological degraded ecosystem with low biodiversity, including low diversity of AMF species. Forest management practices should aim to increase the diversity of tree species of the secondary forest with the aim also to increase the soil microbiological resources like AMF communities.

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