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# High winter diversity of arbuscular mycorrhizal fungal communities in shallow and deep grassland soils

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ABSTRACT

The identity and diversity of the arbuscular mycorrhizal fungal (AMF) symbionts have a large impact on ecosystem functioning and stability, indicating the need to assess their distribution in terrestrial environments. Four temperate grassland sites of low to medium fertility located on a common soil were sampled in winter and summer at two different depths (0–40 cm, 40–80 cm) using a spatially intensive experimental sampling design. Arbuscular mycorrhizal fungal community composition was determined via the amplification of AMF rRNA gene fragments present in fine roots combined with terminal-restriction fragment length polymorphism (T-RFLP) and sequencing analyses. For one out of three endonucleases applied, a site × depth interaction on T-RF richness ($S$) and Shannon's diversity index ($H'$) was observed, indicating differences between sites in the upper soil layer and depth effects on $S$ and $H'$ for the extensively managed, low fertility site. The $S$ and $H'$ of the AMF taxa present in fine plant roots were marginally lower in winter than in summer (−14% and −16% for $S$ and $H'$, respectively). Evenness $E$ did not vary as a function of site, season or depth. The AMF community profiles, as determined by nonmetric multidimensional scaling, differed between sites and seasons, but not among soil depths. The intersite AMF community variations were attributed to niche differentiation based on soil phosphorus availability and pH. Seasonal shifts could not be related to variations in root densities or physico-chemical soil properties measured in this study, suggesting climate and plant regulation as major processes responsible for the variations between winter and summer AMF communities. It is concluded that diverse winter AMF communities are present in temperate grassland soils, and that plant roots colonizing subsoils harbour similar and equally diverse AMF assemblages than those present in topsoil layers.


## 1. Introduction

Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil microbes from the phylum *Glomeromycota* that develop a mutualistic symbiosis with the great majority of land plants (Smith and Read, 2008). Arbuscular mycorrhizal fungi transfer inorganic nutrients and water to the plant and receive carbohydrates in exchange. Soil biogeochemical nutrient cycling (Veresoglou et al., 2011), soil carbon sequestration (Rillig et al., 2001), and plant nutrient and water status (Corkidi et al., 2002; Egerton-Warburton et al., 2007b) are impacted by AMF communities. A major advantage of the arbuscular mycorrhizal symbiosis for plants lies in acquiring phosphorus (P) as AMF provide a very effective pathway by which P is scavenged from large volumes of soil and rapidly delivered to cortical cells within the root (Smith et al., 2011; Smith and Read, 2008). Therefore, the identity and diversity of the mycorrhizal fungal symbionts have a large impact on ecosystem functioning and stability (van der Heijden et al., 1998), indicating the importance of assessing their distribution in P-limited environments.

The identification and taxonomy of the *Glomeromycota* have traditionally been based on spore morphology (Egerton-Warburton et al., 2007a; Oehl et al., 2003), but this method has limitations

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such as species and environment dependent degree of sporulation, spore accumulation over time and erroneous distinctions of AMF genera (Redecker et al., 2003; Schwarzott et al., 2001; Young, 2012). Therefore, a set of DNA-based identification methods, each having their advantages and shortcomings, have been developed to complement morphological studies (Dumbrell et al., 2011; Krüger et al., 2012, 2009; Mommer et al., 2011). The majority of the recent molecular works in grassland ecosystems focused on AMF communities in topsoil layers, seasonal variations in AMF communities along the plant growing season, and the underlying biotic and abiotic conditions that affect AMF distribution. Studies from temperate grasslands with low nutrient inputs have revealed an especially high AMF biodiversity (Lumini et al., 2010), with plant P availability being inversely related to AMF species richness (Covacevich et al., 2007). In addition to niche differentiation based on soil physico-chemical characteristics, climate conditions are also a primary determinant for the AMF community (Dumbrell et al., 2011, 2010; Hazard et al., 2012; Lekberg et al., 2012). High soil moisture contents and low temperatures have, for instance, been indicated to reduce AMF growth (Gavito et al., 2003; Helgason and Fitter, 2009). Likewise, there are indications that plant diversity or functional traits of host plants structure AMF communities (König et al., 2010; van de Voorde et al., 2010). While some studies indicated AMF community composition shifts across the plant growing season (Vandenkoornhuyse et al., 2002; Yang et al., 2010), other works found a more temporally stable AMF community (Santos-Gonzalez et al., 2007). These observations demonstrate the complex interplay of abiotic and biotic factors structuring AMF communities across time.

Yet, there is still a limited understanding on (1) AMF assemblages that are present in the deeper soil layers, and (2) winter AMF communities. Preliminary evidence disclosed a potential diverse and abundant winter AMF community in deep soil layers of grassland ecosystems. In spite of root density decreases with soil depth, AMF spore research pointed towards an extensive AMF community in the 50–70 cm soil layers (Oehl et al., 2005). Nevertheless, as spores may persist a prolonged time in soils, it remains unclear to what extent these results compare with a more dynamic picture of the AMF community determined using molecular techniques. After all, fungal spores may percolate through the soil profile as a function of time leading to their increased presence in deeper soils (Baumgartner et al., 2011). With respect to winter AMF communities, Dumbrell et al. (2011) recently indicated the occurrence of distinct, but equally diverse, winter and summer AMF communities in temperate grassland soils of the United Kingdom using pyrosequencing techniques. A temporally changing supply of host-plant carbon due to seasonal variations in climate was suspected be the driving force in regulating the dynamics of AMF communities (Dumbrell et al., 2011). Earlier spore surveys also documented no shifts in AMF richness, diversity and evenness between summer and winter communities in temperate grasslands, in spite of seasonal AMF species transition (Escudero and Mendoza, 2005; Lugo and Cabello, 2002). Nevertheless, more efforts using molecular techniques are required to gain a deeper understanding in species composition and dynamics of winter communities in deep soil layers of grassland ecosystems.

In this study, we analysed winter and summer AMF communities present in the plant roots of four grassland sites at two different depths (0–40 cm, 40–80 cm) using a spatially intensive experimental sampling design. We selected grassland sites of low to medium fertility located on a common Andisol, characterized by a high P adsorption capacity. The study sites are located in southern Chile and have a temperate rainy climate ($\sim$2500 mm year$^{-1}$) with Mediterranean influences, leading to wide seasonal variations in rainfall amounts. Four grassland sites with a different historical and present land management regime were selected in order to investigate the underlying mechanisms on how land use practices affect AMF communities at different soil depths and seasons. Arbuscular mycorrhizal fungal communities were determined via the amplification of rRNA gene fragments specific for AMF communities and terminal restriction fragment length polymorphism (T-RFLP) analyses present in plant root material. We hypothesized that (1) plant P availability is a major variable explaining intersite variations in AMF diversity and AMF community structure leading to a reduction in AMF diversity as land management intensity increases, (2) roots from deeper soil layers have a reduced diversity in rRNA gene fragments specific for AMF communities than roots from the more shallow soil layer, and (3) winter AMF communities are less diverse than summer communities as high winter soil moisture contents reduce mycorrhizal plant colonization.

## 2. Materials and methods

### 2.1. Study sites

Four grassland sites with different long-term management histories (NPK + FYM, NPK fertilization + farmyard manure application; NPK, NPK fertilization; GACR, grass-arable crop rotation; EXT, extensive management, see below for details) were selected at the *Experimental Centre of Agricultural Fields* (39°47′S, 73°12′W) of the *Universidad Austral de Chile*. All sites are located in close proximity for which factors, other than site management, that affect soil formation (parent material, topography, climate, time) did not significantly differ between the sites. The soil is classified as a Typic Hapludand of the soil series Valdivia. The experimental research station is located in the temperate rainy climate region of south-central Chile. Average air temperatures in the region are 17 °C and 9 °C in the Austral summer (October–March) and Austral winter (April–September), respectively, while average total precipitation amounts to ca. 2500 mm year$^{-1}$, typically concentrated in the winter months (Dirección General de Aeronáutica Civil, 2001).

Long-term records (>30 year) for the first grassland site (NPK + FYM, ±4 ha) indicate that prior to the study it had been fertilized annually with inorganic fertilizers (60–80 kg urea-N, 80–100 kg $P_2O_5$, and 0–40 kg $K_2O$ ha$^{-1}$ year$^{-1}$) supplemented with annual applications (at variable rates) of farmland manure. Dairy cows have rotationally grazed the site, with an average stocking rate of 1.5 cows ha$^{-1}$, and there are no records of tillage activities. The second study site (designated NPK, also ca. 4 ha) had received identical, annual NPK fertilization to NPK + FYM, but there are no records of manure applications at this site. Similarly to the NPK + FYM site, dairy cows had been rotationally grazed at the NPK site, at an average stocking density of 1.5 cows ha$^{-1}$. The third site (designated GACR, ca. 3 ha) has been under grass-arable rotation (grassland rotated with cereal crops once every five years, sowing with *Lolium perenne* and *Agrostis capillaris* in equal ratios after each cereal harvest) for 30 years. Inorganic fertilizers (60–80 kg urea-N, 80–100 kg $P_2O_5$ and 40 kg $K_2O$ ha$^{-1}$) have been applied solely in sowing years. While under grass the site has been mown once a year for silage, and then grazed by dairy cows. At the beginning of the experiment reported here, the site had been under grass for five years. The fourth site (designated EXT, ca. 20 ha) supported permanent grassland that had never been sown, tilled or fertilized and was used for sheep grazing at a stocking rate of 0.5–1 sheep ha$^{-1}$. The only external N inputs at the site originated from cloud and rain water, and have been estimated to vary between 1 and 8 kg N ha$^{-1}$ year$^{-1}$ (Weathers and Likens, 1997).

### 2.2. Sampling

At each site, soil and root samples were taken in the Austral winter (August 2010) and summer (January 2011) period. At each site, a 1 ha